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# INFLUENCE OF WEED CONTROL METHODS AND PLANT SPACING ON THE YIELD OF WHITE JUTE (*CORCHORUS CAPSULARIS*)

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**ABSTRACT**
A field experiment was conducted at Sher-Bangla Agricultural University, Dhaka during April to August, 2009 with a view to find out the influence of weed control methods and plant spacing on the yield of white jute (var.CVL-1). The experiment consisted of four weed control methods *viz.* two times hand weeding with one raking, herbicide Whip Super 9 EC (Fenoxaprop-P-ethyl: $C_{18}H_{16}ClNO_5$) application at 15 DAS, two times hand weeding at 20+40 DAS and three times hand weeding at 15+30+45 DAS and four plant spacing *viz.* 20 cm x 10 cm, 25 cm x 10 cm, 30 cm x 10 cm (20, 25 and 30 rows with plants spaced at 10 cm intervals in the row) and broadcasting. The dominant grass weeds were *Cynodon dactylon* (43%), *Echinochloa colonam* (29%) and *Eleusine indica* (22%). Results showed that plant spacing differed significantly and 25 cm x 10 cm spacing gave highest (3.12 t $ha^{-1}$) fibre yield which was statistically similar with 20 cm x10 cm. Two times weeding and one raking gave highest (3.12 t $ha^{-1}$) fibre yield which was statistically similar with herbicide application (2.97 t $ha^{-1}$). Interaction effect showed highest fiber yield (4.02 t $ha^{-1}$) was obtained from 20 cm x 10 cm spacing with herbicide application. Whip Super 9 EC @ 615 ml $ha^{-1}$ effectively controlled the grass weeds providing higher fibre yield and net 7.13 Taka return per Taka invested whereas 6.51, 5.18 and 5.34 Taka from two times hand weeding with one raking, two times hand weeding and three times hand weeding respectively.

**Keywords**: Jute, Weed control methods, Plant spacing, Herbicide

## INTRODUCTION

Jute (*Corchorus* sp) is a natural long, soft, shiny vegetable fibre that can be spun into coarse, strong threads. It is produced from plants in the genus *Corchorus*, belonging to Malvaceae. Jute is considered as the main cash crop of Bangladesh. Its influence on ecology and economy is so intimate that it's effects are significantly related to the agro-ecology and the socio-economic life of the people. The suitable climate for growing jute (warm and wet climate) is the monsoon season.

Cultural practices are important management factors that affect the yield of a crop. The hot and humid climate coupled with intermittent rainfall during the jute-growing season, however, encourages weed growth resulting in severe crop-weed competition (Saraswat, 1999); yield losses may be up to 75 to 80% (Sahoo and Saraswat, 1988). Weeding is one of the most important cultural practices for the crop plants to take nutrients, moisture, light, space and sometimes controlling many diseases, organisms and insect pest (Alam *et al.*, 2010). But, the most effective and economic cultural practices for weed control in jute crops are not clearly known by our farmers. In Bangladesh, weeds are generally controlled by raking and niri (hand weeding) and weeding and thinning operations involve about 50% or more of the labour cost (Alam, 2003). Grasses constitute the dominant weed flora in jute fields and its management using pre-emergence herbicides is possible (Sarkar *et al.*, 2005), provided the farmers get sufficient time for land preparation and herbicide application before sowing.

Plant density is an important yield contributing factor which can be manipulated in jute to attain higher fibre production per unit area. The yield of many crops is known to be positively correlated with the number of plants per unit area in the field. If the plant population is lower or higher than the optimum, the final output is adversely affected. In order to obtain required plant density, one of the major yield components of jute is optimum seed rate, resulting in proper spacing to maintain the uniformity of stand for better growth and development of plant. Keeping all the points in mind mentioned above, the present piece of research work was under taken with the following objectives:

- Identify the optimum population density on the yield of jute
- Study the effect of weed control methods on the yield of jute
- Find out the interaction effect (if any) of population density and weed control methods on the yield of jute

## MATERIALS AND METHODS

The experiment was conducted at the Agronomy field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during April to July, 2009. The soil of the experimental field belongs to the Shallow Red Brown Terrace Soils. Jute variety CVL-1 was used as the test crop. Two sets of treatments included in the experiment were as follows: A. Factor: weed management (4) $W_1$=Recommended practices recommended by Bangladesh Jute Research Institute (2W+1R), $W_2$=1 herbicide application (2-4 leaf stage of weed), $W_3$=2 weeding (20+40 DAS) and $W_4$=3 weeding (15+30+45 DAS) B. Factor: Plant spacing (4) $S_1$=Line sowing (20 cm x 10 cm), $S_2$=Line sowing (25 cm x 10 cm), $S_3$=Line sowing (30 cm x 10 cm), and $S_4$=Broadcasting. The experiment was laid out in a split plot design with three replications having spacing in the main plots, weeding in the sub-plots. There were 16 treatments combinations. The total numbers of unit plots were 48. The size of unit plot was 4 m x 3 m = 12 $m^2$. The plots were fertilized with the N, $P_2O_5$, $K_2O$ and S at the rate of 76.50, 10.26, 18.00 and 8.09 Kg $ha^{-1}$ respectively. One-third of N and other fertilizers were broadcasted during the time of final land preparation. The remaining two-thirds of N were top dressed in two equal splits on 20 and 35 days after sowing. Respective seed were sown on 6 April, 2009 by following different line sowing and broadcasting method. The seed rate was 7 kg $ha^{-1}$. At harvest each 2 $m^2$ area of one sample was harvested from each plot leaving adequate border for recording data on plant height, top, middle and base diameter of the plants. The plant height and diameters were recorded from 10 randomly selected plants with the help of bamboo scale and slide calipers, respectively. Prior to every harvest ten randomly selected plants from each unit plot were collected to take note of yield components. Two quadrat areas, each measuring 3 rows and 50 cm length along the row were separately harvested from each plot to record plant fibre-stick ratio and harvest index. The plants of the plots were harvested on 21 July. The yields from 10 plants and quadrats were added to the final yield. The data collected on different parameters were statistically analyzed to obtain the level of significance using the MSTAT-C. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

## RESULTS AND DISCUSSION

### Agronomic Performance

Effect of spacing

The plant height, base, middle and top diameter, fibre yield and stick yield were significantly influenced by different spacing (Table 1). Significantly highest fibre yield (3.12 t ha$^{-1}$) and stick yield (7.05 t ha$^{-1}$) were found when spacing was $S_2$ (25 cm x 10 cm). However, as regard to differences in fibre and stick yield between the spacing $S_1$ & $S_2$ were insignificant. Alam *et al.,* (2010) also found similar result.

**Table 1.** Efeect of spacing on plant height, base, middle and top diameter, fibre yield and stick yield of jute

| Treatment | Plant height (m) | Base diameter (mm) | Middle diameter (mm) | Top diameter (mm) | Fibre yield (t ha$^{-1}$) | Stick yield (t ha$^{-1}$) |
|---|---|---|---|---|---|---|
| $S_1$ | 2.73 | 9.38 | 6.78 | 3.83 | 3.09 | 6.16 |
| $S_2$ | 2.69 | 10.02 | 6.63 | 4.27 | 3.12 | 7.05 |
| $S_3$ | 2.69 | 9.88 | 7.07 | 3.23 | 2.37 | 5.63 |
| $S_4$ | 2.10 | 8.47 | 5.38 | 2.72 | 2.62 | 5.95 |
| LSD $_{5\%}$ | 0.1515 | 0.9095 | 0.915 | 0.7680 | 0.5035 | 1.174 |

Effect of weed management

Due to different weed management practice only plant height differed significantly but the diameter of the base, middle and top, fibre yield and stick yield remained unaffected. Highest plant height (2.62 m) was observed from $W_1$ (2W+1R).

**Table 2:** Effect of weed management on plant height, base, middle and top diameter, fibre yield and stick yield of jute

| Treatment | Plant height (m) | Base diameter (mm) | Middle diameter (mm) | Top diameter (mm) | Fibre yield (t ha$^{-1}$) | Stick yield (t ha$^{-1}$) |
|---|---|---|---|---|---|---|
| $W_1$ | 2.62 | 9.20 | 6.62 | 3.78 | 2.97 | 5.65 |
| $W_2$ | 2.61 | 9.64 | 6.54 | 3.56 | 3.12 | 6.75 |
| $W_3$ | 2.46 | 9.55 | 6.53 | 3.23 | 2.34 | 6.41 |
| $W_4$ | 2.52 | 9.35 | 6.16 | 3.48 | 2.76 | 5.96 |
| LSD $_{5\%}$ | 0.1385 | NS | NS | NS | NS | NS |

Effect of spacing and weed management

The plant height, base, middle and top diameter, fibre yield and stick yield were significantly influenced by the combination of different spacing and weed management practice (Table 3). Significantly highest fibre yield (4.02 t ha$^{-1}$) was obtained from the combination of $S_1W_2$ which similar to $S_2W_1$ (3.78 t ha$^{-1}$) and highest stick yield (7.80 t ha$^{-1}$) was observed from $S_2W_1$ which is statistically similar to $S_2W_2$ (7.58 t ha$^{-1}$). Such result was in agreement with those of Hossain *et.al*. (2002).

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**Table 3.** Interaction effect of spacing and weed management on plant height, base, middle and top diameter, fibre yield and stick yield of jute

| Treatment | Plant height (m) | Base diameter (mm) | Middle diameter (mm) | Top diameter (mm) | Fibre yield (t ha$^{-1}$) | Stick yield (t ha$^{-1}$) |
|---|---|---|---|---|---|---|
| $S_1W_1$ | 2.47 | 9.26 | 6.87 | 4.17 | 2.93 | 4.72 |
| $S_1W_2$ | 2.82 | 9.30 | 6.57 | 3.50 | 4.02 | 6.85 |
| $S_1W_3$ | 2.62 | 9.00 | 6.73 | 3.83 | 2.55 | 6.87 |
| $S_1W_4$ | 2.62 | 10.00 | 6.93 | 3.83 | 2.85 | 6.20 |
| $S_2W_1$ | 2.79 | 9.57 | 7.07 | 4.77 | 3.78 | 7.80 |
| $S_2W_2$ | 2.77 | 10.77 | 6.83 | 4.83 | 3.18 | 7.58 |
| $S_2W_3$ | 2.59 | 10.00 | 6.77 | 3.63 | 2.48 | 6.03 |
| $S_2W_4$ | 2.62 | 9.73 | 5.83 | 3.83 | 3.02 | 6.78 |
| $S_3W_1$ | 2.69 | 9.80 | 7.10 | 3.57 | 2.43 | 5.33 |
| $S_3W_2$ | 2.78 | 10.13 | 7.47 | 3.37 | 2.45 | 6.83 |
| $S_3W_3$ | 2.59 | 10.50 | 7.12 | 2.70 | 2.08 | 4.75 |
| $S_3W_4$ | 2.71 | 9.07 | 6.53 | 3.27 | 2.52 | 5.58 |
| $S_4W_1$ | 2.16 | 8.20 | 5.43 | 2.60 | 2.73 | 4.77 |
| $S_4W_2$ | 2.06 | 8.37 | 5.30 | 2.53 | 2.82 | 5.75 |
| $S_4W_3$ | 2.05 | 8.70 | 5.43 | 2.73 | 2.25 | 8.00 |
| $S_4W_4$ | 2.11 | 8.60 | 5.33 | 3.00 | 2.65 | 5.27 |
| LSD $_{5\%}$ | 0.2769 | 1.530 | 1.206 | 1.118 | 0.9562 | 2.718 |

## Economic performance

Gross return was found to be highest (Tk. 257630.55) in the $W_1$ weed management treatment. But in the benefit cost ratio (BCR) this treatment was comparatively higher (7.13) than other weed management practices and also the gross return (Tk. 245037.07) was remarkable. This result is supported by another study as reported by Hossain *et al.*, (2002) and Sarker (2006) who stated that herbicide application effectively controlled grass weeds and gave increased yields with better economic returns.

**Table 4.** Cost of production and benefit cost ratio (BCR) for different weeding management of jute

| Treatment | Cost (Tk. ha$^{-1}$) | | | | | Gross return (Tk. ha$^{-1}$) | | | BCR |
|---|---|---|---|---|---|---|---|---|---|
| | Fixed cost of production | Labor cost | Raking cost | Herbicide cost | Total cost | From fibre | From stick | Total | |
| $W_1$ | 31,790 | 7,000 | 560 | - | 39,560 | 167200.80 | 90429.75 | 257630.55 | 6.51 |
| $W_2$ | 31,790 | 280 | - | 2312.50 | 34382.50 | 159162.30 | 85874.77 | 245037.07 | 7.13 |
| $W_3$ | 31,790 | 7000 | - | - | 38790 | 125400.60 | 75693.05 | 201093.65 | 5.18 |
| $W_4$ | 31,790 | 10500 | - | - | 42290 | 147908.40 | 79846.12 | 227754.52 | 5.34 |

Note: Incase of all weeding method fixed cost was 31790 Tk., 1 Mon= 37.32 Kg., 1 mon Fibre/Bel = 2000 Tk. i.e.,1 ton Fibre price = 2000/37.32x1000= 53590.57 Tk., 1 mon Stick= 500 Tk. i.e., 1 ton Stick = 500/37.32x1000= 13397 Tk.

The dominant grass weeds were *Cynodon dactylon* (43%), *Echinochloa colonam* (29%) and *Eleusine indica* (22%).

It can be concluded that plant spacing 25 cm x 10 cm spacing gave highest (3.12 t ha$^{-1}$) fibre yield which was statistically similar with 20 cm x 10 cm. Two times weeding and one raking gave highest (3.12 t ha$^{-1}$) fibre yield which was statistically similar with herbicide application (2.97 t ha$^{-1}$). Interaction effects showed the highest fiber yield (4.02 t ha$^{-1}$) was

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obtained from 20 cm x 10 cm spacing with herbicide application. Whip Super 9 EC @ 615 ml $ha^{-1}$ effectively controlled the grass weeds providing higher fibre yield and net 7.13 Taka return per Taka invested compared to 6.51, 5.18 and 5.34 Taka from two times hand weeding with one raking, two times hand weeding and three times hand weeding respectively.

## REFERENCES

Alam, A. T. M. (2003). Present status and future strategies of weed management in jute field. *Bangladesh J. Jute. Fabc. Res.* 29(1): 4-6.

Alam. A. T. M. M., Hossain, M. S., Islam M. M. Ahmed, I. and Hamidi, M. A. (2010). Effect of weeding and thinning practices on fibre yield and economics of deshi jute. *Bangladesh J. Weed Sci.* 1(1):31-34.

Hossain, F. M. S. , Majumdar, U. K., Bahadur, M. M. and Islam, M. J. (2002). Effect of tillage, raking and weeding on the growth, yield and economics of aus rice. *Pakistan J. Agron.* 1(2-3): 68-70.

Sarkar, S. (2006). Weed management in jute (*Corchorus olitorius* L.) by post emergence herbicides. *J Tropic Agric.* 44(1/2): 71-73.

Sahoo, K.M. and Saraswat, V.N. (1988). Magnitude of losses in the yields of major crops due to weed competition in India. Pesti. Info.14: 2–9.

Saraswat, V.N. (1999). Weed management in jute and jute based cropping system. In: Jute and Allied Fibres Agriculture and Processing, Palit, P., Pathak, S. and Singh, D.P. (eds.), Central Research Institute for Jute and Allied Fibres, Barrackpore. pp. 193–200.

Sarkar, S., Bhattacharjee, A.K., and Mitra, S. (2005). Weed management in jute by Trifluralin (48% EC) in the early jute-weed competition phase. *J. Crop Weed*. 2: 30–33.

# THE EFFECT OF *PHYLLANTHUS VIRGATUS* EXTRACT ON COMMON WEED SPECIES

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**ABSTRACT**

The potential of allelochemicals from *Pyllanthus virgatus* to inhibit plant germination and growth was tested using 10 common weed species. An aqueous extract of *P. virgatus* (leaf/stem/fruit), was mixed with agar to produce a 5% FW/v substrate concentration. Seeds of *Apium leptophyllum*, *Avena fatua*, *Bidens pilosa*, *Chenopodium album*, *Echinochloa crus-galli*, *Lolium rigidium*, *Parthenium hysterophorus*, *Raphanus raphanistrum*, *Sonchus oleraceous* and *Urochloa pannicoides* were placed on the agar and germinated under controlled conditions for 7-16 days. The extract caused a significant reduction in the germination of *A. leptophyllum*, *L. rigidium*, *P. hysterophorus* and *S. Oleraceous* from 100% down to 63.2%, 88.0%, 49.4% and 40.8%, respectively. Shoot growth was significantly reduced by 8.1% to 65.8% in most weed species. Root growth was so severely affected in all species that the survival of seedlings would be unlikely if the extract environment was maintained.

**Keywords**: allelopathy, allelochemicals, natural herbicide, medicinal plants.

## INTRODUCTION

In the search for better weed management strategies, researchers have been investigating the potential of allelopathic interactions to be developed into a natural herbicide. Many interactions investigated to date have been based on field observations, such as limited growth of plants around particular trees, reduction in crop yields when rotations are not practised and the use of cover crops to reduce weeds. As an alternative approach to identifying allelopathic plants i.e., donor plant selection, this research assesses the potential of medicinal plants. Medicinal plants contain an array of bioactive chemicals, which may have multiple actions.

*Phyllanthus sp*. are widely distributed in tropical and sub-tropical areas and have long been used in traditional medicine in countries such as India (Calixto *et al*., 1998), particularly for liver disorders and urinary tract infection (Chaudhary and Rao, 2002). Studies have shown that the *P. virgatus* extracts have strong antioxidant activity and it has been suggested that this activity helps to scavenge harmful free radicles produced by some diseases and medical disorders. The antioxidant activity has been attributed to the phenolic compounds contained in the plants (Kumaran and Karunakaran, 2007). Further work has identified numerous alkaloids, flavonoids, lignans, phenols and terpenes from plants within this genus (Calixto et al., 1998) with five new compounds identified from *P. virgatus* alone, including a norlignan (virgatyne), a tannin (virganin) and three flavonoid sulfonates (Huang et al., 1998).

*Phyllanthus sp*., like many other traditional medicinal plants have been tested for their pharmacological value, however assessing the potential of bioactive compounds in these plants to inhibit the growth of other plants is rarely reported. In earlier work (Allan and

Adkins, 2007), bioassays of a selection of medicinal plants found that aqueous extracts of *P. virgatus* could inhibit the growth of *Lemna aequinoctialis* by up to 91%. To follow on, this work tests whether aqueous extracts of *P. virgatus* can inhibit the germination and growth of common weed species.

## MATERIALS AND METHODS

### Seed material

Ten common weed species were selected for testing, two summer grasses, two winter grasses and both winter and summer-growing broadleaf weeds. Seeds of *Apium leptophyllum*, *Avena fatua*, *Bidens pilosa*, *Chenopodium album*, *Echinochloa crus-galli*, *Lolium rigidium*, *Parthenium hysterophorus*, *Raphanus raphanistrum*, *Sonchus oleraceous* and *Urochloa pannicoides* were stored at the University of Queensland at approximately 15$^{o}$C and 15-20% RH.

Several methods were used to improve the germination percentage of the seed lots. Seeds of *C. album*, *L. rigidium* and *S. oleraceous* (with the papus removed) were sorted in a New Brunswick General Seed Blower and the lighter unfilled seeds discarded. To overcome physical dormancy, seeds of *A. fatua* were dehusked. Both the pod and seed coat of *R. raphanistrum* were removed as both have been shown to impose dormancy (Young, 2003; Allan, *unpubl.*).

### Plant extract

The above ground parts (leaf/stem/fruit) of healthy *P. virgatus* plants were selected from footpaths and public parks in Bardon, Qld.
Leaf, stem and fruit of *P. virgatus* were collected, weighed, loosely washed, patted dry and then chopped into pieces <1cm$^2$. The chopped material was then placed in a jar of deionised water to produce a 12.5% Fresh Weight/volume (FW/v) concentrate. The sealed jars were kept in darkness for 48h at 22$^{o}$C. The concentrate was then filtered through a fine sieve (mesh <1mm).

Extract agar was made by dissolving 12.5g of Agar (Sigma) into 600ml of heated deionised water. After the agar was dissolved, 150ml of cool deionised water was added and the solution allowed to cool. Once at 55˚C, the *P. viragtus* aqueous extract was added to the agar and stirred vigorously to make a 5% FW/v concentrate. The extract agar was then poured into clear plastic Petri dishes (90mm diameter) to a depth of approximately 5mm.

A control treatment using 10% agar alone was mixed, the pH measured and poured into Petri dishes in the same manner.

### Germination

Seeds were germinated over a 7 to 16 day period in Thermoline Scientific refrigerated incubators (Model 495-1-SD) set on either a summer diurnal regime of 12h (30˚C) in light conditions (PPF of 40µmol m$^{-2}$ s$^{-1}$ supplied by 4 Polylux XL F36W/860 daylight 3250 Lm fluorescent tubes) and 12h (20˚C) darkness or a winter diurnal regime of 12h (25˚C) light conditions (as above) and 12h (10˚C) darkness.

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The treatment and control consisted of four replicates of 25 seeds per Petri dish. Seeds were recorded as germinated when either the radicle of hypercotyl exceeded 1mm.

### Data analysis

The number of germinated seeds were counted over the test period and the treatment germination percentage (T Gm%) was calculated using:

T Gm% = [Treatment germinant No./Control germinant No.] x 100

Shoot and root growth was measured and the shoot inhibition percentage (Shoot In%) and root inhibition percentage (Root In%) calculated using the following:

Shoot In%= 100 – [(Treatment shoot growth/Control shoot growth) x 100]

Root In%= 100 – [(Treatment root growth/Control root growth) x 100]

Statistical analysis was performed via a one-way ANOVA and Tukey-Kramer test (P<0.05), using the program Minitab.

## RESULTS

Control germination exceeded 80% for all species with the exception of *C. album* (77%) and *R. raphanistrum* (73%). This was thought adequate for treatment comparisons (Table 1).

**Table 1.** Germination (Gm %), shoot growth inhibition (Shoot In %) and root growth inhibition (Root In %) of *P. virgatus* extract treatments as a percentage of control germination, shoot and root growth of 10 common weeds. Significant difference (P<0.05) is indicated by * before a value.

| Weed Species | Gm % | Shoot In % | Root In % |
|---|---|---|---|
| *A.fatua* | 99.0 | * 65.8 | * 42.2 |
| *A.leptophyllum* | * 63.2 | 12.0 | * 95.1 |
| *B.pilosa* | 88.4 | * 27.3 | * 78.2 |
| *C.album* | 87.0 | * 25.4 | * 89.2 |
| *E.crus-galli* | 106.7 | 9.0 | * 78.4 |
| *L.rigidium* | * 88.0 | * 46.0 | * 88.6 |
| *P.hysterophorus* | * 49.4 | * 38.7 | * 92.2 |
| *R.raphanistrum* | 98.6 | 25.0 | * 61.2 |
| *S.oleraceous* | * 40.8 | * 53.9 | * 91.7 |
| *U.pannicoides* | 99.0 | * 8.1 | * 62.0 |

## DISCUSSION

The germination of *A. leptophyllum*, *L. rigidium*, *P. hysterophorus* and *S. oleraceous*, was significantly reduced (63.2%, 88.0%, 49.4% and 40.8%, respectively) due to the extract

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treatment. The effect was seen in both broadleaf and grass weeds and both summer- and winter-growing weeds. The reduction in germination percentage implies the seeds had become non-viable or that the extract had imposed dormancy. When the non-germinated seeds were taken off the extract agar, after the 7 to 16 day period and placed on plain agar in the same controlled conditions, the seeds failed to germinate, thereby suggesting that the seeds were non-viable.

Root growth for all species was significantly affected (up to 95.1%), with much of the root growth consisting of numerous small aerial roots avoiding contact with the extract agar. The lack of root growth would ultimately affect shoot growth and seedling survival.

The extract also significantly affected shoot growth of most weed species. It was not just the small seeded weeds which may have been more drastically affected by the poor root growth, but also the large-seeded weeds such as *A.fatua*, which had a significant reduction (65.8%) in shoot growth.

Aqueous extract of *P. virgatus* at a concentration of 5% FW/v had a significant effect on germination and early growth of several weeds species. Future work will focus on identification and isolation of allelochemicals and glasshouse trials assessing the effect of these compounds on germination and growth of weeds in a soil medium.

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**REFERENCES**

Allan, S.M. and Adkins, S. (2007). The effect of medicinal plant extracts on growth of *Lemna aequinoctialis*. Allelopathy Journal 19 (1): 267-274.

Calixto, J.B., Santos, R.S., Filho, V.C. and Yunes, R.A. (1998). A review of plants of the genus *Phyllanthus*: Their chemistry, pharmacology, and therapeutic potential. *Med Res Rev*. 18 (4): 225-258.

Chaudhary, L.B. and Rao, R.R. (2002). Taxonomic study of herbaceous species of *Phyllanthus* L. (Euphorbiaceae) in India. Phytotaxonomy 2: 143-162.

Huang, Y.L., Chen, C.C., Hsu, F.I. and Chen, C.F. (1998). Tannins, flavonol sulphates and a norlignan from *Phyllanthus virgatus*. J Nat Prod. 61 (10): 1194-1197.

Kumaran, A. and Karunakaran, R.J. (2007). In vitro antioxidant activiyies of methanol extracts of five *Phyllanthus* species from India. LWT-Food Science and Technology. 40 (2): 344-352.

Young, K.R., (2003). Germination and emergence of wild radish (*Raphanus raphanistrum* L.). University of Melbourne, Thesis *unpubl*.

# POTENTIAL APPLICATION OF A CROP EXTRACT FOR WEED SUPPRESSION AS AN ALTERNATIVE FOR CHEMICAL WEED CONTROL IN RICE

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## ABSTRACT

An experiment was conducted to assess the feasibility of using a crop extract (bio-herbicide) as an alternative for chemical weed control in rice. The sorghum water extract, used in this experiment, was prepared by soaking sorghum leaves in distilled water in a ratio of 1:5 for 36 h. It was then filtered to collect the extract. After seed bed preparation, seedlings of IR-6 rice were transplanted into the field using a randomized complete block design with four replications. The data showed that hand weeding treatment recorded the maximum fresh and dry weed biomass.

Application of half dose of weedicide ryzelan + half dose of sorghum water extract (sorgaab) produced the maximum number of tillers, panicles and normal kernels. The use of sorghum water extract, alone and in combination with commercial herbicide, produced lower fresh and dry weed biomass than the weedy check and hand weeding treatments. Similarly, the maximum number of spikelets, 1000-grain weight and paddy yield were also obtained in sorghum water extract treatment. Based on the results, it is concluded that the crop extract can be safely used as a bio-herbicide for controlling weeds and obtaining higher yield of rice.

**Key words:** Rice, weeds, crop water extract, weedicide, hand weeding

## INTRODUCTION

Rice (*Oryza sativa* L.) is a main source of nourishment for over half the world's population. Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake. In Asia, about 57% of rice is grown on irrigated land, 25% on rain-fed lowland, 10% on the uplands, 6% in deepwater, and 2% in tidal wet lands.

The annual production of rice in Pakistan is 55 million tons, of which Khyber Pakhtunkhwa (KPK) province contributes 0.128 million tons. The total annual rice cultivated area in the country is 2.5 millions hectares and in KPK 61.7 thousand hectares with an average yield of 2212 kg ha$^{-1}$ (Ministry of Agriculture, Pakistan, 2008).

Rice is grown under diverse climatic and edaphic conditions in Pakistan, from the temperate zones of Swat at high altitudes in mountain valleys to warmer areas of Sindh and Punjab. However, the yield obtained on farmers' fields is low in most parts of the country, including Dera Ismail Khan district, on account of water shortage, high costs of inputs, non-availability of skilled labour during peak planting season, sub-optimal plant population, weeds and pest infestation, high dependence of knowledge on nearby growers and low price of rice in the local market (Baloch *et al*. 2004).

In South East Asia, weeds cause yield losses of 10-46% and occasionally up to 100% in rice. Hand weeding is the most common method of weed control in rice in Pakistan. However it is labour intensive and expensive. The use of mechanical weed control is limited to transplanted rice. Herbicides may provide very good control of weeds in rice. However, they are expensive, and there are continuing concerns about their extensive use and environmental safety.

In this scenario, biological weed suppression appears to be the only viable alternative to reduce weed density to safe limits. In this method, leaf water extracts of allelopathic plants, such as sunflower, sorghum, tobacco, millet, soybean, rice and oat are used. These plants release toxins or allelochemicals to inhibit the growth of certain weeds during the crop cycle and thereby increase yields (Ata and Jamil 2001; Anon 2002; Labrada 2008).

Khanh *et al*. (2007) reported that the synthetic herbicides may eliminate weeds, but their excessive use could adversely affect humans and environment. Thus, controlling weeds by biological methods can alleviate the heavy dependence on synthetic agrochemicals and result in more sustainable agriculture.

Irshad and Cheema (2005) reported that sorghum water extract is a good alternative for chemical weed control in rice, and reduced the biomass of barnyard grass by up to 41% compared to an untreated control.

Earlier research work (Jensen *et al*. 2008; Tanveer *et al*. 2008; Tesio *et al*. 2008) has provided a broad understanding for initiation of the present studies on application of bio-herbicides activities for chemical weed control in rice. The objective of the current research was to investigate the effect of sorghum water extract as an alternative for chemical weed control in rice.

## MATERIALS AND METHODS

The experiment was conducted at the Agricultural Research Institute, Dera Ismail Khan (31º49´ N, 70°55´ E), Pakistan. Soil at the experimental site was silty clay, pH = 8 and organic matter content 0.68%. Electrical conductivity in soil was 400 Ec x $10^{-6}$ (dS/m). Table 1 shows the climatic situation during the crop growth season. The experiment was laid out in a randomized complete block design with four replications. The net plot size was 1.8 m x 5 m, with rows 20 cm apart.

The recommended dose of Ryzelan ® (Penoxulam @ 30 mL a.i.$ha^{-1}$) was applied 15 days after transplanting. This herbicide was introduced by Dow Agro Sciences (USA) for controlling broadleaf and annual grasses in rice.

The sorghum water extract for testing (sorgaab) was prepared by soaking sorghum leaves in distilled water in a ratio of 1:5 for 36 h. It was then filtered to collect the extract.

Weed control treatments in the rice plots included the following:

$T_1$: Weedy check (untreated control)

$T_2$: Ryzelan (full recommended dose 30 mL $ha^{-1}$)

$T_3$: Sorghum water extracts (full recommended dose 15 L $ha^{-1}$)

$T_4$: Ryzelan (15 mL $ha^{-1}$) + sorghum extract (7.5 L $ha^{-1}$)

$T_5$: Hand weeding (50 DAT)

After proper seed bed preparation, seedlings of rice cv. IR-6 were transplanted in the middle of June. Fertilizers were applied @ 120:90:60 NPK kg/ha. Half the nitrogen with the full dose of potash and phosphorus were applied at seed bed preparation, and the remaining half dose of nitrogen was applied in two equal portions 25 and 45 days after transplantation (DAT).

Data were recorded for the number of tillers ($m^{-2}$), number of panicles ($m^{-2}$), number of spikelets ($panicle^{-1}$), normal kernel (%), 1000-grain weight (g), and paddy yield (t $ha^{-1}$). The data obtained were analyzed statistically using the analysis of variance technique. The significant means were separated using the least significant difference test (Steel and Torrie 1980).

**Table 1. Meteorological data recorded at the Agricultural Research Institute, Dera Ismail Khan, during 2009.**

| Month | Temperature ($^0$C) | | Relative Humidity | | Rainfall |
|---|---|---|---|---|---|
| | Max: | Min: | 0800 Hrs. | 1400 Hrs. | (mm) |
| May | 42 | 24 | 69 | 35 | 29 |
| June | 42 | 27 | 60 | 34 | 10 |
| July | 20 | 27 | 66 | 37 | -- |
| August | 39 | 27 | 67 | 38 | 5 |
| September | 34 | 22 | 64 | 37 | 21 |
| October | 32 | 17 | 63 | 40 | 11 |

## RESULTS AND DISCUSSION

### Fresh and dry weed biomass (g)

Data on fresh weed biomass are presented in Table 2, which showed that fresh weed biomass was not significantly affected by weed management practices. However, among treatments, there was a visible difference where $T_5$ had the maximum (132.7 g) fresh weed biomass of common sedges including purple nutsedge (*Cyperus rotundus* L.), umbrella sedge (*Cyperus iria* L.), small-flowered umbrella plant (*Cyperus difformis* L.), and grasses viz. bermuda grass [*Cynodon dactylon* (L.) Pers.], jungle rice [*Echinochloa colona* (L.) Link.] and barnyard grass [*Echinochloa crus-galli* (L.) Beauv] as compared to $T_4$ (81.68 g). This might be the interactive effect of both ryzelan and sorghum water extract. The results are similar to those of Cheema *et al.* (2002), who applied sorghum water extract and recorded minimum fresh weed biomass. Similarly, Parvis and Jessop (1985) found sorghum water extract successfully inhibited the growth of wheat weeds.

Data on dry weed biomass are shown in Table 2. The data showed non-significant difference among weed management practices. Treatment $T_5$ recorded the maximum (36.92 g) dry weed biomass while $T_3$ showed minimum dry weed biomass. The results are similar to those of Cheema *et al.* (2002), who found that dry weed biomass was successfully reduced in plots which received a sorghum water extract.

Khaliq *et al.* (2002) also recorded that a combination of a herbicide and sorghum water extract, in their case, Metolachlor + sorgaab, and pendimethalin + sorgaab, reduced weed dry weight by 78 and 75%, respectively. Anwar *et al.* (2004) also reported that the use of sorghum water extract not only reduced weed number and weed weight but also improved fresh and dry weight of crop.

**Table 2. Effect of weed management practices on fresh and dry weed biomass in rice.**

| Treatment | | Fresh weed biomass (g) | Dry weed biomass (g) |
|---|---|---|---|
| $T_1$ | Weedy check | 113.4$^{NS}$ | 27.32$^{NS}$ |
| $T_2$ | Ryzelan (30 mL $ha^{-1}$) | 86.29 | 22.93 |
| $T_3$ | Sorghum water extract (15 L $ha^{-1}$) | 97.90 | 21.89 |
| $T_4$ | Ryzelan (15 mL $ha^{-1}$) + Sorghum water extract (7.5 L $ha^{-1}$) | 81.68 | 24.09 |
| $T_5$ | Hand weeding (50 days after transplanting) | 132.7 | 36.92 |

NS = Non-significant

**Number of tillers ($m^{-2}$)**

Data revealed that weed management practices had significant effect on number of tillers (Table 3). It is obvious from the data that the maximum number of tillers (358.5) was recorded in $T_4$ followed by $T_5$ (329.0). However, the results were not statistically different in both the treatments. The minimum number of tillers (284.3) was recorded in the weedy check due to higher weed density. The higher number of tillers in $T_4$ and $T_5$ was probably due to the increased availability of nutrients for the rice plants from reduced competition between crop plants and the weed flora.

The higher number of tillers in $T_4$ indicated that the use of herbicide plus a reduced concentration of bio-herbicide could increase the number of tillers in rice. These findings agree with Cheema *et al.* (2003), who obtained increased number of tillers when herbicide was applied in combination with natural allelopathic water extract (sorgaab).

**Number of panicles ($m^{-2}$)**

The number of panicles was significantly affected by weed management practices (Table 3). The highest number of panicles was obtained in $T_4$ (360.8) whereas the weedy check produced the lowest number of panicles (257.3). The higher number of panicles in $T_4$ (half dose of ryzelan with half dose of sorghum water extract) suggested that the combination of ryzelan plus sorghum water extract was as effective as the application of the herbicide alone.

**Number of spikelets ($panicle^{-1}$)**

Data on the number of spikelets are presented in Table 3, and revealed that weed management practices had a significant impact on number of spikelets. The maximum number of spikelets (157.8) was recorded in $T_3$ (sorghum water extract), which was not

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statistically different from $T_2$ (full dose of ryzelan). This demonstrates that sorghum water extract is a good alternative for chemical weed control in rice. The minimum number of spikelets (135.1) was obtained in the weedy check.

The higher number of spikelets recorded in the sorghum water extract treatment demonstrated that the use of this bio-herbicide is potentially a good alternative for chemical weed control and can result in increased number of spikelets in rice. The weedy check treatment gave the minimum number of spikelets probably due to higher weed population. The results are similar to those of Irshad and Cheema (2005), who also reported that sorghum water extract significantly reduced barnyard grass as compared to weedy check, and led to maximum number of spikelets.

**Table 3. Effect of weed management practices on plant characteristics of rice.**

| Treatment | | Tillers/ $m^2$ | Panicles/ $m^2$ | Spikelets / panicle | Normal kernels (%) | 1000-grain weight (g) | Paddy yield (t/ha) |
|---|---|---|---|---|---|---|---|
| $T_1$ | Weedy check | 284.3 c | 257.3 d | 135.1 b | 75.00 a | 25.61 b | 5.988$^{NS}$ |
| $T_2$ | Ryzelan (30 mL/ha) | 309.5 bc | 298.8 c | 154.0 a | 74.75 a | 24.64 b | 6.637 |
| $T_3$ | Sorghum water extract (15 L/ha ) | 318.8 bc | 317.5 bc | 157.8 a | 73.50 ab | 27.60 a | 6.738 |
| $T_4$ | Ryzelan (15 mL/ha) + Sorghum water extract (7.5 L/ha) | 358.5 a | 360.8 a | 148.2 ab | 75.50 a | 25.38 b | 6.238 |
| $T_5$ | Hand weeding (50 days after transplanting) | 329.0 ab | 330.0 b | 141.9 ab | 70.25 b | 23.94 b | 6.262 |
| | $LSD_{0.05}$ | 34.77 | 28.39 | 18.77 | 4.428 | 1.760 | --- |

Means followed by different letter(s) in a column are significant at 5% level of probability.

NS = Non-significant

### Normal kernel (%)

The data pertaining to normal kernels are given in Table 3, and shows a higher percentage of normal kernels in $T_4$, but not statistically different from $T_1$ and $T_2$. The lowest normal kernel percentage was recorded in $T_5$.

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The higher normal kernels recorded in $T_4$ indicated that the application of bio-herbicide along with commercial herbicide is a useful proposition for increasing normal kernels in rice. The use of sorghum water extract alone gave comparatively fewer normal kernels, that is, the use of sorghum water extract alone had a reduced positive effect on kernel production.

### 1000-grain weight (g)

Data on 1000-grain weight of paddy as affected by different weed control treatments are presented in Table 3. Grain weight is the key to higher yield. Analysis of the data revealed that weed control treatments had significant effect on grain weight. All weed control treatments significantly increased grain weight; however, $T_3$ recorded the maximum (27.60g) grain weight. Minimum grain weight (23.94 g) was obtained from hand weeding. The weedy check (25.61 g), full dose of ryzelan (24.64 g) and half dose of ryzelan plus half dose of sorghum water extract (25.38g) were not statistically different.

The higher grain weight recorded from the sorghum water extract might be due to presence of essential nutrients which significantly increased spike density and resulted in higher grain weight. Xuan *et al.* (2004) reported that many plant water extracts successfully enhanced the 1000- grain weight of rice. Similar results were obtained by Salisbury and Ross (1978), who found that sorghum extract increased grain weight of maize by suppressing the vegetative growth.

### Paddy yield (t/ha)

Data recorded on paddy yield are presented in Table 3. The use of different weed control treatments had no significant effect on paddy yield. Among treatments, weedy check recorded the minimum (5.988 t/ha) paddy yield while the maximum (6.738 t/ha) was produced in the sorghum extract treatment. These results agree with Cheema *et al.* (1997), who showed that increasing the concentration of sorghum extract from 50% to 100% improved the yield by 14%.

The increased yield might be due to the fact that all yield components were higher in plants treated with herbicide and sorghum water extract. The likely reason for high paddy yield in the different weed control treatment might be the increased availability of nutrients and the reduced competition which stimulated vegetative growth and resulted in better spike population and more grains spike$^{-1}$ and ultimately higher paddy yield.

### Conclusions

This paper suggests that the application of allelopathic crop water extracts not only reduces the reliance on synthetic herbicides but can also reduce their indiscriminate use. The application of sorghum water extract alone or in combination with a 50% reduced dose of ryzelan was found to be the best treatment for suppressing weeds and producing higher paddy yield. Reduced herbicide doses, in combination with allelopathic crop water extracts, also minimize the cost of production thereby increasing the farm income.

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## REFERENCES

Anon, I. (2002). Multifaceted approach to study allelochemicals in an ecosystem. In Reigosa, M.J. and Pedrol N. (eds.). *Allelopathy, from Molecules to Ecosystems*. Science Publishers, Enfield, New Hampshire.

Anwar, S., Shah, W.A., Bakht, J. and Jabeen, N. (2004). Comparison of sorghum extracts chemical and hand weeding management in wheat crop. *Pakistan Journal of Agronomy* 3(1): 59-67.

Ata, Z. and Jamil, M. (2001). Allelopathic suppression of weeds: a new field in need of attention. *Daily Dawn*, December 31, 2001.

Baloch, M.S., Awan, I.U., Hassan, G., Khan, M.A., Ahmad, K. and Sulemani, M.Z. (2004). Quantitative assessment of social and some input variables relating to rice production in Dera Ismail Khan. *Pakistan Journal of Agronomy* 3(1): 52-58.

Cheema, Z.A., Luqman, M. and Khaliq, A. (1997). Use of allelopathic extracts of sorghum and sunflower herbage for weed control in wheat. *Journal of Animal and Plant Sciences* 7: 91-93.

Cheema, Z.A., Khaliq, A. and Ali, K. (2002). Efficiency of sorgaab for weed control in wheat grown at different fertility levels. *Pakistan Journal of Weed Science Research* 8(1-2): 33-39.

Cheema, Z.A., Khaliq, A. and Mubeen, M. (2003). Response of wheat and winter weeds to foliar application of different plant water extracts of sorghum. *Pakistan Journal of Weed Science Research* 9(1-2): 89-97.

Irshad, A. and Cheema, Z.A. (2005). Comparative efficacy of sorghum allelopathic potential for controlling barnyardgrass in rice. Proc. 4th World Congress on Allelopathy, "Establishing the Scientific Base", Wagga Wagga, New South Wales, Australia, 21-26 August 2005.pp: 508-513.

Jensen, L.B., Courtois, B. and Olofsdotter, M. (2008). Quantitative trait loci analysis of allelopathy in rice. *Crop Sciences* 48: 1459-1469.

Khaliq, A., Aslam, Z. and Cheema, Z.A. (2002). Efficacy of different weed management strategies in mungbean (*Vigna radiata* L.). *International Journal of Agriculture and Biology* 4(2): 237-239.

Khanh, T. D., Elzaawely, A.A., Chung, I.M., Ahn, J.K., Tawata, S. and Xuan, T.D. (2007). Role of allelochemicals for weed management in rice. *Allelopathy Journal* 19(1): 85-95.

Labrada, R. (2008). Allelopathy as a tool for weed management. *Allelopathy Journal* 22(2): 283-287.

ISBN Number: 978-0-9871961-0-1

Ministry of Agriculture, Pakistan. (2008). Agricultural Statistics of Pakistan. Ministry of Food and Agriculture. Govt. of Pakistan, Islamabad.

Parvis, C. E. and Jessop, R. S. (1985). Biochemical regulation of wild oat germination and growth by wheat crop residues. Proc. British Crop Protection Conference- Weed 2: 661-668.

Salisbury, F. B. and Ross, C. W. (1978). Allelochemicals and allelopathy in plant physiology 2nd eds. Wadsworth Pub. Co. California. pp: 357.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics: A biological approach. 2nd eds. McGraw Hill Book Co. Inc. New York, USA.

Tanveer, A., Tahir, M., Nadeem, M.A., Younis, M., Aziz, A. and Yaseen, M. (2008). Allelopathic effects of *Xanthium strumarium* L. on seed germination and seedling growth of crops. *Allelopathy Journal* 21(2): 317-327.

Tesio, F., Vidotto, F., Weston, L.A. and Ferrero, A. (2008). Allelopathic effects of aqueous leaf extracts of *Helianthus tuberosus* L. *Allelopathy Journal* 22(1): 47-58.

Xuan, T.D., Eiji, T., Shinkichi, T. and Khanh, T.D. (2004). Methods to determine allelopathic potential of crop plants for weed control. *Allelopathy Journal* 13(2): 149-164.

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# HERBICIDE RESISTANCE IN ANNUAL RYEGRASS (LOLIUM RIGIDUN GAUD) POPULATIONS IN WHEAT FIELDS OF FIROOZABAD

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## ABSTRACT

An experiment was conducted to detect herbicide resistance in ryegrass (*Lolium rigidum Gaud*) populations infesting wheat (*Triticum aestivum L.*) fields across the Firoozabad city, Fars Province, during 2008-2009. Each ryegrass population in each agronomic zone was sprayed with several herbicides with different modes of action with upper recommended dose. Some of these ryegrass populations were found to be resistant to the ACCase – inhibitor herbicides: diclofop-methyl 60% and clodinafop-propargyl 50%. These populations were completely susceptible to herbicides with other modes of action. For example, ALS-inhibitor herbicide, mesosulfuron+iodosulfuron, inhibitors of tubuline formation, Trifluralin and Phenylpyrazolin, and Pinoxaden. We did not detect any Point mutation 2078-Gly in ryegrass populations through the molecular dCAPS approach.

**Keywords**: Cross resistance, dCAPS, mutation, ryegrass.

## INTRODUCTION

Evolved herbicide resistance is affected by the genetic, weed biology, herbicide mode of action and application methods. In conventional agriculture, weed control is based on herbicide use. Worldwide, Herbicide resistance is an increasing concern, and more weed species have developed resistance to group B herbicides than to any other mode of action group (Mcgillion and Storrie 2006). Rates of spontaneous mutation vary from organism to organism and from locus to locus, ranging from 7-10 mutations per base pair per replication in some bacteriophages to 10-11 per base pair per replication in humans (Drake *et al.*, 1998). Recently, researchers have identified molecular mutations in the ACCase gene endowing target site based herbicide resistance in some ACCase herbicide-resistant *Lolium rigidum* populations .The aim of this research was to test, some of the ACCase herbicides in controlling annual ryegrass resistant biotype and widespread. This study also examined the target site 2078-Gly resistance to Diclofop-methyl and other ACCase-inhibiting herbicides in populations of *L. rigidum* by use of (dCAPS) method.

## MATERIALS AND METHODS

Crop fields were surveyed over a three-week period just before the 2009 wheat grain harvest. Crop fields in three regions (A, high use of single mode of action herbicides, B, medium and C, low) were randomly visited. Fields were surveyed by two people walking in an inverted ‘V’ 100m into the crop. Fully mature annual ryegrass spikes were collected and bulked from mature plants in the sampling path. Immediately after collection, the ryegrass seed samples were stored in a non air-conditioned glasshouse over summer to break seed dormancy, ensuring maximum germination (Steadman *et al*. 2003). The methods described are similar to those used in the 1998 survey (Llewellyn and Powles 2001).

## Resistance Testing

During the 2010 growing season, 50 seeds of each of the 42 ryegrass populations were planted in plastic seedling trays containing peat. Trays were kept outdoors at the University of Firoozabad, Iran and were watered and fertilized as required. For almost all populations, germination and seedling emergence was high (>90%), ensuring that 40 individual seedlings in each population were screened to each herbicide. Seedlings were treated with herbicide (upper recommended field rates) using an 8002 Tee-jet nozzlessprayer delivering herbicide in 200 L/ha water at 210 kPa. Known resistant and susceptible ryegrass populations were used as controls for each herbicide treatment. In all experiments, 100%mortality occurred in the known susceptible population.

**Table 1.** Herbicides and adjuvants applied to ryegrass populations during the 2010 growing season

Herbicide effect was assessed by determining seedling mortality 21 days after herbicide treatment. Ryegrass populations were classed as resistant if 20% or more of the individuals in the population survived the herbicide. Where there was 1–19% survival, the population was classed as developing resistance. Where there was less than 1% survival, the population was classed as susceptible. (Tardif and Powles 1994). Seedlings that survived diclofop-methyl at 21 days after treatment were cut back to a height of 20 mm, allowed to regrow for 1 week, and then treated with the sethoxydim 12.5%EC (2- L/ha). Sethoxydim was applied to diclofop-methyl survivors because previous work showed that although ryegrass can metabolise diclofop-methyl, it cannot metabolise sethoxydim (Tardif

| Chemical Class | Common name | Formulation | Rate / Adjuvants |
|---|---|---|---|
| Aryloxyphenoxy-propionate | Diclofop-methyl | 36% EC | 3 lit/ha cytogate 0.2% |
| Aryloxyphenoxy-propionate | Clodinafop-propargyl | 8% EC | 1.5lit/ha cytogate 0.2% |
| Phenylpyrazolin | Pinoxaden | 10% EC | 600ml/ha Adigore 0.5% |
| Sulfonylurea | Mesosulfuron+iodosulfuron | 30%+30% WG | 500g/ha cytogate 0.2% |
| Triazines | Atrazine | 80% WP | 1500g/ha |

and Powles 1994). Therefore resistance to sethoxydim indicates target site resistance.

## Detection of 2078-Gly Mutation

Fresh leaf material (~1cm2) was harvested from young leaves of a single resistant plant for each resistant population; snap frozen in liquid nitrogen and stored at 80°C. DNA was extracted using the cetyl terimethyl ammonium bromide (CTAB ) (Doyle *et al*. 1987). The concentration of nucleic acids was determined spectrophotometrically on a NanoDrop ND-

1000 (Thermo scientific, USA) at 260 nm. The PCR was conducted in a 25 mL volume that consisted of about 300 ng of genomic DNA, 0.5 mM of each primer, and 12.5 mL of Taq polymerase. (Cinagen, Iran). The PCR reaction was run in a (Techne, UK) with the following profile: 94 $^{o}$C 4 min, 35 cycles of 94$^{o}$C 30 s, 62$^{o}$C 30 s, and 72$^{o}$C 30 s, followed by a final extension step of 5 min at 72$^{o}$C.

### dCAPS Analysis

A dCAPS marker for the 2078 mutation (Asp to Gly) was developed in this research to facilitate rapid and accurate identification of mutant 2078-Gly alleles in resistant ryegrass populations. The primer pair ACCF1/EcoRV2078r (Table 2) amplifies a 353-bp fragment using the same PCR conditions. Following EcoRV digestion, individuals with homozygous-resistant 2078-Gly alleles would have an uncut band of 353 bp, while individuals with homozygous susceptible 2078-Asp alleles would have a digested band of 323 BP. Individuals with both susceptible and resistant alleles would have a combination of two resolvable bands.

**Table 2.** Primer for PCR reaction

| Primer | Sequence 5 -3 |
|---|---|
| ACCF1 | CACAGACCATGATGCAGCTC |
| EcoRV2078r | GCACTCAATGCGATCTGGATTTATCTTGAT |

## RESULTS AND DISCUSSION

### Resistance to ACCase herbicides

Diclofop-methyl resistance was common in the ryegrass populations randomly collected from Firoozabad wheat fields. Of the 42 populations treated with diclofop-methyl, 60% displayed some level of resistance (Table 3).

**Table 3.** The number and (percentage) of resistant ryegrass populations in each category for each herbicide populations (TR) and the total number of populations tested (TP) are shown. Zero indicates fully susceptible populations, 1–19% survival results in classification as populations developing resistance, and >20% survival results in classification as resistant populations.

| category | Diclofop | Sethoxydim | Clodinafop | Mesosulfuron | Trifluralin | Pinoxaden |
|---|---|---|---|---|---|---|
| 0 | 16(40) | 15(62.5) | 19(50) | 42 | 36 | 42 |
| 1 -19 | 9(22.5) | 6(25) | 7(18.4) | 0 | 0 | 0 |
| > 20 | 15(37.5) | 3(12.5) | 12(31.5) | 0 | 0 | 0 |
| TR | 24(60) | 9(37.5) | 19(49.5) | 0 | 0 | 0 |
| TP | 40 | 24 | 38 | 42 | 36 | 42 |

As expected, the degree of resistance varied across populations, with 37.5% classified as resistant (>20% survival). A further 22.5% of populations were classified as developing resistance (1–19% survival) and 40% of populations were diclofop-methyl susceptible (Table 3).This indicates that diclofop-methyl resistance is now much more common in ryegrass populations across high herbicide use with single mode of action zone. This may be attributed to the different herbicide use and characteristics among agronomic zones which in turn may explain the different diclofop- methyl selection pressure. Regions with greater diclofop-methyl usage may experience a higher frequency of resistant populations. This pattern was also found in the 2007 survey of Owen *et al*. 2007. For example, region C has less herbicide usage which is reflected by the lowest degree of resistance. In this region, 19% of the ryegrass populations were resistant ryegrass compared with high herbicide use areas (e.g. A, B) where 56% and 45% of the ryegrass populations had resistant plants respectively (Table 4).

**Table 4.** The percentage of annual ryegrass populations that are resistant (R) or developing resistance (DR) to herbicides, and total resistance (TR) in each agronomic zone.

| zone | Diclofop | | | Sethoxydim | | | Clodinafop | | |
|---|---|---|---|---|---|---|---|---|---|
| | DR | R | TR | DR | R | TR | DR | R | TR |
| A | 17 | 39 | 56 | 8 | 23 | 31 | 8 | 36 | 44 |
| B | 15 | 30 | 45 | 12 | 11 | 23 | 14 | 24 | 38 |
| C | 10 | 9 | 19 | 5 | 7 | 12 | 5 | 9 | 14 |

### Testing for Target Site Resistance With the Sethoxydim Herbicide

In general, resistance may be due to target site or non-target sites resistance as well as to occurrence of different ACCase mutations in ryegrass populations. (Tardif and Powles 1994; Tardif *et al*. 1996; Zhang and Powles 2006*a*, 2006*b*, Mathews *et al*. 1991; Holtum *et al*. 1992; Preston *et al*. 1996; Preston and Powles 1998). Previous research has established that resistant ryegrass populations can not metabolise sethoxydim which may lead to sethoxydim resistance which indicates target site resistance. (Tardif and Powles 1994). Therefore, resistance to sethoxydim, can be endowed by mutations at the amino acid position 1781(Delye *et al*. 2003; Menchari *et al.*2006; Zhang and Powles 2006a). Although resistance to diclofop-methyl can be target or non-target site resistance. Our study revealed 24 diclofop-methyl resistant ryegrass populations. Of these populations, 37.5% were also found to be resistant to sethoxydim (Table 3). The majority of these populations originated from the A and B agronomic zones (Table 4). Thus, at least 37.5% of the ACCase herbicide resistant populations are likely to have target site resistance.

### Resistance to Clodinafop-propargyl

Almost 50% of ryegrass populations collected from the Firoozabad wheat farms were found to exhibit resistance to the clodinafop-propargyl herbicide. Table 3 showed that 50% of ryegrass populations were resistant to clodinafop-propargyl (31.5% resistant and 18.4% developing resistance). In the A and B zones, 44 and 38% of populations were resistant to clodinafop-pro pargyl respectively (Table 4).

### Substitution of Amino Acid Asp-2078-Gly and Ile-1781-Leu Endowing Resistance to Accase Herbicides in Ryegrass Populations

*Ryegrass* populations resistant to ACCase inhibiting herbicides are widespread in the Firoozabad site. Target site mutation Ile-1781-Leu was detected in the majority of the

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resistant individuals surveyed, ranging from 31% in A agronomic zone to 12% in C agronomic zone (Table 4). This study suggests that target site mutation within ACCase is common in populations of ryegrass. Mutation ranged from 37.5% in the target site to 62.5% in the non-target site. In this research only those ryegrass resistant (R) populations to diclofop (Table 3) were tested for mutation and we did not detect any point mutation 2078 – Gly in ryegrass populations through the molecular dCAPS approach (Figure 1).

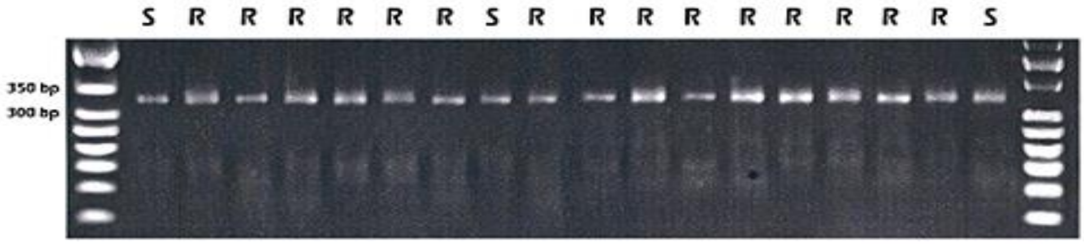


**Figure1.** DCAPS analysis individual *L.rigidum* Plants. The sizes of restriction enzyme 2078-Gly (EcoRV) digested fragments are 323bp.

## CONCLUSION

During the last decades, more herbicides in controlling weeds in wheat fields of Iran had a same mode of action (ACCase-inhibiting herbicides) and this situation has caused emerging herbicide resistance in some weeds such as ryegrass. Continued selection of these populations with ACCase. - inhibiting herbicides will cause, more mutation and resistance in ACCase group. The present study suggests demonstrated that mesosulfuron+iodosufuron, trifluralin and pinoxaden herbicides may control most of the annual ryegrass populations. Both new herbicides (i.e. mesosulfuron+ iodosufuron and pinoxaden) are considered a low risk for resistance evolution and so will need to be used in rotation with other modes of action to delay the onset of resistance to the new herbicides. Resistance to diclofop-methyl and clodinafop-propargyl in annual ryegrass population is a serious problem for wheat producers across Firoozabad region. This study recommends management of ryegrass populations through herbicide rotation and adoption of integrated weed management strategies to prolong the efficacy of these herbicides.

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## REFERENCES

Delye, C., Zhang, X., Chalopin, C., Michel, S., Powles ,S.B. (2003). An isoleucine residue within the carboxyl-transferase domain of multi domain acetyl-Coenzyme A carboxylase is a major determinant of sensitivity to aryloxyphenoxy propionate inhibitors but not to cyclohexanedione inhibitors. *Plant Physiology* 132, 1716–1723.

Drake, J.W., Charlesworth, B., Charlesworth, D., Crow, J.F. (1998). The plant glutathione transferase gene family: genomic structure, functions, expression and evolution. *Physiology Plantarum* 119: 469–479.

Holtum, J.A.M., Matthews, J.M., Liljegren, D.R., Powles, S.B. (1992). Cross resistance to herbicides in annual ryegrass (*Lolium rigidum*) on the mechanism, of resistance to diclofop-methyl. *Plant Physiology* 97, 1026–1034.

Llewellyn, R.S., Powles, S.B. (2001). High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technology* 15, 242–248.

Matthews, J.M., Holtum, J.A.M., Liljegren, D.R., Furness, B., Powles, S.B. (1991).Cross-resistance to herbicides in annual ryegrass (*Lolium rigidum*).1. Properties of the herbicide target enzymes acetyl-CoA carboxylase (ACC) and acetolactate synthase (ALS). *Plant Physiology* 94, 1180–1186.

Mcgillion, T., Storrie, A. (2006). Integrated weed management in Australia cropping systems – A training resource for farm advisors. CRC for Australian Weed Management. 247p

Menchari, Y., Camilleri, C., Michel, S., Brunel, D., Dessaint, F., Le Corre, V., Delye, C. (2006).Weed response to herbicides: regional-scale distribution of herbicide resistance alleles in the grass weed *Alopecurus myosuroides. New Phytolologist* 171, 861–874.

Owen, M.J., Walsh, M.J., Llewellyn, R.S., Powels, S.B. (2007). widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum) populations. Australian Journal of Agricultural Research. 58, 711-718.*

Preston, C., Powles, S.B. (1998). Amitrole inhibits diclofop metabolism and synergises diclofop-methyl in a diclofop-methyl-resistant biotype of *Lolium rigidum. Pesticide Biochemistry and Physiology* 62, 179–189.

Preston, C., Tardif, F.J., Christopher, J.T., Powles, S.B. (1996). Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. *Pesticide Biochemistry and Physiology* 54, 123–134.

Steadman, K.J., Bignell, G.P., Ellery, A.J. (2003). Field assessment of thermal afterripening time for dormancy release prediction in *Lolium rigidum* seeds. *Weed Research* 43, 458–465.

Tardif, F.J., Powles, S.B. (1994). Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: Isolation of a subset with resistant acetyl-CoA carboxylase. *Physiologia Plantarum* 91, 488–494.

Tardif, F.J., Preston, C., Holtum, J.A.M., Powles, S.B. (1996). Resistance to acetyl coenzyme A carboxylase inhibiting herbicides endowed by a single major gene encoding a resistant target site in a biotype of *Lolium rigidum*. *Australian Journal of Plant Physiology* 23, 15–23

Zhang, X., Powles, S.B. (2006*a*). The molecular basis for resistance to acetyl co-enzyme A carboxylase (ACCase) inhibiting herbicides in two target based resistant biotypes of annual ryegrass (*Lolium rigidum*). *Planta* 223, 550–557.

Zhang, X., Powles, S.B. (2006*b*). Six amino acid substitutions of the plastidic ACCase gene endow resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in a *Lolium rigidum* population. *New Phytologist* 172, 636–645.

# STUDIES ON THE PERSISTENCE OF DICLOSULAM IN SOYBEAN CROP

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## ABSTRACT

A three season field trial was conducted under West Bengal conditions during 2007–2009 to evaluate the dissipation pattern of Diclosulam (84 WDG) in soybean at two application rates (26 and 52 g a.i./ha). The quantitative analysis of the herbicide residues was performed using HPLC with UV-VIS detector. The average recovery was found to be 90.67%, 88.67%, 88.33% and 89.33% for soybean cropped soil, soybean plant, soybean oil and de-oil cake, respectively. Following the first order kinetics the herbicide dissipates in soybean cropped soil with half-life ($T_{1/2}$) value ranges between 5.28-6.02 days, 7.52-8.36 days and 6.27-6.84 days in three consecutive seasons irrespective of doses.

Diclosulam residues were below detectable level (BDL) in plant samples irrespective of the treatment doses and days in all seasons. No residues were detected in untreated control samples of field soil and plant during the entire study period. Furthermore, soybean oil and its deoil cake were also analyzed and Diclosulam residues were found well below the detectable limit irrespective of season and treatments. So it may be concluded from the study that Diclosulam will not pose any residual toxicity problem in soybean crop.

**Key words:** Persistence, dissipation, herbicide, Diclosulam

## INTRODUCTION

Diclosulam, a novel Triazolopyrimidine sulfonamide class of herbicide, is one of the new molecules which are highly effective for controlling broad-leaved weeds. It has been studied in field trials since 1990 and was first registered in 1997 in Argentina and Brazil. It was later registered in Bolivia (1998), Paraguay (1998) and the U.S. (2000). It is classified as a "not likely" human carcinogen. Diclosulam is not a developmental or reproductive toxicant. Based on the results of several subchronic, chronic and developmental reproductive toxicity studies, there was no evidence of neurotoxicity (source: http://www.epa.gov/opprd001/factsheets/appen_j.pdf).

Diclosulam inhibits acetolactate synthase (ALS), the enzyme responsible for biosynthesis of branch-chain amino acids and thereby cell division and growth of the weeds are quickly arrested. As an active ingredient, Diclosulam has activity both soil applied and post-emergence. Diclosulam can be soil applied in any tillage system since it does not require incorporation. The herbicide is a highly active, low dose compound. Its longevity in the soil makes Diclosulam ideal for control of broadleaf weeds in soybean and peanuts (Sheppard et. al., 1997).

In soybean and peanut, Diclosulam is rapidly metabolized by facile conjugation with homoglutathione, which displaces the 7-fluoro substituent (Owen 2000). The objective of the present work was to study the dissipation and the fate of Diclosulam residue in/on soybean in different seasons under West Bengal condition.

## MATERIALS AND METHODS

Field study was conducted at University Experimental Field, Mohanpur, BCKV for three consecutive seasons from August 2007 to November 2007 ($1^{st}$ season), August 2008 to November 2008 ($2^{nd}$ season) and August 2009 to November 2009 ($3^{rd}$ season) on soybean [variety- PK-472].

The climatic parameters of the soybean field in different seasons are presented in the following table (Table 1).

**Table-1:** Climatic condition during the field study

| Parameters | Kharif,2007 | Kharif,2008 | Kharif,2009 |
|---|---|---|---|
| **Average Maximum Temperature ($^0$C)** | **31.85** | **32.38** | **32.18** |
| **Average Minimum Temperature ($^0$C)** | **23.98** | **23.38** | **23.35** |
| **Average RH I (%)** | **94.33** | **94.05** | **94.65** |
| **Average RH II (%)** | **70.85** | **68.53** | **69.45** |
| **Average Rainfall (mm)** | **236.85** | **130.28** | **169.45** |
| **Other pesticides applied to trial plots** | **-** | **-** | **-** |

### Application details and sampling details

The formulation Diclosulam 84 WDG was applied with a knapsack sprayer equipped with WFN 40 nozzle @ 26 g a.i./ha ($T_1$) and @ 52 g a.i./ha ($T_2$) in Randomized Block Designed (RBD) plots and untreated control ($T_3$) plots. Three replications were used for each treatment. Spraying of herbicide was done once one month after sowing of the soybean crop for three consecutive seasons. Soybean field soil and plant samples were collected at 0, 1, 3, 7, 15, 30 and 60 days after application of the herbicide for dissipation study in all the seasons.

Soybean plant, soybean seed and cropped soil samples (for all seasons) were also collected at the time of harvest following standard sampling procedures. Soybean plant and seed (250 g) and field soil (1 kg) samples were collected from 5-7 places randomly in each treatment plot replication on each date of sampling. Samples from untreated control plots were collected in the same way. Soil samples were collected from a depth of 6″ with the help of soil auger.

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## RESIDUE ANALYSIS

Soil samples for the respective sampling dates were added to100 mL of a mixture of acetone: water (8:2), kept overnight and then shaken for 30 minutes using a mechanical shaker at 25˚C. They were then filtered and the extract collected and the sample re-extracted using a further 100 mL mixture of acetone: water (8:2). The combined filtrate was concentrated by evaporating the acetone portion and then transferred to a 500 mL separatory funnel. Then 100 mL of distilled water was added to it. This mixture was partitioned thrice (100+50+50) with dichloromethane, and the dichloromethane fraction was collected through anhydrous $Na_2SO_4$. This combined fraction was concentrated to 1-2 mL in a Rotary Vacuum Evaporator at 40˚C.

A chromatographic column was packed up with a mixture of 10 g Silica gel and Florisil (1: 1). Anhydrous sodium sulphate was placed at the bottom and top of the column using n-hexane. The residue was transferred into the column. Elution was done with 100 mL hexane followed by 100 mL of hexane: dichloromethane (8:2) mixture and then 100 mL methanol. Methanol fraction was evaporated to dryness in a rotary vacuum evaporator at 40˚C and the volume was reconstituted in HPLC grade methanol for HPLC analysis.

Plant samples were homogenized with 100 mL mixture of acetone: water (8:2). They were then filtered and the extract collected, and re-extracted using 100 mL mixture of acetone: water (8:2). The combined filtrate was concentrated to evaporate the acetone portion and then transferred to a 500 mL separatory funnel. This mixture was partitioned thrice (100+50+50) with a hexane: ethyl acetate (9:1) mixture. The aqueous phase was then partitioned thrice (100+50+50) with dichloromethane. The same process as mentioned above was followed for the field soil.

The soybean seed samples (50 g) were ground in a grinder and subjected to Soxhlet extraction with 150 mL of hexane for 6 hrs. The oil portion dissolved in hexane was then partitioned thrice (100+50+50) with acetonitrile and the acetonitrile fraction collected over anhydrous $Na_2SO_4$ and the combined organic phase was evaporated in a rotary vacuum evaporator. The residue was collected in 1-2 mL of dichloromethane and then the same procedure followed for column chromatography as described above for the field soil. The deoil cake (10 g) obtained from the oil extraction step was analysed with the same procedure as for the field soil.

### Instrumental Parameters

| | | | |
|---|---|---|---|
| i) | Column | : | Thermo $C_{18}$, 250 mm 4.6 mm |
| ii) | Mobile phase | : | Methanol: Water (1:1) |
| iii) | Flow rate | : | 0.5 mL/ min |
| iv) | Detector | : | UV-VIS detector |
| v) | Wavelength ($\lambda_{max}$) | : | 235 nm |
| vi) | Retention time | : | 5.36 ± 0.20 min |
| vii) | Injection volume | : | 20 µL |
| viii) | LOD (Limit of Detection) | : | 0.02 ppm |
| ix) | LOQ (Limit of Quantification) | : | 0.05 ppm |

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**Linearity Check**

A calibration curve (Figure 1) was constructed by plotting seven concentrations (0.05–2.00 μg/g) of standard Diclosulam versus absorption. Also, to determine the interference of each substrate, a matrix match calibration standard for each substrate was prepared. In this study, the calibration curve was prepared by taking the areas corresponding to different concentrations of the matrix match calibration standard, against which final quantification was done.

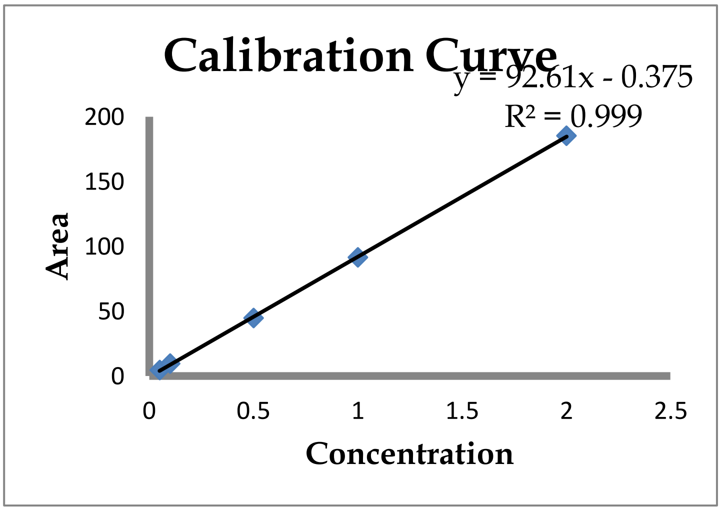


**Figure 1.** Analytical standard calibration curve of Diclosulam

## RESULTS AND DISCUSSION

Recovery studies were carried out to establish the reliability of the analytical method and to determine the efficiency of extraction and clean up steps employed for the present study. The average recovery was found to be 90.67%, 88.67%, 88.33% and 89.33% for soybean cropped soil, soybean plant, soybean oil and de-oil cake, respectively (Table 2). As the recovery percentage is more than 85% for all the substrates, the method can be adopted for residue and dissipation study of Diclosulam in different substrate of soybean.

The results of this field study of persistence of Diclosulam in soybean cropped soil have been summarized in Table 3 (season-I), Table 4 (season-II) and Table 5 (season-III). A straight line was found in all cases, when the log of residue was plotted against time, thereby establishing that first order reaction kinetics were involved in the dissipation process. The half-life values ($T_{1/2}$) in soybean cropped soil were in the range of 5.28-6.02 days, 7.52-8.36 days and 6.27-6.84 days in three consecutive seasons irrespective of dose used. More than 75% of the initial deposit was dissipated within 15 days irrespective of doses and seasons. Murdock et. al. (1999) reported that Diclosulam followed first order rate kinetics, the dissipation was relatively rapid, and half-life values ranged from 7-16

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days in various seasons. Diclosulam residues were below detectable level (BDL) in plant samples irrespective of the treatment doses and days in all the seasons.

Diclosulam residues were found well below the detectable limit irrespective of seasons and treatments in all the substrates at harvest time. This may be due to rapid metabolism of Diclosulam in the soybean plant leaving non-toxic metabolites in the plant system as described by Kramer et. al. (2007). The MRL value of Diclosulam in soybean in India has not yet been established. On the basis of above facts it may be concluded that Diclosulam in this formulation does not pose any residue toxicity problem in soybean at harvest.

**Table 2.** Recovery study of Diclosulam in different substrates

| Substrate | Amount fortified (ppm) | Amount recovered (ppm) | % Recovery | Average % recovery |
|---|---|---|---|---|
| Field Soil | 0.05 | 0.046 | 92.00 | 90.67 |
| | 0.10 | 0.089 | 89.00 | |
| | 1.00 | 0.910 | 91.00 | |
| Plant | 0.05 | 0.043 | 86.00 | 88.67 |
| | 0.10 | 0.091 | 91.00 | |
| | 1.00 | 0.880 | 89.00 | |
| Oil | 0.05 | 0.043 | 86.00 | 88.33 |
| | 0.10 | 0.088 | 88.00 | |
| | 1.00 | 0.910 | 91.00 | |
| Deoil Cake | 0.05 | 0.045 | 90.00 | 89.33 |
| | 0.10 | 0.089 | 89.00 | |
| | 1.00 | 0.890 | 89.00 | |

*** Average of three replicates**

**Table 3.** Dissipation of Diclosulam in soybean cropped soil in season-I

| Days after application | Treatment | Residues in ppm. | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | $T_1$ | 0.86 | 0.81 | 0.77 | 0.81 ± 0.037 | - |
| 1 | | 0.74 | 0.68 | 0.72 | 0.71 ± 0.025 | 12.34 |
| 3 | | 0.61 | 0.54 | 0.57 | 0.57 ± 0.029 | 29.62 |
| 7 | | 0.27 | 0.23 | 0.26 | 0.25 ± 0.017 | 69.13 |
| 15 | | 0.13 | 0.11 | 0.12 | 0.12 ± 0.008 | 85.18 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | $T_2$ | 1.52 | 1.58 | 1.48 | 1.53 ± 0.041 | - |
| 1 | | 1.36 | 1.31 | 1.34 | 1.34 ± 0.021 | 12.41 |
| 3 | | 1.11 | 1.18 | 1.15 | 1.15 ± 0.029 | 24.83 |
| 7 | | 0.71 | 0.65 | 0.62 | 0.66 ± 0.037 | 56.86 |
| 15 | | 0.31 | 0.24 | 0.26 | 0.27 ± 0.029 | 82.35 |
| 30 | | BDL | BDL | BDL | - | - |
| **$T_1$: Y = -0.057x+2.897** <br> **$T_{1/2}$ = 5.28 Days** | | | **$T_2$: Y = -0.050x+3.186** <br> **$T_{1/2}$ = 6.02 Days** | | | |

*BDL below detectable limit

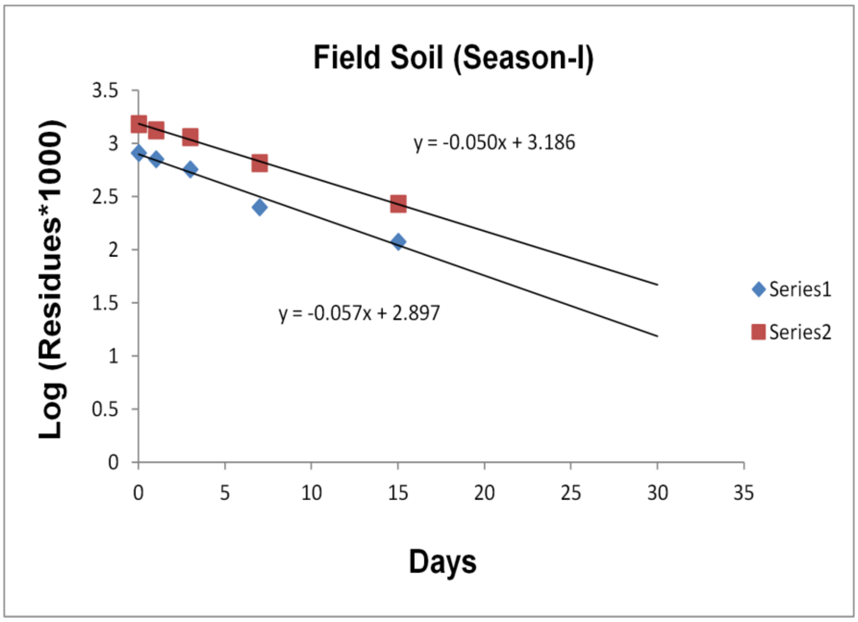


**Figure 2.** Linear plot of dissipation of Diclosulam in field soil in season-I

**Table 4.** Dissipation of Diclosulam in soybean cropped soil in season-II

| Days after application | Treatment | Residues in ppm. | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | $T_1$ | 0.93 | 0.95 | 0.89 | 0.92 ± 0.025 | - |
| 1 | | 0.75 | 0.70 | 0.73 | 0.73 ± 0.021 | 20.29 |
| 3 | | 0.67 | 0.59 | 0.62 | 0.63 ± 0.033 | 31.88 |
| 7 | | 0.47 | 0.43 | 0.45 | 0.45 ± 0.016 | 51.45 |
| 15 | | 0.18 | 0.25 | 0.20 | 0.21 ± 0.029 | 76.81 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | $T_2$ | 1.63 | 1.60 | 1.54 | 1.59 ± 0.037 | - |
| 1 | | 1.45 | 1.39 | 1.38 | 1.41 ± 0.031 | 11.11 |
| 3 | | 1.33 | 1.28 | 1.32 | 1.31 ± 0.022 | 17.82 |
| 7 | | 0.81 | 0.72 | 0.75 | 0.76 ± 0.037 | 52.20 |
| 15 | | 0.48 | 0.44 | 0.46 | 0.46 ± 0.016 | 71.28 |
| 30 | | BDL | BDL | BDL | - | - |
| **$T_1$: Y = -0.040x+2.931**<br>**$T_{1/2}$ = 7.52 Days** | | | **$T_2$: Y = -0.036x+3.192**<br>**$T_{1/2}$ = 8.36 Days** | | | |

*BDL below detectable limit

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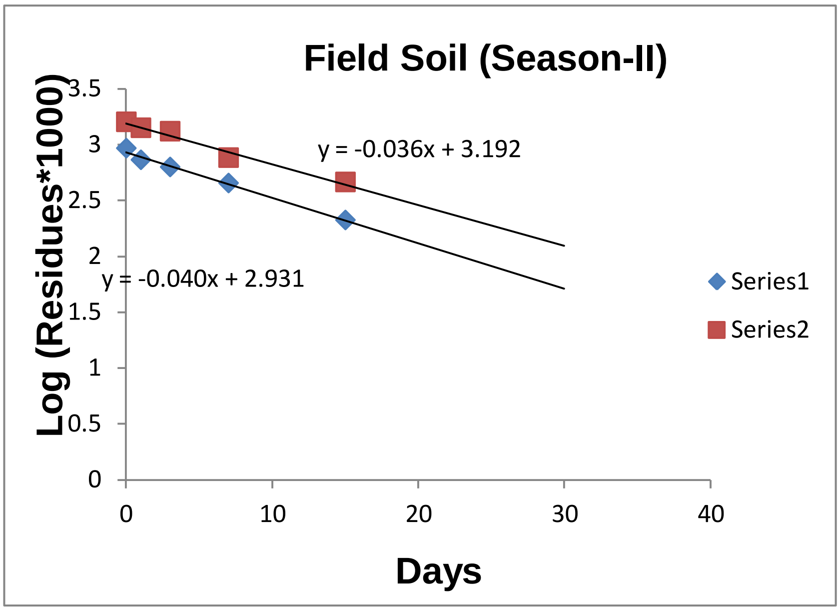


**Figure 3.** Linear plot of dissipation of Diclosulam in field soil in season-II

**Table 5.** Dissipation of Diclosulam in soybean cropped soil in season-III

| Days after application | Treatment | Residues in ppm. | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | $T_1$ | 0.84 | 0.89 | 0.86 | 0.86 ± 0.021 | - |
| 1 | | 0.78 | 0.73 | 0.75 | 0.75 ± 0.021 | 12.79 |
| 3 | | 0.65 | 0.60 | 0.61 | 0.62 ± 0.022 | 27.91 |
| 7 | | 0.41 | 0.33 | 0.35 | 0.36 ± 0.034 | 58.14 |
| 15 | | 0.18 | 0.15 | 0.16 | 0.16 ± 0.012 | 81.40 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | $T_2$ | 1.65 | 1.59 | 1.63 | 1.62 ± 0.025 | - |
| 1 | | 1.37 | 1.44 | 1.41 | 1.41 ± 0.029 | 12.76 |
| 3 | | 1.20 | 1.15 | 1.18 | 1.18 ± 0.021 | 26.95 |
| 7 | | 0.62 | 0.56 | 0.60 | 0.59 ± 0.025 | 63.79 |
| 15 | | 0.34 | 0.36 | 0.37 | 0.36 ± 0.012 | 77.57 |
| 30 | | BDL | BDL | BDL | - | - |
| **$T_1$: Y = -0.048x+2.927 $T_{1/2}$ = 6.27 Days** | | | **$T_2$: Y = -0.044x+3.184 $T_{1/2}$ = 6.84 Days** | | | |

*BDL below detectable limit

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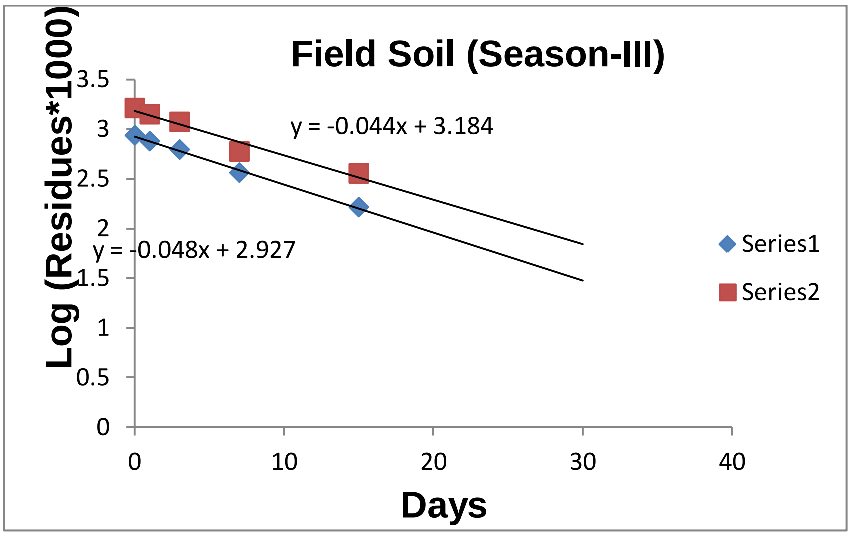


**Figure 4.** Linear plot of dissipation of Diclosulam in field soil in season-III

## References

Kramer, W. and Schirmer, U. (2007), Modern Crop Protection Compounds, Willey VCH, 3: 816-819.

Murdock, S.W. and Witt, W.W. (1999), Degradation of Diclosulam in tilled and non-tilled soil. Proceedings, Southern Weed Science Society, 52:198.

Owen, W.J. (2000), Herbicide metabolism as a basis for plant selectivity, Metabolism of agrochemicals in plants, Roberts T(Ed.), John Willey & Sons Ltd., London, 240-249.

Sheppard, B.R., Braxton, L.B., Barrentine, J.L., Geselius, T.C., Grant, D.L., Langston, V.B., Redding, K.D., Richburg, J.S.III, and Roby, D.B. (1997), Diclosulam: a new herbicide for broadleaf weed control in soybeans and peanuts, Proc. South. Weed Sci. Soc, 50:161.

http://www.epa.gov/opprd001/factsheets/appen_j.pdf – last accessed on 07.08.2010.

# EFFECT OF PARTHENIUM WEED (*PARTHENIUM HYSTEROPHORUS* L.) ON GRAZED PLANT COMMUNITIES DURING A PERIOD OF CONCERTED MANAGEMENT

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## ABSTRACT

The response of a rangeland community to different parthenium weed (*Parthenium hysterophorus* L.) management regimes is being assessed in a pastoral area at Kilcoy, Queensland, Australia. The area selected to do this work is on a gentle slope with good drainage and has a typical soil for this region (i.e. a brown-grey Dermosol). This area has been divided into four sites to undertake the following treatments: 1) non-grazed with chemical control applied; 2) non-grazed without chemical control; 3) grazed with chemical control applied and 4) grazed without chemical control. In the previous season, prior to the application of these treatments (i.e. in summer 2009/2010) the species composition of the community was recorded and used as the community benchmark. This paper reports upon that community structure. In total, 48 plant species were recorded within the above-ground community and 64 species within the soil seed bank. The above-ground vegetation was dominated by stoloniferous grass species, but there was also a high frequency of species belonging to the Malvaceae, Chenopodiaceae and Amaranthaceae families. Parthenium weed was found in high abundance and frequency within all of the four sites (i.e. 100 % and 63 % respectively). A correlation analysis showed that parthenium weed frequency was negatively associated with the Shannon index, as well as with the dry matter of the remaining species. A similar trend was found within the soil seed bank, with the species diversity being lower when parthenium weed's frequency was higher. These preliminary results provide a background against which the effect of chemical management of parthenium weed and/or a reduction in grazing pressure might improve the species composition of the community, which will be best seen in the years to come.

Keywords: parthenium weed, seed bank, grazing management, chemical control

## INTRODUCTION

Parthenium weed (*Parthenium hysterophorus* L.) is thought to significantly influence both the diversity and productivity of grassland plant communities (Evans 1997; Navie *et al*. 2004; Nguyen *et al.* 2010; Nigatu *et al*. 2010). Currently in Australia, parthenium weed is mostly a grassland weed, affecting cattle production in up to 20 million hectares of Queensland (Adkins 2010, personal communication). Previous studies of parthenium weed and its effect on plant biodiversity have reported a total habit alteration a rapid replacement of native grasses and other herbaceous species that were valuable grazing plants (Evans 1997), a reduction in the diversity of other plant species as well as their seed banks (Navie *et al*. 2004) and the replacement of native vegetation in several ecosystems (Yaduraju *et al*. 2005). The replacement of native species usually leads to a

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loss in the quality of the grazing land as less palatable species colonize. This in turn, reduces herbage palatability and digestibility and leads to a lowering of the carrying capacity of the grassland. As rangelands in Queensland cover more than 70% of the total state area, representing about 49% of the national cattle production, parthenium weed causes a serious threat to domestic stock yields and native plant community biodiversity.

The response of plant communities to livestock grazing differs across environments, especially when other factors such as the presence of an invasive species, are considered. An increase in community biodiversity, as a consequence of grazing, is often observed when domesticated large grazers are managed at low stocking rates on productive grasslands (Petraitis *et al.* 1989). While other studies have shown that grazing has a slight or negative impact upon biodiversity (Kelt and Valone 1995). In general, it has been found that diverse communities (i.e. those with high species richness) are more resistant to invasion (Richardson and Pysek 2006). Therefore, grasslands with a long history of grazing cattle usually present low resistance to invasion by an aggressive weed species such as parthenium weed. Up to now, little has been reported about the changes in species richness within a plant community when grazing is reduced and/or the invasive species are managed. In addition, very little has been reported concerning this topic when parthenium weed is the invasive species (Nguyen *et al.* 2010).

The aim of the present study is to characterize, then determine the response of a rangeland community to different parthenium weed management and grazing strategies, both when assessed in the above-ground and in the below-ground communities. This will provide an insight into the effect of parthenium weed upon the species composition and richness of these plant communities which can then be used to determine the speed of recovery of the community once the weed is removed and/or the grazing rate is reduced.

## MATERIALS AND METHODS

### Study location

This investigation was conducted at a property located in the Kilcoy district (27.11$^{o}$ S, 152.56$^{o}$ E) in South-East Queensland, Australia. The altitude of the site ranged from 225 to 238 m above sea level. The site has a gentle slope with good drainage and a typical soil of the region (i.e. brown-grey Dermosol, pH 6). The characteristic vegetation of the region was that of a native grassland but under continuous grazing, this has led to the replacement of certain desirable native species, such as black speargrass (*Heteropogon contortus* L.), with other less desirable species (Loi and Malcom 1998). The site has also been infested by parthenium weed for at least 25 years (Youles 2011 personal communication). Surveys were undertaken in the summer of 2009/2010 (i.e. February 2010) and the winter of 2010 (i.e. August 2010). Before and during these studies, the land was grazed by cattle at a typical stocking rate of *ca.* 0.5 cows ha$^{-1}$ during the drier winter months to 0.8 cows ha$^{-1}$ (Youles 2011 personal communication). For the past few years, the land was also subjected to an annual aerial application of mixture of Brush-Off ® at 10g/100 L water and 2,4-D at 320mL/100 L for the management of parthenium weed, except for 2009 when a shortage of rainfall meant that there was no need to undertake this management program (Lampard 2011, personal communication). The climate of the site is characterized by sub-tropical conditions, with a 40 year average annual precipitation of 950 mm occurring mainly in the summer (i.e. January and February). However, the annual rainfall was well above average during the surveyed year (i.e. 1619 mm for 2010). The

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mean night time temperatures are lower than 7°C in the winter nights and above 29°C during the day in the summer (Australian Bureau of Meteorology 2011).

Within this location, two rectangular plots (*ca.* 300 $m^2$) were selected; both with similar densities of parthenium weed infestation. One plot was protected by erecting a fence so no cattle or wild life could graze the land for the duration of the study (i.e. February 2010 to February 2012). The other site continued to be subjected to grazing (*ca.* stocking rate of 0.5 cows $ha^{-1}$ during the drier winter months to 0.8 cows $ha^{-1}$). Within each plot, two equally sized sub plots were defined: one having a herbicide application (i.e. mixture of Brush-Off ® at 10g/100 L water and 2,4-D at 320mL/100 L) applied twice a year in autumn, and the other two sub plots without any herbicide application. This created four treatments: 1) non-grazed with chemical weed control applied; 2) non-grazed without chemical weed control; 3) grazed with chemical weed control applied and 4) grazed without chemical weed control.

In the previous season, and prior to the application of the treatments (i.e. in summer 2009/2010), the species composition of the community was assessed for each sub plot. This community analysis will be used as the benchmark for all future community comparisons. The response of plant communities to each treatment over time will be determined with subsequent assessments on the site using the same sampling methodology.

**Assessment of the above-ground species diversity**

The above-ground vegetation was determined through sampling using 40 quadrats (1 $m^2$; 10 for each treatment). Within each quadrat, the above-ground plant community composition and species density of all species were determined by counting the number of individual species present. If plants were at the edge of a quadrat, only those rooted within the quadrat were counted. However, the density of two stoloniferous species: blue couch (*Digitaria didactyla* Willd.) and green couch (*Cynodon dactylon* (L.) Pers.) were estimated in the field by a coverage method which involved a visual estimate of the proportion of the quadrat occupied by these two species (Smart *et al*. 2006). Therefore, a conversion to individual plants was necessary for data analysis. It was assumed that each 2% of cover corresponded to an individual (i.e. 50% of coverage = 25 individuals). The determination of the above-ground biomass was taken from only a quarter of the quadrats (i.e. 0.25 $m^2$) and divided into parthenium weed and all other species (i.e. forbs, legumes, grasses and weeds). Upon cutting, these two classes of plants were put into separate brown paper bags and, upon returning to the University of Queensland, dried in an oven at 90 ± 5 °C for *ca.* 72 hours.

**Assessment of the soil seed bank**

To assess the soil seed bank in the four plots, two soil cores were collected from each of the 40 quadrats with a metal soil corer which has a diameter of 10 cm and could sample to a depth of 15 cm. The cores remained intact when removed from the soil and the two samples from each quadrat were mixed together. Hence, a total of 10 soil seed bank samples were collected from each plot during each survey season. The soil samples collected in the field were then spread thinly (5-7 mm layer) over a sterilized soil (University of California mixture; 3 cm thick layer) that was contained within a shallow plastic tray (20 × 25 × 6 cm, w/l/h; one quadrat per tray). These trays were distributed randomly on benches within a glasshouse at the University of Queensland, Brisbane, with

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a temperature maintained as close as to the ambient temperature outside using exhaust fans and an evaporative wet wall cooling system. Two control trays of sterilized soil alone were placed among the experimental trays to monitor for any seedlings that may have arisen from the soil or from the glasshouse environment. All trays were watered daily to maintain soil moisture content approximately at field capacity. Trays were observed weekly for any newly emerging seedlings. Once they had emerged, seedlings were counted and removed as soon as possible, depending on identification complexity. In the case where easy identification was not possible, representative individuals were planted into small pots and grown to maturity, to allow for later taxonomic identification. When no further emergence was recorded, the soil was stirred and watering stopped for a week, then rewatered to trigger further germination. Each seed bank assessment, from every survey season, was run over a six month period to allow for all of the species in the seed bank to be identified, including those with long-term seed dormancy. The 'unidentified species' term was used for several seedlings that died before they could be identified.

### Statistical analysis

The data collected was used to characterize the vegetation composition using the following parameters calculated for individual species using the following formulae:

1) $$\text{Presence of species A (\%)} = \frac{\text{Number of sub plots where species A occurs}}{\text{Total number of sub plots}} \times 100$$

2) $$\text{Frequency of species A (\%)} = \frac{\text{Number of quadrats where species A occurs}}{\text{Total number of quadrats sampled}} \times 100$$

3) $$\text{Density of species A} = \frac{\text{Number of individuals of species A}}{\text{Total number of quadrats where the species A occurs}}$$

The species diversity within the above-ground vegetation and the seed bank was assessed using the Shannon-Weiner index ($H' = -\sum_{i=1}^{S} p_i \log_e p_i$, where $S$ is the number of species or species richness, $N$ is the total number of all individuals and $p_i$ is the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: $p_i = n_i / N$, where $n_i$ is the number of individuals in species $_i$ i.e. the abundance of species $_i$ -Krebs, 1989-). All data sets were analysed by an Analysis of Variance using a General Linear Model procedure in Minitab, version 16 (Minitab Inc., USA). No data transformation was needed. The general linear model was set up with two seasons (summer and winter) and two grazing and herbicide treatments as factors for the analysis of the Shannon index and species density data. They were analysed using an Adjusted Sum of Squares approach using 95.0 % confidence intervals. A correlation analysis was used to study linear associations between the measured variables and the calculated parameters.

## RESULTS

### Above-ground species diversity

In total, 48 species were recorded in the above-ground plant community and 64 species in the soil seed bank prior to the application of the treatments (Table 1). In the above-ground community, 12 species belonged to the Poaceae, three to the Asteraceae and the remaining 37 species came from 16 other families. Native species represented 48% of those present in the above-ground plant community. The remainder were introduced weed species, most of which were broadleaf species (18 species). The majority of the species identified in the above-ground community were perennials and/or annuals that could behave as perennials depending on weather conditions (45.8 and 22.9 % respectively).

The most dominant species in the above ground plant community were the weed species *Portulaca oleracea* L. (100 and 85% respectively) and *Dysphania carinata* (R.Br.) Mosyakin & Clemants (100 and 70% respectively) and the grass species *Digitaria didactyla* Willd. (100 and 78% respectively) and *Paspalidium distans* Trin. (100 and 68% respectively). The presence, frequency and density of parthenium weed were high to moderate across the whole site (i.e. 100%, 62.5%, 5.69 plants/m$^{-2}$ respectively). The dry matter of all other species in the quadrat was negatively correlated to the dry matter ($r^2$ = -0.35; $P$ = 0.029), density ($r^2$ = -0.56; $P$ = 0.001) and frequency ($r^2$ = -0.44; $P$ = 0.05) of parthenium weed. However, the Shannon-Wiener index was not significantly correlated to the frequency, density or dry matter of parthenium weed.

Table 1. The presence, frequency and density of plants in the above-ground or seeds in the below-ground plant community at a pastoral site in Kilcoy during the summer of 2009/2010.

| **Species** | **Presence (%)** | | **Frequency (%)** | | **Density (pl m$^{-2}$)** | |
|---|---|---|---|---|---|---|
| | Above-ground | Below-ground | Above-ground | Below-ground | Above-ground | Below-ground |
| *Alternanthera nana* R.Br. | 25 | 25 | 5.0 | 5.0 | 0.05 | 1.03 |
| *Alternanthera pungens* Kunth | 100 | 25 | 45.0 | 10.0 | 1.10 | 4.13 |
| *Amaranthus spinosus* L. | 25 | 50 | 2.5 | 2.5 | 0.03 | 7.23 |
| *Amaranthus viridis* L. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Anagallis arvensis* L. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Aristida* sp. | 50 | 0 | 5.0 | 0.0 | 0.08 | 0.00 |
| *Boerhavia dominii* Meikle & Hewson | 25 | 0 | 2.5 | 0.0 | 0.03 | 0.00 |
| *Bothriochloa decipiens* (Hack.) C.E.Hubb. | 100 | 75 | 57.5 | 7.5 | 2.23 | 0.00 |
| *Chloris divaricata* R.Br. | 75 | 100 | 12.5 | 27.5 | 0.18 | 23.77 |
| *Chloris ventricosa* R.Br. | 0 | 25 | 0.0 | 7.5 | 0.00 | 8.27 |
| *Conyza bonariensis* (L.) Cronq. | 0 | 100 | 0.0 | 37.5 | 0.00 | 63.06 |
| *Conyza sumatrensis* (Retz.) E.H. Walker | 50 | 75 | 7.5 | 17.5 | 0.15 | 17.57 |
| *Crassula sieberiana* (Schult. & Schult.f.) Druce | 0 | 75 | 0.0 | 7.5 | 0.00 | 3.10 |
| *Cyclospermum leptophyllum* (Pers.) Sprague | 0 | 75 | 0.0 | 20.0 | 0.00 | 12.40 |
| *Cynodon dactylon* (L.) Pers. | 50 | 50 | 17.5 | 5.0 | 0.99 | 4.13 |
| *Cyperus brevifolius* (Rottb.) Hassk. | 0 | 100 | 0.0 | 30.0 | 0.00 | 28.94 |
| *Cyperus gracilis* R.Br. | 100 | 100 | 55.0 | 97.5 | 1.28 | 1515.7 |
| *Cyperus iria* L. | 0 | 50 | 0.0 | 10.0 | 0.00 | 5.17 |
| *Datura ferox* L. | 25 | 0 | 2.5 | 0.0 | 0.05 | 0.00 |
| *Digitaria didactyla* Willd. | 100 | 100 | 77.5 | 90.0 | 14.20 | 625.51 |
| *Dysphania carinata* (R.Br.) Mosyakin & Clemants | 100 | 100 | 70.0 | 50.0 | 3.20 | 107.52 |
| *Dysphania pumilio* (R.Br.) Mosyakin & Clemants | 0 | 100 | 0.0 | 70.0 | 0.00 | 200.57 |
| *Einadia polygonoides* (Murr.) Paul G. Wilson | 75 | 100 | 20.0 | 72.5 | 0.20 | 122.00 |

| **Species** | **Presence (%)** | | **Frequency (%)** | | **Density (pl m$^{-2}$)** | |
|---|---|---|---|---|---|---|
| | Above-ground | Below-ground | Above-ground | Below-ground | Above-ground | Below-ground |
| *Einadia trigonos* (Schult.) Paul G. Wilson | 100 | 100 | 20.0 | 45.0 | 0.40 | 46.52 |
| *Eleusine indica* (L.) Gaertn. | 50 | 75 | 22.5 | 22.5 | 0.33 | 18.61 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| *Eragrostis cilianensis* (All.) Janch. | 25 | 100 | 2.5 | 32.5 | 0.03 | 16.54 |
| *Galactia tenuiflora* (Willd.) Wight & Arn. | 75 | 0 | 17.5 | 0.0 | 0.25 | 0.00 |
| *Gamochaeta pensylvanica* (Willd.) Cabrera | 0 | 75 | 0.0 | 32.5 | 0.00 | 42.39 |
| *Glycine* sp. | 75 | 0 | 22.5 | 0.0 | 0.33 | 0.00 |
| *Gomphrena celosioides* Mart. | 75 | 25 | 12.5 | 5.0 | 0.18 | 2.07 |
| *Heliotropium amplexicaule* Vahl | 0 | 50 | 0.0 | 5.0 | 0.00 | 6.20 |
| *Hydrocotyle acutiloba* (F.Muell.) Wakef. | 0 | 50 | 0.0 | 27.5 | 0.00 | 18.61 |
| *Ipomoea* sp. | 25 | 0 | 2.5 | 0.0 | 0.03 | 0.00 |
| *Juncus usitatus* L.A.S.Johnson | 0 | 25 | 0.0 | 5.0 | 0.00 | 2.07 |
| *Lepidium africanum* (Burm.f.) DC. | 75 | 100 | 15 | 37.5 | 0.23 | 80.64 |
| *Lepidium bonariense* L. | 0 | 100 | 0.0 | 45.0 | 0.00 | 34.12 |
| *Lepidium didymum* L. | 0 | 100 | 0.0 | 57.5 | 0.00 | 172.66 |
| *Macroptilium atropurpureum* (DC.) Urb. | 25 | 0 | 2.5 | 0.0 | 0.03 | 0.00 |
| *Malva parviflora* L. | 50 | 25 | 5.0 | 5.0 | 0.08 | 2.07 |
| *Malvastrum americanum* (L.) Torr. | 25 | 75 | 2.5 | 10.0 | 0.03 | 5.17 |
| *Malvastrum coromandelianum* (L.) Garcke | 100 | 25 | 25.0 | 2.5 | 0.40 | 1.03 |
| *Ophioglossum reticulatum* L. | 50 | 0 | 5.0 | 0.0 | 0.13 | 0.00 |
| *Oxalis exilis* A. Cunn. | 100 | 100 | 50.0 | 95.0 | 0.95 | 687.55 |
| *Oxalis purpurea* L. | 50 | 100 | 7.5 | 57.5 | 0.13 | 139.58 |
| ***Parthenium hysterophorus* L.** | **100** | **100** | **62.5** | **67.5** | **6.33** | **1298.5** |
| *Paspalidium distans* (Trin.) Hughes | 100 | 100 | 67.5 | 60.0 | 3.83 | 89.95 |
| *Plantago debilis* R.Br. | 75 | 100 | 7.5 | 25.0 | 0.10 | 17.58 |
| *Polygonum aviculare* L. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Portulaca oleracea* L. | 100 | 100 | 85.0 | 95.0 | 3.43 | 911.91 |
| *Portulaca pilosa* L. | 50 | 100 | 5.0 | 37.5 | 0.05 | 33.09 |
| *Pterocaulon redolens* (Willd.) Fern.-Vill. | 0 | 50 | 0.0 | 5.0 | 0.00 | 2.07 |
| *Rumex brownii* Campd. | 75 | 50 | 15.0 | 12.5 | 0.15 | 12.41 |
| *Schenkia spicata* (L.) Mansion | 0 | 100 | 0.0 | 87.5 | 0.00 | 385.64 |
| *Sida cordifolia* L. | 50 | 25 | 5.0 | 2.5 | 0.05 | 2.07 |
| *Sida rhombifolia* L. | 100 | 50 | 32.5 | 7.5 | 0.50 | 3.10 |
| *Sida spinosa* L. | 100 | 75 | 37.5 | 17.5 | 0.53 | 12.41 |
| *Sida subspicata* F.Muell. ex Benth. | 100 | 0 | 50.0 | 0.0 | 2.58 | 0.00 |
| *Sisyrinchium* sp. | 0 | 25 | 0.0 | 2.5 | 0.00 | 2.07 |
| *Solanum americanum* Miller | 75 | 50 | 30.0 | 5.0 | 0.65 | 2.07 |
| *Soliva* sp. | 0 | 50 | 0.0 | 17.5 | 0.00 | 369.11 |
| *Sonchus oleraceus* L. | 0 | 25 | 0.0 | 5.0 | 0.00 | 2.07 |
| *Sporobolus creber* De Nardi | 25 | 100 | 2.5 | 32.5 | 0.03 | 63.07 |
| *Sporobolus elongatus* R.Br. | 25 | 75 | 2.5 | 27.5 | 0.03 | 32.05 |
| *Stachys arvensis* (L.) L. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Tribulus micrococcus* Domin | 100 | 0 | 45.0 | 0.0 | 1.10 | 0.00 |
| *Urochloa panicoides* Beauv. | 25 | 25 | 2.5 | 2.5 | 0.03 | 2.07 |
| *Urtica incisa* Poir. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Verbena litoralis* Kunth | 25 | 50 | 2.5 | 7.5 | 0.03 | 5.17 |
| *Verbena rigida* (Hayek) Moldenke | 25 | 75 | 2.5 | 20.0 | 0.08 | 11.37 |
| *Vittadinia sulcata* N.T.Burb. | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Wahlenbergia gracilis* (G.Forst) A.DC. | 0 | 100 | 0.0 | 67.5 | 0.00 | 111.66 |
| *Unknown 1(shrub)* | 75 | 0 | 42.5 | 0.0 | 1.30 | 0.00 |
| *Unknown 2* | 0 | 75 | 0.0 | 10.0 | 0.00 | 4.14 |
| *Unknown 3* | 0 | 25 | 0.0 | 2.5 | 0.00 | 1.03 |
| *Unknown 4* | 0 | 25 | 0.0 | 2.5 | 0.00 | 2.07 |

## Soil seed bank diversity

The seed bank analysis showed a slightly more diverse flora than was seen in the above-ground vegetation (average Shannon-Wiener index: 2.2 and 1.8 respectively), with an additional 11 botanical families represented there. Similar to the above-ground plant community, the seed bank had 12 species belonging to the Poaceae, however there were eight species belonging to the Asteraceae and 44 species from 25 other families. Native species represented only 39% of those present in the below-ground community. The remainder were introduced species, and 88.2% of these were considered to be pasture weeds. The seed bank correlation analysis did not show the same significant negative trends as the above-ground results.

## DISCUSSION

At the start of the study, the above-ground plant community was clearly dominated by species considered to be weeds (i.e. 38 weed species with only 12 grass species present). Only three grasses showed 100% presence (*i.e. Digitaria didactyla* Willd., *Paspalidium distans* (Trin.) Hughes and *Bothriochloa decipiens* (Hack.) C.E.Hubb.). According to previous observations of the region's native pastures by Loi and Malcom (1998), this kind of plant community composition was indicative of a history of heavy grazing. Thus, this community probably presents a low resistance to invasion by weeds, including parthenium weed and may explain why there is a high presence and frequency of the weed at the site.

Although the frequency and presence of the dominant species recorded in the survey changed in magnitude for the seed bank, *Portulaca oleracea* L., *Digitaria didactyla* Willd, *Dysphania carinata* (R.Br.) Mosyakin & Clemants, *Paspalidium distans* (Trin.) Hughes, *Parthenium hysterophorus* L., *Cyperus gracilis* R.Br. and *Oxalis exilis* A. Cunn. were still the most common species (Table 1).

Conversely to what has been reported before (Nguyen *et al.* 2010; Nigatu *et al*. 2010) the plant diversity, either in the above or in the below ground community did not show a decrease under high frequencies of parthenium weed. However, greater biomass of the weed did show a negative effect on the community's biomass production.

The greater number of species and families found in the below-ground community, and the lack of negative correlations with parthenium weed frequency and density at the seedling stage, may indicate that it will be possible to recover this grassland community once the invader is better managed. Further data collection over the coming year will show if there is any recovery of the biodiversity and biomass production of the community due to the chemical control of parthenium weed and which functional groups (i.e. forbs, weeds, and woody species) prosper after the application of the treatments.

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## REFERENCES

Adkins, S.W. and Navie, S.C. (2006) Parthenium weed: a potential major weed for agro-ecosystems in Pakistan. *Pakistan Journal Weed Science Research* 12 (1-2): 19-36.

Adkins, S.W. (2010) Pers. comm.

Bostock, P.D. and Holland, A.E. (eds) (2010). *Census of the Queensland Flora 2010*. Queensland Herbarium, Department of Environment and Resource Management, Brisbane.

Bureau of Meteorology (2011). National Climatic Database http://www.bom.gov.au.

Evans, H.C. (1997) *Parthenium hysterophorus*: a review of its weed status and the possibilities for biological control. *Biocontrol News and Information* 18: 89N-98N.

Kelt, D.A. and Valone, T.J. (1995) Effects of grazing on the abundance and diversity of annual plants in Chihuahuan desert scrub habitat. *Oecologia* 103 (2): 191-195.

Krebs, C.J (1989) *Ecological methodology*. Harper Collins, New York.

Lampard, S. (2011), Kilcoy Somerset Council, pers. comm.

Loi, J.K. and Malcom, D.T. (1998) *Land resource assessment SEQ 2001 REPORT 6. Soils and land suitability. The Kilcoy – Woodford Area*. Department of Natural Resources, Brisbane Queensland.

Navie, S.C., Panetta, F.D., McFadyen, R.E. and Adkins, S.W. (2004) Germinable soil seedbanks of central Queensland rangelands invaded by the exotic weed *Parthenium hysterophorus* L. *Weed Biology and Management* 4: 154-167.

Nguyen, T., Navie S. and Adkins, S.W. (2010) The effect of parthenium weed (*Parthenium hysterophorus* L.) on plant diversity in pastures in Queensland, Australia. In *Proceedings of the 17 th Australasian Weeds Conference,* ed. Zydenbos, p. 138. Cristchurch, New Zeland.

Nigatu, L., Hassen, A., Sharma, J. and Adkins, S.W. (2010) Impact of *Parthenium hysterophorus* on grazing land communities in north-eastern Etiopia. *Weed Biology and Management* 10: 143-152.

Olff, H. and Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* 13 (7): 261-265.

Petraitis, P. S., Latham, R. E. and Niesenbaum, R.A. (1989) The maintenance of species-diversity by disturbance. *Quarterly Review of Biology* 64 (4): 393-418.

Richardson, D.M. and Pysek, P. (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography* 30: 409-431.

Smart, J., Sutherland, W.J. and Watkinson, A.R. (2006) Grassland-breeding waders: identifying key habitat requirements for management. *Journal of Applied Ecology* 43(3): 454-463.

Yaduraju, N.T., Susshilkumar, Prasad Babu, M.B.B. and Gogoi, A.K. (eds.) (2005) *Parthenium hsyterophorus - distribution, problem and management strategies in India,* University of Agricultural Sciences, Bangalore.

Youles, D. (2011) Landowner, pers. comm.

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# CONTROL OF WEEDY RICE IN DIRECT-SEEDED RICE USING THE CLEARFIELD PRODUCTION SYSTEM IN MALAYSIA

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## Summary

Weedy rice (*Oryza sativa* complex) is a serious threat to direct-seeded culture in Malaysia because of its taxonomic and physiological similarities to cultivated rice. This weedy rice problem was first observed in 1988 but later became widespread and prevalent in rice cultivation in the 2000's. Farmers have considered weedy rice as of significant importance and a serious threat since no selective herbicide was available for controlling it prior to the advent of an imidazolinone tolerant variety (IMI-TR). The development of local IMI-TR rice, a collaborative project between MARDI and BASF (Malaysia) Sdn Bhd, started in 2003 at MARDI Station in Penang. IMI-TR Line No. 1770 from USA was crossed with a popular local rice cultivar MR 220 using conventional breeding techniques. The goal of the project is to offer farmers an effective and farmer friendly solution to the weedy rice problem with minimal changes to their normal practices. Two locally developed IMI-TR varieties namely MR 220CL1 and MR 220CL2 together with the technique known as Clearfield Production System (CPS) were officially launched on the 8th July 2010 in FELCRA Seberang Perak rice granary. This CPS for rice was the first launch in Malaysia as well as in the Asia Pacific Region. This system is justified by the need to offer an innovative alternative method to manage weedy rice in wet-seeding culture. The CPS package for rice consists of three main components namely Clearfield certified seeds, OnDuty® herbicide (imazapic/imazapyr) and the Stewardship Guide. The seeds are supplied in standard 20 kg bags while the OnDuty® herbicide is packed in a box of 4 sachets of 31 g each. The seed rate per ha is 7 bags (140 kg/ha) which is the normal seed rate used by farmers. The seeds and OnDuty herbicide are sold together as a package to ensure that; a) both seeds and herbicides are available together at all times, b) OnDuty herbicide and seeds are applied at the correct rate, and c) the correct variety together with the correct herbicide are used. To ensure successful crop establishment and attainment of high yield of Clearfield rice varieties, standard good agricultural practices have to be implemented and followed from sowing to harvesting. All these practices are basically similar to practices currently adopted by farmers in Malaysia.

**Keywords**: Clearfield Production System, weedy rice, imidazolinone, direct-seeded rice

## Introduction

A major factor that contributes to a higher production cost for rice is weed control. Currently, rice farmers throughout the world face a unique weed problem. A weedy relative of cultivated rice known as weedy rice has invaded and severely infested rice

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fields. Weedy rice has long been a major threat in the direct-seeded rice culture in Asia, especially in Malaysia. Because of the close genetic relation to commercial rice, weedy rice has proved to be difficult to control, as it cannot be controlled with conventional rice herbicides. Weedy rice biotypes have morphology similar to cultivated rice varieties and, therefore, are more difficult to control compared to other weeds. Weedy rice cannot be harvested and reduces yield because it matures earlier than cultivated rice, shatters and lodges easily. Under moderate weedy rice infestation (15-20 panicles/$m^2$), yield loss is approximately 12 to 15%; under high infestation (21 to 30 panicles/$m^2$) yield loss is 15 to 22%; while under heavy infestation (more than 50 panicles/$m^2$), lodging of weedy rice plants may occur and can cause total yield loss under tropical climatic conditions (Azmi and Abdullah 1998, Azmi and Karim, 2008).

Herbicide-tolerant rice cultivars may hold the answer to effective management of problem weeds especially weedy rice (*Oryza sativa* complex) in direct-seeding in Malaysia (Azmi and Karim, 2008). It is estimated that USD 40 million in rice yield value is lost to weedy rice competition every season. Following the discovery of weedy rice in 1988, it has rapidly become an important and prevalent weed in all grain areas in Malaysia.

The combination of imidazolinone tolerant varieties (MR 220CL1 and MR 220CL2) with imidazolinone herbicides is known as the Clearfield Production System (CPS). Clearfield Rice Production System is able to effectively control weedy rice, which no other herbicides or system can control in wet-seeding culture. The use of the CPS will directly benefit the rice industry in Malaysia by providing an effective chemical control for the management of weedy rice and other noxious paddy weeds. The CPS is the first herbicide tolerant rice to be introduced in Malaysia that is non GMO, and signifies the beginning of a paradigm shift in modern agriculture for effective weedy rice control.

## The Clearfield Production System

The use of a herbicide tolerant rice variety will benefit the rice industry and farmers:

- √ Herbicide tolerant cultivars will help to lower the cost of weed management making rice cultivation even more cost effective.
- √ The use of the imidazolinone tolerant variety has been demonstrated to bring fields, that are under performing due to heavy infestation of weedy rice (1-2 ton/ha), back into higher levels of productivity (>5.5 t/ha)
- √ This technology is cost efficient, where returns ranging from 5 to 8 times, equivalent to USD 1000 to USD 1600, could be expected from investment on this technology of about USD 200/ha.
- √ It allows good flexibility in the timing of herbicide application to control weeds not controlled by conventional means or products
- √ Replacing the larger volume herbicides with imidazolinone herbicides which are applied in much lesser volumes, will result in the reduction of herbicides released into the environment and the eco-system
- √ This technology saves water through delayed flooding because this herbicide works best and is highly effective under saturated or minimal water condition. This itself indirectly prevents Golden Apple Snail attack on rice crop as the snails become inactive under minimal water condition

## Market potential

- √ It is estimated that at least 10% of the granary areas in Malaysia (20,500 ha) have a severe weedy rice problem which results in a total loss of 50,000 t/season (RM55 million). As many as 20,000 farmers could gain by using a imidazolinone-tolerant rice variety as a result of weedy rice control.
- √ Dedicated rice seed producers were appointed to produce the Clearfield certified seeds to be commercialised under the CPS. Under this system, farmers will be offered an imidazolinone tolerant rice seed package containing certified seeds, imidazolinone herbicide and Stewardship Guide

## Comparisons between Clearfield Production System and major crop establishment methods

The CPS is basically the same as wet seeding technique. The field is thoroughly puddled and leveled before sowing. Sowing can be carried out using motor-blower or line seeders such as drum seeders or knapsack row seeders. Under water seeding culture, water is retained in the field for seeding. On the other hand, transplanting requires saturated soil without standing water for good crop establishment. Other comparisons are in Table 1.

**Table 1.** Comparisons between Clearfield Production System, mechanical transplanting, water seeding and wet seeding technique

| | Clearfield Production System | Mechanical transplanting | Water seeding | Wet seeding |
|---|---|---|---|---|
| Optimal field conditions | Saturated | Saturated | 5-10 cm water depth | Saturated |
| Crop establishment | Low incidence of golden apple snail | Low incidence of weedy rice and golden apple snail | High incidence of golden apple snail attack | High infestation of weedy rice and low incidence of golden apple snail |
| Cost of establishment | Low | High | Low | Low |
| Cost of weed control | Moderate - High | Low | Low | Moderate - High |
| Optimal season | Off season | Main season | Main season | Off season |
| Equipment | Motor-blower, drum seeders, knapsack row seeder | Transplanter | Motor-blower | Motor-blower, drum seeders, knapsack row seeder |
| Weeds associated with planting method | A wider range of weeds i.e. grasses, broadleaves and sedges. | Broadleaves but other weeds i.e. grasses and sedges depend on time of flooding | Broadleaves are the dominant weeds | A wider range of weeds i.e. grasses, broadleaves and sedges |
| Weed control | Total weed control with imidazolinone herbicides with minimum roguing of weedy rice. | 2,4-D, Sulfonyl ureas, pre-emergence herbicide and roguing of weedy rice. | 2,4-D, Sulfonyl ureas and roguing of weedy rice. | Graminicide, 2,4-D and sulfonyl urea product followed by roguing of weedy rice. |

## Large scale evaluation

A large scale evaluation of CPS cultivation (47.62 ha) was carried out in the off season 2010 in fields seriously affected by weedy rice infestation in the previous season (main

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season 2009) in FELCRA Seberang Perak rice estate. Weedy rice populations were effectively controlled in the fields resulting in an average yield increment of 0.76 ton/ha from 4.93 ton/ha (main season 2009) to 5.69 ton/ha (off season 2010) giving a better B/C ratio from 2.55 to 3.42 (Table 2).

**Table 2**. Financial analysis of Clearfield Production System in the 2010 off-season compared with Felcra management at Felcra Seberang Perak Rice Estate in the main 2009 season

| Block | Area (ha) | Main Season 2009/10 | Off Season 2010 | Yield Increment | Increase In Income |
|---|---|---|---|---|---|
| | | Yield (t/ha) | | (t/ha) | (RM/ha) |
| T 5A | 10.68 | 4.11 | 5.43 | 1.32 | 659.16 |
| T 5B | 10.88 | 3.84 | 4.61 | 0.77 | 384.51 |
| T 6A | 14.76 | 5.61 | 6.51 | 0.90 | 449.43 |
| T 6B | 11.30 | 6.14 | 6.20 | 0.06 | 29.96 |
| Av/ha | | 4.93 | 5.69 | 0.76 | 380.76 |
| B/C ratio | | 2.55 | 3.42 | | |
| B/C ratio* | | 2.29 | 3.08 | * Sensivity analysis - return 10% | |
| B/C ratio** | | 2.32 | 3.11 | ** Sensitivity analysis - cost increase 10% | |

## References

**Azmi, M.** and Abdullah, M. Z. (1998). *A manual for the identification and control of padi angin (weedy rice) in Malaysia*. MARDI Publication, Serdang. pp. 18.

**Azmi, M**. and Rezaul, K. (2008). *Weedy Rice - Biology, Ecology and Management*. MARDI Publication. p. 56.

# NEW FORMULATION OF GLYPHOSATE AND RECENT DEVELOPMENTS ON THE USES OF GLYPHOSATE IN INDIA

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**Abstract**

Glyphosate usage is much lower in developing countries. Labour availability for weed control is a big question. Time available for de-weeding operations is also very less. Cost involved in manual weed control is very high. *Cyperus* and *Cynodon* multiply very fast after manual weed control.

Successful weed control depends on interactions between weeds (growth habit/size), environmental factors (light, temperature, moisture, wind) and quality of the spray solution. The environment plays major role in the physical development of plants. The size, shape, and thickness of leaves, cuticle, wax deposition, and changes in the water and nutrient status within plants are crucial. These changes affect glyphosate efficacy in several ways viz., interception, retention, penetration, translocation to the site of action. Prevailing environmental conditions also, before, at and after application affect glyphosate performance. Actively growing plants are ideal for glyphosate penetration and retention. Rain and high wind at time of application are not desired at all.

Glyphosate inhibits the shikimate pathway enzyme EPSPSase, and enzyme that acts late in that pathway. Glyphosate reduces the weed population and their decomposition makes the soil porous.

Glyphosate usage other than in orchards are pre tillage / post harvest, pre harvest, after sowing before germination, before sowing/germination, inter-row and non-crop area. Through Herbidisk in segments like PT/PH, time, labour and water can be saved. Sure shot (Adjuvant) gives better rainfastness.

**Introduction**

Excel Mera 71(a new 71% WDG formulation of Ammonium Salt of Glyphosate ) gives quicker and higher absorption of glyphosate. Better management of broad leaf & hardy weeds and good rain fastness. It is effective at lower dosage / ac. Product can be easily transported to the point of consumption.

This formulation can be better used in cotton for weed management in interrows with the help of foam nozzle and guard. Cleaning of bunds & channels and surroundings helps in reducing the alternate hosts of mealy bug and CLCV. S. Sanbagavalli et.al.,2009b in an experiment observed that presowing weed control through SSB (Stale Seed Bed) by glyphosate with post sowing method of hand weeding twice recorded significantly higher seed cotton yield (1815 kg / ha ) and was comparable with pendimethalin 1.0 kg / ha followed by one HW on 45 DAS (1798 kg / ha). For effective control of *Rottboellia* *cochinchinensis*, pre – emergence application of pendimethalin 1.5 kg / ha in cropped fields and post emergence spraying of glyphosate 3.0 kg / ha under non crop or fallow seasons effectively manages *Rottboellia cochinchinensis*, a thorny problematic grassy weed in black clay soils of Tamil Nadu( C. Nithya et. al.,2009). D.Ravisankar et. al., 2009 reported that tuber treatment with cytokinin (0.01%) followed by application of glyphosate @ 3.0 kg / ha on third day controlled the tuber emergence by 92%. Whereas, N.K. Prabakaran et.al., 2009 observed directed application of glyphosate 41% SL @ 15 ml / lit of water could reduce the density of *Cyperus rotundus* effectively. Higher yield and economic returns were obtained in glyphosate41% SL 15 ml /lit of water.

## Weed Management with Glyphosate: Major Highlights 2009-10

Very recently a lot of work on Glyphosate has been carried out in India especially in Southern and Western parts. Results are promising and farmers are using this herbicide for the weed management in their crop fields on large scale. However, it has been noticed that a few hard –to- kill weed species have started showing resistance against IPA salt of Glyphosate 41 SL formulation . The application of Excel Mera 71 ( Ammonium salt of Glyphosate 71% SG ) have shown remarkably better result against such hard –to-kill weed species.

## Cotton:

Wider spacing of cotton and slow growth during initial stages results in luxurious growth of weeds. Timely weeding after the crop emergence is not feasible due to demand and cost for agricultural labourers and frequent rainfall in the Monsoon season. These warrant the adoption of pre-sowing weed control method i.e., Stale Seed Bed (SSB) in order to reduce the weed competition after the establishment of the crops.

Stale seedbed may be defined as a seedbed prepared several days, weeks or months prior to sowing or planting a crop. Weed seeds in the surface layer of the soil are induced to germinate, emerge and controlled before cropping so that a part of weed population could be eliminated by pre-plant shallow tillage or by post emergence non selective herbicide spray. Yield loss of 56-85% was observed in cotton grown under un-weeded condition. Weeds remove 30-50% of applied nutrients and 20-40% of available moisture besides reducing the yield and quality of the produce.

S. Sanbagavalli et.al., 2009a reported SSB by Glyphosate either with hand weeding (HW) or Pendimethalin 1.0 kg / ha followed by one HW on 45 DAS recorded maximum B:C ratio. SSB by Glyphosate + Pendamethalin 0.5 kg / ha followed by one HW on 45 DAS registered comparatively higher B:C ratio of 2:30 than SSB by cultivation (2:05) and conventional seedbed preparation (1:83) with all the post sowing weed management treatment combinations. So, there is the possibility of reducing half of the recommended pendimethalin to cotton crops and one hand weeding to green gram by adopting pre sowing SSB technique. In another study, S. Sanbagavalli et.al., 2009b concluded that pre-sowing weed control through SSB by Glyphosate with post-sowing hand weeding twice recorded significantly higher seed cotton yield (1815 kg / ha) and was comparable with Pendimethalin 1.0 kg / ha followed by one HW on 45 DAS (1798 kg / ha). Pendamethalin 0.5 kg / ha followed by one HW on 45 DAS with SSB Glyphosate exhibited seed cotton yield (1701 kg / ha) higher than that of conventional seedbed + HW twice (1435 kg / ha).

*Solanum elaeagnifolium* (Silver Leaf) is a perennial weed that has become increasingly troublesome over the past several decades. Extensive use of soil-applied herbicides, accompanied by a reduction in annual weed competition and reduced tillage, has contributed to its spread and establishment as a noxious weed in cultivated fields. Cotton yields have been reduced by 75% and in cereals 12% grain yield reduction. Its problem is more aggressive in black clay soils where the dominant crops are cotton, maize and sunflower. R.Sathya Priya et.al.,(2009) have reported that post-emergence application of glyphosate 10 ml + 2,4-D 6 g + soap solution 2 ml per litre of water reduces the infestation of *Solanum elaeagnifolium* distinctly.

**Banana:**

Bananas are planted in wider spacing and being perennial, slow growing at early stages give scope for stiff weed competition. *Portulaca quadrifida* is an annual weed which multiplies with seeds as well as vegetative fragments with inter-nodes. It is a common weed in garden land crops like banana. S. Srinivasan *et.al*.,(2009) observed higher yield and economic returns in bananas when weed management was carried out with glyphosate.

**Hard to Kill - Weeds:**

***Tithonia rotundifolia***

*Tithonia rotundifolia* is a noxious weed which belongs to the family Asteraceae , a native of Northern America named as Mexican-sunflower. It is an annual monocarpic plant and seeds exhibit a period of dormancy before germinating. *Tithonia rotundifolia*, is an agricultural weed, casual alien, cultivation escape and noxious weed.

The invaded weed into the black soils of Slur areas of Coimbatore district has been identified as *Tithonia rotundifolia* (Mill.) Blake. This produces small-size seeds with high reproductive ability. Entire or lobed dark green leaves (3-6” long) with hairy undersides are generally ovate to triangular in shape with serrated margins. Leaves are spindled shape with shallowly incised at the base and thin hairy in nature with parallel venation. Leaf arrangements are alternate and opposite. Stem is succulent and angular and roots are adventitious and runners. Flowers have yellow colour petals and ray florets are solitary and terminal heads. V.S.Mynavathi et. al.,(2009) have reported that post-emergence application of Glyphosate @10 ml/L + 2, 4-D 6 g/L + soap solution 2 ml / litre of water could reduce the *Tithonia* density as well as biomass considerably. For effective control of *Tithonia rotundifolia*, repeated spraying of glyphosate @ 10 ml/L + 2, 4-D 6 g + soap solution 2 ml / litre of water at an interval of 30-40 days under non-crop situation is recommended. Excel Mera 71 ( A.S. of Glyphosate 71% SG ) @ 10g / L of water has shown considerably better control over a period of 45-50days .

### *Rottboellia cochinchinensis*

*Rottboellia cochinchinensis* is a C4 species is a most problematic weed in rainfed crops and common on contour banks and roadsides. This is one of the primary colonizers of disturbed ground. Rapid growth and sharp irritating hairs, make it very competitive. The thorny nature of this weed hinders manual removal warranting herbicidal control.

*Rottboellia cochinchinensis* is observed to invade black soils of Madurai, Coimbatore and Thirunelveli districts. It reproduces entirely by seeds and continues to flower and seed year-round and a single plant can bear 2,000 seeds. The weight of 1,000 seeds is 10.6 g. Leaf blades broadly linear, up to 45 cm long and 2 cm wide, rough on both sides, sheath wide open, lower part of central nerve inflated, with bristle like hairs along sheath. Ligule membranous and short,1 mm long. Culms stout, growing to a height of 1–3 m, occasionally branching. Inflorescence a simple raceme, spike like, contracting at the tip, cylindrical. Fruit is a cylindrical caryopsis. C.Nithya et. al.,(2009) have reported pre-emergence application of pendimethalin 1.5 kg / ha in cropped fields and post-emergence spraying of IPA salt of Glyphosate 3.0 L / ha under non crop or fallow seasons could control the weed effectively. Spraying of Excel Mera 71 @ 2 Kg / ha has shown better control over a period of 45-50days .

### *Cyperus rotundus* i

*Cyperus rotundus* is one of the most harmful perennial weed species with rapid growth that causes hindrance to agronomic practices of crops grown in tropics. The weed multiplies through rhizomes and tubers that of underneath the ground up to the depth of 90 cm. All the commercial herbicides available in the market aimed to control or kill growing above ground part of the weed plants. None of the herbicides inhibit active viable underground plant parts like rhizome or tubers which act as a source for new plants in the next season. D.Ravisankar et.al., (2009) observed that tuber treatment with cytokinin at 0.01 per cent followed by herbicide spray with glyphosate 3.0 kg/ha (3 days after tuber treatment) killed the induced bud sprout (by cytokinin) by 92 per cent under laboratory condition. Excel Mera 71 @ 2Kg / ha has remarkably better control of Cyperus rotundus under field condition .

*Cyperus rotundus* is a perennial from rhizomes and tubers and thrives in moist soils and is spread by flood water and cultivation. It is a common weed in garden land crops like banana, grapes, tapioca, cotton, chillies and other vegetable crops.

It is not controlled by most commonly used herbicides. N.K.Prabhakaran et. al.,(2009) reported effective control by glyphosate 41% SL @15 ml / litre of water. It reduced the weed density and weed dry weight considerably.

### Conclusion

A number of diseases and insect-pests first appear on perennial weeds and then they migrate to main crops. Sheath blight and false smut first invade *Cynodon* and then infect paddy. Similarly loose smut gets transferred from *Cynodon* to wheat. Mealy bug has been noticed on *Parthenium* when cotton is not there in the field. These hard to kill weeds can be managed successfully by the application of Excel Mera 71 (Ammonium salt of

Glyphosate 71% SG ) . We need to keep surrounding of the crop fields weed free. Farmers are advised to go for weed control on bunds & channels and nearby fallow land.

Thus the new formulation of Glyphosate (Excel Mera 71) shows a good prospect in the management of a broad spectrum of weed flora and specially hard-to-kill weeds. Results from Excel Mera 71 have been far superior to other formulations of post-emergent herbicide at farmers' field due to its different chemistry and formulation. The farmers also find it easier to handle due to the nature of formulation ( water soluble granule ).

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**Bibliography**

S. Sanbagavalli, O.S.Kandasamy, K. Sathyamoorthi and V. Ganesaraja.2009a.Effect of stale seedbed technique of weed management on yield and economics of cotton-green gram cropping systems. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

S. Srinivasan, N. K. Prabhakaran and C. Nithya. 2009.Efficacy of post emergence Non – Selective herbicidal management of Portulaca quadrifida in Banana.2009. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

V.S.Mynavathi, C.Chinnusamy and P. Muthukrishnan.2009.Tithonia rotundifolia an invasive poisonious weed and its post emergence herbicidal management in western zone of Tamil Nadu. TNAU,Coimbatore. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

C.Nithya, V.S. Mynavathi and C. Chinnusamy. 2009.Rottboellia cochinchinensis A thorny problematic grassy weed in black clay soils of Tamil Nadu. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

D. Ravisankar, C.R.Chinnamuthu and P. Srimathi.2009.Pre – emergence control of purple nut sedge (*Cyperus rotundus* L.) using growth promoting substances in combination with herbicides. Poster in weed symposium, 02.08.09,TNAU, Coimbatore.

N.K.Prabakaran,C. Nithya and C. Chinnusamy.2009. Post emergence herbicidal management of perennial sedge (*Cyperus rotundus* L.) in banana. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

R.Sathya Priya, C. Chinnusamy and N.K.Prabhakaran.2009.Post – emergence herbicidal management of Solanum elaegnifolium in crop fallow conditions under rainfed ecosystem. Poster in weed symposium, 02.08.09, TNAU, Coimbatore.

# TESTING THE EFFICACY OF LOW VOLUME HERBICIDE APPLICATIONS ON *CHROMOLAENA ODORATA*

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## ABSTRACT

Siam Weed (*Chromoleana odorata)* is the target of an eradication program in north Queensland; however some infestations occur on ground inaccessible to high volume, ground based herbicide spray equipment. Four foliar herbicides were applied to dense infestations of mature Siam Weed in March 2009, near Townsville, north Queensland. Low volume, high concentration solutions containing 40 g $L^{-1}$ a.i. glyphosate, 1.2 g $L^{-1}$ a.i metsulfuron-methyl, 10 g $L^{-1}$ a.i. fluroxypyr + 0.7 g $L^{-1}$ a.i. aminopyralid and 15 g $L^{-1}$ a.i. triclopyr + 5 g $L^{-1}$ a.i. picloram + 0.4 g $L^{-1}$ a.i. aminopyralid were applied using a 5 L backpack and hand gun (or splatter gun). Relatively small amounts (approximately 24-28 mL) of the high concentration solutions were applied to each bush and assessments of the replicated treated and untreated control plots were conducted 76, 207 and 356 days after treatment. These assessments demonstrated that the fluroxypyr and triclopyr based herbicides controlled 96 to 100% of plants. The metsulfuron-methyl and glyphosate based herbicides controlled 40 and 57% of plants respectively 12 months after treatment, when 3% of untreated control plants were dead. The trial demonstrated that this application method and either of two herbicides provides an additional tool for controlling Siam weed in remote areas, which are inaccessible to traditional higher volume foliar herbicide applications. Lower volume herbicide solutions reduce the volume of water and thus the effort needed to effectively treat less accessible infestations.

**Keywords**; *Chromolaena odorata*, Australia, splatter gun, fluroxypyr and triclopyr

## INTRODUCTION

Siam weed (*Chromolaena odorata* (L.) King & Robinson) is a large multi-stemmed perennial shrub in the Asteraceae family. It was first discovered on mainland Australia in 1994 near the towns of Mission Beach and Tully on the tropical coast of north east Queensland. Infestations of Siam weed have also been found in other tropical coastal areas of Queensland, including the Johnstone River and Maria Creek catchments since 1994, Murray River catchment (1997), Russell River catchment (2005) and near the town of Mossman (2003). Siam weed has also been found in the drier inland areas of the Upper Herbert catchment south of Mount Garnet since 1997, and in the Black and Ross River catchments west of Townsville since 2003.

In recognition of the serious problem, a weed eradication program was established targeting Siam weed commenced in 1994 (Waterhouse 2003) and continues today. The eradication program is managed by Biosecurity Queensland (within the Department of Employment, Economic Development and Innovation) with funding received via national cost share arrangements from the Federal, Queensland and other state governments potentially affected by Siam weed. The availability of effective measures to control Siam weed in a range of situations is an integral component of the eradication program, as for the program to be successful the entire population has to be effectively treated (Panetta and Timmins 2004).

Eradication program field crews annually survey thousands of hectares on foot to control scattered seedlings and survey in riparian or hilly areas that are not readily accessible to spray equipment; as a result most plants are physically controlled. Where herbicides are used they are usually high volume foliar applications of fluroxypyr- or triclopyr-based. Such applications are effective but they rely on being able to transport sufficient volumes of water and herbicide to the infestations by vehicle or on foot to treat the plants.

Many Siam weed infestations, particularly near Townsville, occur on steep rocky ground and are hundreds of metres away from tracks, so they can not be accessed by the ground based equipment used to apply high volume foliar herbicides. In these situations eradication field staff were walking into remote infestations and physically digging out plants, including the basal ball, from rocky soils on steep hills in humid tropical conditions, a particularly slow and arduous activity, especially when the first treatments may need to be applied to high densities of large plants. To investigate potentially more efficient control measures a trial was conducted to determine if Siam weed can be effectively controlled by a low volume foliar herbicide spray, containing a higher concentration of active ingredients than would be applied in high volume sprays.

Low volume treatments are applied in small amounts broadly across patches of weeds with a hand held 'splatter', 'drenching' or 'gas' gun attached to a 5 L backpack of herbicide solution**.** Owing to the small areas treated in this trial, a 20 mL applicator with a manual trigger was used. Manual operation also allows the operator to vary the amount of herbicide solution deployed onto smaller plants up to the maximum of 20 mL per shot. An alternative applicator can be attached to a small propane gas cylinder via a regulator to power the trigger and fire the gun, the gas driven trigger deploys a set shot of up to 50 mL of herbicide.

Low volume applicators such as 'splatter' guns have been recognised as an effective and efficient way of treating woody shrubs with systemic herbicides for a number of years (e.g. Toth and Smith 1984). Recently, more research and publicity has led to more widespread use on weeds such as lantana (*Lantana camara* L.) (State of Queensland 2006). The advantages of the splatter gun herbicide application method include: more specific targeting of vegetation to be treated, thereby reducing off-target damage, application of small volumes of high concentration herbicide mixture to plants to reduce chemical usage, no requirement to cover all foliage, and use in areas of difficult access or sensitive vegetation. Application recommendations include a marker dye to identify splattered bushes, squirting large droplets from 6–10 m away and applying approximately 15–20 mL per splatter to achieve a recommended application rate of 2 x 2 mL per 0.5 m of bush height.

## MATERIALS AND METHODS

### Location

A trial was established on the western side of the 'Pinnacles' (steep hills to the west of Townsville) in the Alice River catchment in March 2009.. The trial was located at 19°23'56 "S, 146°35'54"E and approximately 230 m above sea level. The vegetation is predominantly a sparse *Eucalyptus* or *Corymbia* woodland with open mid story trees and shrubs and a grassy ground dominated by *Heteropogon* species (Queensland Herbarium 2011). The site has a locally common duplex soil, specifically soil type Dy3.43 (map unit Va78), a hard setting soil with mottled yellow clayey subsoils and a moderately deep A

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horizon (Isbell *et al.* 1968). It is occasionally grazed and burnt but otherwise largely unmanaged. A controlled burn of the site was done on 2/10/2008, the effects of which are being monitored in parts of this infestation. The fire reduced the cover of lantana on the trial site, but the grass layer and larger diameter Siam weed plants recovered quickly and grew well over the 2008-9 wet season. Though drier in March 2009, good rainfall in the preceding four months ensured that the soil was moist and the plants actively growing when treated (Table 1).

**Table 1**. Total monthly rainfall (mm) from the 5th of September 2008 at 'The Pinnacles' rainfall alert station. Data collated from Bureau of Meteorology (2011).

| Year | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | 27 | 0 | 194 | 131 | 352 |
| 2009 | 671 | 880 | 13 | 59 | 11 | 3 | 0 | 0 | 0 | 0 | 28 | 174 | 1839 |
| 2010 | 560 | 343 | 219 | 38 | 7 | 40 | 2 | 117 | 44 | 46 | 316 | 501 | 2233 |

**Trial Design and Assessment**

The trial included three replicate blocks with four herbicide treatments and an untreated control plot, all randomised within each block. Each of the 15 plots was established around 10 large tagged Siam plants, seven days prior to treatment. Tagged plants were assessed prior to treatment and at 35, 207 and 356 days after treatment (DAT). The pre-treatment and final two assessments included maximum live leader length and average plant height, two diameters of the basal ball at right angles, and total number of leaders. An assessment of herbicide damage on a 1 to 9 scale, adapted from Vitelli (1990) (Table 2) was conducted 35, 207 and 356 DAT. In some cases it was noted that the initial herbicide damage varied between leaders on the same plant; in these cases the damage score reflected the least damaged leader. During the 207 and 356 DAT assessments, plants were scored as dead (=9) if they had no leaves, appeared rotten and were very easily pulled from the ground. If none of the leaders were green, even when the bases were scraped with callipers but the plant was still standing, it was scored 8, especially in the assessment at 207 DAT.

**Table 2**. Herbicide damage assessment score (modified from Vitelli (1990)).

| Score | Damage description |
|---|---|
| 1 | No effect |
| 2 | Leaf yellowing, up to 50%leaf drop |
| 3 | 50-75% Leaf drop |
| 4 | 75-100% Leaf drop |
| 5 | 100% Leaf drop, lateral branches damaged |
| 6 | Lateral branches dead, some leaders still alive |
| 7 | All leaders damaged, probably die |
| 8 | All leaders appear dead |
| 9 | All leaders dead and base rotting |

The presence of regrowth from the base and along leaders was recorded on a 1 to 5 scale (1= abundant, 2= frequent, 3= common, 4= rare and 5= none). Regrowth that exhibited herbicide damage was also noted, although there were only a couple suspected cases of regrowth injury.

The number of plants with developing flower buds was also recorded 76 DAT. To prevent seed production, all flower buds were removed from plants in the trial area and incinerated. The number of seedlings in each plot was also recorded 356 DAT and divided by the plot area to determine seedling density.

**Treatments**

All plots contained a sprawling mass of intertwined Siam weed leaders and some contained more than 10 plants; the approximate total area and amount of each herbicide used in the trial are shown in Table 3. Treatments were applied in 20 mL shots from a 'Forestmaster' applicator plus a 'lantana' nozzle manufactured by N.J. Philips, on the morning of 20/3/2009. The equipment was rinsed with clean water between applications. Two litres of solution were prepared for each treatment and leftover herbicide was used to create a buffer around the trial so the area was obvious to the eradication program field crew working in the area. The non-ionic wetter/spreader/penetrant Pulse® (Nufarm)(1020 g/L polyether modified polysiloxane) along with red Spraymate™ Spray Marker Dye (150 g/L Rhodamine B) was added to each treatment at a rate of 2 mL and 1mL per litre of solution respectively. When purchased in small quantities at 'over the counter rates', these additives cost $0.18/L of splatter gun solution. No rain was recorded at the Pinnacles flood alert station for three weeks after treatment application (Bureau of Meteorology 2011). All Siam weed in the trial area was controlled after the final assessment.

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**Table 3**. Herbicide treatment details.

| Herbicide trade name | Active ingredient | Active concentration | Active rate (a.i g/L) | Herbicide mix rate | Cost of herbicide in 1 L solution at trial rate* ($AUD) | Amount of herbicide applied in 20 mL 'shots' (mL) | Sum of 3 plot areas ($m^2$) |
|---|---|---|---|---|---|---|---|
| Brush-off® | metsulfuron-methyl | 600 g/kg | 1.2 | 2 g/L | 0.4 | 840 | 79.6 |
| Grazon® Extra | triclopyr + picloram + aminopyralid | 300 g/L + 100 g/L + 8 g/L | 15 5 0.4 | 1:20 | 1.75 | 800 | 75.8 |
| Hotshot® | fluroxypyr + aminopryalid | 140 g/L + 10 g/L | 10 0.7 | 1:14 | 1.49 | 740 | 72.8 |
| Weed-master® Duo | glyphosate | 360 g/L | 40 | 1:9 | 0.68 | 740 | 75.1 |
| Control | | | | | | | 101.7 |

*Based on commercial prices of 20 L of herbicide or 200 g container of Brush-Off between the trial and publication. Costs will vary with alternative brands, quantities and over time. 1 L of splatter gun solution will supply 50 x 20 mL shots.

## RESULTS

One way ANOVA with randomised blocks was used to analyse the mean plot data, which was normally distributed. However, most of the data presented is the net effect of the herbicides summed across three plots and 30 plants per treatment. There were no significant differences (P=0.05) between mean plant sizes prior to treatment, with plants averaging 2.1 m tall and with 4.8 leaders per plant (Table 4) across all treatments. 76 DAT there were flower buds on all the untreated control plants and six of the herbicide treated plants (Table 4).

**Table 4.** Mean pre-treatment plant morphology data and flowering occurrence in each treatment.

| Treatment | Number of live leaders (pre-treatment) | Average of maximum leader length (m) | Average plant height (m) | Mean sum plot basal area ($cm^2$) | Number of plants flowering (76 DAT) |
|---|---|---|---|---|---|
| Control | 154 | 2.47 | 2.20 | 59.02 | 30 |
| Brush-Off | 127 | 2.70 | 2.13 | 57.43 | 5 |
| Weed-master Duo | 144 | 2.37 | 1.99 | 55.00 | 1 |
| Grazon Extra | 122 | 2.13 | 1.82 | 41.40 | 1 |
| Hotshot | 182* | 2.62 | 2.29 | 60.93 | 0 |

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*One large plant had 35 leaders.

Larger differences between herbicide treatments were evident at 207 DAT than in the herbicide damage assessment 35 DAT. Therefore, only 207 DAT data is summarised in Table 5. Despite very dry conditions in Autumn 2009 (Table 1), all of the control plants were alive 207 DAT, though one of the control plants on the boundary of a Weed-master Duo plot was treated and incurred herbicide damage (score 7). At the other end of the damage scale, most of the Grazon Extra and all of the Hotshot treated plants were dead or close to dead (Table 5).

**Table 5.** Summary of herbicide damage assessment (207 DAT) in each treatment, using score of 1 (no effect) to 9 (dead).

| | Herbicide damage score | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Control | 29 | | | | | | 1 | | |
| Brush-Off | | 3 | 2 | 3 | 4 | 8 | 5 | 5 | |
| Weed-master Duo | 1 | 1 | 1 | 5 | 3 | 1 | 2 | 16 | |
| Grazon Extra | | | | | | 1 | | 24 | 5 |
| Hotshot | | | | | | | | 27 | 3 |

The increase in number of live leaders in the untreated control plots and remaining live Weed-master Duo treated plants (Table 6) showed that the heavy wet season rainfall (Table 1) in the three months prior to the final assessment (March 2010) provided good conditions for weed recovery in the absence of effective treatment. The final assessment (356 DAT) thus highlighted the ability of some treated Siam weed plants to recover from severe damage and produce new leaders from the basal ball.

**Table 6.** Summary of treatment effects on leaders, live plants and seedling recruitment.

| Treatment | Number of live leaders (207 DAT) | Number of live leaders (356 DAT) | Number of plants alive (356 DAT) | Mean seedling density plants/m$^2$ (356 DAT) |
|---|---|---|---|---|
| Control | 153 | 190 | 28 | 5.53 |
| Brush-Off | 96 | 79 | 17 | 2.10 |
| Weed-master Duo | 39 | 59 | 13 | 4.42 |
| Grazon Extra | 3 | 1 | 1 | 5.70 |
| Hotshot | 0 | 0 | 0 | 2.73 |

The splatter application of smaller amounts of herbicides to the leaves is not likely to result in notable suppression of seedling emergence from the seed bank (i.e. pre-emergent herbicide activity). No significant difference (P=0.05) in mean seedling density was recorded in the plots 356 DAT (Table 6). Observations and photos indicated that the existing grass cover remained in the Hotshot, Grazon Extra and Brush-Off treated plots during the course of the trial.

**Hotshot**

All of the tagged plants treated with Hotshot died. These included the largest plant in the trial, with 35 leaders up to 3.5 m long and a basal ball 22 cm in diameter. There was no flowering or regrowth recorded or observed in the Hotshot treated plots at any of the assessments. High levels of herbicide damage were evident in all the assessments, culminating in 100% mortality 356 DAT. Although one of the more expensive treatments, it was the most successful. Further research is being conducted to investigate whether lower rates of Hotshot are as effective. Since 2009 splatter gun applications of Hotshot have been used to control Siam weed seedlings around plots retained for fire research at this trial site and around other research trials.

**Grazon Extra**

The one tagged plant that flowered and survived until the final assessment was on the edge of one of the plots; it showed some herbicide damage but was not effectively treated. The remaining 29 tagged plants were all effectively treated with Grazon Extra and showed no regrowth in the final two assessments. This was the most expensive treatment, although it provides an alternative to Hotshot if the price of either alters, or in situations where an alternative treatment is preferred by field crews. The results of this trial also suggest that other post emergent herbicides containing 15 g/L a.i. of triclopyr may also be effective when applied via a splatter gun.

**Brush-Off**

The Brush-Off treatment prevented flowering on 90% of tagged plants (Table 4) but ultimately only 43% of tagged plants were controlled (Table 6). The reduction in the number of live leaders between the last two assessments (Table 6) reflects the transition of 13 plants from damage score 6 or greater 207 DAT to the dead category 356 DAT. Over the same timeframe, 11 plants from damage categories 6 or lower 207 DAT (Table 5) were undamaged 356 DAT and had produced some new leaders. Overall, the time to mortality of some of the Brush-Off treated plants was greater than those in other treatments. Six plants remained in a partial damage category 356 DAT, although all six showed some fresh regrowth. As the Brush-Off treatment had some effect when applied via a splatter gun (Table 5) and is by far the cheapest treatment, rates greater than 2 g/L used in this trial are the subject of further research. As mortality in this treatment was spread evenly across the basal diameter sizes of the tagged plants (data not shown), care may need to be taken to ensure good coverage along most of leaders on each plant when treating Siam weed with Brush-Off applied via a splatter gun.

**Weed-master Duo**

Although only one of the tagged plants was flowering 76 DAT, the overall results for the Weed-master Duo plots were mixed. Most of the plants that received high scores (especially 8) at 207 DAT (Table 5) were dead by 356 DAT. However, three highly damaged plants (207 DAT) had recovered by the time of the final assessment through regrowth from the basal ball. Some plants that had partially damaged leaders 207 DAT had recovered and were producing new leaders by 356 DAT (Table 6). Although the low volume application was aimed at the Siam weed foliage, a considerable reduction in live grass cover in the Weed-master Duo treated plots was particularly evident 76 and 356

DAT. Such off-target damage was not observed in the other plots and is undesirable. Bare ground is more susceptible to soil erosion and the replacement of Siam weed with more Siam seedlings or different weeds, rather than the largely native grass cover, which is also essential fuel for the controlled fires planned for this infestation. With an overall control of 57% of tagged plants and notable off target damage, this treatment will not be trialled further.

## DISCUSSION

Two of the herbicides tested in this trial provided excellent control of Siam weed. Therefore this serious weed can be effectively treated with a low volume herbicide application administered via a splatter gun. Although the splatter gun can be used to target weeds amongst native vegetation (State of Queensland 2006) field crews will still need to be careful to avoid non target vegetation or over-treating plants particularly with higher concentrated solutions of herbicide. As this application method is designed to treat only foliage, follow-up control of any missed plants and seedling regrowth will be necessary. In addition to the splatter gun, tools such as high volume spraying from an aerial platform and cutting leaders at the base and treating with a picloram based gel have also recently been permitted under a minor use permit (APVMA 2011) and provide more alternatives to the physical control of Siam weed in remote areas.

Since the inclusion of ‘splatter gun’ applications in a minor use permit (APVMA 2011), the Siam Weed field crew based in Townsville have been using the splatter gun to treat some infestations (S. Brooks, unpublished database data 2011). As crews use multiple 5 L herbicide solutions each day, there is still a need to carry sufficient water, gas and herbicide for a crew of three or four to treat an infested area (A. Clarke pers comm. 2010). To reduce the effort required to carry water to some infestations, the eradication field crews filter water from nearby creeks during the summer wet season and are investigating whether water drops via helicopter to some infestations are feasible (R. Winton pers comm. 2011). Since this initial trial, a subsequent trial was established in May 2010 to determine if lower rates of fluroxypyr and/or herbicides containing fluroxypyr, without aminopyralid, are as effective as the Hotshot treatment in this trial. If lower rates of fluroxypyr are found to be equally effective, then significant reductions in the amount of herbicide used and cost will result. A further trial has been established (May 2011) to investigate whether higher rates of the granular Brush-Off could be more effective than the 2 g/L used in this trial. Identifying effective alternative treatments from these subsequent trials would reduce the volume or remove the need to carry any liquid herbicides to infestations.

All the information from this series of trials and field crew records will be used to build a more detailed picture of the relative costs and efficiencies that result from splatter gun herbicide applications, as compared with other control measures. In the case of this first trial, conventional spraying is not possible so the comparison would be with manual control or spraying from an aerial platform. Spraying from an aerial platform was first trialled in this area in 2010 and its effectiveness has not yet been assessed. However, due to the cost of helicopter hire, this treatment may be limited to the initial management of the very dense and the least accessible infestations. Manual control is an effective control measure, but it is slow, strenuous, disturbs the soil and workers may need to excavate the Siam weed basal ball from between or beside rocks. Splatter gun applications effectively treat large plants up to 10 m away, whereas some alternative treatments, such as physical removal and cut stump, field staff are required to access the base of each plant, irrespective of the surrounding vegetation or terrain.

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## REFERENCES

Australian Pesticides and Veterinary Medicines Authority (2011). Minor use permit 11833–Siam Weed. http://permits.apvma.gov.au/PER11833.PDF (Australian Government, Canberra).

Bureau of Meteorology (2011). Daily Rainfall Bulletin for Pioneer, Proserpine, Don, Burdekin, Haughton, Ross, Bohle; The Pinnacles Alert Rainfall Station data (532041) operated by Townsville City Council. Accessed and updated weekly. http://www.bom.gov.au/cgi-bin/wrap_fwo.pl?IDQ60364.html (Bureau of Meteorology, Melbourne)

Isbell, R.F., Webb, A.A. and Murtha, G.G. (1968). Atlas of Australian Soils, Explanatory data for sheet 7 North Queensland. (CSIRO and Melbourne University Press, Melbourne.)

Panetta, F.D. and Timmins, S.M. (2004). Evaluating the feasibility of eradication for terrestrial weed invasions. *Plant Protection Quarterly (*19): 5-11.

Queensland Herbarium (2011). Regional Ecosystem Description Database (REDD) (RE 9.12.22). Version 6.0b - January 2011 (Department of Environment and Resource Management: Brisbane).

State of Queensland (Department of Natural Resources and Water) (2006). Using Herbicides on Lantana – a guide to best practice management practices. http://www.weeds.org.au/WoNS/lantana/resources.htm (Department of Natural Resources, Mines and Water, Brisbane).

Toth, J. and Smith, L.W. (1984). A low-volume, gas powered, spray gun for application of herbicides to blackberry and other woody perennials. Proceedings of the 7th Australian weeds conference. Vol 1. Ed by R.W Madin. Perth, Western Australia, 17-21 Sept 1984. pp. 56-63.

Vitelli, J. S. (1990). Effect of soil moisture on the efficacy of 2, 4-D and metsulfuron-methyl on rubber-vine – preliminary investigation. Proceedings of the 9th Australian Weeds Conference. Adelaide, South Australia. Ed by J.W. Heap. 6-10th Aug 1990. Crop Science Society of South Australia Inc. Adelaide pp. 407-410.

Waterhouse, B. M. (2003). Know your enemy: recent records of potentially serious weeds in northern Australia, Papua New Guinea and Papua (Indonesia). *Telopea* 10(1):477-485.

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# SOIL SEED BANK LONGEVITY INFORMATION FOR WEED ERADICATION TARGET SPECIES

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## ABSTRACT

The longevity of seed in the soil is a key determinant of the cost and length of weed eradication programs. Soil seed bank information and ongoing research have input into the planning and reporting of two nationally cost shared weed eradication programs based in tropical north Queensland. These eradication programs are targeting serious weeds such as *Chromoleana odorata*, *Mikania micrantha*, *Miconia calvescens, Clidemia hirta* and *Limnocharis flava*. Various methods are available for estimating soil seed persistence. Field methods to estimate the total and germinable soil seed densities include seed packet burial trials, extracting seed from field soil samples, germinating seed in field soil samples and observations from native range seed bank studies. Interrogating field control records can also indicate the length of the control and monitoring periods needed to exhaust the seed bank. Recently, laboratory tests which rapidly age seed have provided an additional indicator of relative seed persistence. Each method has its advantages, drawbacks and logistical constraints.

**Keywords**: seed banks, eradication, seed packet burial, Clidemia, Miconia, Chromolaena

## INTRODUCTION

Eradication or the elimination of all vegetative individuals of a weed species and exhaustion of its seed bank is the well-intentioned goal of many weed control programs, but can be difficult to attain. When all infestations and all the plants within the infestation are located and effectively treated, so that infestations are managed to prevent fresh seed input, the duration of the program is determined by the persistence of seed in the soil.

Two tropical weed eradication programs are targeting serious tropical weeds such as Siam weed (*Chromoleana odorata* (L.) King & Robinson), clidemia (*Clidemia hirta* L. D Don), limnocharis (*Limnocharis flava* Buchenau), miconia (*Miconia calvescens* DC.) and mikania vine (*Mikania micrantha* Kunth), principally in north Queensland. The Siam weed eradication program commenced in 1994 when this serious weed was discovered on mainland Australia. The ‘Four Tropical Weeds’ Eradication Program (targeting clidemia, miconia, mikania vine and limnocharis) commenced operations in late 2003 (Erbacher *et al.* 2008). Single infestations of the shrubs *Miconia racemosa* (Aubl.) DC. and *Miconia nervosa* (J.E. Smith) Triana. were identified in north Queensland in 2002 and 2004 respectively, and are included in eradication program as they co-occur with *M. calvescens* infestations.

Both eradication programs receive funding via national cost share arrangements from the Federal, Queensland and other state governments potentially affected by the weeds. The programs are required to estimate a timeframe and resources required for eradication, and knowledge of seed persistence is an important component of such estimates. Ongoing research into seed persistence also has input into activities of and reporting for the two programs. Information on soil seed bank persistence may also help inform decisions about when infestations are declared eradicated. This paper summarises sources of seed bank information, what is known about the main target species and what are some of the constraints in investigating seed persistence. Since much of this research in ongoing, an overview of trials is presented.

**Sources of Seed Bank Information**

Information on soil seed bank persistence can be sought from the invasive and native ranges across of the targeted species.

Repeated collection of soil samples from within infestations is a common method for estimating rates of seed bank depletion. This process is suited to eradication programs where field crews aim to prevent fresh seed production by controlling plants. Ideally, the sampled areas should contain a high initial seed density and they need to be accessible and locatable for the duration of the sampling, which may take many years. Such studies are enhanced by data on the initial weed population and ongoing seedling emergence. Methods to estimate the total seed bank include physical (including sieving) or chemical extraction of seed from field soil samples. Soil samples can also be collected from infestations over time and kept under favourable conditions, such as in a glasshouse, to determine the germinable seed bank.

Seed can also be buried in permeable packets filled with field soil and the packets retrieved over time to test seed viability. These trials eliminate the factors of seed immigration and emigration, so depletion of a set number of seeds can be investigated (Van Mourik *et al.* 2005). Depletion can occur through seed death or germination. Germination can hasten the decline, particularly in packets exposed to light on the soil surface. Biotic agents such as soil fauna, bacteria and fungi may cause seed depletion, but the packets may concentrate seed, making fungal attack more likely (Van Mourik *et al.* 2005). Seed is germinated at the commencement of packet trials to provide data on initial viability and germinability.

Both eradication programs maintain databases which record field visit information (including plant absence). To report on eradication progress each infestation or management area is allocated to a ‘control’ status if any plants were present at any time during the year. If no plants were found in a year then a ‘monitoring’ status is allocated. The time spent in the control phase provides an indication of the persistence of viable seeds over a variety of infestations. The length of the monitoring phase provides a buffer period between the control phase and declaring eradication, during which seedlings could emerge from the seed bank. Interrogating field control records can indicate the length of these phases across multiple infestations. Seed persistence can vary between populations and locations (Panetta 2004).

Recently, a procedure undertaken in a controlled laboratory environment has provided an additional indicator of weed seed persistence (Long *et al*. 2008). The controlled aging test (CAT) determines number of days it takes for the viability of seeds to decline to 50% ($P_{50}$) when exposed to 65% humidity and 45$^{o}$C. The following seed persistence terms, $P_{50}$ values and equivalent periods of field persistence are adapted from Long *et al.* (2008):

- Transient seed banks, $P_{50}$<20, field persistence <1 year,
- Short term seed banks, $P_{50}$ = 20 to 50, field persistence 1 to 3 years,

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- Long lived seed banks, $P_{50}$ = >50 field persistence 3+ years.

**Investigating the Seed Longevity of Eradication Target Species**

Siam Weed

Witkowski and Wilson (2001) studied increasingly older Siam weed infestations in KwaZulu-Natal and found a higher seed bank density in sunny than shaded micro-sites. In a glasshouse trial, they found that less than 9% of seeds (surface and buried at 1 cm) were viable, but failed to germinate in 12 months. Overall they concluded that; although most seeds are short lived, some buried seeds formed a persistent seed bank.

Siam weed was first discovered in 1994 near Bingil Bay on the wet tropical coast of north Queensland. It has since been found in other coastal areas of north Queensland, including the Johnstone and Tully River catchments and near Mossman. Siam weed has also been found in the drier inland Tableland areas of the upper Herbert River and west of Townsville. There have been several local investigations of soil seed bank persistence of Siam weed on the wet tropical coast. Soil samples were collected from one of the first Siam weed locations (Bingil Bay) identified in 1994 and germinated in a glasshouse. Seedling emergence equivalent to a seed density of 9050 seeds/m$^2$ was recorded (M. Setter, unpublished data). Samples collected and germinated from the same area seven years later revealed 99.9% fewer seeds (12.5 seeds/m$^2$); during this time seed input was believed to have been prevented. No seed germinated from samples collected 12 years after discovery. In 2000, a trial in which permeable packets were filled with 50 locally obtained Siam Weed seeds and buried at 0, 2 and 10 cm was established near South Johnstone (M. Setter pers comm. 2008). In the first four years of the trial the surface-situated seed showed the greatest run down, after five years of annual retrievals 1.5% of seeds (across all depths) was viable and no viable seed was found after seven years when the final sample was taken.

There has also been interest in Siam weed seed longevity in the drier tropical Tableland and Townsville areas. As the source of these infestations is not confirmed, there may be genetic or environmental differences to the wet tropics infestations that could influence seed longevity. From 2003 annual soil samples were collected from an infestation in the Upper Herbert catchment and seedling recruitment was recorded on transects in this infestation. No seedlings emerged from the 5th year of sampling and no seedlings were noted after four years of monitoring the field transects. A buried seed longevity trial was established at Charters Towers in December 2008. Seed from a Townsville infestation was buried in fine nylon mesh packets placed at 0, 3 and 13 cm within a holed pipe (container) filled with soil. The top of the containers was covered with 30% shade cloth to contain the packets and lower the effect of sunlight on the nylon surface packets. The trial includes four soil types with grassed or bare ground sub-plots. Sufficient containers were buried to allow for six monthly retrievals for two years, annual retrievals between two and seven years and three extra retrievals if required after seven years.

Field data, field samples and a buried seed trial indicate that Siam weed develops a long lived seed bank, with most viable seed exhausted between four and eight years.

Miconia

Meyer (2010) reported on the density of Miconia seeds germinated from soil samples collected on Raiatea in 1992, 1993, 1995 and 2008. From 4500 seeds/m$^2$ in 1993 there

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was a rapid decline in germinable seed density to below 1000 seeds/m$^2$ in 1995; however, seeds germinated in the 2008 samples. The emergence of seedlings 16 years after the removal of locally reproductive plants indicates a very persistent seed bank. Meyer (2010) also reports soil seed densities approaching 50 000 seed/m$^2$ under Tahitian infestations.

Miconia has been identified at 57 locations in Queensland and New South Wales, though naturalisation has only been recorded at 28 locations (Brooks and Jeffery 2010). Most naturalised infestations are in a control phase, some for at least 10 years. For example, following the removal of mature miconia from a small infestation near Julatten in 2001, local seedling recruitment has been observed in 2011 (K. Erbacher, pers. comm. 2011). Additional seed input is unlikely as field crews search hundreds of hectares annually around this location for another eradication target without finding any mature miconia (Brooks *et al*. 2009). Control records indicate that *M. nervosa* and *M. racemosa* have developed persistent seed banks (Brooks *et al.* 2009), but no further seed studies have been undertaken and no studies of seed persistence have been identified in their native ranges.

At a large ‘El Arish’ miconia infestation, sampling (20 surface cores each from 1 m$^2$ plots replicated across six blocks) and sieving of the soil samples was conducted annually between 2004 and 2008. Samples can be taken five more times at greater than yearly intervals. There has been no decline in the number of seeds extracted (via sieving) from samples collected between 2004 and 2008, though the viability of the extracted seeds is being assessed; the seeds extracted equate to a total field density of less than 2000 seeds/m$^2$ (S. Brooks unpublished data). A few seedlings emerged in the sampled area annually from 2005 to 2010. The closest mature trees were removed in September 2004.

A buried seed packet trial was established in October 2010 in the area used for the wet tropics Siam weed buried packet trial at South Johnstone. Fifty miconia seeds were placed into 180 µm stainless steel mesh packets. Packets were placed a holed container filled with soil at the soil surface and buried 3 and 10 cm below the soil surface. Containers will be removed every six months for two years and annually between 2 and 16 years. The trial design allows for seven additional retrievals after 16 years if viable seed remains and the retrieval schedule can be altered during the trial. The containers were covered with 30% shade cloth to contain the packets and lower the effect of sunlight on the surface packets until grasses shaded the containers.

<u>Clidemia</u>

Mendes-Rodrigues *et al.* (2008) assessed the viability of four clidemia seed samples collected near Uberlandia (Brazil), two fresh collections, one sample stored under laboratory conditions for two years and one buried in permeable packets in local soil for two years. Germination from all samples was similar and high (range 87.7 to 94.2%); 93.1% of seed germinated after being recovered from the packets. In the same area of Brazil, Pereira-Diniz and Ranal (2006) found small numbers of germinable clidemia seed at various soil depths from the surface to 30-35 cm in microhabitats of a gallery forest; concluding that species found in the deeper samples formed a persistent seed bank. In the Bragantina area of Brazil, Vieira and Proctor (2007) found clidemia seed made considerable contributions to the total soil seed bank and seed rain in a primary forest and in 5, 10 and 20 year old secondary forest plots, although clidemia was not recorded in censuses of nearby plants. Medeiros (2004) reported evidence of a widespread and persistent seed bank, when clidemia seedlings emerged in plots on Hawaii with limited seed input. Further anecdotal reports indicate that clidemia develops a seed bank that persists for at least 3 years (Medeiros 2004) and seedling emergence may occur up to 10 years after the removal of mature plants (Smith 1992). Overseas field observations indicate that clidemia can quickly develop a persistent seed bank in a variety of vegetation types, even when the frequency of mature plants is low.

Clidemia is known only at one location in Australia. This infestation was discovered and first controlled in 2001, with seedling recruits from an active seed bank recorded in 2011 (K. Erbacher pers comm. 2011). Within the core area of highest recruitment there has been limited fresh seed produced since 2004 and probably back to 2001 (Brooks *et al.* 2009). Clidemia seed was included in the buried packet trial established with miconia seed in October 2010 and using the same methodology described above. This trial will complement field population run down data and provide information about seed persistence at recently discovered patches.

<u>Mikania vine</u>

It was generally thought that Mikania seeds would persist for around seven years (Brooks *et al.* 2008); however, few published studies have been identified. Buried seed trials have not been conducted locally as seed is rarely encountered in the field and is destroyed immediately when found. A few samples have been collected in the past 13 years but their viability has been low (1 to 18%). There is also a reluctance to cultivate specimens under quarantine conditions, given the seeds are wind dispersed and known distribution in Australia is limited to 15 infestations around three locations. With few new discoveries and over half of the infestations in a monitoring phase, the eradication of this weed is going well (Brooks *et al.* 2008). Recently, Macanawai *et al.* (2010) conducted a controlled aging test (CAT) on Mikania vine seed from Fiji and found it fitted into the short term persistence category, with $P_{50}$ = 48 days.

<u>Limnocharis</u>

Ortiz Domínguez and González (2001) collected soil seed bank samples from nine rice paddy areas near Calabozo, Venezuela. Some of their samples were germinated in trays in a humid laboratory environment and some where chemically separated to determine the total seed bank. They reported that limnocharis formed a large proportion of total seeds present (55.7% of total weeds) in the chemically separated samples but a lower proportion of germinable weeds present (12% of total weeds). Ismail and Phaik-Kong (2004) did not record limnocharis plants at one of their study sites in Malaysian rice paddies, although limnocharis accounted for 4.1% of the seed bank. Across several sites limnocharis also made a significant contribution (1.9 to 16%) to the total seed bank at soil depths to 15 cm. Both the studies above indicate a greater presence of limnocharis seed in the seed bank than is evident in rice field populations.

Between 2001 and 2010, 15 naturalised populations of limnocharis (occurring in unconfined habitats such as drains and creeks) were detected on the north east coast of tropical Queensland. A further 13 infestations were plantings confined in urban water features (Brooks *et al*. 2008). A quick transition to monitoring status has been recorded amongst 11 confined infestations and three unconfined infestations.

The number of seeds extracted (sieved) from 40 soil samples collected at a limnocharis infestation in a perennial spring fed stream (near Feluga) in 2003 and annually from 2005 to 2010 is shown in Table 1. Despite no confirmed records of seed input since November 2003, viable seed was found in one of the 2010 soil samples. The silt level at the site varies and there has been year to year variation in the number of seeds. Thus it remains to be seen whether the decline in 2010 is maintained in later samples. Although the number of plants removed during control activities has declined between 2005 and 2010 (Table 1), viable seed remains at this site and indicates that limnocharis forms a long lived soil seed bank. This site is constantly wet and limnocharis depletion may be faster with annual dry periods. The quarantine risks in conducting a buried seed trial in a flowing water setting are too large, but a glasshouse immersion trial with varied periods of immersion is being planned. A germination test has been determined for limnocharis seed, but the results can be inconsistent.

**Table 1.** Summary of bank seed samples collected from a Limnocharis infestation in north Queensland and approximate number of plants controlled from November 2003.

| | Year | | | | | | |
|---|---|---|---|---|---|---|---|
| Data | 2003 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
| Seeds extracted from 40 samples | 623 | 358 | 1252 | 489 | 530 | 323 | 8 |
| % of samples with seeds | 70.7 | 77.5 | 82 | 55.5 | 67.5 | 47.5 | 2.5 |
| Average seeds per sample | 15.2 | 9.0 | 31.3 | 12.2 | 13.3 | 8.1 | 0.4 |
| Average seed viability (%) | | 64.7 | 54.4 | 59.5 | 80.7 | 57.9 | 100 |
| Number of plants controlled* | 397 | 1037 | 426 | 416 | 99 | 22 | 23 |

* 593 plants were controlled in 2004 but no soil samples were collected.

**Comments on the Practicalities of Estimating Seed Bank Persistence**

The eradication target species present such entrenched problems across much of their invasive ranges that the focus of management is often on suppression and eradication is usually not an objective on any large scale. Studies of seed persistence seem less of an imperative for suppression campaigns, particularly for environmental weeds. However, some studies have been identified where target weeds, such as limnocharis and mikania vine, occur in agricultural situations.

Seed bank dynamics are also studied in the neo-tropical native ranges of the target species, and several studies serve to reinforce the greater occurrence in seed bank samples than in the vegetative phase. Native range seed bank studies typically report on different habitats, vegetation assemblages or soil depths on a range of species, but over limited time frames.

When effective survey and control activities prevent seed production then field records can also provide be an indication, over a range of infestations, of the time frame over which plants establish from the seed. However, such data can be influenced by variation in past visit frequencies, data gaps or inaccuracies and changing the scale of the reporting units.

A number of buried packet trials have been established on the target species. Such trials can investigate treatments such as soil depth, different soil types and altered moisture regimes. Buried packet trials can be labour intensive to establish, but they are easy to maintain. Packet trials require sufficient flexible retrieval times and hardy permeable materials to cover the possible duration of seed persistence. The buried seed trials have been conducted on land owned by government research centres, as the monitoring and containment of target species seed is essential to the eradication programs. The piped burial containers have been used to reduce root growth into packets, making them easier to extract, and they could be relocated if the need arose. The containers also create and maintain consistent burial depths. However, increases in soil moisture in the base of the containers may also increase seed depletion rates (Bebawi, F. pers. comm. 2009).

Seed batches used in the recent Siam weed, miconia and clidemia buried packet trials have been set aside for a CAT. Running the CAT on the same seed lots as those used in the buried seed trial will complement the results of field studies and assist in validating the CAT on tropical weeds. Importantly the CAT provides a standardised laboratory methodology to quickly obtain an indication of relative persistence; although there is already evidence of long lived seed banks from field data on all the target species.

Seed bank determination from field samples can be via seed extraction, typically by sieving in these cases to estimate of the total seed bank. Sieving soil samples is labour intensive and some year to year variation may result from different levels of diligence in processing the samples, particularly where minute seeds are involved. Alternatively, larger field samples are often germinated under consistent, quarantine conditions to assess the 'germinable' seed bank over time. The seed densities obtained from germinating samples may be less than the total seed bank if a proportion of the seed bank remains dormant.

For each of the eradication target species information from field populations, field soil samples and laboratory studies will continue to be collated to add to the baseline estimates of seed persistence provided by the buried packet trials. While there is already field evidence of long lived seed banks in all the target species, more precise information on seed bank persistence will help to refine estimates of the length of the eradication programs.

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## REFERENCES

Brooks, S.J. and Jeffery, M. (2010). Status of *Miconia calvescens* and the eradication program in Australia. In: Loope, L.L., J.-Y. Meyer, B.D. Hardesty and C.W. Smith (eds.), *Proc of the International Miconia Conference*, Keanae, Maui, Hawaii. Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. http://www.hear.org/conferences/miconia2009/proceedings/

Brooks, S.J., Panetta, F.D. and Galway, K.E. (2008). Progress towards the eradication of mikania vine (*Mikania micrantha*) and limnocharis (*Limnocharis flava*) in northern Australia. *Invasive Plant Science and Management* 1: 296-303.

Brooks, S.J., Panetta, F. D. and Sydes, T. A. (2009). Progress towards the eradication of three melastome shrub species from northern Australian rainforests. *Plant Protection Quarterly* 24(2): 72-8.

Erbacher, K., Sydes, T.A., Galway, K.E. and Brooks, S.J. (2008). The National Four Tropical Weeds Eradication Program: a case study for future weed eradication projects in the wet tropics. In R.D. Van Klinken, V.A. Osten, F.D. Panetta and J.C. Scanlan (eds). '*Proc of the 16th Australian Weeds Conference'* (Qld Weeds Society, Brisbane). pp. 430-2.

Ismail, B. S. and Phaik-Hong, K. (2004). A Study of Weed Populations and Their Buried Seeds in the Soil of MARDI Research Station and at Farmers' Rice Fields in Sungai Burung, Selangor, Malaysia. *Pertanika J. Trop. Agric. Sci,* 27(2): 113– 120.

Long, R.L., Panetta, F.D., Steadman, K.J., Probert, R., Bekker, R.M., Brooks, S. and Adkins, S.W. (2008). Seed persistence in the field may be predicted by laboratory-controlled aging. *Weed Science* 56: 523-8.

Macanawai, A. R., Day, M.D.,Tumaneng-Diete, T. and Adkins, S.W. (2010). Some factors that may influence the invasiveness of *Mikania micrantha* Kunth. ex. H.B.K. in Fiji. edited In S.M. Zydenbos (ed). Proceedings *17th Australasian Weeds Conference.* Christchurch, New Zealand. New Zealand Plant Protection Society. pp. 95-8.

Medeiros. A.C. (2004). Phenology, reproductive potential, seed dispersal and predation and seedling establishment of three invasive plant species in a Hawaiian rain forest. Ph.D. Thesis, Department of Zoology, University of Hawaii at Manoa, Honolulu.

Mendes-Rodrigues, C., Ranal, M. and Oliveira, P.E. (2008). Could seed dormancy and polyembryony explain the success of *Clidemia hirta*? *IX Simposio Nacional Cerrado, II Simposio Internacional Savanas Tropicais*. (ParlaMundi, Brasilia).

Meyer, J-Y. (2010). The *Miconia* saga: 20 years of study and control in French Polynesia (1988-2008) In: Loope, L.L., J.-Y. Meyer, B.D. Hardesty and C.W. Smith (eds.), *Proceedings of the International Miconia Conference*, Keanae, Maui, Hawaii. Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. http://www.hear.org/conferences/miconia2009/proceedings/

Ortiz Domínguez, A. and González, L. (2001). Preliminary study of weed seed banks of certain soils of rice areas of Calabozo, Guárico. *Agronomía Tropical* (Maracay), 51/4, 501-517. In Spanish with English summary.

Panetta, F.D. (2004) Seed banks: the bane of the weed eradicator. *Proceedings of the 14th Australian weeds conference* (ed. By B.M. Sindel and S.B. Johnson), Weed Society of New South Wales, Wagga Wagga, New South Wales, Australia. pp. 523–526.

Pereira-Diniz and Ranal (2006). Germinable soil seed bank of a gallery forest in Brazilian Cerrado. *Plant Ecology* 183: 337–348.

Smith, C.W. (1992). Distribution, status, phenology, rate of spread and management of *Clidemia* in Hawai'i. In C.W. Smith, C.P. Stone, and J.T. Tunison. (eds) *Alien plant invasions in native ecosystems in Hawaii*. (University of Hawaii. Honolulu). pp 241-253.

Van Mourik. T.A, Stomph, T.J. and Murdoch A.J. (2005). Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks. *Journal of Applied Ecology* 42: 299–305.

Witkowski, E. T. F. and Wilson, M. (2001). Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata,* along a 15 year chronosequence. *Plant Ecology* 152: 13–27.

Vieira, I.C.G. and Proctor, J. (2007). Mechanisms of plant regeneration during succession after shifting cultivation in eastern Amazonia. *Plant Ecology* 192: 303-315.

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# NEW ZEALAND'S BIOSECURITY RESPONSE SYSTEM - A CASE STUDY ON THE RESPONSE TO *PASSIFLORA APETALA*

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## ABSTRACT

New Zealand's Ministry of Agriculture and Forestry (MAF) is responsible for leading the country's biosecurity system. In July 2008, MAF introduced a single system to respond to all organisms or goods that pose a biosecurity risk to the values of New Zealand (economic, environmental, human health and socio-cultural). This system is used for responses from all sectors, of all sizes, resulting from a new incursion or an established risk organism. One such example is MAF's response to an emerging threat, the bat-wing passion flower *(Passiflora apetala)* in December 2009. This plant is believed to have been introduced into the country around the mid 1990s for its ornamental value. *P. apetala* is currently known from discrete populations in the Northland and Auckland regions. Its spread is attributed to subtropical plant enthusiasts and avian vectors. A weed risk assessment indicates the potential for *P. apetala* to be as invasive as other established *Passiflora* species in New Zealand. There is a reasonable likelihood of management success for local elimination as *P. apetala* is in the early stages of naturalisation. A summary of response actions undertaken to date is discussed.

**Keywords:** *Passiflora apetala*, biosecurity system, management, weed, naturalisation, emerging threat.

## INTRODUCTION

New Zealand's Ministry of Agriculture and Forestry (MAF) is the lead government agency for agriculture, food safety, biosecurity and forestry matters. MAF's focus is on enhancing the integrity and performance of the biological value chain, which covers animals, plants, food and related sectors, and their contribution to New Zealand's economy, environment and social well-being.

### Biosecurity Response System

In July 2008, MAF introduced a single system to respond to all organisms or goods that pose a biosecurity risk to the values[1] of New Zealand (Ministry of Agriculture and Forestry 2008). This generic management approach covering all sectors applies to both new-to-New Zealand and established risk organisms. The response system can be scaled up or down as appropriate for almost any situation.

The response system is aligned to the response policy, *Preparing for and responding to risk organisms* (MAF Biosecurity New Zealand (2) 2008), and sets out what the Crown will

[1] Economic, environmental, human health and socio-cultural.

do and what people can expect in respect of responses to pests and diseases (risk organisms). It reaffirms MAF's leadership role, while anticipating that there are other stakeholders who will participate in a response.

## Underpinning Principles

The key underlining principles of MAF's biosecurity response system include:

- *Risk-based decision making*: decisions made based on risks to the values[1] of New Zealand at each stage of the response;
- *Whole-of-government approach:* follows New Zealand's Coordinated Incident Management System (CIMS) management structure, terminology and processes for interagency co-ordination and planning;
- *Scalable and consistent:* response phases (Figure 1) and core management approach are the same for a large response as for a small response (3-3,000 person response);
- *Project management:* underpins the approach with a focus on planning the work and working to the plan;
- *A response organisation structure dictated by the work:* organisation charts are based on response activities, not on role-holders. This allows responses to be easily scaled up or down;
- *Activities:* defined by the work that is required to be completed, not by the responsibilities of role-holders.

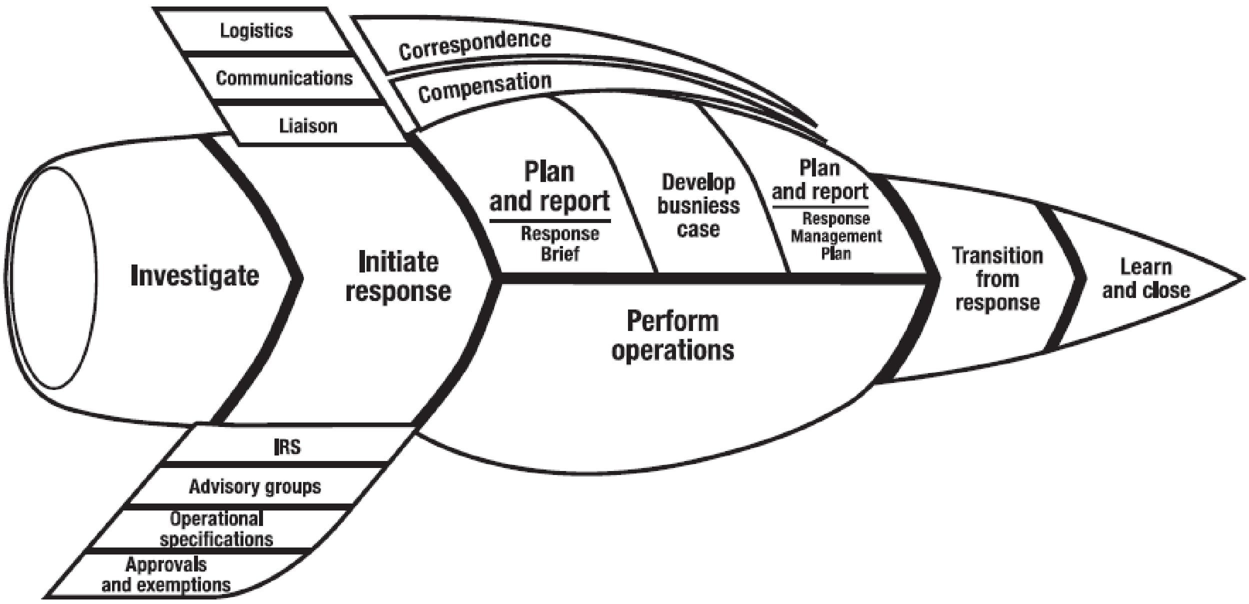


**Figure 1.** A diagram representing MAF processes and procedures for responding to all organisms or goods that pose a biosecurity risk to the values of New Zealand.

## Maf's Response to *Passiflora Apetala*

Investigate Phase

MAF was notified of this previously undetected plant in September 2009 and initiated an investigation following concerns of vines of an unknown *Passiflora* sp. growing prolifically in a council-owned, regenerating native reserve in Kamo, Whangarei in northern New Zealand. The vines were strangling and generally smothering other plants.

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A rapid assessment report was produced as a biosecurity issue was confirmed and the biosecurity risk remained. Following formal identification as *Passiflora apetala*, MAF initiated a response in December 2009 due to the invasive properties *P. apetala* exhibited.

<u>Initiate Response Phase</u>

A Planning and Intelligence workstream was established with an Intelligence Portfolio to provide information required to make initial decisions on the response options. MAF undertook tracing activities to determine the history and extent of the *P. apetala* spread in New Zealand by interviewing residents in the immediate area of the infested reserve. These investigations revealed that *P. apetala* may have been present in New Zealand for up to 20 years, and had been distributed widely amongst sub-tropical plant enthusiasts.

With the assistance of Auckland Council, Northland Regional Council and the Department of Conservation, eight locations in the Northland, Auckland and Waikato regions of New Zealand were identified as having *P. apetala* present. Initial delimitation surveys at each location revealed varying states of infestation. The plant has been found in regenerating native forests and scrub, home gardens and amongst hedges and fence lines. Seedlings are usually found under places where birds perch. Only seven of these sites (Figure 2) in the Northland and Auckland regions currently have *P. apetala.* The site in Waikato, a butterfly farm, had *P. apetala* plants growing in pots in a secure environment; these have subsequently been destroyed (Pearson *et al.* 2011). The presence of *P. apetala* at each of the seven locations can be attributed to present or former property owners who introduced and cultivated this species.

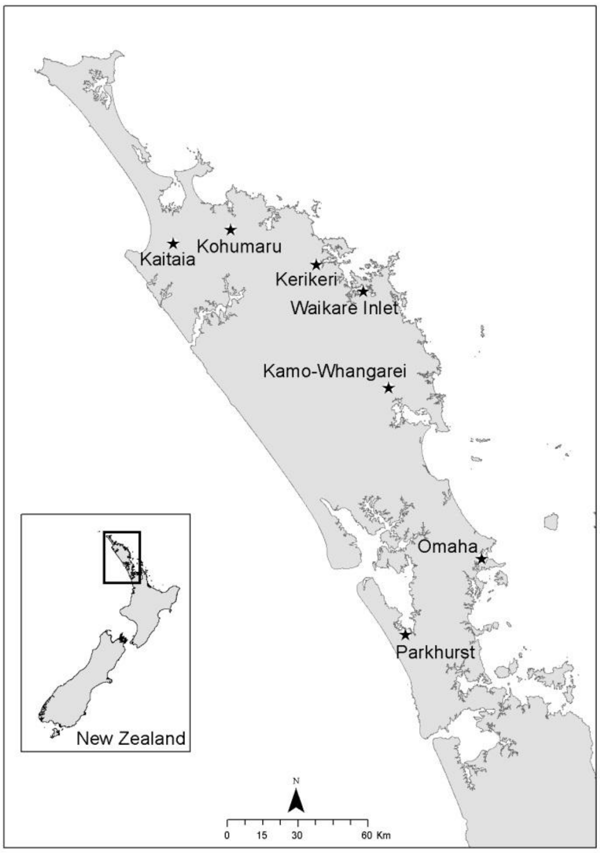


**Figure 2.** Locations of *P. apetala* infestations in New Zealand

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In November 2009, *P. apetala* was given the legal status of an Unwanted Organism under New Zealand's Biosecurity Act 1993. Under this status it is an offence to breed, knowingly communicate, exhibit, multiply, propagate, release, or sell an Unwanted Organism unless permission is obtained from a Chief Technical Officer e.g. MAF (Pearson *et al.* 2011).

*P. apetala* is seen as an emerging threat to New Zealand's environment, with the potential to smother regenerating forests and forest margins. *P. apetala* may also impact upon the native flora and fauna that are culturally significant to New Zealand's indigenous Māori, and impact on people's recreational experience.

Intelligence gathered on the biology of *P. apetala.* revealed that it is native to central Costa Rica and Panama, where it grows at elevations of 1300-2200 m (Ulmer and MacDougal 2004). In New Zealand it appears to be shade tolerant and grows at lower altitudes. The deeply cut two lobed leaves have the appearance of a bat's wing. As the specific epithet suggests, the flowers of this plant do not generally have petals. Flowers are yellow/light green in colour, 1.2–2 cm diameter (Ulmer and MacDougal 2004). Fruit are purplish black, subglobose, 0.7-1.5 cm diameter (Ulmer and MacDougal 2004) and are attractive to birds and suitable to feed on. The minimum temperature for growing *P. apetala* is 5°C (Ulmer and MacDougal 2004). Flowers and fruit have been found all year round in New Zealand. A mature *P. apetala* vine 2-3 years old produces > 3000 fruit with an average of 15 seeds per fruit (Pearson 2010).

## Plan and Report

A response brief was produced based on the intelligence collected, which outlined three management strategies for the management of *P. apetala* in New Zealand. These included the 'do nothing approach'; 'eradication from New Zealand' and 'local elimination' from known areas.

Eradication is defined as the "removal of every individual and propagule of a species from New Zealand so that only reintroduction from beyond New Zealand's borders would enable the re-emergence of the species. Achievement of eradication would be demonstrated by surveillance" (MAF Biosecurity New Zealand 2008). This response option was dismissed and considered unlikely to be feasible due to the unknown extent of the *P. apetala* infestation in New Zealand. Tracing activities into the distribution of *P. apetala* have been difficult due to the lack of records kept and a reliance on the memory recall of key persons, along with a reluctance to provide the relevant information. A public awareness campaign has resulted in no further locations being discovered, and undertaking a national surveillance strategy for this pest was considered uneconomical.

The preferred option of local elimination from known areas is currently being pursued by the Response Team. Local elimination is defined as "the removal of all known propagules of a population from an area, including a period of observation (monitoring) to see if the pest re-occurs" (Knegtmans 2007). This definition is similar to that of Panetta (2007) for the term 'extirpation' to denote local, as opposed to global, elimination of a species.

As for other weed management programmes, understanding the seed dynamics of *P. apetala* is seen as an important determinant for setting response goals and objectives, to determine the duration of an eradication management programme, and determine the total effort required to achieve the objectives (Panetta 2004). Further intelligence projects were initiated to inform a business case, which is being prepared, to make a final commitment (or not) to the local elimination option. These projects included a pilot treatment programme, a cost benefit analysis and research into seed dynamics (viability, longevity and time to maturity of *P. apetala* in New Zealand).

Pilot treatment programme

Pearson *et al.* 2011 describe the one-off pilot treatment programme which took place in March 2011, at two of the seven locations where *P. apetala* is present. The objectives of the pilot treatment programme were to test the feasibility, effectiveness and costs of local elimination attempts, by locating and destroying all *P. apetala* plants within a 500 m radius from previously known mature *P. apetala* plants at Parkhurst (North of Auckland) and Kerikeri (Northland).

*P. apetala* plants at Parkhurst and Kerikeri were found in the same vicinity of plants recorded during previous delimiting surveys (Pearson *et al.* 2011). Approximately 88 kg of plant material was removed from the heavily infested Parkhurst location; whilst the infestation at the Kerikeri location was considerably smaller, with only c. 3-5 kg of plant material removed (Coates 2011).

Follow-up monitoring performed one month later revealed the presence of three undetected mature fruiting vines at the Parkhurst site, and a single mature fruiting vine at Kerikeri (Pearson *et al.* 2011). At both locations, seedlings and juveniles were also previously undetected or had sprouted since completion of the pilot treatment programme.

Seed dynamics study

Pearson *et al.* 2011 describes the early results of a two year seed dynamics study, which commenced in 2010. Preliminary research results of the programme have shown that seeds collected from Northland, New Zealand had a viability of around 90%. Two accelerated aging experiments using Kew Gardens Protocols (Newton *et al.* 2009) were run. Results were similar between the two experiments (Fig. 3A & 3B) showing that seed persisted in the high humidity, high temperature environment for between 30 and 50 days, with germination dropping off more rapidly in the second experiment (Fig. 3B). Extrapolation of the data shows that seeds are likely to remain viable in the natural environment for more than 10 years (Dowsett and James 2011).

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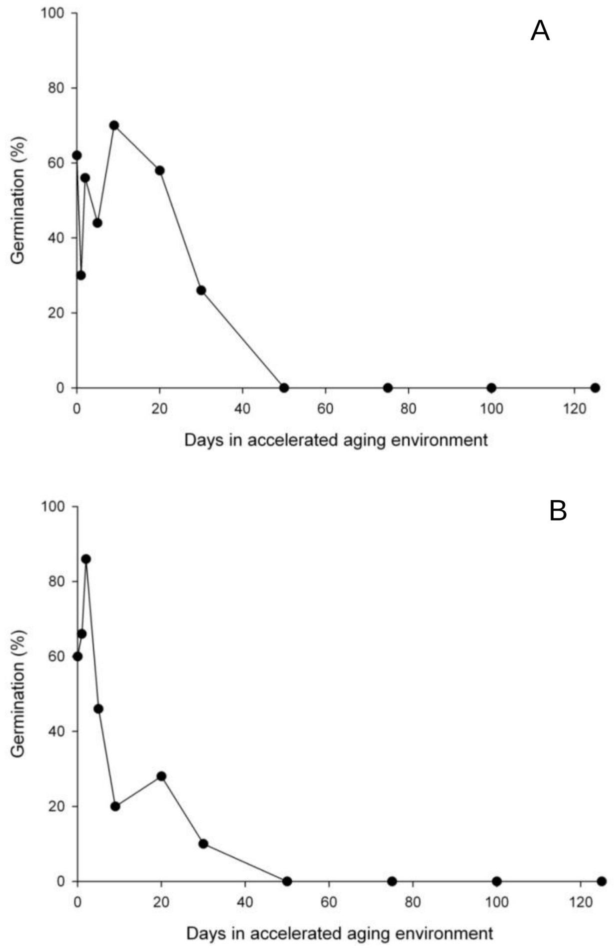


**Figure 3:** Germination of *Passiflora apetala* seed after various durations in the Accelerated Aging environment – First experiment (A) and Second Experiment (B)

The time for *P. apetala* seeds to reach maturity was assessed in both glasshouse and secured outdoor environments where plants grew vigorously and were trained up on stakes (Pearson *et al.* 2011). By January 2011 (24 weeks) the outdoor plants were flowering, followed by the glasshouse plants about a month later. By early March 2011, the fruits on the outdoor plants were maturing. As of 18 April 2011 plants in the glasshouse were still flowering vigorously and fruiting (Dowsett and James 2011).

## DISCUSSION

The response to *P. apetala* is following MAF's generic response framework that is used for responses in all sectors covering both new to New Zealand and existing risk organisms or goods. A structured approach has been followed in accordance with the MAF response model (see Ministry of Agriculture and Forestry 2008).

The response to *P. apetala* is an example of an active response that has successfully progressed through three phases of the response system. The investigation phase allowed the relevant information to be collected to determine whether or not a response should be initiated. The response initiation phase then provided further information through activities such as tracing of movement of *P. apetala* within New Zealand as well as information on the biology and ecology of the plant.

The response brief prepared under the planning and reporting phase contained sufficient information to develop different response options as well as to highlight areas where extra research was required (e.g. seed dynamics study and pilot treatment programme). At present, the information obtained is being used to develop a business case for the next phase of a response based on the preferred management strategy of local elimination from each of the seven known areas.

To assist in the preparation of a business case, an analysis of potential economic impacts of *P. apetala* establishment as an invasive weed in New Zealand is in development. The analysis will include the evaluation of the economic feasibility of the preferred management strategy.

Other eradication programmes for weeds that develop long-lived seed populations require longer-term funding and institutional commitment (Panetta and Timmins 2004). Such programmes typically require 10 years or more to complete (Panetta 2007). Preliminary results of the research programme currently underway suggest that the seed is quite resistant to the aging process and therefore is likely to remain viable in the natural environment for more than 10 years (Dowsett and James 2011). The seed longevity results will determine the length of the proposed local elimination programme which is currently planned to run for 15 years. The current proposal is similar to the two operational phases of the extirpation criterion described in Panetta (2007).

The discovery of new locations of *P. apetala* will result in a review of the local elimination programme. The final results of the seed dynamics research may also require a reassessment of the duration and effort required for the proposed programme, as the research has only been underway for one year of the two year programme. It is still to be determined how environmental conditions in New Zealand trigger plant growth and affect fertility (Dowsett and James 2011).

The MAF response system has provided a structured process which has enabled a consistent, transparent approach based on project management principles, to the management of an emerging pest plant in New Zealand.

## ACKNOWLEDGEMENTS

We would like to thank Northland and Auckland Regional Councils and the Department of Conservation for their assistance in locating and treating of *P. apetala* infestations. AsureQuality and Targeted Pest Solutions for managing and conducting field operations for the pilot treatment programme, and the numerous property owners who have assisted MAF with this response. Jaap Knegtmans, Blake Dearsly and Karyn Froud commented on the draft of this paper and Hernando Acosta and Bobby Yee assisted with the Figures.

## REFERENCES

Coates, P. (2011). Passiflora Response – Pilot Treatment Programme. AsureQuality Limited. *A report prepared for the Ministry of Agriculture and Forestry*. 31 pp.

Dowsett, C. A. and James, T. K. (2011). Biological Management studies into Bat-wing Passion Flower (*Passiflora apetala*). *Report to MAF Biosecurity New Zealand.* (AgResearch Client report number: SC110/2011/215). 10 pp.

Knegtmans, J. W. (2007). Biosecurity Pest Management Programme Operational Plan White Bryony (*Bryonia cretica* subsp. *dioica, syn. Bryonia dioica*). (MAF Biosecurity New Zealand Internal Report. 19 pp.

MAF Biosecurity New Zealand (2008). Welcome to the Biosecurity Response Knowledge Base. http://brkb.biosecurity.govt.nz/index.htm (Ministry of Agriculture and Forestry).

MAF Biosecurity New Zealand (2) (2008). Policy for MAF's Responses to Risk Organisms. (Ministry of Agriculture and Forestry). 42 pp.

Newton, R. Hay, F. and Probert R. 2009. Protocol for comparative seed longevity testing *Technical information sheet_01.* Seed Conservation Department. Millennium seed bank project Kew Gardens.
http://www.kew.org/ucm/groups/public/documents/document/ppcont_014163.pdf

Panetta, F. D. (2004). Seed banks: the bane of the weed eradicator. *Weed management: balancing people, planet, profit*. 14th Australian Weeds Conference, Wagga Wagga, New South Wales, Australia, 6-9 September 2004: papers and proceedings 2004 pp. 523-526.

Panetta, F. D. (2007). Evaluation of weed eradication programmes: containment and extirpation. *Diversity and Distributions* 13:33-41.

Panetta, F. D. and Timmins, S. M. (2004). Evaluating the feasibility of eradication for terrestrial weed incursions. *Plant Protection Quarterly* 19:5-11.

Pearson, H. G. (2010). *Passiflora apetala* risk assessment. MAF Biosecurity New Zealand Internal Report. 17 pp.

Pearson, H. G. Christian, S., Baldwin, A., Yard, D. (2011). Naturalisation of yet another *Passiflora* species in New Zealand. *New Zealand Plant Protection* 64: (in press).

Ulmer, T. and MacDougal, J. M. (2004). *Passiflora: passionflowers of the world.* Timber Press, Inc. Portland Oregon, USA. 340 pp.

Williams, P. A. Nicol, E. and Newfield, M. (2000). Assessing the risk to indigenous New Zealand biota form new exotic plant taxa and genetic material. *Science for Conservation* 143. (New Zealand Department of Conservation, Wellington) 42 pp.

# LONG TERM HERBICIDAL WEED MANAGEMENT INTEGRATED WITH NITROGEN NUTRIENT IN TRANSPLANTED RICE-RICE CROPPING SYSTEM OF TAMIL NADU, INDIA

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**ABSTRACT**

With a primary objective to evaluate the long-term herbicide application integrated with nitrogen management on weed shift, weed control efficiency, soil micro flora, herbicide residue and productivity of transplanted rice-rice cropping system, field experiments were conducted for ten years from 2000 to 2010. Field experiments with hand weeding (HW) twice, pre-emergence butachlor 0.75 or pretilachlor 0.75 kg/ha or in rotation + post emergence 2,4, D, 0.4 kg/ha along with inorganic and organic nitrogen(N) at 75 and 25 percent were conducted with four replications arranged in a randomized blocks design.

Decreased grass weeds density from 53.0% with first rice crop during *kharif* 2000 to 42.1% with eighteenth rice in HW with 100% N as inorganics, was observed. Broad leaved weed (BLW) density was higher with HW in eighteenth and nineteenth rice crops compared to first rice crop. *Ludwigia parviflora*, *Eclipta alba* and *Marselia quadrifoliata* were BLWs in nineteenth and twentieth rice crops. Weed shift from *Echinochloa crusgalli* to *Panicum distachyon* was observed, which was more in rotational use of herbicides. BLW density was higher in HW with nineteenth and twentieth rice crops compared to first crop. Reduced weed density and dry weight were observed with herbicidal weed control and it was well pronounced under rotational use of herbicides (butachlor in *kharif* and pretilachlor in *rabi* with 2,4-D). Herbicides application recorded significantly higher yield in nineteenth and twentieth rice crops and the yield increase was higher with continuous and rotational use of herbicides. Residues of butachlor, pretilachlor and 2,4-D were below deductable level from 45 days after application in soil and crop. Improvement in soil actinomycetes, fungi and bacteria was observed with herbicides application.

**Key words:** Transplanted rice, weed shift, weed density, dry weight, soil micro flora, herbicide residue.

## INTRODUCTION

Control of weeds at the early rice growth stage before they compete with crop is essential to restrict nutrient depletion by weeds and its availability to crop for higher yields. Manual weeding could be done only when the weed growth is to a size large enough for hand removal, by that time weeds would have competed with crop. Herbicide use for the control of weeds, especially in intensive rice-rice cropping system, is at higher rates as cost and demand for manual labour is increasing. Further, long term continuous use of selective herbicides in rice may cause a shift in weed flora, from annuals to perennials, which are difficult to control. Other crop management practices, especially nitrogen use (organic / inorganic / integrated nutrient management) is likely to change the crop-weed ecology as well as herbicide activity and herbicide residue in the soil. With these in view, a long term herbicide experiment on fixed plot basis has been initiated from *kharif* 2000 with the following objectives:

- To assess the effect of continuous use of herbicides on the shift in weed flora, control of weeds and yield of rice
- To estimate the fate of herbicides and level of herbicide residue in the soil and crop produce; and
- To study the impact of long term use of herbicides on the soil microbial population.

## MATERIALS AND METHODS

Field experiment was initiated at the Wetlands of Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, is the southernmost State in the Indian Union. The experimental farm is located at 77 E 11°N latitude 426 metre above mean sea level, and the farm receives the normal total annual rainfall of 674.2 mm in 45.8 rainy days. Trial was conducted in soil with fine clay loam type of soil and belonging to *Typic chromusterts* soil group and *noyyal* soil series. This soil is medium in organic carbon content (0.64 per cent) and the available nutrient status is low in nitrogen, medium range of phosphorus and the potassium status is high with neutral to alkaline in soil reaction and soluble salt content is within the permissible limits.

The first crop of rice was planted during *kharif* season 2000 (sowing/planting from June to August months) and in rotation during *rabi* season (sowing / planting from September to November months) of every year. Field trials were carried out for ten years with twenty rice crops in rice-rice cropping system during *karif* and and *rabi* seasons up to 2009-10. Two sources of nitrogen (N) viz.,organic (in situ green manuring with *Sesbania rostrata* to supply 25% of N) and inorganic (prilled urea with 46% N) were used as per the treatment schedule(Table 1). Weed management treatments include hand weeding, pre (PE) and post emergence (POE) herbicides in combination and pre emergence herbicides in rotation as detailed below.

**Table 1.** Nitrogen and weed management treatments in transplanted rice-rice cropping system

| Weed control methods | Source of nitrogen | |
|---|---|---|
| | **Inorganic** | **Organic** |
| W1N1 - Hand weeding on 20 and 40 days after transplanting | 100 % | - |
| W1N2 - Hand weeding on 20 and 40 days after transplanting | 75% | 25% |
| W2N1 - PE Butachlor 0.75 + POE 2,4-DEE 0.4 kg / ha | 100% | - |
| W2N2 - PE Butachlor 0.75 + POE 2,4-DEE 0.4 kg / ha | 75% | 25% |
| W3N1 - PE Butachlor 0.75 + POE 2,4-DEE 0.4 kg / ha( *kharif*) PE Pretilachlor 0.75 + POE 2,4 DEE 0.4 kg / ha( *rabi*) | 100% | - |
| W3N2 - PE Butachlor 0.75 + POE 2,4-DEE 0.4 kg / ha( *kharif*) PE Pretilachlor 0.75 + POE 2,4 DEE 0.4 kg / ha( *rabi*) | 75% | 25% |

W1 & W2 : Same pre emergence herbicde for *kharif* & *rabi* rice crops

W3 : Rotation of pre emergence herbicide for *kharif* and *rabi* rice crops

Design : RBD; Replication: Four

Observations were made on predominant weed flora and weed density and dry weight, rice grain yield, herbicide residue in post harvest soil, grain and straw and post harvest soil microbial population (bacteria, fungi and actinomycetes) in every trial.

## RESULTS AND DISCUSSION

### PREDOMINANT WEED FLORA OF THE EXPERIMENTAL FIELD

In the first rice crop (*kharif,* 2000) the general weed flora (Table 2) was *Echinochloa crusgalli* (41.1%), *Echinochloa colona* (8.1%) and *Leptochloa chinensis* (3.8%) among grasses (53.0%), while sedge (18.5%) mainly comprised of *Cyperus difformis* (15.7%) and *Cyperus iria* (2.8%). Broad leaf weeds observed were *Ludwigia parviflora* (12.3%), *Ammania baccifera* (9.4%), *Eclipta alba* (3.7%), *Marselia quadrifoliata* (1.9%) and others (1.2%). The absolute weed density was 41.7m$^2$ as observed in hand weeding twice applied with 100% N by inorganic source.

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**Table 2.**Summed dominance ratio (SDR) of predominant weed species in first rice

| SDR of predominant weeds in first rice (*kharif* 2000) | | | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | **W1N1** | **W1N2** | **W2N1** | **W2N2** | **W3N1** | **W3N2** |
| **Weed species** | | | | | | |
| *Echinochloa crus-galli* | 41.1 | 38.6 | 33.8 | 33.3 | 38.9 | 34.0 |
| *Echinochloa colona* | 8.1 | 6.4 | 7.1 | 8.2 | 6.8 | 7.4 |
| *Leptochloa chinensis* | 3.8 | 2.2 | 2.5 | 4.1 | 3.7 | 2.9 |
| *Panicum distachyon* | - | - | - | - | - | - |
| Total grasses | 53.0 | 47.2 | 43.4 | 45.6 | 49.4 | 44.3 |
| *Cyperus difformis* | 15.7 | 14.6 | 14.1 | 14.8 | 15.3 | 15.0 |
| *Cyperus iria* | 2.8 | 2.7 | 1.5 | 1.6 | 2.1 | 1.2 |
| Total sedges | 18.5 | 17.3 | 15.6 | 16.4 | 17.4 | 16.2 |
| *Ludwigia parviflora* | 12.3 | 18.7 | 21.9 | 19.4 | 17.8 | 22.3 |
| *Ammania baccifera* | 9.4 | 10.1 | 8.9 | 10.4 | 7.3 | 8.4 |
| *Eclipta alba* | 3.7 | 3.4 | 4.7 | 4.2 | 3.6 | 4.3 |
| *Marselia quadrifoliata* | 1.9 | 2.3 | 3.1 | 2.2 | 2.1 | 2.7 |
| Others | 1.2 | 1.0 | 2.4 | 1.8 | 2.4 | 1.8 |
| Total BLW | 28.5 | 35.5 | 41.0 | 38.0 | 33.2 | 39.5 |
| Absolute weed density / $m^2$ | 41.7 | 35.1 | 32.8 | 27.5 | 31.5 | 26.4 |

Analysis of Summed Dominance Ratio of weed species in the first and twentieth rice crop (60 days after transplanting -DAT) showed that the grass weed density decreased from 53.0% to 40.1 % (60 DAT), where hand weeding on 20 and 40 DAT method of weed control was practiced with 100% nitrogen nutrient as inorganics (Table 3). In all the treatments grass weeds density decreased from first rice crop to $20^{th}$ rice crop. However the *Echinochloa colona* and *Leptochloa chinensis* which were present in first rice crop were completely absent in twentieth rice crop. Increase in total sedge weed density was observed in all the treatments when compared to the first rice crop except in the case of rotational use of pre emergence herbicides with butachlor for *kharif* rice and pretilachlor for *rabi* rice ($W_3$) and hand weeding for both seasons rice in rice-rice cropping system. Broad leaved weed density was higher in hand weeding treatments of twentieth rice crop (60 DAT) compared to first rice crop. *Ludwigia parviflora*, *Eclipta alba* and *Marselia quadrifoliata* were the predominant broad leaved weeds present in the twentieth rice crop at both stages.

An increase in total broad leaved weed density was observed at 60 DAT in twentieth rice crop. Among the grasses, *Echinochloa colona* and *Leptochloa chinensis* and *Cyperus iria* among the sedges recorded in the first rcie crop were absent in twentieth rice crop.

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**Table 3. Long-term weed control methods and source of N on weed flora of transplanted rice-rice cropping system**

| Treatments | Summed Dominance Ratio(SDR) of weed species–60 DAT | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | First crop (*kharif*, 2000) | | | | | | Twentith rice crop (*rabi*, 2009-10) | | | | | |
| | W1 N1 | W1 N2 | W2 N1 | W2N 2 | W3 N1 | W3 N2 | W1 N1 | W1 N2 | W2 N1 | W2 N2 | W3 N1 | W3 N2 |
| *Echinochloa crus-galli* | 41.1 | 38.6 | 33.8 | 33.3 | 38.9 | 34.0 | 29.5 | 24.6 | 16.1 | 6.8 | 10.9 | 12.2 |
| *Echinochloa colona* | 8.1 | 6.4 | 7.1 | 8.2 | 6.8 | 7.4 | - | - | - | - | - | - |
| *Leptochloa chinensis* | 3.8 | 2.2 | 2.5 | 4.1 | 3.7 | 2.9 | - | - | - | - | - | - |
| *Panicum distachyon* | - | - | - | - | - | - | 13.3 | 15.5 | 15.5 | 24.7 | 16.6 | 14.4 |
| Total grasses | 53.0 | 47.2 | 43.4 | 45.6 | 49.4 | 44.3 | 42.8 | 40.1 | 31.6 | 31.5 | 27.5 | 26.6 |
| *Cyperus difformis* | 15.7 | 14.6 | 14.1 | 14.8 | 15.3 | 15.0 | 28.6 | 18.1 | 12.4 | 25.1 | 6.1 | 9.7 |
| *Cyperus iria* | 2.8 | 2.7 | 1.5 | 1.6 | 2.1 | 1.2 | - | - | - | - | - | - |
| Total sedges | 18.5 | 17.3 | 15.6 | 16.4 | 17.4 | 16.2 | 28.6 | 18.1 | 12.4 | 25.1 | 6.1 | 9.7 |
| *Ludwigia parviflora* | 12.3 | 18.7 | 21.9 | 19.4 | 17.8 | 22.3 | 40.1 | 11.7 | 11.7 | 15.6 | 20.9 | - |
| *Ammania baccifera* | 9.4 | 10.1 | 8.9 | 10.4 | 7.3 | 8.4 | 14.5 | 19.1 | 19.1 | 0.0 | 19.1 | 28.2 |
| *Eclipta alba* | 3.7 | 3.4 | 4.7 | 4.2 | 3.6 | 4.3 | - | - | - | - | - | - |
| *Marselia quadrifoliata* | 1.9 | 2.3 | 3.1 | 2.2 | 2.1 | 2.7 | 12.1 | 16.3 | 22.2 | 25.1 | 12.1 | 12.1 |
| Others | 1.2 | 1.0 | 2.4 | 1.8 | 2.4 | 1.8 | - | - | - | - | - | - |
| Total BLW | 28.5 | 35.5 | 41.0 | 38.0 | 33.2 | 39.5 | 66.7 | 47.1 | 53.0 | 40.7 | 52.1 | 40.3 |

## WEED DENSITY AND DRY WEIGHT

During 60 DAT increase in grass weed density when compared to the first rice crop was observed in the plots which received herbicidal weed management treatments (Table 4.). The same trend was observed during harvest stage also. Treatments which received 100% inorganic nitrogen recorded higher grass weed density. The total sedge density was more in all the treatments when compared to the first rice crop. Broad weed density was more in the treatments which received hand weeding and also higher in the 100 % inorganic nitrogen applied plots.

In both rice crops, hand weeding treatments recorded significantly higher weed dry weight than in chemical treatments. Treatments with rotational application of herbicides (butachlor + 2,4-D (*kharif*) and pretilachlor + 2,4-D (*rabi*) and integration of organic and

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inorganic source of nitrogen nutrient recorded significantly lesser weed dry weight at 60 DAT and harvest stage in both the first and twentieth rice crops.

**Rice grain yield**

Rice grain yield was not significantly influenced by the interaction of source of N and hand weeding method of weed management in first as well as twentieth rice crops (Table 4.). The effect of weeding methods on grain yield was more pronounced during *rabi*,2009-10 compared to *kharif,* 2000. Manual weeding recorded higher grain yield in the first crop. Whereas, treatments involving pre emergence and post emergence herbicides recorded significantly higher yield in twentieth rice crop. Among the herbicidal weed management treatments which received rotational use of pre emergence herbicides recorded higher grain yield than single herbicide of butachlor for both *kharif* and *rabi* seasons rice.

**Table 4.** Weed density and dry weight and rice grain yield as influenced by long-term herbicide application and sources of N in transplanted rice-rice cropping system

| Treatments | Weed density 60 DAT ($m^{-2}$) | | Weed dry weight 60 DAT (g $m^{-2}$) | | Rice grain yield (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|
| | I rice crop | XX rice crop | I rice crop | XX rice crop | I rice crop | XX rice crop |
| W1N1 | 41.7 | 46.7 | 18.4 | 48.3 | 5784 | 4937 |
| W1N2 | 35.1 | 37.3 | 13.9 | 38.6 | 5542 | 5300 |
| W2N1 | 32.8 | 29.8 | 20.9 | 30.5 | 5626 | 5225 |
| W2N2 | 27.5 | 30.0 | 16.3 | 29.4 | 5426 | 5000 |
| W3N1 | 31.5 | 25.9 | 21.0 | 27.1 | 5676 | 5362 |
| W3N2 | 26.4 | 33.6 | 15.8 | 32.5 | 5570 | 5700 |
| CD (P=0.05) | 7.9 | 5.6 | 3.4 | 4.9 | 136 | 315 |

**Table 5.** Persistence and residues of pre emergence herbicide butachlor in transplanted rice-rice cropping system

| Treatments | Days after herbicide application | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 7 | 15 | 30 | 45 | At Harvest |
| **First rice (*kharif*, 2000)** | | | | | | |
| W2N1 | 0.301 | 0.212 | 0.116 | 0.025 | 0.002 | 0 |
| W2N2 | 0.289 | 0.174 | 0.101 | 0.022 | 0.0011 | 0 |
| W3N1 | 0.312 | 0.194 | 0.112 | 0.0106 | 0.001 | 0 |
| W3N2 | 0.278 | 0.186 | 0.106 | 0.012 | 0.008 | 0 |
| **Twentieth rice (*rabi*, 2009-10)** | | | | | | |
| W2N1 | 0.526 | 0.324 | 0.201 | 0.087 | 0.054 | BDL |
| W2N2 | 0.451 | 0.285 | 0.169 | 0.065 | 0.025 | BDL |
| W3N1 | 0.421 | 0.265 | 0.197 | 0.085 | 0.025 | BDL |
| W3N2 | 0.358 | 0.218 | 0.19 | 0.078 | 0.031 | BDL |

**Table 6.** Persistence and residues of post emergence herbicide **2,4-D** in transplanted rice-rice cropping system

| Treatments | Days after herbicide application | | | | |
|---|---|---|---|---|---|
| | **1** | **15** | **30** | **45** | **At Harvest** |
| | | | **First rice (*kharif*, 2000)** | | |
| W2N1 | 0.94 | 0.061 | 0.003 | 0 | |
| W2N2 | 0.083 | 0.068 | 0.004 | 0 | 0 |
| W3N1 | 0.079 | 0.059 | 0.003 | 0 | 0 |
| W3N2 | 0.075 | 0.067 | 0.003 | 0 | 0 |
| | | | **Twentieth rice (*rabi*, 2009-10)** | | |
| W2N1 | 0.214 | 0.125 | 0.054 | BDL | BDL |
| W2N2 | 0.215 | 0.108 | 0.048 | BDL | BDL |
| W3N1 | 0.21 | 0.124 | 0.048 | BDL | BDL |
| W3N2 | 0.15 | 0.115 | 0.04 | BDL | BDL |

Continuous application of butachlor + 2,4-DEE herbicide mixtures in every season or rotational application of butachlor + 2,4-DEE during *kharif* and pretilachlor + 2,4-DEE during *rabi* seasons did not show build up of these herbicides in the post harvest soil or grain and straw of the first (*kharif,* 2000) and twentieth (*rabi,*2009-10) rice crops as observed in hand weeding treatment in all the twenty rice crops in rice-rice cropping system(Table 5 and 6).

## SOIL MICROBIAL POPULATION

The soil microflora was not quantitatively affected as far as the bacterial and fungal populations are concerned. The normal range of population of bacterial and fungi in a flooded soil was present throughout without any alarming changes. The actinomycetes population was very low during the entire duration of crop growth, but a slight increase was observed after harvest of the crop (Table 7.). The microbial population among different treatments varied during *kharif* 2000 and *rabi* 2009-10 seasons experiments. Higher bacterial population was recorded with rotational pre emergence herbicidal weed management integrated with organic source of N in twentieth rice (*rabi* 2009-10) which was comparable with first rice crop(*kharif*, 2000) with rotational herbicidal and hand weeding weed management. Whereas, fungi population was consistently higher with hand weeding combined with inorganic N and integrated N management in both the rice crops. Similarly, actinomycetes population was also higher in hand weeding integrated with either the sources of N (organic and inorganic) management in twentieth rice crop (*rabi*, 2009-10).

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**Table 7.** Soil microbial population at post harvest stage in transplanted rice-rice cropping system

| Treatments | First rice crop(*kharif,* 2000) | | | Twentieth rice crop (*rabi*, 2009-10) | | |
|---|---|---|---|---|---|---|
| | **Bacteria x $10^6$/g ODS** | **Fungi x $10^3$/g ODS** | **Actinomycetes x $10^3$/g ODS** | **Bacteria x $10^6$/g ODS** | **Fungi x $10^3$/g ODS** | **Actinomycetes X $10^3$/g ODS** |
| W1N1 | 37.15 | 52.99 | 17.17 | 32.46 | 56.32 | 18.45 |
| W1N2 | 58.33 | 53.80 | 19.79 | 50.35 | 48.62 | 25.92 |
| W2N1 | 43.05 | 23.06 | 4.86 | 39.45 | 28.54 | 3.56 |
| W2N2 | 36.81 | 27.51 | 6.25 | 41.56 | 32.92 | 7.03 |
| W3N1 | 56.04 | 40.45 | 7.64 | 61.34 | 45.31 | 8.94 |
| W3N2 | 22.57 | 32.76 | 9.38 | 45.37 | 42.54 | 14.58 |

g ODS: gram of oven dry soil

## DISCUSSION

Rotational application of pre emrgence herbicides (butachlor + 2,4-D (*kharif*) and pretilachlor + 2,4-D (*rabi*) and integration of nitrogen nutrientwith inorganic and organic sources recorded significantly lesser weed density and weed dry weight at 60 DAT in both first and twentieth rice crops. In general weed dry matter accumulation was significantly lower under combined use of organic and inorganic sources of nutrients as compared to inorganic alone irrespective of the method of weed management as earlier observed by Rajkhowa *et al*.(2001). Rotational use of selective pre emrgence herbicide might have helped in broad spectrum weed control avoiding chances of herbicide resistant weed biotypes while using a single herbicide for a longer period.

Normally herbicide applied treatments observed with lesser weed density and dry weight compared to hand weeding treatments. According to Jacob *et al*.(2005) who had reported that pre-emergence application of anilofos + 2,4-D EE supplemented with 2,4-D sodium drastically reduced the weed density and dry weight when compared to anilofos + 2,4- D EE supplemented with hand weeding. Integration of weed control by butachlor + 2,4-DEE followed by pretilachlor 0.75 + 2,4-DEE 0.4 kg ha$^{-1}$ with 100% inorganic nitrogen recorded maximum yield in twentieth rice crop and the application of butachlor + 2,4-DEE during both the seasons recorded maximum yield in twentieth rice crop. Similar increase in grain yield of tenth rice grown in succession was reported by Kandasamy and Chinnusamy (2005) with rotational use of pre emergence herbicide integrated with organic and inorganic source of N in transplanted rice. Rice grain yield increase in effective weed management methods involving rotational pre emergence herbicides usage integrated basal application of organic source on N and split application of inorganic source of N in rice could be attributed to lesser weed density and dry weight recorded consistently with all the twenty rice crops in transplanted rice-rice cropping system. These results corroborate with the findings of Singh *et al*. (2003) who had observed similar increase in rice grain yield with split application of nutrients along with effective weed management increased the crop yield due to lower density, dry matter accumulation in weeds which helped in providing favorable growing environment resulting into better expression of potential yield.

Mean half life of the butachlor and 2, 4-DEE during *kharif* was found to be 9 and 7 days respectively and 13 days during *rabi* for both the molecules. Comparing pooled data of

2000-05 with 2005-2010, butachlor build up was 31 per cent high irrespective of continuous or rotational application and was also influenced by the N sources. 2,4-DEE persistence and build up was influenced by the N sources was in the range of 14 to 19 percent. However at harvest the residues were not detected in grain, straw or in soil. These results are in line with the findings of Shanmughasundaram *et al.(2005) and* Janaki *et al*.(2010) who had reported below deductable levels of butachlor, pretilachlor and 2,4-DEE applied in rotation integrated with organic and inorganic source of N in transplanted rice-rice system.

## REFERENCES

Jacob, D., Elizabeth. K. Syriac and Pushpakumari, R. (2005). Spacing and weed management in transplanted basmati rice. *Madras Agricultural Journal.* 92 (4-6): 224-229.

Janaki, P., Chinnusamy, C., Meena, S.and Shanmugasundaram, R. (2010). Persistence And Degradation Behavior Of Butachlor And 2,4-D In Rice Soil Under Continuous And Rotational Use: Effect Of Nitrogen Sources And Seasons. In: *Proceedings of the International Rice Research Conference held at Hanoi, Vietnam*, November 8-12th, 2010: pp.3817

Kandasamy,O.S. and Chinnusamy,C. (2005). Studies on the effect of weed management practices in two transplanted rice crops grown in sequence. *Proc. National Biennial Conference of Indian Society of Weed Science, PAU, Ludhiana*, India(April,6-9,2005):pp;1-2.

Rajkhowa, D.J., Gogoi, A.K. and Kandali, R. (2001). Effect of weed control and nutrient management practices in Rice. *Indian Journal of Weed Science*. 33(1&2): 41-45.

Shanmughasundaram, R., Kandasamy, O.S. and Chinnusamy, C. (2005).Impact of herbicide application on soil physico-chemical properties and its residue in rice-rice cropping system. *Proc. National Biennial Conference of Indian Society of Weed Science, PAU, Ludhiana*, India (April,6-9,2005):pp;290-91.

Singh, R.P., Singh, C.M. and Singh, A.K. (2003). Effect of crop establishment methods, weed management and splitting of N on rice and associate weeds. *Indian Journal of Weed Science*. 35(1&2): 33-37.

# EFFECTS OF POST- EMERGENCE HERBICIDES ON WATER HYACINTH (*EICHHORNIA CRASSIPES*) -TANK CULTURE EXPERIMENT

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## ABSTRACT

Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms) has become a major weed of rivers and dams, and a problem weed in southern parts of India. Water hyacinth has many adverse impacts on waterways, includes reduced access to water and poor quality of available drinking and irrigation water. Hence, a study was initiated to quantify the characteristics of Water Hyacinth and to evaluate the effect of post-emergence herbicides management methods on Water Hyacinth in tank culture in the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, during 2007 to 2009. Tank culture experiments were carried out to quantify the characteristics and to evaluate the effects of post emergence herbicides on the management of Water Hyacinth in a randomized block design with three replications.
Water Hyacinth mother plants grew very fast from one week after inoculation up to six weeks, after which the growth rate decreased and it involved in seed maturation, senescence and ramet production. Ramets emerged from the third week onwards and showed fast growth up to six weeks. This continuous growth process and multiplication by ramet production at a very high rate are the main reasons why Water Hyacinth causes enormous problems to the environment.

A tank culture experiment was conducted to evaluate the efficacy of different post emergence herbicides (i.e. Paraquat, Glyphosate and 2, 4-D Na salt) on both Water Hyacinth and on the aquatic ecosystem. The results revealed that spraying of glyphosate at 10 mL/L + ammonium sulphate 20g/L + Triton 1mL/L effectively reduced the Water Hyacinth density as well as Water Hyacinth dry weight considerably.

**Key words:** Water Hyacinth, Tank culture, post emergence herbicides, paraquat, glyphosate 2, 4-D Na salt

## INTRODUCTION

Invasive aquatic weeds have spread throughout the world’s waterways as a result of anthropogenic activities. The most problematic species among water body weeds is Water Hyacinth. It is perennial fresh water aquatic plant that doubles its number within four weeks under favourable conditions (Akinyemiju and Bewaji, 1990). Originally introduced to India in Bengal as an aquatic ornamental plant, Water Hyacinth has become a major aquatic weed of rivers and dams. Not only does it destroy native habitats, but it also seriously depletes water bodies of oxygen, increases water loss and provides a breeding ground for mosquitoes. Cultural eutrophication of lakes is also a major problem around the world and this can amplify the problem due to Water Hyacinth. Nowadays Water Hyacinth becomes a notorious aquatic weed in western parts of Tamil Nadu. Moreover for human population living around such water bodies, it reduces access to and quality of available drinking and irrigation water, prevents fishermen from making a living, clogs water intakes at hydro-electric dams, increases vector-borne diseases, eutrophication and generally leads to an increase in human suffering. This free floating monocotyledous plant obstructs fishing, water transportation and recreation uses in many riverines (Adekoya, 1992).

Considering the gravity of problems posed by the aquatic weed; Water Hyacinth on human health and on environment, tank culture experiment was conducted with the objectives to quantify the characteristics of Water Hyacinth and to evaluate the effects of post emergence herbicides on the management of Water Hyacinth.

## MATERIALS AND METHODS

### Characteristics of Water Hyacinth

Water Hyacinth plants were collected uniformly at four leaf stages and inoculated in ten pre-fabricated cement tanks (dimensions-5 feet x 5 feet x 2 feet) at the rate of twenty plants per tank and watering was done periodically. Then ten plants per tank were tagged for biological studies. Observations on Water Hyacinth characteristics were recorded at weekly intervals and up to six weeks after inoculation. Mean data over ten plants each in a tank were arrived and presented in the results and discussion.

### Herbicidal management of Water Hyacinth

The experiment was conducted at controlled condition using cement tanks with the size of 10 feet x 5 feet x 4 feet. Water Hyacinth plants were collected from local lakes and 75 number of Water Hyacinth plants have been inoculated in each water filled cement tanks. The tank culture experiment was laid out in randomized block design with treatments replicated thrice. Initial fresh biomass of 75 plants was recorded. Water Hyacinth plants were allowed as such to establish in the tanks for a period of one month. Then the calculated quantity of herbicides was sprayed in the respective cement tanks as per the treatment schedule. Plant height, number of leaves, wet weight and dry weight of the Water Hyacinth plants have been recorded at weekly intervals. Visual scoring for qualitative assessment of effect of herbicides has been done at weekly intervals after herbicide treatment for three months. Simultaneously visual scoring on regeneration of the water hyacinth has also been done up to four months after treatment. The data on plant dry weight were subjected to logarithmic transformation.

### Treatment details

$T_1$ - Paraquat 6 mL/ L of water
$T_2$ - Paraquat 8 mL/ L of water
$T_3$ - Glyphosate 10 mL/ L of water
$T_4$ – Glyphosate 15 mL/ L of water
$T_5$ - 2,4-D Na salt 6 g / L of water
$T_6$ - 2,4-D Na salt 8 g / L of water

$T_7$ - Coleous plant water extract (1:1 w/w) 300 mL/ L of water
$T_8$ - Unsprayed control
$T_1$ to $T_6$ ammonium sulphate 20 g/L and Triton 10 mL / L of water were tank mixed before spraying.

## RESULTS AND DISCUSSION

### Characteristics of Water Hyacinth

Plant growth steadily increased and attained the maximum height on six weeks after inoculation, where large number of ramets production also occurred (Table 1). From the day of inoculation, it took 18 days for the initiation of inflorescence. Each plant produced an average of two inflorescences. Root growth also attained its maximum on sixth week of inoculation and declined there after but the rate of decline was low when compared to shoot portion. Number of stolons and leaves increased gradually up to sixth week of inoculation and then decreased. After sixth week small reduction in the ramets production due to the ramets remain attached to the parent plant. Inflorescence initiation in ramets started in fifth week having an average of one inflorescence per ramet and an average of seven flowers per inflorescence. Higher rate of multiplication of Water hyacinth was earlier observed that ten water hyacinth plants could thus produce six lakhs plants covering0.4 hectare water spread area within months and the individual plant starts to double its number in merely six days (Akinyemiju and Bewaji, 1990). All the characters contributing to dry matter production were at peak on sixth week after inoculation. Total dry weight of the plant reached higher on sixth week after inoculation.

**Table 1.** Characteristics of mother plant and ramets of Water Hyacinth

| Observations | 0 DAT | 14 DAT | 28 DAT | 42 DAT |
|---|---|---|---|---|
| Plant height (cm) | 11.40 | 22.80 | 56.60 | 62.00 |
| Number of leaves / plant | 4.00 | 6.20 | 9.00 | 5.00 |
| Number of Inflorescence / plant | 0.00 | 0.00 | 2.00 | 2.00 |
| Number of flowers / Inflorescence | 0.00 | 0.00 | 12.00 | 12.00 |
| Root length (cm) | 7.40 | 21.40 | 44.50 | 45.60 |
| Total dry weight (g) | 1.19 | 26.08 | 64.20 | 83.80 |
| Number of ramets | 0.00 | 2.00 | 7.00 | 6.00 |
| Number of leaves / ramets | 0.00 | 5.00 | 7.00 | 7.00 |
| Number of Inflorescence | 0.00 | 0.00 | 1.00 | 1.00 |
| Number of flowers in ramets | 0.00 | 0.00 | 7.00 | 7.00 |

DAT: Days after treatment (inoculation); Data not statistically analysed

### Effect of Herbicides On Water Hyacinth

Plant Height

The height of the plants recorded before herbicide spray did not show any significant variations among the treatments (Table 2). Lower plant height was observed in Glyphosate at 10 mL/L ($T_3$) and 15mL/L ($T_4$) treatments followed by 2,4-D at 6g/L($T_5$) and 8g/L ($T_6$). Whereas the unsprayed control ($T_8$) and *Coleus* sp. plant extract spray ($T_7$) registered the maximum height (102.4 cm and 91 cm) followed by paraquat at 6mL/L($T_1$) and 8mL/L($T_2$) treatments at later stages of observation. The reduction in plant height in

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paraquat spray ($T_1$ and $T_2$) might be due to quick knock down effect at initial stages and later stages being a contact herbicide it losses its effect on Water Hyacinth.

**Table 2.** Effect of herbicides on plant height of Water Hyacinth.

| Treatments | Plant height (cm) | | | | |
|---|---|---|---|---|---|
| | BHT | 3 DAT | 7 DAT | 14 DAT | 21 DAT |
| $T_1$-$Par_6$ | 74.0 | 58.0 | 61.0 | 34.0 | 57.0 |
| $T_2$-$Par_8$ | 76.0 | 56.0 | 60.0 | 32.0 | 52.6 |
| $T_3$-$Gly_{10}$ | 72.0 | 72.0 | 14.0 | 5.80 | 0.00 |
| $T_4$-$Gly_{15}$ | 74.0 | 74.0 | 12.5 | 4.50 | 0.00 |
| $T_5$-2,4-$D_6$ | 73.0 | 48.0 | 12.0 | 0.00 | 14.0 |
| $T_6$-2,4-$D_8$ | 70.0 | 40.0 | 14.0 | 0.00 | 13.5 |
| $T_7$-$Col_{300}$ | 77.0 | 83.0 | 87.0 | 83.0 | 91.0 |
| $T_8$-Con | 72.0 | 86.0 | 91.0 | 89.0 | 102.4 |
| SEd | 3.21 | 5.8 | 4.8 | 2.01 | 1.48 |
| CD(5%) | NS | 12.4 | 10.2 | 4.32 | 3.17 |

BHT: before herbicide treatment; DAT: days after treatment

Number of Leaves

At the time of spraying, the number of leaves did not show any significant variation among treatments (Table 3). Paraquat spray at 6mL/L($T_1$) and 8mL/L($T_2$) recorded lower number of leaf during initial stages and started increasing on 7 days after treatment(DAT)and increased up to 21 DAT. This might be due to Paraquat being a contact non-selective weed killer it might have allowed regeneration of Water Hyacinth at later stages, which was reflected through higher density of Water Hyacinth with more number of leaves recorded at 7 DAT onwards.

**Table 3.** Effect of herbicides on number of leaves of Water Hyacinth

| Treatments | Number of leaves/plant | | | | |
|---|---|---|---|---|---|
| | BHT | 3 DAT | 7 DAT | 14 DAT | 21 DAT |
| $T_1$-$Par_6$ | 9.00 | 0.00 | 2.00 | 6.00 | 8.00 |
| $T_2$-$Par_8$ | 8.00 | 0.00 | 2.00 | 6.00 | 8.00 |
| $T_3$-$Gly_{10}$ | 9.00 | 9.00 | 4.00 | 4.00 | 0.00 |
| $T_4$-$Gly_{15}$ | 8.00 | 8.00 | 3.00 | 4.00 | 0.00 |
| $T_5$-2,4-$D_6$ | 9.00 | 2.00 | 0.00 | 0.00 | 3.00 |
| $T_6$-2,4-$D_8$ | 8.00 | 2.00 | 0.00 | 0.00 | 3.00 |
| $T_7$-$Col_{300}$ | 8.00 | 9.00 | 9.00 | 9.00 | 11.0 |
| $T_8$-Con | 9.00 | 11.00 | 11.0 | 11.0 | 12.0 |
| SEd | 0.36 | 0.60 | 0.50 | 0.27 | 0.31 |
| CD(5%) | NS | 1.25 | 1.01 | 0.58 | 0.67 |

BHT: before herbicide treatment; DAT: days after treatment

Treatments with Glyphosate at 10 mL/L ($T_3$) and 15mL/L ($T_4$) initially recorded higher number of leaves (9/plant and 8/plant) and started decreasing from 7 DAT and resulted in lower number of leaves 21 DAT. Glyphosate at 2.38 kg/ha effectively controlled the Water Hyacinth densities at 20 kg/m$^2$ in 51 days and the second dose of 0.95kg/ha was required for the complete plant control (Eric, 1993). The slower rate of control of Water Hyacinth with Glyphosate might be due to the slow translocation of Glyphosate herbicide which was effective only after 15 DAT. Treatments with 2,4-D at 6g/L($T_5$) and 8g/L ($T_6$)resulted in a gradual decrease in number of leaves from initial stages and recorded lower ratings on 14 DAT and afterwards it showed minimum increase ($T_5$ and $T_6$) on 21 DAT. Water Hyacinth plants sprayed with 2, 4-D showed epinastic symptoms and brittle stems and leaves. This

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effect might be due to differential turgidity and unequal rates of cell division and cell enlargement. More number of leaves (12/plant) was observed in unsprayed control ($T_8$) throughout the experiment followed by coleus plant extract ($T_7$) from the day of herbicide spraying up to 21 DAT.

Plant Dry Weight

Glyphosate treatments at 10 mL/L ($T_3$)and 15mL/L ($T_4$) recorded higher dry weight in the initial stage and have shown gradual reduction and resulted in lower dry weight (2 g / plant) at later stages(Table 4). Glyphosate being a non-selective systemic herbicide readily absorbed by leaves and throughout the symplast. All Water Hyacinth plants can be eliminated after three weeks after Glyphosate application (Gutiérrez *et al.,* 1996). Similarly, Ashwini Jadhav (2007) reported that lower dose of Glyphosate 0.8 %( 1.36 L/ha) retarded the growth of parent plants of Water Hyacinth and inhibited the production of daughter plants. Similarly, earlier Singh and Muller (1979) also obtained 100% control of Water Hyacinth with 2.0 kg/ha Glyphosate within three weeks after application and noted that most plants decayed 56 days after treatment. The herbicide, 2, 4-D at 6g/L ($T_5$) and 8g/L ($T_6$) treatments showed epinastic symptoms in the earlier stage and cause death of plants. However, at later stages, there was some evidence of regrowth of Water Hyacinth from partially decaying stolons, which may be due to diluted effects of herbicide. However, Mallya *et al*. (2001) observed that integrated management of water hyacinth was more effective than individual methods. Whereas, untreated control ($T_8$) and those plants treated with the *Coleus* sp. plant extract ($T_7$) registered the maximum dry weight of Water Hyacinth at all the stages of observation, followed by the Paraquat at 6mL/L($T_1$) and 8mL/L($T_2$) treated plants at 14 and 21 DAT.

**Table 4.** Effect of herbicides on dry weight of Water Hyacinth

| Treatments | Dry weight (g/plant) | | | | |
|---|---|---|---|---|---|
| | BHT | 3 DAT | 7 DAT | 14 DAT | 21 DAT |
| $T_1$-$Par_6$ | 1.64(41.4) | 1.25(18.0) | 1.34(22.0) | 1.54(34.8) | 1.54(34.6) |
| $T_2$-$Par_8$ | 1.67(44.6) | 1.22(16.5) | 1.32(21.0) | 1.51(32.4) | 1.49(30.9) |
| $T_3$-$Gly_{10}$ | 1.65(42.8) | 1.67(46.4) | 0.95(8.90) | 0.39(2.45) | 0.30(2.00) |
| $T_4$-$Gly_{15}$ | 1.62(40.2) | 1.65(45.2) | 0.87(7.50) | 0.36(2.30) | 0.30(2.00) |
| $T_5$-2,4-$D_6$ | 1.69(46.8) | 1.16(14.5) | 0.54(3.50) | 0.30(2.00) | 0.59(3.90) |
| $T_6$-2,4-$D_8$ | 1.65(42.6) | 1.09(12.3) | 0.48(3.00) | 0.30(2.00) | 0.52(3.30) |
| $T_7$-$Col_{300}$ | 1.64(41.5) | 1.71(51.5) | 1.81(64.4) | 1.85(71.4) | 1.90(79.9) |
| $T_8$-Con | 1.66(43.6) | 1.75(56.4) | 1.85(70.8) | 1.87(73.9) | 1.94(87.5) |
| SEd | 0.01 | 0.04 | 0.04 | 0.04 | 0.04 |
| CD(5%) | NS | 0.08 | 0.08 | 0.08 | 0.08 |

Figures in parenthesis are original values; BHT: before herbicide treatment; DAT: days after treatment

## Summary of results

Mother plants grow very fast from one week after inoculation up to six weeks and then growth rate decreased and involved in seed maturation and senescence and ramet production. Ramets emerged from third week onwards and grow fast up to six weeks because of this continuous growth process the multiplication of water hyacinth resulted in very high rate

The tank culture experiment was conducted to evaluate the effect of different post emergence herbicides on Water Hyacinth plant revealed that spraying of glyphosate at 10 mL/L. + ammonium sulphate 20g/L + Triton 10mL/L as a surfactant effectively controlled

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the Water Hyacinth plants. It can be concluded that spraying of glyphosate at 10 mL/L + ammonium sulphate at 20g/L+ Triton at10mL/L as a surfactant may be recommended for the control of *Eichhornia crassipes* in large stagnated water bodies.

## References

Adekoya,B.B.(1992). Herbicidal control of Water Hyacinth (Fisheries Adaptive Research): Report presented to the World Bank Thematic Mission, OGADEP, Abeokuta.

Akinyemiju,O.A. and Bewaji, F.A.(1990). Chemical control of Water Hyacinth and associated aquatic weeds at Itoikin near lagos. Poceeedings of 8th EWRS Symposium on Aquatic Weeds, 1990. pp.3-8**.**

Ashwini Jadhav, Anthony King, Ryan Brudvig, Martin Hill and Marcus Byrne.(2007). Integrated weed control using a retardant dose of Glypjhosate: A new mangment tool for Water Hyacinth?. *Outlook on Pest Management-October 2007*: 213-216

Eric,G.L.(1993).Effect of Glyphosate on different densities of Water Hyacinth. *J.Aquat.Plant Manage*. 31:255-27.

Gutiérrez López, E., Huerto Delgadillo, R. & Martínez Jiménez, M. (1996). Waterhyacinth problems in Mexico and practiced methods for control. *In* Charudattan, R., Labrada, R. Center, T.D. & Kelly-Begazo., C. eds. *Strategies for Water hyacinth Control Report of a Panel of Experts Meeting*, 11-14 September**,** 1995. Fort Lauderdale, Florida USA. FAO, Rome, pp. 125-135

Mallya, G. , Mjema, P.and Ndunguru, J. (2001). Water Hyacinth Control through Integrated Weed Management Strategies in Tanzania. In M.H.Julien,M.P.Hill, T.D.Center and Ding Jianqing (eds.) *Biological and Integrated Control of Water hyacinth(Eichhornia crassipes)*. *ACIAR Proceedings.* 102. pp: 120-122.

Singh,P.S. and Muller, F. (1979). Efficacy, uptake and distribution different herbicides in the Water Hyacinth. *Weed Sci*.23:235-240

# DEVELOPING HYDROGEL® FOR SELECTIVE MANAGEMENT OF SUBMERGED AQUATIC WEEDS

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## ABSTRACT

A new technique for applying the aquatic herbicide - diquat for the control of submerged aquatics was developed in New Zealand and is now being used widely in both New Zealand and Australia. This method involves the use of guar gum, and formulating a diquat gel form (Hydrogel®), which can then be applied to water as a surface spray. The gel droplets sink rapidly on to submerged plant beds, releasing diquat at in the vicinity of target plants. This allows cost-effective 'spot treatments', targeting both containment of large infestations of an undesirable species, and eradication of small patches.

Several case studies from Australia are discussed, in which diquat, used with Hydrogel®, provided cost-effective control of large infestations of submerged aquatics – hornwort (*Ceratophyllum demersum*), Egeria (*Egeria densa*), sago pondweed (*Potamogeton pectinasus*) and naiads (*Najas tenuifolia* and *Najas marina*) in urban wetlands, large and small lakes and a river. Recent trials in shallow ponds indicate that dense infestations Hydrilla (*Hydrilla verticillata*) infestations could also be reduced by approximately 50-70% with one or two treatments. However, in deeper and larger lakes, hydrilla control has been variable. In addition, Hydrogel® provides excellent control of filamentous green algal scum and submerged beds of Charophytes – *Chara* and *Nitella* at nominal costs.

Results indicate that diquat residues rapidly dissipate from treated waterbodies after Hydrogel®treatments. The advantages of using Hydrogel® include reduced herbicide loads, savings in cost, increased confidence in treatment outcomes, and reduced risks of undesirable impacts on non-target species and aquatic ecosystems.

**Keywords:** Aquatic weeds, Diquat, Hydrogel

## INTRODUCTION

A number of native and introduced submerged aquatic weed species have increasingly colonized Australia's waterways, assisted mainly by human activities. The main species of concern include: *Egeria densa* Planch. (egeria), *Cabomba caroliniana* Gray (cabomba), *Hydrilla verticillata* (L. f.) Royle (hydrilla), *Ceratophyllum demersum* L. (hornwort), several pondweeds (*Potamogeton pectinasus* L.; *P. crispus* L. and other species), *Elodea canadensis* Michx. (elodea) and *Najas tenuifolia* R. Br. and *Najas marina* L. (naiads). Among green algae, *Chara* sp. L. and *Nitella* sp. Agardh. and several filamentous species (e.g. *Cladophora* Kutzing, *Pithophora* Whittrock, and *Spirogyra* Link) also pose problems in many nutrient-enriched waterbodies.

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It is known that submerged aquatic plants mediate ecological processes in aquatic habitats, such as predator-prey interactions involving macroinvertebrates and food webs. They are also important in reducing nutrients from waterways, through luxury consumption. However, their excessive growth and biomass production often lead to adverse effects on aesthetic, recreational and economic values of waterways, and their management in waterways results in significant economic costs (Wells and Clayton, 2005).

Over the past 50 years many techniques have been used for control of submerged aquatic weeds and for local eradications, where this has been necessary. Aquatic herbicides have been at the forefront of these efforts, because they afford the opportunity to achieve the objectives effectively and cheaply in most situations, when compared with mechanical control and other methods. However, the time and method of herbicide application varies with the type of weed and the habitat in which they are to be controlled.

Diquat (Reglone®) has been used for over 40 years in New Zealand and Australia for the control of submerged species. Diquat does little harm to non-nuisance native species, such as charophytes, and native potamogetons and milfoils (Wells and Clayton, 2005). Endothall (Aquathol® and Aquathol Super K®) has recently been registered for use in New Zealand, but significant restrictions remain on its use. Endothall is superior to diquat for controlling some species, such as Hydrilla (Hofstra and Clayton, 2001, Hofstra *et al,* 2001).

Both diquat and endothall have sound environmental profiles, and at concentrations required for control of aquatic weeds, they are relatively safe for humans, fish and other aquatic fauna. They are also not very persistent in the environment. However, when applied correctly, both chemicals have a high degree of phytotoxicity to kill aquatic weeds fast and then rapidly degrade in the water. 'Bi-Active Glyphosate', which also has a high degree of aquatic safety, is widely used for controlling a variety of emergent and floating aquatic weeds, but glyphosate is not commonly used for controlling submerged aquatics.

The mode of delivery of herbicides is very important for the effectiveness of treating submerged aquatic weeds. Various gel adjuvants have been mixed with diquat, such as alginate gum (Torpedo®), guar gum (Aquagel®) and methocel (hydroxypropyl methylcellulose, marketed as Depth Charge®). All are formulated to mix with diquat, and applied at 60 - 80 L $ha^{-1}$. When applied as a steady stream, the mixtures sink and attach onto submerged weeds and the released diquat then desiccates aquatic weeds.

The most widely used gel adjuvant is a guar gum-based product, marketed in Australia as Hydrogel®. Chemically, guar gum is a polysaccharide starch, obtained from the endosperms of seeds of a legume – Cluster Bean (*Cyamopsis tetragonoloba* (L.) Taub). The carbohydrate polymer contains galactose and mannose sugars as the structural building blocks. Food grade guar gum is used in a variety of foods as a thickener and gelling agent. It is also widely used in industry as an emulsifier, stabilizer, bonding agent, hydrocolloid, soil stabilizer and a flocculent. One of the strengths of guar gum is that it can be mixed on site to any desired viscosity. In that sense, guar gum is superior to alginate gum, as it retains a consistent viscosity at any temperature. If viscosity varies with temperature, the delivery equipment requires recalibration throughout the day (Chisholm, *et al*., 2007).

The objectives of this paper are to present several case studies of using Hydrogel® to deliver underwater diquat treatments to submerged aquatic invaders in Australian waterways. These case studies demonstrate the possibilities and constraints of controlling some sensitive species, and also exemplify key factors that affect the level of aquatic weed control that can be achieved.

## METHODS AND RESULTS

Diquat, mixed with the carrier Hydrogel® is applied into water from a knapsack sprayer, gun and hose, boat-mounted boom or helicopter-mounted boom. Dispersal and drift of diquat in water is significantly reduced by the gelling process, and aerial spray drift is reduced to near zero. The relatively heavy nature of the gel carrier prevents diquat from being instantly dispersed, as gel droplets sink in the water column and land on target foliage. This allows the targeting of submerged aquatic species, at specific locations in a waterway, without the need for treating the whole waterbody.

Phytotoxicity responses and control of submerged weeds were evaluated using visual observations from a boat, scooping up samples using a hook, and in other cases, with an underwater camera. Estimates of control in treated areas were expressed as percent control relative to untreated areas, four, 12 or 16-weeks after treatment (WAT).

### Field Case Studies

Given below are case studies of the Diquat-Hydrogel® treatments at various locations in NSW and Queensland, targeting different submerged species. Relevant observations and management experiences are provided, and the overall results are summarised in Table 1.

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**Table 1**. Summary of results of recent Diquat-Hydrogel® Treatments at various locations

| Location | Target Species | Treatment | % Control 4 WAT | % Control 12 WAT | % Control 16 WAT |
|---|---|---|---|---|---|
| Botany Wetlands, Sydney, NSW | *Ceratophyllum demersum* | Single treatment; 600 $m^2$ infested area | 80% | 100% | 100% |
| Del Rio Resort, Wisemans Ferry, NSW | *Ceratophyllum demersum* | Split treatment; 15000 $m^2$ infested area treated with a 4-week interval | 50% | 95-98% | 98% |
| Georges River, Liverpool, NSW | *Egeria densa* | Single treatment; 2500 $m^2$ infested area | 80% | 98% | 98% |
| Forest Lake, Brisbane, QLD | *Egeria densa* | Single treatment; 600 $m^2$ infested area | 90% | 100% | 100% |
| Halcyon Waters, Gold Coast, QLD | *Potamogeton pectinatus* | Single treatment; 600 $m^2$ infested area | 90% | 100% | 100% |
| Cable Ski Penrith, NSW | *Najas tenuifolia*<br>*Filamentous algae* | Split treatment; 4.5 ha<br>• 1st treatment 2.5 ha<br>• 2nd treatment 2.0 ha | 60% | 95% | 95% |
| Aqua Golf Penrith, NSW | *Najas tenuifolia* | Single treatment; 2.5 ha total area | 80% | 95% | 95% |
| Port Ash | *Nitella* sp.<br>*Najas marina* | Multiple treatments; 600 $m^2$ infested area | 40%<br>60% | 40%<br>80% | 40%<br>80% |
| Penrith Lakes, NSW | *Hydrilla verticillata* | Single treatment; 2 x 1000 $m^2$ infested areas | >50% | 40-50% | 40% |
| | | Multiple treatments | >50% | >50% | 40% |
| Hyatt Coolum, Sunshine Coast, Qld | *Hydrilla verticillata* | Multiple treatments; several infested ponds; total of 2 ha treated | >50%<br>>65% | 40-50%<br>>70% | 40-50%<br>>65% |

Hornwort infestations, Botany Wetlands, Sydney, New South Wales

Botany Wetlands (S 33$^0$ 56’ 01.25”; E 151$^0$ 13’ 01.99”) are the largest freshwater lakes in the Sydney Basin. The large wetland/lake system is nationally-listed as important for migratory birds, and is recognised as regionally significant aquatic habitat. The water quality in the lakes has been poor for decades, as a result of urban runoff. Elevated concentrations of nitrogen and phosphorus resulted in recurrent, toxic Cyanobacterial blooms. The wetlands’ aquatic habitat became degraded and weed infested over time, and the lakes were also invaded by the pest fish species – European Carp (*Cyprinus carpio*).

Carp are bottom-feeders, and their feeding behaviour continually disturbs lake bed sediments, leading to poor establishment of submerged aquatic plants in carp-infested lakes. However, after a sustained Carp removal program (Pinto *et al.*, 2007), a dense, almost monotypic, Hornwort (*Ceratophyllum demersum*) infestation covered the largest of the lakes – Pond 5. The density of the infestation caused a decline in all other submerged species, including both native species (e.g. *Hydrilla*) and exotic species (e.g. *Cabomba*). The change in submerged aquatic plant composition was regarded as undesirable.

The hornwort infestation was treated with a single treatment of Diquat-Hydrogel® at 30 L/ha in December 2006. Phytotoxicity was spectacular, and the dense beds collapsed within four WAT to 100% control by 16 WAT (Table 1). Water quality in the lake at the time of treatment was particularly good with low turbidity (less than 5 NTU) and average water clarity of 2.0 m. Since the treatment and control of Hornwort, the lake has developed a mix of submerged species more representative of what existed prior to treatment.

In the Mill Pond, at the downstream sections of Botany Wetlands (S $33^0$ 56' 18.70"; E $151^0$ 11' 42.98"), *Cabomba* infestations were also treated with single treatments of diquat-Hydrogel®. However, these treatments had no effect, leading to the conclusion that *Cabomba* may not be sensitive to diquat. However, being at the downstream reaches of the chain of ponds, water quality in the Mill Pond was particularly poor with very high turbidity (above 30 NTU), which may have at least partially affected the treatments.

<u>Hornwort infestation, Del Rio Resort, Wisemans Ferry, NSW</u>
A dense hornwort infestation in a large pond within the Del Rio Resort (S $33^0$ 24' 10 .92"; E $150^0$ 58' 02.77"), on the banks of the Hawkesbury-Nepean River in NSW, was treated with Diquat-Hydrogel® at 30 L per ha in March 2011. The treatments were conducted as split treatments, which were three weeks apart, due to the size and infestation and almost 100% cover of dense *Ceratophyllum*. The first treatment caused a near complete collapse, and the second treatment ensured that remaining fragments did not regrow. Within 8 WAT, control was 98% and the lake was almost completely clear (Table 1). Hornwort is particularly sensitive to Diquat-Hydrogel® and can therefore be very reliably controlled even with a single treatment with no known adverse effects.

<u>Egeria infestations, Georges River, Liverpool NSW</u>
The Georges River is a major waterway that flows through densely-populated parts of south-west Sydney. As a consequence, it is highly nutrient-enriched, due to urban growth over more than 50 years. The river suffers from serious aquatic weed infestations, including those of alligator weed, *Salvinia*, water hyacinth and submerged *Egeria*.

Once colonisation begins, *Egeria* can rapidly cover large areas of a waterway, leading to reduction in water flows. Observations in the field indicate that *Egeria* invades areas with low light availability, but is found at highest density in areas of high Secchi depth (clearer water). Dense growth of *Egeria* tends to cause laminar flow of fast moving water above the infestation, and such flows lead to undercutting of banks and increased erosion.

Diquat-Hydrogel® applications were conducted at a reach severely infested with *Egeria* (S $33^0$ 56' 11.80"; E $150^0$ 55' 14.90"), in January 2007. The area treated was about 2500 $m^2$. One treatment of 30 L Diquat-Hydrogel® completely eradicated the infestation within about two months (Table 1). Minor regrowth appeared within 12 months, possibly due to establishment of fragments from *Egeria* beds in upstream reaches of the river. At the time of treatment, the flow in the river was minimal, and conditions were calm with minimal turbulence due to wind. Turbidity in the water column was also quite low. Such conditions assist Diquat-Hydrogel® treatments, due to reduced underwater dissipation and reduced inactivation of the herbicide by suspended particles in the water column.

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Egeria infestations, Forest Lake, Brisbane

Forest Lake, in north-west Brisbane (S $27^0$ 37' 48"; E $152^0$ 57.5' 51") is a popular suburb that was developed about 15 years ago. The large recreational lake and cascade system of smaller lakes are key features of the development. Over the years, urban influences negatively impacted on the lakes, resulting in elevated nutrient levels, algal growth and increased aquatic plant growth, including infestations of *Egeria*, *Hydrilla* and *Vallisneria*. The excessive aquatic plant growth made some of the ponds in the system unsightly, reducing aesthetic quality, and aquatic habitat quality.

In 2009, Brisbane City Council commissioned a Trial to determine the effectiveness of Diquat-Hydrogel® to control *Egeria* at the site. The extensive, submerged beds of *Egeria* in a relatively shallow section (about 1.3 m deep) were treated with a single application of Diquat-Hydrogel® 30 L/ha on 7th May 2009. The treatments completely removed the algal scum and eradicated the *Egeria* beds within four weeks (Table 1).

Sago Pondweed infestations, Halcyon Waters, Gold Coast, Queensland

Halcyon Waters, a newly developed residential resort in the Gold Coast (S $27^0$ 52' 52.41"; E $153^0$ 21' 50.79"), south-east of Brisbane in Queensland, has a pond system, which is part of its water management system. Within three years, the ponds became nutrient-enriched, due to runoff from the newly developed precinct. Some ponds developed extensive beds of sago pondweed (*Potamogeton pectinasus*), covered with filamentous algal scum, causing concerns to residents. The ponds were treated with a single application of Diquat-Hydrogel® 30 L/ha in April 2009. The treatments cleared algal growth within a few days, followed by 100% control of pondweed beds within 4 WAT (Table 1). The ponds remained clear of pondweed and algae for the ensuing 12 months.

Najas and algal infestations, Aqua Golf and Cable Ski Lakes, Penrith, NSW

Large lakes of two popular, recreational resorts in Penrith, NSW were infested with *Najas tenuifolia* and filamentous algae. The lakes were treated with Diquat-Hydrogel® during the summer of 2010-11. A 2.5 ha lake at the Aqua Golf resort received a single treatment of Diquat-Hydrogel® 30 L per ha in February 2010. Diquat was quite effective on Najas, and the treated lake was cleared of *Najas* and algae within 4 WAT (Table 1).

The lake at Cable Ski received Diquat-Hydrogel® split treatments, four weeks apart, due to the large size of the water body (4.5 ha). The heaviest infestation areas (approximately 2.5 ha) were treated in November 2010, leaving the centre of the lake untreated. No adverse effects were recorded in the untreated areas, where Najas flourished until the second treatment. The lake has remained largely free of Najas for the past six months. Spot treatments ensured that other native vegetation – sporadic patches of Hydrilla and Vallisneria (*Vallisneria americana* Michx.) were left largely unaffected.

*Nitella* and *Najas marina* infestations, Port Ash Training Facility, NSW

The Port Ash Training facility is a system of lakes, located north of Newcastle (S32 39 05.70 E 151 51 43.05). It has been designed for training for naval and seagoing vessels, using large model boats, and tidal and turbulent, flowing water conditions, representative of major sea ports of Australia. In 2009, the waterbodies became seriously infested with *Nitella* sp. in the shallower sections and *Najas marina* in the deeper sections.

Two applications of Diquat-Hydrogel® 30 L/ha, given during the summer of 2009, achieved excellent control of *Najas marina* (Table 1). However, despite initial desiccation, Nitella infestations declined only partially in some sections and were relatively unaffected in other sections (Table 1). The poor control of Nitella with Diquat-Hydrogel® could be related to high amounts of sediments and particulate matter, which coated the *Nitella* infestations.

Hydrilla infestations, Penrith Lakes

Penrith Lake, north-west of Sydney (S 33$^0$ 43’ 27.16”; E 150$^0$ 40’ 34.60”), is the site at which the Sydney Olympics 2000 were held. The main regatta lakes – Rowing Lake and Warm Up Lake, both have extensive beds of submerged beds of *Hydrilla* (95%). The excessive growth of *Hydrilla* has caused serious impediments for recreational water users. The minimum requirement for international competitions is clear water up to 3.5 m depth.

In Trial 1, in May 2010, selected sections of the lakes were treated with single treatments of Diquat-Hydrogel® mixture at a rate of 30 L/ha. Effectiveness was assessed by visual rating of phytotoxicity. *Hydrilla* beds were only partially affected by these treatments, and control was estimated to be 40-50%, compared with untreated control beds.

Diquat residues were also analysed as a part of the Trial with a detection limit of 0.5 µg/L (0.5 ppb). Water samples were taken at various time intervals after treatment from the water column. Diquat was detected at approximately 75-125 µg/L for up to about 1 h within the treated zones; at 6 h after treatment, the concentration was 12–32 µg/L. No diquat was detected in treated zones beyond 18 h, which indicated dissipation and dilution.

Multiple treatments, with a gap of 4-5 weeks between treatments, were applied in a second series of trials, during the summer of 2010-11. The reduction of *Hydrilla* by these treatments was also variable and ranged 40-50% at most treated locations (Table 1).

In the in relatively deep regatta lakes, the effectiveness of Diquat-Hydrogel® appeared to be influenced by several ‘site-specific factors’. The *Hydrilla* bed density and biomass was very high due to several years of ‘pruning’ by mechanical harvester (e.g. wet weight of several samples ranged from 5.4 kg to 9.8 kg per m$^2$). With elongated stems, comprising long stringy strands and branches, sparsely populated with leaves, the architecture of *Hydrilla* was also somewhat different from those in shallower lakes. Epiphytic, filamentous algal growth also covered the beds. Significant turbulence, due to wind and wave action caused by the boat conducting the treatments, was unavoidable. Some, or all, of these factors may have directly contributed to the reduced performance of diquat on *Hydrilla*.

Hydrilla infestations, Hyatt Regency, Coolum, Sunshine Coast, Queensland

The water levels in several ponds at Hyatt Regency, at Coolum (S 26$^0$ 33’ 32.00”; E 153$^0$ 05’ 33.75”), fluctuate during dry periods. Hence, the ponds are replenished with partially-treated wastewater from the Coolum Sewage Treatment Plant. The waste water enriches the ponds with nutrients, as a result of which, in 2009, extensive, submerged beds of *Hydrilla* and algal scum infested the ponds, reducing aesthetic quality. The ponds were treated with a single application of Diquat-Hydrogel® 30 L/ha in May 2009, which eradicated the algal scum and reduced *Hydrilla* beds by about 50% (Table 1). Additional treatments were given four weeks after the first treatment, reducing *Hydrilla* further.

## DISCUSSION AND CONCLUSIONS

Infestations of invasive submersed plants are increasing in Australia’s rivers, large and small lakes, reservoirs, tidal systems and irrigation canals. These infestations are posing serious threats to the use of waterways by blocking water movement, obstructing intakes, reducing biodiversity, and degrading habitat of threatened and endangered species.

Although the ‘aquatic weed problem’ is perceived as large, the solutions are relatively few. For instance, there are fewer aquatic weed control options in Australia, compared to USA or other developed countries, on account of a small population base, restricted market for herbicides or for mechanical control solutions, and legislation restricting the ability to experiment with new herbicides or bio-control agents.

Aquatic herbicides are invaluable tools, which provide cost-effective means for controlling plants submerged in water. However, presently, there are only three herbicides (e.g. diquat, glyphosate and acrolein) registered for this use, and strategies for using them effectively have not been fully developed. Ignoring this emerging problem will result in the inability to effectively respond and manage new invasive, aquatic plant infestations.

Control of submerged aquatic weeds in waterways presents particular challenges, because many are poor in water quality, which affects control, and some are used as drinking water supplies, and others for irrigation. Varying and often strong water flow patterns, turbulence caused by wind, sediment composition, changing water temperature and turbidity, and other factors can all influence the efficacy of an aquatic herbicide and the actual weed control achieved by a chemical. Treatment timing is also a key factor that influences the success of aquatic weed control with herbicides. The information available on most of the above factors is often dubious, largely due to insufficient research, and this leads to uncertainty in the minds of those who have to manage waterways.

In our view, there is a significant body of evidence from both New Zealand and Australia that the effectiveness of aquatic herbicides can be improved, to suppress extensive areas of critical aquatic weeds infestations quickly at a relatively low cost. Use of smart delivery systems, such as Hydrogel®, allows for this, particularly to accurately deliver the required dosage of diquat over a treatment area, without wasting chemicals. Diquat-Hydrogel® treatments make the control significantly more cost-effective than control by other methods. Additional advantages are that these treatments do not generate unsightly piles of aquatic weeds on shorelines, and applications require a much smaller suitable weather window, because of the speed of application and action, and the result is often long lasting. The differential response in submerged plants is at least partly related to their architecture and other mitigating factors in the field. For instance, the reduced effectiveness of diquat on *Cabomba* and *Hydrilla* could be related to less retention of the herbicide on the fan-like *Cabomba* leaves, or on the sparsely-leaved *Hydrilla* shoots.

The strength of an additive, such as Hydrogel® is that it allows spot treatments to be made targeting specific submerged infestations, without whole-lake poisoning. The spot treatments, applied using a small backpack sprayer or hand-gun can more precisely target invasive plants than do herbicide applications from hoses trailing from an airboat or from aerial applications. The use of appropriate sprayer nozzles and pressure assists the Diquat-Hydrogel® application, reducing spray drift to negligible levels, and thereby improving the selectivity of the applications with a high degree of environmental safety.

We believe that although herbicides are the most cost-effective method of aquatic weed control, there is an understandable general community aversion for using chemicals in water. This aversion can often prevent the use of herbicides over large areas. In this situation, Hydrogel® is extremely useful, because it allows less number of treatments and specific targeting, reducing herbicide loads and offsite drift. The development of new techniques for aquatic weed control needs to continue, despite the relatively small market in this field. The potential environmental impacts and monetary costs of many of the other control methods mean that more attention is needed for aquatic herbicides and smart delivery systems to achieve superior results.

## REFERENCES

Chisolm, B., Harper, P & Chandrasena, N. (2007). Hydrogel for Management of Aquatic Weeds - Possibilities and Constraints. Proceedings 21st Asia-Pacific Weed Science Soc. Conf., Colombo, Sri Lanka. 143-148.

Hofstra D. E. and Clayton J. S. (2001). Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: I. The use of endothall, triclopyr and dichlobenil. *J. Aquatic Plant Management* 39: 20–24.

Hofstra D. E.; Clayton J. S. and Getsinger K. D. (2001). Evaluation of selected herbicides for the control of exotic submerged weeds in New Zealand: II. The effects of turbidity on diquat and endothall efficacy. *J. Aquatic Plant Management* 39: 25–27.

Pinto L., Chandrasena N., Pera J., Hawkins P., Eccles D. and Sim R. (2005). Managing the invasive cyprinids at Botany Wetlands for habitat enhancement. *Journal of Aquatic Conservation: Marine & Freshwater Ecosystems*. 15: 447-462.

Wells R. D. S. and Clayton J. S. (2005). Mechanical and Chemical Control of Aquatic Weeds: Costs and Benefits. In: Encyclopedia of Pest Management DOI: 10.1081/E-EPM-120024643. Copyright # 2005 by Taylor & Francis.

# CAN WE MANAGE ALLIGATOR WEED BETTER IN AUSTRALIA? LESSONS FROM HERBICIDE TRIALS

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**ABSTRACT**

Alligator Weed [*Alternanthera philoxeroides* (Mart.) Griesb] is acknowledged as the aquatic invader that poses the largest threat to Australian waterways and moist, terrestrial habitats. Despite control efforts over several decades, alligator weed is now widespread across NSW, and occurs as sporadic infestations in Queensland, ACT and Victoria, as well. It has the potential to cause losses of millions of dollars from agricultural, tourism and extractive industries across major waterways and catchments in Australia.

Control methods for alligator weed include mechanical control, classical bio-control and herbicides, and combinations of these. Of these, mechanical control may provide immediate control, but also results in fragmentation and increased risks of further spread, and bio-control agents have not been particularly successful on aquatic or terrestrial infestations. Chemical control has been successful, but only short term, and several repeat applications have been required per season to contain or eradicate local infestations.

Recent glasshouse trials evaluated the efficacy of a range of herbicides (glyphosate, metsulfuron, 2, 4-D, triclopyr, a mixture of mecoprop and dicamba, and imazamox) on Alligator Weed, with and without adjuvants. Field trials conducted in Fairfield and Liverpool Local Government Areas in NSW, demonstrated that effective reduction of alligator weed infestations in urban creeks requires multi-year, multiple herbicide treatments. This research provides new insights into alligator weed control using herbicides, and should offer more effective options for managing both aquatic and terrestrial infestations.

**Keywords:** Aquatic weeds, Alligator Weed, Aquatic Herbicides

## INTRODUCTION

Most weed managers agree that alligator weed [*Alternanthera philoxeroides* (Mart.) Griesb] is the No. 1 aquatic invader in Australia, which poses the largest threat to Australian waterways and moist, terrestrial habitats. It is widespread across two major regions in the State of New South Wales (NSW) - the Sydney basin and Hunter region, with deeply entrenched infestations (referred to as 'core' infestation areas). In NSW, it is declared as a Class 2 or Class 3 Noxious Weed. Relatively small, sporadic infestations (referred to as 'non-core' infestation areas) are also present in Queensland, Victoria, and the Australian Capital Territories (ACT).

Over the past two decades, significant research has been conducted in Australia on alligator weed, improving the 'knowledge base' for integrated management (Sainty *et al*., 1998; Schooler *et al*. 2008). Other management experiences (Chandrasena *et al*. 2004;

Chandrasena and Pinto, 2007) also provide significant insights in to factors that affect alligator weed management. Since 2000, a national plan and several state-wide alligator weed management plans have been developed for implementation (CRC, 2003 and NSW DPI, 2007 and references therein).

In 2002, a research program, sponsored by the *CRC for Australian Weed Management* commenced and research continued until 2008. The main strategies of alligator weed management in Australia, advocated by the above plans, have been to prevent its spread into new areas by: (a) maintaining quarantine, (b) managing current infestations, and (c) educating people to recognise and respond quickly to outbreaks. Despite these considerable efforts, alligator weed has continued to invade more territory in NSW and elsewhere, as highlighted in a recent review (Chandrasena, 2009). This view was confirmed by Burgin *et al*. (2010), who stated: "…*we are no closer to control of alligator weed in local government areas of NSW in 2007 than in 2001, despite substantially more monetary resources contributed in 2007 compared with 2001...*"

Whilst the herbicides effective against alligator weed have been known for some time, there is need to make them more effective by understanding how to improve treatment regimes (Chandrasena *et al*. 2004). The objective of the current research was to evaluate a selection of herbicides, rates, adjuvants and treatment regimes that could provide some answers and improve the overall alligator weed management in Australia.

## MATERIALS AND METHODS

### Glasshouse Studies

Glasshouse trials were conducted at the University of Western Sydney, Richmond, over two years. Trial 1 (September 2008 to April 2009) and Trial 2 (September 2009 to April, 2010) both used uniform batches of alligator weed plants, raised from stem cuttings (about 25-30 pieces, 3-4 cm long) planted in nursery trays (0.5 x 0.3 m) in top soil and potting mix. At the time of treatments (i.e. 4-5 months from planting), the cuttings had produced a dense growth of plants in each tray. Four trays constituted a single replicate (laid out as ~1 $m^2$). There were three replicates per treatment, which were completely randomised.

Trial 1

In Trial 1, six herbicides were evaluated: glyphosate (Roundup® Biactive™, 360 g/L), an aquatic glyphosate formulation (Country Glyphosate®, 360 g a.i./L), metsulfuron-methyl (Brush-Off® 600 g a.i./kg), 2,4-D amine (500 g a.i./L), triclopyr (Garlon®, 600 g a.i./L) and a commercial turf weed control mixture of mecoprop (336 g a.i./L) and dicamba (40 g a.i./L). Only glyphosate and metsulfuron-methyl currently have label recommended rates for alligator weed control, which were used in the trials (Table 1). The other broad-leaf herbicides: 2,4-D, triclopyr, mecoprop and dicamba, which do not have a label recommendation for alligator weed control, were included in the study for comparison, as they were likely to significantly affect alligator weed at the right concentrations. These herbicides were tested at label recommended rates to control hard-to-kill weeds. Some treatments had additional adjuvants: either an alkyl ethoxylate surfactant (BS1000®), or Canola Oil (Synetrol®). A total of nine treatments were evaluated (Table 1).

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Trial 2

In Trial 2, four herbicides were tested: Biactive™ glyphosate, metsulfuron-methyl, triclopyr and imazamox (Raptor®, 120 g ai.l/L), with 12 treatments (Table 2). The adjuvant guar gum (Hydrogel®) was incorporated in to spray treatments as a thickener and sticker.

In both glasshouse Trials, herbicide treatments were applied with a 2 L hand-held, pressurised sprayer, using a high carrier volume equivalent to 1000 L/ha. At this volume, the spray treatments wetted all the plants in the trays with some runoff.

Alligator Weed control was visually evaluated at weekly intervals, by following plant death (% death and necrosis) until the end of trial. Control ratings were a percentage scale with 0 = no injury and 100 = complete killing. Alligator Weed regrowth data were recorded 16 weeks after treatment (16 WAT) and expressed as regrowth percentage (i.e. (Number of shoots, which emerged at 60 DAT/Number of shoots, originally planted) x 100).

**Field Studies – Multiple Treatments**

Field Trials were conducted over two summers (2008 and 2009) at Fairfield and Liverpool Local Government Areas (LGAs) in the Sydney basin. Within the Fairfield LGA, field sites were located at downstream Cabramatta Creek, Prospect Creek and Orphan School Creek. Within the Liverpool LGA, the sites were at upstream Cabramatta Creek and Brickmakers Creek. At all locations, the aquatic infestations were on water, extending from shoreline edges, and also spreading on to upper riparian zones. Average size of a treatment replicate was 25-30 $m^2$. Treatments were replicated (minimum three replicates) at different locations in the different creeks.

Herbicide treatments in the field studies were limited to glyphosate (Roundup® Biactive™) and metsulfuron-methyl (Brush-Off®) with either BS1000® or Hydrogel® adjuvants. Herbicides were applied to mature and dense alligator weed infestations, which showed lush growth, with a 15 L back-pack, to achieve maximum foliar coverage. Treatments were applied three times with a gap of four weeks during November and December 2009 and January 2010. Effectiveness on both alligator weed, and on riparian zone vegetation was assessed over the following two to six months, and subsequently, 12 months after treatment, by visual rating of phytotoxicity and photographic recordings.

A one way Analysis of Variance (ANOVA) with repeated measures was performed with each set of visual rating and regrowth control results. Mean comparisons were conducted by a Tukey's Multiple Comparison Test. All analyses were conducted at a $p = 0.05$ level of significance using GraphPad Prism 5.0 Statistical software (GraphPad Prism™ 2010).

## RESULTS

### Glasshouse Trials

The phytotoxicity ratings and regrowth after eight weeks (Table 1) indicated that the aquatic glyphosate formulation, Biactive™ glyphosate and metsulfuron-methyl were highly effective at the label recommended rates in controlling alligator weed. The aquatic glyphosate formulation was particularly effective, as it killed the treated plants almost totally, with very little regrowth occurring at 16 WAT. Based on visual phytotoxicity and percent regrowth results, the effects of adjuvants were not significant on Biactive™

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glyphosate, although, at the time of treatment, the foliar sprays with adjuvants achieved better coverage than the spray without an adjuvant.

Triclopyr achieved the next best initial control, and also suppressed regrowth to a level achieved by the glyphosate and metsulfuron-methyl. Overall, initial control was relatively low with 2, 4-D and the dicamba-mecoprop mixture, although both treatments achieved partial control of alligator weed, evident through leaf and shoot death and necrosis, and stunting of shoot growth. However, inadequate control was reflected in significant regrowth, which was produced by the surviving shoots, by 16 WAT.

**Table 1.** Effect of selected herbicides and adjuvants on Alligator Weed – Trial 1

| Herbicide and Rate of products | Adjuvant (% v/v) | % Control | | % Regrowth |
|---|---|---|---|---|
| | | 4 WAT | 8 WAT | 16 WAT |
| Biactive™ glyphosate (100 mL/10L) | None | 88 a | 94 a | 13.3 c |
| Biactive™ glyphosate (100 mL/10L) | BS1000 (0.05%) | 92 a | 95 a | 6.7 c |
| Biactive™ glyphosate (100 mL/10L) | Synetrol (0.1%) | 92 a | 96 a | 10.0 c |
| glyphosate (Aquatic) (100 mL/10 L) | None | 95 a | 100 a | 1.7 d |
| metsulfuron-methyl (1 g/10 L) | BS1000 (0.05%) | 91 a | 95 a | 6.7 c |
| metsulfuron-methyl (1 g/10 L) | Synetrol (0.1%) | 89 a | 91 a | 10 c |
| 2,4-D amine (50 mL/10 L) | BS1000 (0.05%) | 63 b | 77 b | 60 b |
| triclopyr (17 mL/10 L) | BS1000 (0.05%) | 73 b | 75 b | 16.7 c |
| dicamba-mecoprop (80 mL/10 L) | BS1000 (0.05%) | 68 b | 63 c | 45 b |
| Untreated control | - | 0 d | 0 d | 100 a |

Means in each column, followed by the same letter are not significantly different at 0.05 level of significance, according to Tukey's Multiple Comparison Test

In Trial 2, the effects of incorporating Hydrogel® as a sticker to improve spray retention on alligator weed foliage were not significant (Table 2). As in Trial 1, Biactive™ glyphosate and metsulfuron-methyl at the full rates were highly effective in controlling alligator weed. Even their half rates provided a high degree of regrowth control.

The control achieved by triclopyr and imazamox, at the tested rates, was somewhat less, which was reflected in regrowth at 16 WAT from the treatments (Table 2). However,

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slightly higher rates of triclopyr and imazamox may achieve a more comprehensive initial kill and more effective control of regrowth.

Field Trials

When a single treatment of a given herbicide does not provide a high level of season-long control of a target weed, it is necessary to consider multiple treatments. Climatic conditions often interfere with the timing of herbicide applications, and difficulties in access to infestations also make herbicide treatments less effective.

Figure 1 and 2 present the results of alligator weed control achieved by single field treatments of specific sites in the urban creeks with glyphosate and metsulfuron-methyl. Control, rated by visual phytotoxicity, indicated that the effects of both herbicides were slow to develop, but by 4 WAT, significant effects were visible and infestations were breaking up. However, significant regrowth occurred at nearly all treated locations at 12 WAT, indicating that the treatments were only partially effective.

Overall, the single treatments led to only 50-60% control with glyphosate (Figure 1) and slightly higher control with metsulfuron-methyl (Figure 2). As in the glasshouse trials, the effects of adjuvants were not significant. However, adjuvant incorporated sprays visibly achieved better coverage.

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**Table 2**. Effects of selected herbicides and adjuvants on Alligator Weed – Trial 2

| Herbicide and Rate of Product | Adjuvant (% v/v) | % Control | | % Regrowth |
|---|---|---|---|---|
| | | 4 WAT | 8 WAT | 16 WAT |
| Biactive™ glyphosate half rate (50 mL/10L) | None | 66 cd | 61.7 c | 18 b |
| Biactive™ glyphosate half rate (50 mL/10 L) | Hydrogel (0.05%) | 62.7 cd | 66.7 c | 16 b |
| Biactive™ glyphosate full rate (100 mL/10 L) | None | 90 a | 95 a | 6.7 d |
| Biactive™ glyphosate full rate (100 mL/10 L) | Hydrogel (0.05%) | 90 a | 91.7 a | 6.7 d |
| metsulfuron-methyl half rate (0.5 g/10 L) | None | 56.7 d | 70 c | 18 b |
| metsulfuron-methyl half rate (0.5 g/10 L) | Hydrogel (0.05%) | 60 cde | 75 bc | 16.7 bc |
| metsulfuron-methyl full rate (1 g/10 L) | None | 86.7 a | 95 a | 6.7 d |
| metsulfuron-methyl full rate (1 g/10 L) | Hydrogel (0.05%) | 87.7 a | 95 a | 5.7 d |
| triclopyr half rate (8.5 mL/10 L) | Hydrogel (0.05%) | 56.7 de | 64.3 c | 25 b |
| triclopyr full rate (17 mL/10 L) | Hydrogel (0.05%) | 67.7 bc | 71.7 bc | 20 b |
| imazamox half rate (75 g/10 L) | Hydrogel (0.05%) | 54.3 e | 65 c | 17.3 bc |
| imazamox full rate (150 g/10 L) | Hydrogel (0.05%) | 76.7 b | 83.3 ab | 13.3 cd |
| Untreated control | - | 0 d | 0 d | 100 a |

Means in each column, followed by the same letter are not significantly different at 0.05 level of significance, according to Tukey's Multiple Comparison Test

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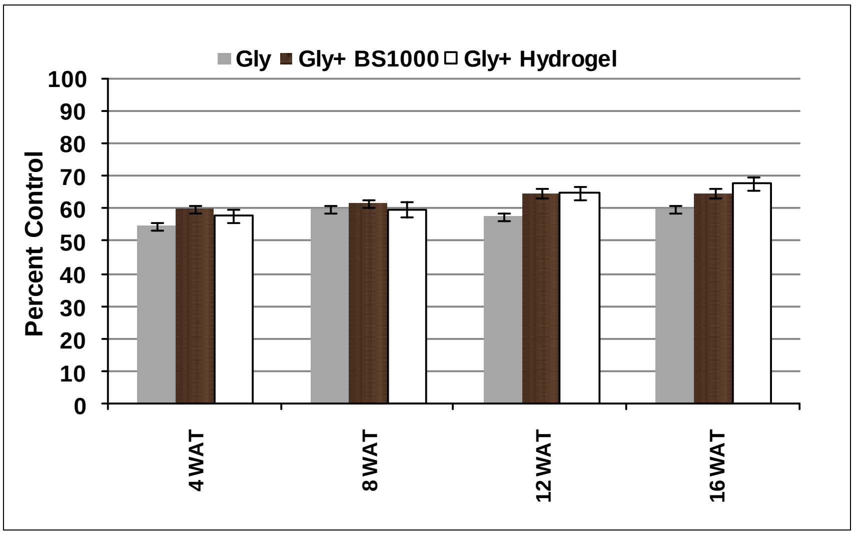


**Figure 1**. Effect of single treatments of Glyphosate and adjuvants on Alligator Weed field infestations

Figure 3 presents the combined results of multiple treatments on alligator weed infestations, conducted at several locations in the urban creeks. These infestations were robust and dense over water at the outset (e.g. approximately, 10-11 kg wet weight of shoot and root systems per $m^2$).

The first treatment caused a spectacular collapse of infestations, and phytotoxicity and percent control obtained at each of the treated sites increased with time. With each subsequent treatment, there was significant suppression and increasing collapse of the initial dense infestations at most sites. The second and third treatments were actually given to collapsed infestations at most sites, which still showed significant numbers of live shoots, which would have regrown. Very little alligator weed was found regrowing at the treated sites one year after treatment, indicating the success of the multiple treatments. At some locations, the multiple treatments led to complete eradication of the original patches.

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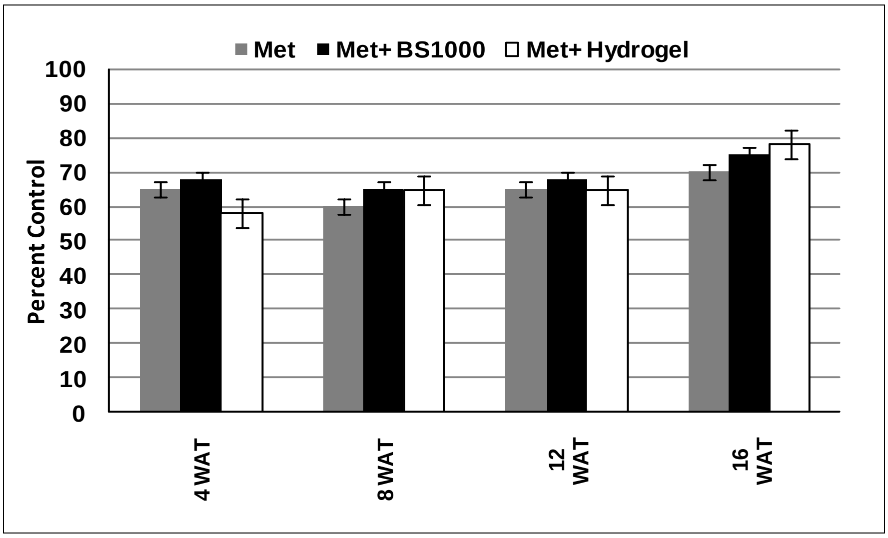


**Figure 2**. Effect of single treatments of Metsulfuron-Methyl and adjuvants on Alligator Weed field infestations

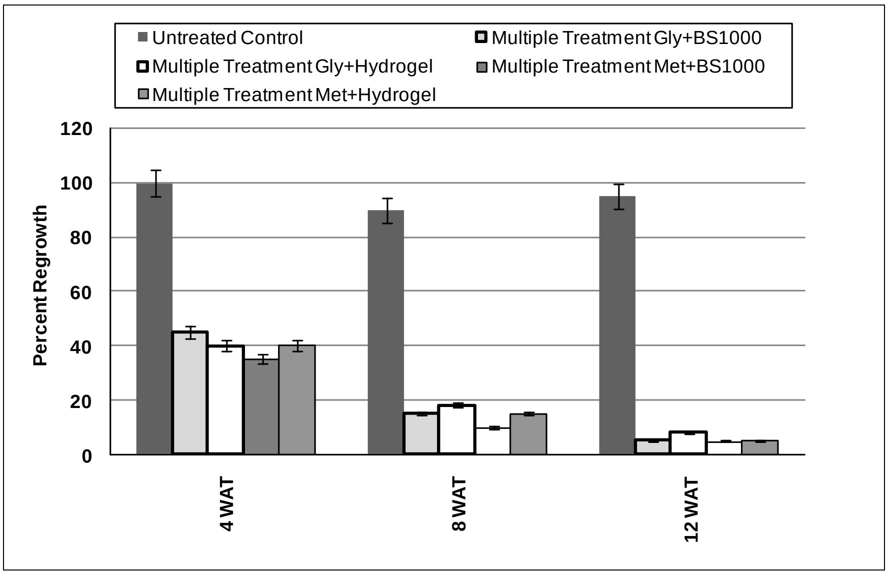


**Figure 3**. Effect of multiple treatments at select locations of urban creeks in NSW

Nearly two years after the multiple treatments, the levels of infestation were very low at the treated locations and in the creeks, in general. However, there was evidence of re-infestation of the creeks by alligator weed, and these were evidently from upstream untreated areas, particularly from riparian areas. There were no noticeable adverse effects on the upper and lower riparian vegetation except for a decline in some bull rushes (*Eleocharis* sp.) at one location.

## DISCUSSION AND CONCLUSIONS

Alligator Weed is regarded in Australia as a remarkably hard-to-control weed, which is often recalcitrant to herbicide treatments. This view has led to a widely expressed sentiment that alligator weed management over large areas is rarely successful or even possible. However, our experiences indicate a more optimistic view. As shown in this research, glyphosate and metsulfuron-methyl are exceptionally effective herbicides against alligator weed. In addition, other selective herbicides are also effective to varying degrees.

The use of metsulfuron-methyl near water or over water is under a special, off-label permit in Australia. However, this herbicide is not registered in USA and other developed countries for applications in water, primarily due to its adsorption and persistence in some soils, and resistance development that has occurred in some weeds. Therefore, increased use of metsulfuron-methyl may not be desirable in the long run. The selectivity of metsulfuron-methyl allows riparian treatments to be conducted without killing all types of reeds and rushes. This is clearly an advantage in many situations. Glyphosate, on the other hand, may kill or reduce most native macrophytes, opening up an infested area, which allows effective targeting of the infestations for re-treatment.

Adequate spray coverage and foliar wetting are important issues when dealing with alligator weed, which can often form multiple layers of thick vegetation, lying over each other. Therefore, treatments must be made at a high enough water volume to carry the herbicides down into all layers of the alligator weed canopy. Often the failures in field control are attributable to access difficulties into infested creeks to achieve good spray coverage. We believe that the biodegradable surfactants, vegetable oils or a natural product like Hydrogel® will overcome this issue. Incorporating an adjuvant is a must to achieve better foliar coverage, and make the alligator weed treatments more reliable.

A contentious issue in managing alligator weed in the field has been the timing of multiple treatments of herbicides. The long-standing practice in LGAs has been to implement three rounds of herbicide applications per year, usually applied early-season (i.e. September-October), mid-season (i.e. February-March) and late-season (April-May). The basis for this practice is actually not scientific, but operational reasons (i.e. limited funding and availability of contractors to adequately cover the treatment areas in a single growing season). However, alligator weed regrowth in between these treatments is typically substantial and often could be such that that season after season, there appears no actual reduction in the infestations. When a treatment does not adequately cover the infested area, alligator weed will re-infest such areas, and the progress is lost.

An integrated strategy for LGAs with large infestations of alligator weed must include several years of sequential herbicide treatments to achieve a successful eradication or a drastic reductionin a given area. In almost all cases, shortening the gap between treatments will reduce the opportunity for treated infestations to ‘escape’ and regrow. With the currently registered herbicides, four to five consecutive years of multiple treatments of glyphosate or metsulfuron-methyl would be typically required, with monitoring of treated areas and follow-up treatments (if required) for up to about 10 years.

However, there will be a need to increase selectivity, if multiple treatments with relatively short gaps are to be implemented. Applying herbicides at the correct time will reduce the rates of herbicide use or the need for multiple treatments in a season. Proper timing may also lessen impacts on non-target vegetation, because the differences in life-cycles between alligator weed and non-target plants. We believe that other selective herbicides, such as triclopyr and imazamox, and possibly others, should be part of the overall effort to manage alligator weed. Other ways of improving treatment regimes include manipulating half-dose treatments of the effective herbicides, as sequential treatments and combinations of herbicides. A judious integration of methods, effective application and possibly, a rotation of herbicides, as best practice, will prevent any possibility of alligator weed developing resistance to herbicides.

We believe that alligator weed managers in Australia should not wait until more effective biological control agents are discovered and developed. The march of alligator weed across many landscapes has been steady and increasing in the last 5-10 years. In some creeks, such as South Creek in Sydney, the situation is dire. Effective treatment regimes with herbicides are essential to contain alligator weed. In our view, more dedicated effort and leadership is needed to garner commitment from all stakeholders and to better access funding, expertise and public involvement - to effectively implement the existing alligator weed control plans.

## REFERENCES

Burgin, S., Norris, A. and D. Karlson (2010). *Alternanthera philoxeroides* in New South Wales, Australia: Are We Closer to Control of Alligator Weed? Weed Technology, 24: 121-126.

Chandrasena, N. (2009). Report prepared for Department of Agriculture, Forestry and Fisheries (DAFF) under 'Defeating the Weed Menace' Funding.

Chandrasena, N. and L. Pinto (2007). Integrated Management of Alligator Weed [*Alternanthera philoxeroides* (Mart.) Griseb] at Botany Wetlands, Sydney- A Case Study. *Proceedings of 21st Asia-Pacific Weed Science Society Conference,* Colombo, Sri Lanka. 59-64.

Chandrasena N., Gunasekera L. and R. Coventry (2004). Improved Strategic Planning for management of Alligator Weed- Myth or Reality? *Proceedings of 14th Australian Weeds Conference*, Wagga-Wagga. 214-217.

CRC (2003). Weed Management Guide - Alligator Weed. CRC Australian Weed Management.

NSW DPI (2007). Alligator Weed Control Manual, Weeds of National Significance, NSW Department of Primary Industries.

Sainty, G., McCorkelle, G. and M. H. Julien (1998). Control and Spread of alligator weed, *Alternanthera philoxeroides*, in Australia: lessons for other regions. *Wetlands Ecology Management*, 5: 195–201.

Schooler, S., Cook, T. Bourne, A. Prichard, G. and M. H. Julien (2008). Selective Herbicides Reduce Alligator Weed (*Alternanthera philoxeroides*) Biomass by Enhancing Competition. *Weed Science*, 56: 259-264.

# UTILIZATION OF AN INVASIVE SPECIES (*SPARTINA ALTERNIFOLIA)* IN THE MOLDED PULP INDUSTRY

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## ABSTRACT

As the world's forest resources become increasingly scarce, there is a serious shortage of fiber raw materials for the pulp industry. As a result, non-wood fibers are becoming more and more widely used as raw materials in this industry. In addition, non-wood fibers may be obtained from increasing supplies of biological wastes, and rapidly spreading invasive plants, such as monocotyledonous grasses.

A good management practice for many invasive plants with huge biomass potential is to maximize use of them, in order to reach a position of balance, with regard to the control of the species. As an example, this paper discusses the utilization potential of *Spartina alternifolia*, a grass species, which has invaded large areas of coastal regions of, Eastern China. Our present studies show that *Spartina*'s fiber composition includes: cellulose 35.9%, hemicelluloses 27.2%, lignin 15.9% and ash 9.98%. The length of *Spartina* fibers is shorter and narrower than bamboo fibers, which. to a certain extent, makes it hard for its stems to be developed into chemical pulp. However, for this wild grass, a thermo-mechanical pulping (TMP) process was found to be easier to develop and mix with chemical pulps to manufacture molded pulp products.

With new innovations in the world-wide packaging industry, molded pulp products (MPP) may be used in larger quantities and in a broader range of fields. Our present study aims to make TMP fibers from *Spartina* composites with other chemical pulps to produce MPP. Utilization of wild grasses, such as *Spartina*, has the following advantages: 1) It provides a new use for biomass of invasive species, which are considered problematic in many situations; 2) The species, with its large potential for biomass production, provides the opportunity to store $CO_2$ and biomass on a large scale; 3) Such biomass provides new industrial raw materials for replacing wood fiber-based chemical pulps and petroleum-derived plastics; 4) The technology allows a reduction of pollution, which could arise from wood fiber-based chemical pulping processes; and 5) The materials derived from these species allows new innovations, including the creation of new products with improved characteristics, such as hardness and strength.

**Key words**: Invasive grass, *Spartina alterniflora*, Utilization of weeds, pulp molding products, mechanical pulp.

## INTRODUCTION

*Spartina alterniflora* and related grass species are native to certain coastal areas and have been introduced to many new coastal countries. Their seeds are dispersed primarily by water and they can also be spread long distances by floation of, pieces of stems. As an invasive species, *Spartina* spp. have adapted well and spread rapidly in intertidal flats of many regions in China (Sally *et al*. 2001). In 2004, *S. alterniflora* infestations in salt marsh areas in Jiangsu Province reached a total of about 150 $km^2$. As herbaceous plants, they can reach heights of 1.5 to 2.1 meters in salty and wet coastal areas. There are 14 to 17 species in the genus *Spartina* (Curtis and Strong 1997). Biogeographical patterns suggest that the genus *Spartina* originates from the Atlantic and Gulf Coasts of North America. Most species are native to America, while only *S. maritime* is native to Europe. *Spartina* species are highly invasive, as demonstrated by their successful introduction in many locations around the world. (Loebl *et al*., 2006).

This extensive invasion and replacement of native wetland vegetation has resulted in the loss of habitat for aquatic species such as salmon and oysters, resulting in economic losses to those who rely on these species. In China, *Spartina* species have been shown to have significant negative impacts on native coastal ecosystems. Many native species, including plants, endangered birds and human food mollusks, are threatened by *Spartina* invasions (Kaparaju and Felby 2010). *Spartina* can out-compete almost all native plants, including *Phragmites australis* and *Scirpus mariqueter,* that originally dominated the coastal wetlands and even invaded fish ponds and young mangrove swamps (Squiers and Cordeiro 2004).

Molded pulp products (MPP) have undergone increasing usage for many kinds of packaging, such as industrial and food packaging. With new technical developments, MPP have become increasingly popular in a variety of manufacturing industries for their advantages in environmental compatibility, biodegradability, low production costs, recyclability, and safe methods of production. The traditional raw material for making pulp is wood fiber; however, with the increasing reduction of available traditional wood fiber sources, increasing attention has been given to non-wood fiber sources. At the present time, conventional MPP are made from diverse materials, including newspapers, waste cardboard, and cartons.

The present study was aimed at developing an industrial system to utilize the large biomass of *Spartina*, an invasive species, in the molded pulp industry, based on its fiber characteristics. An analysis of its fresh fiber characteristics provides a basis for initial utilization, and ultimately, permits the use of its large biomass in the manufacture of larger packaging containers with improved strength properties. It is suggested that industrial utilization of such novel materials is an important management tool for those invasive plants with large biomass. This would allow for a balanced approach to invasive species management, within the "growth-collection-reduction" model for invasive species.

## MATERIALS AND METHODS

### Samples

Samples were harvested in October from the upper intertidal area of a muddy salt marsh located in Dafeng Port on the easterns coast of Jiangsu Province, China. The stems of *S. eastenflora* were firstly cut into 1-2 cm pieces (i.e. Figure 1.). These pieces were then sieved, and samples from the 40–60 mesh fractions were selected in order to determine their chemical composition, and for further use.

**Figure 1.** Stem sample of *Spartina*

**Fiber Chemical Analysis**

Stem samples of *S. alterniflora* were characterized chemically in accordance with the applicable national standard methods (GBs) for different components, including lignin (GB/T10337-1989), hot water solubles (GB/T2677.4-1993), 1% NaOH solubles, ethanol/benzene extractables (GB/T2677.6-1994) and ash (GB/T2677.3-1993). The holocellulose, celullose, hemicellulose, and lignin contents of the material were determined using an Ankom Fiber Analyzer (Ankom Corp. USA), with up to 24 samples treated individually enclosed in filter bags. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) contents were determined at the same time, and the percentages of cellulose, hemicelluloses, and lignin then calculated. Fiber length was determined biometrically, using an optical microscope, after micro-cooking the raw material with 10% soda at 80℃ for 1 h and staining the fibers with 1% safranin, according to Rodríguez et al. (2010).

**Molded Pulp Production**

Fruit trays were selected as molded pulp products, which were made from pulps, which consisted of various mixtures of recycled newspaper pulp, bamboo pulp, corrugated cardboard box pulp and thermo mechanical pulp (TMP) from *Spartina* fibers, respectively. The properties of these molded pulp products are shown in Table 1.

**Table 1.** Properties of fruit trays prepared from different pulp fiber sources.

| | Sample Name | Fiber source | Average quantity（g） | Average thickness (mm) | |
|---|---|---|---|---|---|
| | A | Bamboo pulp | 68.5 | 0.865 | |
| | B | Bamboo & TMP（30%wt） | 76.55 | 0.877 | |
| | C | Bamboo & TMP（40%wt） | 69.36 | 0.753 | |
| **Fruit** | D | Newspaper pulp | 66.5 | 0.85 | **Tray** |
| | E | Newspaper & TMP(40%wt) | 68.5 | 0.803 | |
| | F | Corrugated box pulp | 81.8 | 0.83 | |

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## Manufacturing Process

There were three main steps in the manufacture of molded pulp products. Firstly, the addition of a volume of water in the pulp pool was followed by the addition of a pre-weighed amount of composite TMP/chemical pulp. The raw materials in the pulp pool were then dissolved for 20-30 minutes using a Hydropulper. The pulp consistency in the pulp pool was approximately 2%. Normally, in this preparation step, the chemical agents were added into the pulp pool to impart special properties. Secondly, a metal mold for the fruit trays of the MPP machine was submerged in the pulp slurry, which was prepared in previous process, and then the slurry was pulled into the mold by vacuum to form the shape of the MPP fruit trays. After this step, the MPP was ejected out of the mold. The fruit tray products were then deposited on a conveyer that moves through a drying oven (Cao and Zhang 2006). Lastly, the products were dried and then used for further analysis.

## Testing of Fruit Trays

Tensile strength tests were undertaken on product samples using a horizontal paperboard tensile tester (WZL-B type horizontal paperboard tensile tester, Hangzhou, China), with ISO 1924.2-1994 as the methodology used in this test (Hoffmann 2000; Kibirkštis 2006). Puncture resistance tests were carried out with a board puncture strength tester (Huawei Company, Hangzhou, China) following the ISO 3036 standard method. Board determination of puncture resistance was the methodology for these tests. Air permeability testing was performed with a ZQA-1000 type air permeability monitor (Huawei Company, Hangzhou, China), using methodology with GB458-89 (Gallstedta and Hedenqvistb 2006). The compression study was divided into two different parts: test of compression resistance of different types of fruit trays, and determination of the cushioning properties. For both parts, a compression tester (DYSY-1 Type compression tester) was utilized (Hornsby, et al. 1997).

## RESULTS

Different types of molded pulp products can be made. Two such examples are fruit trays and wheel hub containers, which were both made from *Spartina* fiber raw materials and chemical pulps (Figure 2.), which allows these pulp-based products to achieve high impact strength. These and other pulp products which are green and environmentally safe products made from plant/weed fibers and bamboo pulp as raw material, have potential for wide use in industry and agriculture, The present study is focused on the fruit trays.

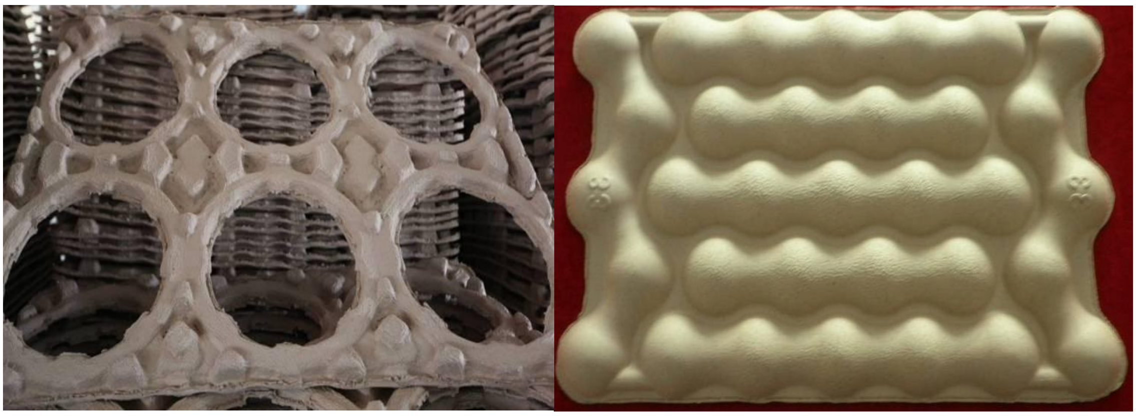

**Figure 2**. Wheel hub container (left) and fruit tray (right) made of mixed pulps.

## Composition of *Spartina* Stem Tissue

The average values of each parameter determined in the chemical characterization of *S. alterniflora* stem tissue are given in Table 2. A comparison of these values with those previously obtained for other agricultural residues, alternative non-wood plants or agricultural residues is also provided in Table 2. The holocellulose content for *S. alterniflora* stem tissue was higher than that of other agricultural residues, except wheat straw, while the cellulose content was lower than the values of the other materials. The hemicellulose content was higher than the values for rice straw, *Phragmites* and Sorghum stalks. The hemicellulose/cellulose ratio of *S. alterniflora* is suitable for the production of pulp, to increase the fiber's plasticity and flexibility. The lignin content was similar to Sorghum stalks, but lower than those of *Phragmites*, rice straw and wheat straw. Therefore, *S. alterniflora* provides an effective raw material for making mechanical pulp, since it contains 70.1% holocellulose, 35.9% cellulose and 15.9% lignin.

**Table** 2. Chemical Characterization of Various Alternative Raw Materials.

| Material | Holocellulose (%) | Celullose (%) | Hemicellulose (%) | Ligin (%) |
|---|---|---|---|---|
| *S. alterniflora* | 70.1 | 35.9 | 34.2 | 15.9 |
| Wheat straw | 76.2 | 39.7 | 36.5 | 17.3 |
| Rice straw | 60.7 | 41.2 | 19.5 | 21.9 |
| *Phragmites* | 64.2 | 39.8 | 24.4 | 23.7 |
| Sorghum stalks | 65.9 | 41.5 | 24.4 | 15.6 |

## Spartina Fiber Properties

Fiber and cell photomicrographs of *S. alterniflora* fibers are shown inFigure 3 for fibers and cells released after mechanical processing. The fiber size distribution plot (Figure 4) indicates that the majority of the fibers are distributed in the 0.50-0.60 mm length range.

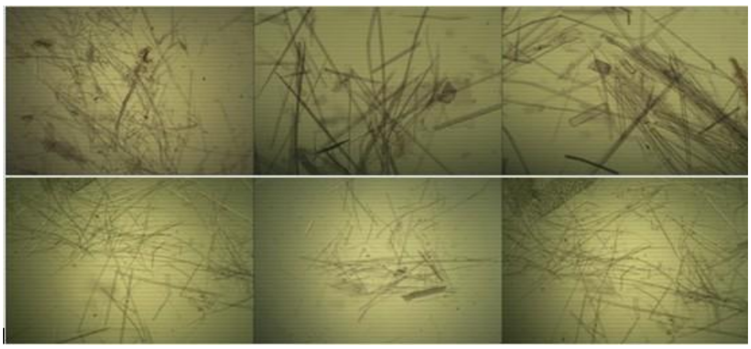

**Figure 3**. Fiber types of *S. alterniflora* by light microscopy.

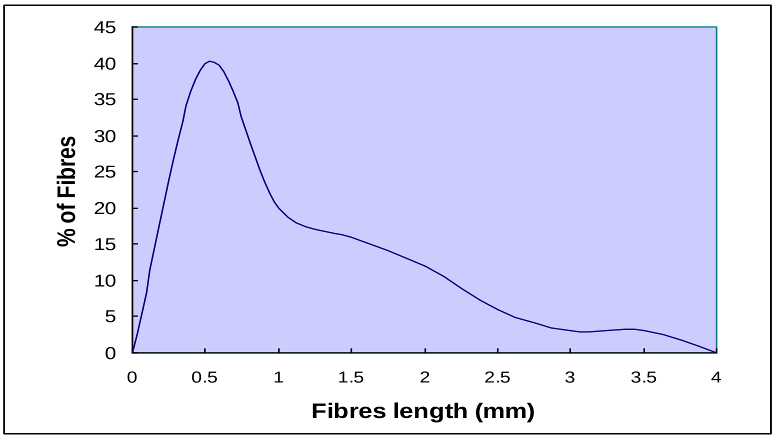


**Figure 4**. Distribution plot of *S. alterniflora* fiber size.

## Mechanical Properties of the Molded Pulp Products

Compression resistance is one of the most important physical properties in the packaging industry. Accordingly, the compression resistance properties of the MPP obtained in this study were examined. The relationship between time and applied pressure was obtained for six types of fruit trays, under 120 mm/min uniform loading pressure, within 1 minute. The results (Figure 5.) indicate that the type B sample (30% TMP/bamboo pulp from Table 1) exhibits the best compression resistance performance amongst the tested samples. Type C, which was made of 40 wt% TMP/bamboo pulp, exhibited the worst compression resistance overall. From these results, it can be concluded that, when using TMP from *Spartina* mixed with bamboo pulp as a composite pulp to produce fruit trays, the *Spartina* fibers performed an important role in these composite fiber materials. When the weight percentage of *Spartina* fiber was less than 30%, the *Spartina* fibers can play and effective supporting role in TMP/bamboo pulp raw materials. However, if the weight ratio of TMP fiber increased to 40%, the compression resistance was less than that of the sample using 100wt% bamboo pulp.

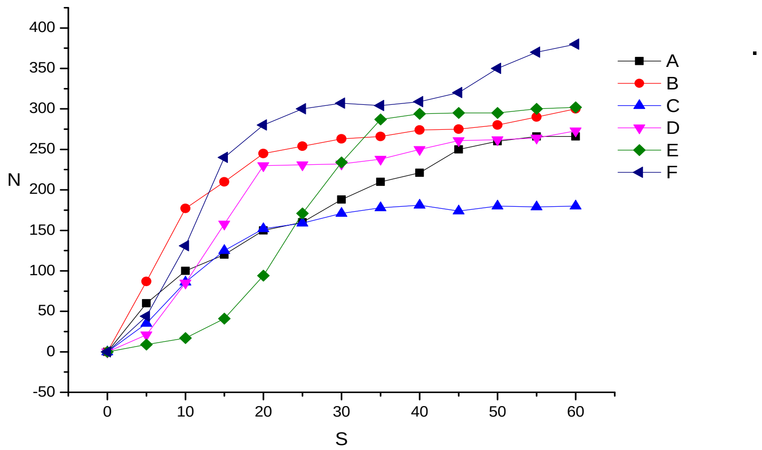


**Figure 5**. The resistance of compression properties of 6 types of fruit trays (Left). (A, B, C, D, E, F as shown in Table 1 above)

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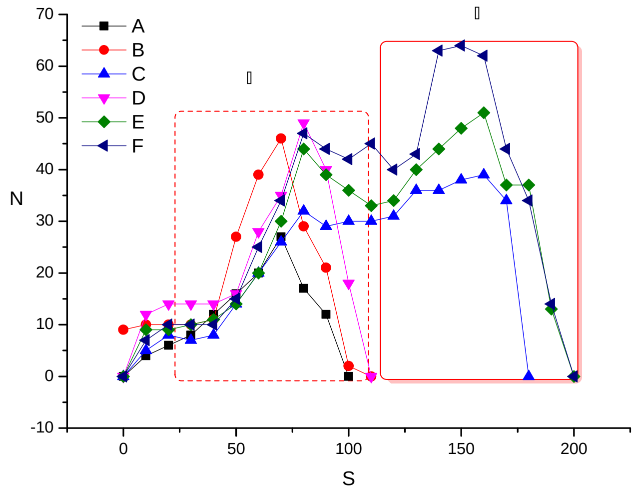


**Figure 6.** The cushioning properties of cushion structure in six types of fruit trays (Right). (A, B, C, D, E, F as shown in Table 1 above)

### Cushioning Properties Of Molded Pulp Products

One of the important characteristics of fruit trays is the resistance to vertical load, or cushioning. For instance, by stacking packed fruit trays for storage, there are cumulative vertical forces acting downwards on the trays. Good cushioning properties of fruit trays mean that they can achieve good loading capacity, thereby effectively reducing potential damage to the contained fruits. In this study, the resulting cushioning properties of fruit trays prepared from different pulp mixtures are shown in Figure 6.

As observed in Figure 6, the pressure loading of fruit trays of type A, B, and D increased with compression times in the range from 0-75seconds. However, for compression times from 75-100 seconds, the cushioning characteristics of these same 3 sample types decreased rapidly. These observations may due to the presence of different deformation processes in the materials, including elastic deformation to plastic deformation transitions. From these observations, it may be concluded that, types C, E, and F have better overall cushioning performance than the types A, B, and D.

## CONCLUSIONS

Based on the chemical and biometric studies of *S. alterniflora* and the corresponding molded pulp products, it is concluded that pulp molding provides an effective means of utilization of invasive species consisting of non-wood fibers. Mechanical pulping of *S. alterniflora,* mixed with chemical pulp, can make useful molded pulp products with good mechanical properties. Based on the yield values obtained, nearly one half of the raw material can be efficiently converted into pulp for molding. This provides a good way to turn *S. alterniflora* into a useful raw material, while also finding a new balance between control and utilization of this invasive plant species. When two kinds of fibers are mixed as composite fiber materials, the TMP fibers can increase the hardness of the composite fiber, and the chemical pulp can increase the extension properties of the composite pulp. In conclusion, the present work represents the first reported utilization of *Spartina* spp. in the molded pulp industry. Using this weed as an industrial raw material to make packaging products provides a basis for further utilization of other straw and weed plants in the future.

## REFERENCES

Cao G and Zhang Y (2006). Progress in the development of moulded pulp products, *Packaging Engineering*, 27:21-23.

Cordeiro, N, Belgacemc, M.N., Torres, C. and. Moura, J.C.V.P (2004). Chemical composition and pulping of banana pseudo-stems. *Industrial Crops and Products* 19: 147–154.

Curtis, C.D. and Strong, D.R. (1997). Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (S-foliosa) in San Francisco Bay. *American Journal of Botany* 84: 607–611.

Gallstedta, M. and Hedenqvistb, M.S. (2006). Packaging-related mechanical and barrier properties of pulp-fibre-chitosan sheets, *Carbohydrate Polymers*, 63: 46-53.

Hoffmann J. (2000). Compression and cushioning characteristics of molded pulp packaging, *Packaging Technology and Science* 13: 211-220.

Hornsby, P.R., Hinrichsen, E., and Tarverdi, K. (1997). Preparation and properties of polypropylene composites reinforced with wheat and flax straw fibres. Part II Analysis of composite microstructure and mechanical properties, *Journal of Material Science* 32: 1009-1015.

Kaparaju, P. and Felby, C. (2010). Hydrothermal pre-treatment of rapeseed straw. *Bioresourource. Technology*.101: 3175–3181.

Kibirkštis, E., Lebedys,A.,. Kabelkaitė, A., and Havenko, S. (2006). Experimental study of paperboard package resistence to compression, *Mechanika*, 63: 27-33.

Loebl, M., Beusekom, J.E.E.V., and Reis, K. (2006). Spread of the neophyte *Spartina* anglicarecently enhanced by increasing temperatures. *Aquatic Microbiology and Ecology*. 40: 315–324.

Rodríguez, A., Sánchez, R., Requejo A., and Ferrer, A. (2010), Soda pulp and fuel gases synthesis from Hesperaloe funifera. *Journal of Cleaner Production*. 18:, 1084–1091.

Sally, D.H,. David, H., and Hellquist C. E. (2001). A Marine Plant (*Spartina Anglica*) Invades Widely Varying Habitats: Potential Mechanisms of Invasion and Control, *Biological Invasions.* 3: 211-217

Squiers, E.R. & Good, R.E. (1974). Seasonal changes in the productivity, caloric content, and chemical composition of a population of salt marsh cord grass (*Spartina alterniflora*). *Chesapeake Science*. 15: 63-71.

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# POTENTIAL HERBICIDE TARGET, 7-KETO-8-AMINOPELARGONIC ACID SYNTHASE

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## ABSTRACT

The validation of potential herbicide target, 7-keto-8-aminopelargonic acid synthase (KAPAS) in the early step of biotin biosynthesis pathway, was performed *in vitro* and *in vivo* with lead chemical triphenyltin acetate (TPTA). KAPAS activity was completely inhibited by TPTA with an $IC_{50}$ of 19.85 $\mu$M. 40-day-old *Arabidopsis thaliana* plants were killed with foliar treatment of 125 g/ha TPTA under the greenhouse conditions. The germination of *A. thaliana* seeds was also completely inhibited with 62.5 $\mu$M TPTA, but it was rescued by 85–92% with the supplement of biotin biosynthesis intermediates such as 0.5 mM of biotin, dethiobiotin, and 7, 8-diaminopelargonic acid, but not by 7-keto-8-aminopelargonic acid (KAPA). However, additional supplement of 0.5 mM S-adenosyl-L-methionine (SAM) with 0.5 mM KAPA rescued up to 91% of the germination previously inhibited by the 50 $\mu$M TPTA. Also, biotin supplements alleviated the growth inhibition of 40-day-old *A. thaliana* plant. Foliar application of TPTA induced 8-fold higher substrate (L-alanine) accumulation in the treated *A. thaliana* plants. RNA expression for KAPAS transcripts were much low in leaf tissue treated with TPTA. With these results, we report that SAM is an essential donor of amino groups for synthesis of the biotin precursor KAPA to 7, 8-diaminopelargonic acid (DAPA) synthesis in plants that KAPAS is a potential herbicidal target site in the biotin biosynthesis pathway, and that TPTA might be one of the potential non-comparative KAPAS inhibitors.

**Keywords**: biotin, KAPAS, SAM, Target, TPTA

## INTRODUCTION

We have described the effects of expressing anti-sense RNA of cloned plant genes encoding for potential herbicide target enzyme 7-keto-8-aminopelargonic acid synthase (EC 2.3.1.47, KAPAS, also known as 8-amino-7-oxononanoate synthase) in stably transformed transgenic test plants (Hwang *et al*. 2003). Individual biotin auxotrophs for KAPA synthase, transformed with anti-sense *Arabidopsis thaliana* KAPAS (*AtKAPAS*) construct, exhibited considerable phenotypic alterations such as growth inhibition, severe growth retardation, yellow–green cotyledons and leaves as well as lethal phenotype (Figure 1). These results suggest that the anti-sense disruption of *AtKAPAS* gene causes lethality in the early stage of plant development. 7-Keto-8-aminopelargonate synthase is a pyridoxal 5'-phosphatedependent enzyme which catalyzes the decarboxylative condensation of L-alanine with pimeloyl-CoA in a stereospecific manner to form KAPA, coenzyme A, and carbon dioxide in the first committed step of biotin biosynthesis. Perhaps the most important role of biotin is in the carboxylation of acetyl-CoA to give malonyl-CoA, which is the first step in fatty-acid biosynthesis. Since fatty-acid synthesis is essential for the growth and development of most organisms, biotin is thus an essential nutrient for plants and animals. Plants, micro-organisms, and some fungi biosynthesize their own biotin, while animals necessarily require trace amounts of the vitamin in their diet. Therefore, inhibition of the enzymes involved in the biotin biosynthesis pathway can cause irreparable damage to plants, and for this reason, such enzymes can be useful targets for the rational design of inhibitors in the hopes of finding new herbicides (Webster *et al*. 2000; Nudelman *et al*. 2004).

The aim of our investigation is to confirm that the genetic validation of KAPAS as a potential herbicide target enzyme, and chemical validation of TPTA as a lead compound for the potential KAPAS inhibiting herbicide derivatives *in vitro* and *in vivo*.

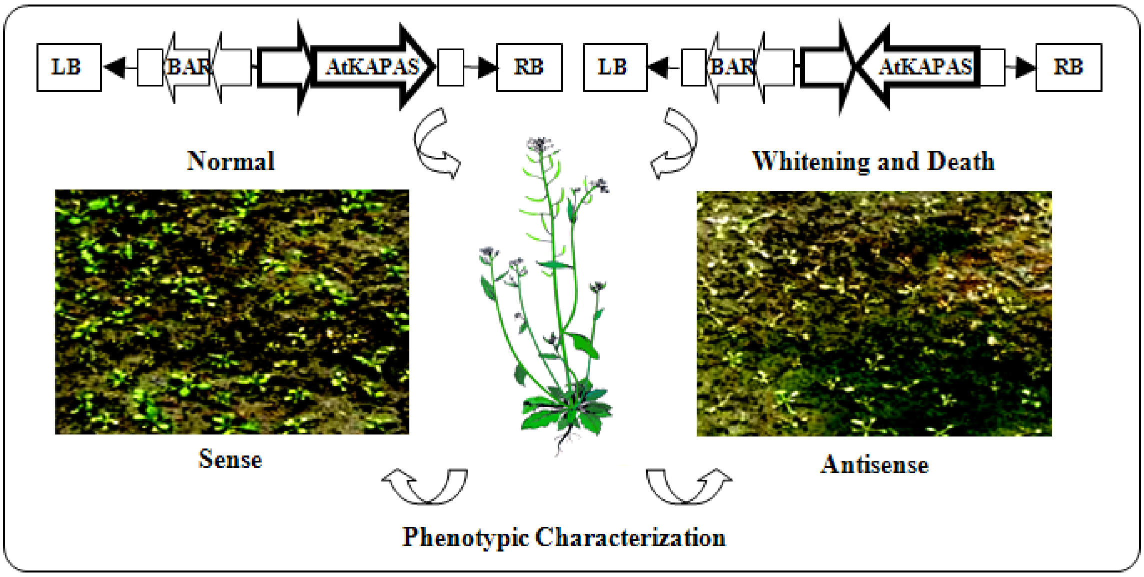


**Figure 1.** Sense and anti-sense expression of target gene in *Arabidopsis*

## MATERIALS AND METHODS

### Cloning and Expression of *AtKAPAS*

Double-stranded cDNA was constructed from 5 µg of poly(A)+mRNA with the Time Saver cDNA synthesis kit (Pharmacia, Piscataway, NJ, USA), using Oligo(dT)18 as a primer. By performing PCR (polymerase chain reaction) with the two primers (KAPAFB, 5'-CAAAAAGAATTCGACGACGACGACAAGATGGCGGAT CATTCGTGGGATAAA-3' and KAPARH, 5'-GTGCACCTCGAGTTATAATTTGGGAAA TAGAAAGGA-3'), the full-length AtKAPAS cDNA was amplified and isolated from *A. thaliana* cDNA library prepared. The resulting PCR fragment was digested with *Eco*RI and *Xho*I, and cloned into MBP (maltose binding protein) fusion vector (Bioprogen Co., Ltd., Korea) to generate construct pEMBPek-KAPAS (Fig. 2). *Escherichia coli* BL21-Gold(DE) (Stratagene, USA) was transformed with expression vector pEMBPek-KAPAS and then cultured in LB (Luria–Bertani broth, USB, USA) medium containing 100 µg/ml of ampicillin at 37°C (150 rpm) until the value of $OD_{600}$ reached 0.6. In order to induce the expression of the target protein in *E. coli* cells, isopropyl-D-thiogalactoside was added to the suspension at a final concentration of 1 mM, and further cultured for 3 h.

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### Substrate Synthesis and Enzyme Assay *in Vitro*

Pimeloyl CoA was synthesized according to the method of Ploux and Marquet (1992). TPTA was purchased from Sigma (USA) and used as a KAPAS-inhibitor. KAPAS activity was determined according to the method of Webster *et al* (2000). The procedure was the same apart from the reaction volume of 250 μl instead of 1 ml. The KAPAS concentration in all analysis was 10 μM in 20 mM potassium phosphate (pH 7.5) and the concentrations of TPTA were 3.125, 6.25, 12.5, 25, 50, and 100 μM. Reference cuvettes contained all other compounds except inhibitor.

### Herbicidal Activity under Greenhouse Condition

Seeds of *A. thaliana* were sown in plastic pots (24 $cm^2$ surface area) filled with artificial nursery soil (Boo-Nong Soil, Seoul, Korea), and the plants were grown to the required growth stage for TPTA application in a greenhouse maintained at 25–30$^oC$ during the day and 20–25$^oC$ at night. The plants were TPTA treated (16, 32, 62.5, 125, 250, and 500 g/ha with laboratory spray gun (spray volume of 1,000 l/ha) 40 days after seeding. The herbicidal spectrum of TPTA was investigated in 10 weed species (*Sorghum bicolor*, *Echinochloa crus-galli*, *Agropyron smithii*, *Digitaria sanguinalis*, *Panicum dichotomiflorum*, *Solanum nigrum*, *Aeschynomene indica*, *Abutilon avicennae*, *Xanthium strumarium*, *Calystegia japonica*) with foliar application of 0.25, 0.5, 1, 2, and 4 kg/ha using a laboratory spray gun two weeks after sowing (in plastic pot (350 $cm^2$ surface area filled with upland soil). Visual injury was determined at 2 weeks after application with a scale of 0 (no injury) to 100 (complete death).

### Rescue of Seed Germination and Plant Growth

Germination test: Seeds of *A. thaliana* were germinated in 55 mm plastic Petri-dish lined with one-layer filter paper (Advantec No. 2). About 1 ml of each TPTA solution dissolved in absolute acetone with various concentrations of 0, 0.0063, 0.0125, and 0.025 mM was spread evenly onto the filter paper (Ø 5 cm), respectively and allowed to dry in a laboratory fume hood. After that, 1 ml of distilled water with or without supplement of 0.5 mM biotin (Sigma, USA), dethiobiotin (Sigma, USA), 7,8-diaminopelargonic acid (DAPA, Synthesis), and KAPA (TRC, Inc., Canada) was added, and 30-seeds were placed onto the filter paper in Petri-dish. Each Petri-dish was sealed with laboratory film and held in an incubator at 25$^oC$, 14/10 h (Light/Dark). Plant growth test: *A. thaliana* of 40-day-old plants as reported above were used. 1 mM biotin was supplemented by foliar laboratory spray gun with spray volume of 5,000 L/ha at each 1 or 2 days prior to 100 g/ha TPTA application. At 5 days after TPTA application, plant leaves were harvested and chlorophyll content was determined following the method reported by Hiscox and Israelstam (1979). Chlorophyll content was calculated following the equation used by Arnon (1949).

### L-Alanine accumulation

Alanine was determined using a detection system of copper complex with L-alanine described by Nakao et al (1986) with some modifications (Weinstein, 1984; Lin and Wu, 2005). Forty-day-old *Arabidopsis* plants grown as above were treated with TPTA (200 g/ha) by foliar application with laboratory spray. Plant leaves were harvested at 3 days after TPTA application. The copper complex of L-alanine was determined by the optical density at 620 nm of the supernatant (200 µl) using a microplate spectrophotometer (Benchmark Plus, Bio-Rad, USA). The concentration of L-alanine was determined by standard curve prepared from the same method with various concentrations of L-alanine. The standard curve was calculated as $Y = 0.4695X + 0.0146$, $r^2 = 0.9993$.

**RNA Isolation and RT-PCR Analysis**

RT-PCR (Reverse transcription-polymerase chain reaction) amplifications were performed with an iCycler™ Thermal Cycler (BIO-RAD, http://www.bio-rad.com/), according to the manufacturer's instructions. RNA was prepared from various tissues of *Arabidopsis* that had been immediately frozen in liquid nitrogen under RNase-free conditions. The RNA was isolated with the Qiagen RNeasy Plant Mini Kit (Qiagen, http://www.giagen.com/) for subsequent reverse transcription reactions. First-strand cDNA was synthesized with 1 µg of total RNA using the Oligo(dT)12–18 primer and the SuperScript™ III Reverse Transcriptase (Invitrogen, http://www.invitrogen.com/), following the manufacturer's instructions. One microliter of cDNA was used for PCR reactions. The PCR conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 26 cycles of 94°C for 2 min, 55°C for 40 s and 72°C for 1 min. KAPAS-specific primers for RT-PCR were: KAPAS-F, 5'-GCTGAACGACAAGGA AATGTTG-3'; KAPAS-R, 50-GAGTGGCTGTGTTGTCAAAG-30. Primers for amplification of reference gene, tubulin was: TUB-F, 5'-CTCAAGAGGTTCTCA GCAGTA-3'; TUB-R, 5'-TCACCTTCTTCATCC GCAGTT-3'.

**RESULTS**

The *AtKAPAS* cDNA was cloned into MBP fusion vector to generate the *E. coli* expression construct pEMBPek-KAPAS. SDS–PAGE analysis revealed that *E. coli* transformed with MBP fusion vector showed the expression of a very strongly induced fusion protein of ca. 98.2 kDa, which consisted of the *AtKAPAS* protein of 51.3 kDa and the maltose binding peptide MBP affinity tag of 46.9 kDa. For purification of *AtKAPAS* protein, the lysates from IPTG-induced *E. coli* containing pCKAPA as well as from *E. coli* harboring control vector MBP fusion vector were loaded onto maltose affinity column (1.1 cm x 30 cm, Millipore, USA). Elutes of *E. coli*-expressed *AtKAPAS* protein contained the induced fusion protein of ca. 98.2 kDa while those of *E. coli* control did not. *AtKAPAS* protein was expressed in *E. coli* at a very high level, and a significant portion of these proteins was soluble, and their affinity-purified preparations contained a single major polypeptide (Figure 2). The dose-dependent *in vitro* inhibition of KAPAS activity by TPTA was examined and the $IC_{50}$ was calculated as 19.85 µM. The foliar-treatment of 16, 32, 62.5, 125, 250, and 500 g/ha TPTA to the 40-day-old *A. thaliana* plants caused visual injury of 8.3, 20, 47, 90, 97, and 100%, respectively, and herbicidal activity increased with time. The application rate of more than 125 g/ha caused almost complete death at 1 week after application. The main symptoms were tissue collapse and desiccation. Symptoms began to appear within several hours after treatment, and the application region in the leaf was desiccated at 1 day after treatment of higher than 250 g/ha. Foliar application of TPTA to 10 weed species showed good herbicidal activities. The most sensitive species was *Xanthium strumarium* which was completely dead at 250 g/ha of TPTA foliar application. *Abutilon avicennae*, *Calystegia japonica*, and *Aeschynomene indica* were also controlled by 500 g/ha of TPTA foliar application (Table 1). Grass weeds such as *Sorghum bicolor*, *Echinochloa crus-galli*,

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*Agropyron smithii*, *Digitaria sanguinalis*, and *Panicum dichotomiflorum* were tolerant to TPTA foliar application compared to the broad-leaf weeds.

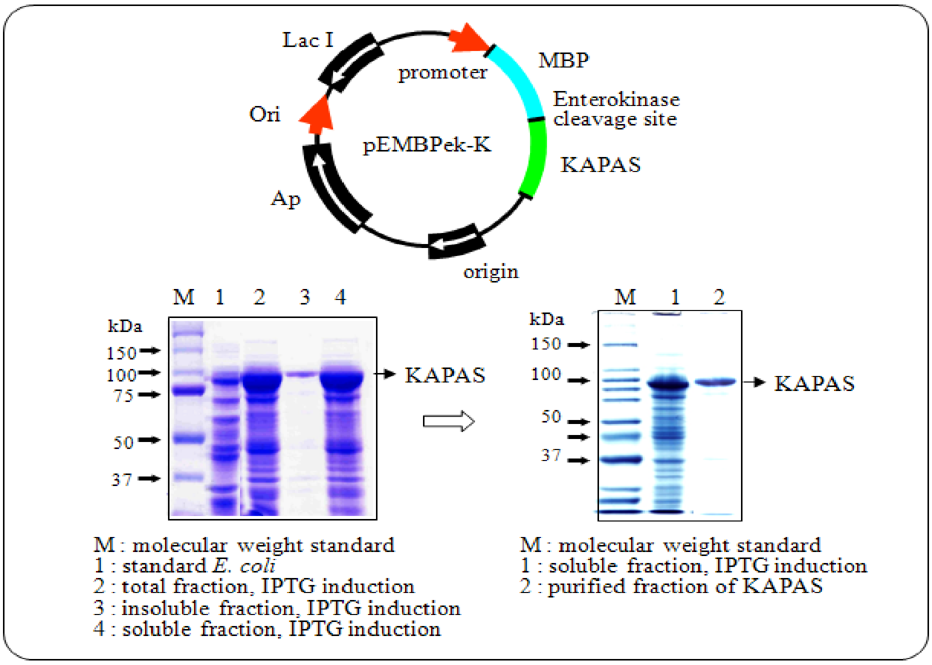


**Figure. 2.** KAPAS over expression and purification from transgenic *E. coli*.

**Table 1.** Herbicidal activity of triphenyltin acetate (TPTA) on several weed species under greenhouse conditions

| Rate (kg/ha) | Control value (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SORBI | ECHCG | AGRSM | DIGSA | PANDI | SOLNI | AESIN | ABUTH | XANSI | CAGEH |
| 0.25 | 30 | 40 | 0 | 30 | 50 | 30 | 70 C | 40 C | 100 | 30 |
| 0.5 | 40 | 40 | 0 | 50 | 60 | 80 | 95 | 100 | 100 | 100 |
| 1 | 40 | 40 | 0 | 50 | 60 | 80 | 95 | 100 | 100 | 100 |
| 2 | 40 C | 60 | 60 | 50 | 80 | 100 | 100 | 100 | 100 | 100 |
| 4 | 70 CI | 70 BC | 50 BC | 60 N | 100 | 100 | 100 | 100 | 100 | 100 |

**SORBI, *Sorghum bicolor*; ECHCG, *Echinochloa crus-galli*; AGRSM, *Agropyron smithii*; DIGSA, *Digitaria sanguinalis*; PANDI, *Panicum dichotomiflorum*; SOLNI, *Solanum nigrum*; AESIN, *Aeschynomene indica*; ABUTH, *Abutilon avicennae*; XANSI, *Xanthium strumarium*; CAGEH, *Calystegia japonica*. Pre, pre-emergence application; Post, post-emergence application. Description of footnotes: B, stunting; C, desiccation; I, chlorosis or abnormal color of plant; N, bleaching (lack of pigmentation). Visual injury was determined at 2 weeks after application with a scale of 0 (no injury) to 100 (complete death).**

The germination of *A. thaliana* seeds was almost completely inhibited by 0.05 mM TPTA. Also, TPTA at > 0.125 mM significantly reduced the plant growth at early stage after seed germination. However, the inhibited germination by 0.05 mM TPTA was recovered by 85–92% with the supplement of 0.5 mM biotin, dethiobiotin, or DAPA, but not by KAPA, one of the biotin biosynthesis intermediates (Figure 3). Additional supplement of 0.5 mM SAM with 0.5 mM KAPA increased up to 91% of the germination inhibited by 0.05 mM TPTA (Figure 4).

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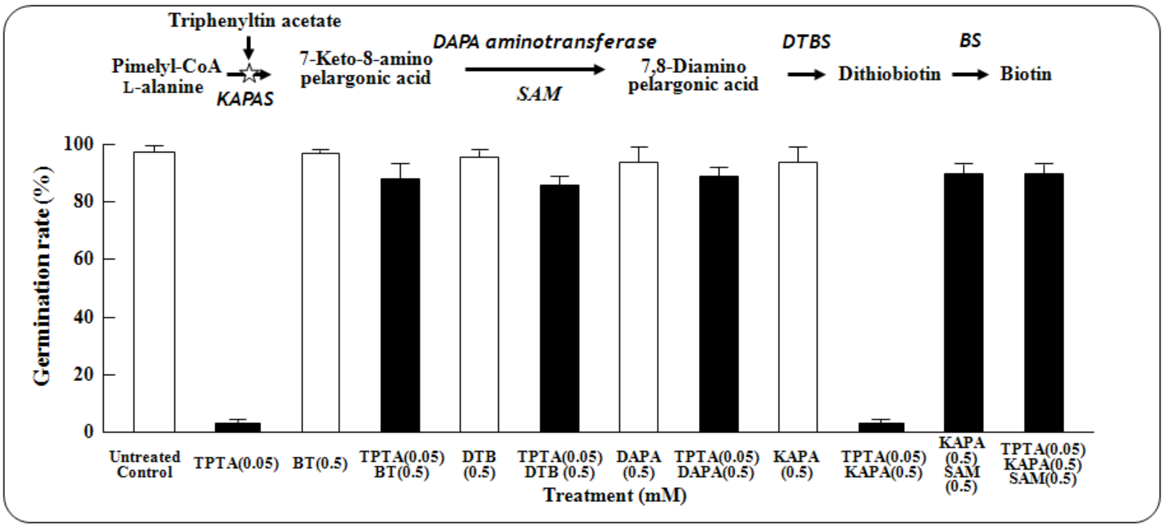


**Figure 3.** Reversal of *A. thaliana* seed germination with biotin biosynthesis intermediates supplement. KAPAS, 7-keto-8-aminopelargonic acid synthase; DAPA, 7,8-diamino pelargonic acid; DTBS, dithiobiotin synthase; BS, biotin synthase; TPTA, Triphenyltin acetate; BT, Biotin; DTB, dithiobiotin; SAM, *S*-adenosyl-L-methionine.

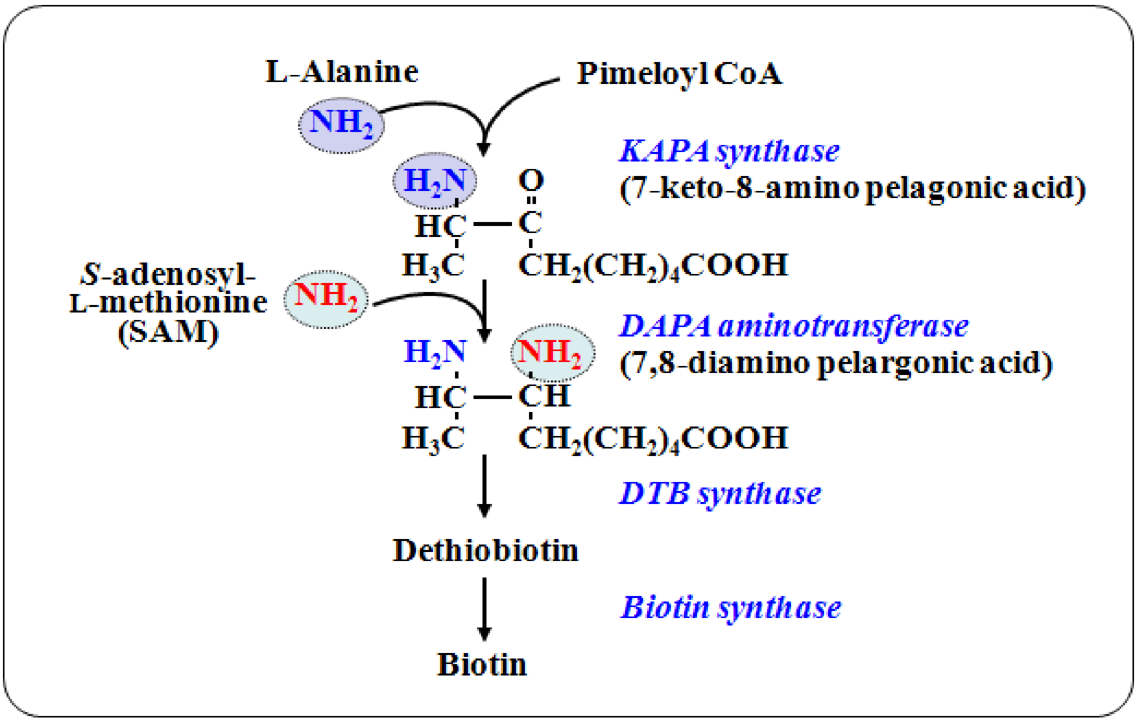


**Figure 4.** Proposed pathway of biotin biosynthesis containing SAM as amino group donor.

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Chlorophyll content in *A. thaliana* plant treated TPTA without biotin pretreatment was 10.7 mg/l, and that in untreated control *A. thaliana* plant was 20.5 mg/l. In contrast, the amount of chlorophyll extracted from the *A. thaliana* plant treated TPTA at 1 and 2 days after biotin pretreatment was 19.5 and 19.8 mg/l, respectively. It is clear that chlorophyll loss of *A. thaliana* plant treated with TPTA was recovered by biotin pretreatment at 1 and 2 days before TPTA application (Figure 5).

Consequently, biotin pretreatment reversed the growth inhibition of *A. thaliana* plant by TPTA at the similar extent to the untreated control plants. 1.28 mM of L-alanine was detected from *A. thaliana* plants treated with 200 g/ha of TPTA, in contrast to 0.16 mM of L-alanine from untreated plants. The TPTA application induced 8-fold greater L-alanine accumulation in the plants (Figure 6).

To expand our understanding on the role of TPTA, KAPAS gene expression in the root, leaf, stem, and whole plant of *A. thaliana* was analyzed by RT-PCR at 1 day after treatment with or without 100 g/ha TPTA (Figure 7). KAPAS was expressed in most tissues, with the highest levels either in stems or roots of the untreated plants. Tubulin was used as a reference for gene expression in *A. thaliana*. However, RNA expression of KAPAS band was much fainter in the lane representing leaf tissue of TPTA (+) plants. Also, less RNA appears to the tublin band than in the other lane. This result implies that TPTA treatment reduces KAPAS expression in the leaf within 1 day of treatment like *bio1* mutants.

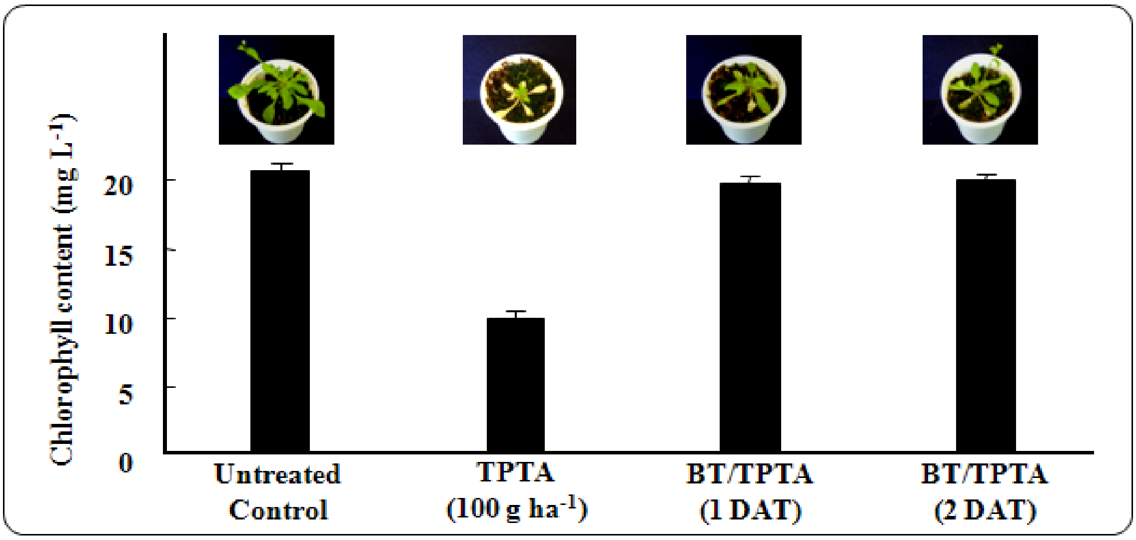
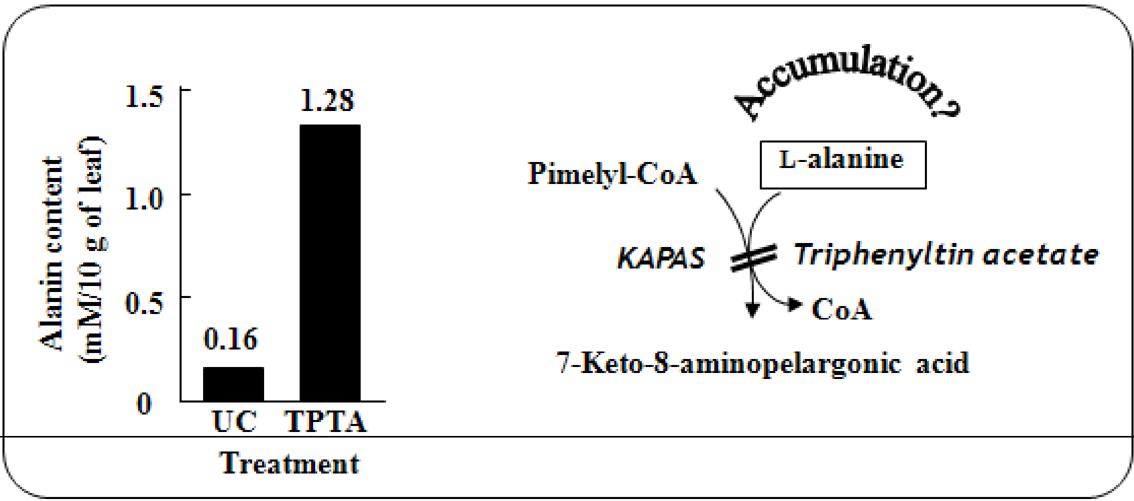



**Figure 5.** Reversal of *A. thaliana* growth inhibition with biotin supplement. TPTA, Triphenyltin acetate; BT, Biotin; BT/TPTA, BT treatment followed by TPTA; DAT, day after



treatment.

**Figure 6.** L-alanin accumulation in *A. thaliana* plants treated with triphenyltin acetate. KAPAS, 7-Keto-8-aminopelargonic acid synthase; UC, untreated control; TPTA, triphenyltin acetate.

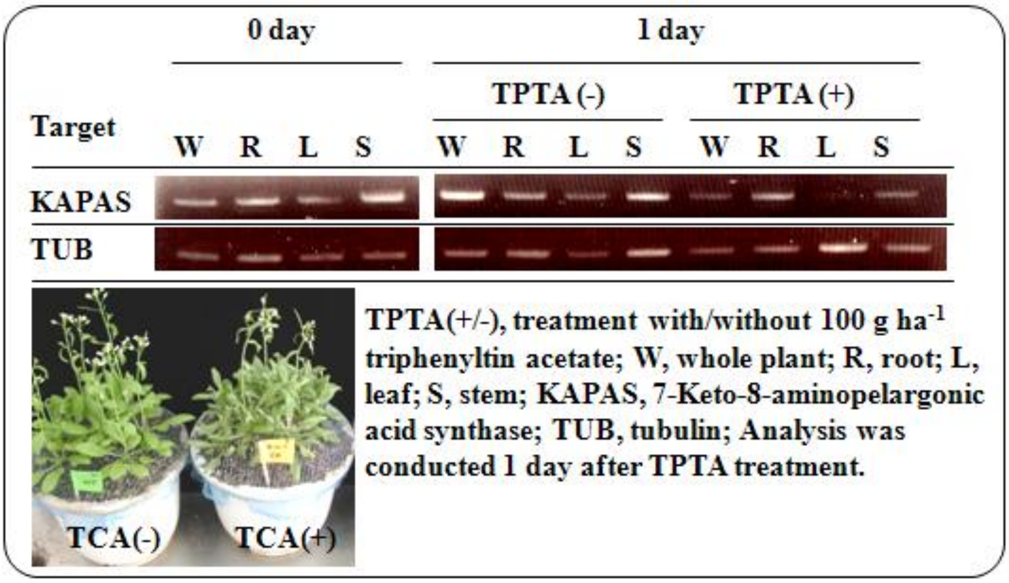



**Figure 7.** Semi-quantitative RT-PCR analysis of KAPAS gene expression in *A. thaliana* plants treated with or without 100 g/ha triphenyltin acetate.

## DISCUSSION

As a number of enzymes in the metabolic pathways of plants are essential for growth and development, those can be utilized as potential herbicide targets. We performed molecular genetics dissection using reverse genetics of anti-sense approach to identify *AtKAPAS* gene encoding KAPA synthase in the pathway of biotin biosynthesis and to characterize the phenotypic consequences of loss-of-function mutations. Many researchers have investigated the KAPAS in micro-organisms and the most of these reports were focused on the biosynthesis in micro-organisms (Eisenberg and Star, 1968), purification and characterization (Ploux and Marquet, 1992; Stoner and Eisenberg, 1975), crystal structure (Alexeev *et al*. 1998; Kack *et al*. 1999), binding and kinetics (Ploux *et al.* 1999), point mutation (Andrew *et al.* 2002) and stereospecificity (Vikrant *et al.* 2006).

Among them, Ploux *et al.* (1999) reported that the KAPAS catalyzes the first committed step of biotin biosynthesis in micro-organisms and plants, and suggested that the inhibitors of this pathway might lead to antifungal or herbicide agents. Webster *et al* (2000) also reported that biotin is an essential enzyme cofactor for carboxylase and transcarboxylase reactions. The biosynthesis of biotin appears to follow similar pathways in both plants and micro-organisms, and thus, inhibition of the enzymes involved in the pathway is potentially an attractive target for both herbicide and antibiotics development.

Herbicidal symptoms after foliar treatment with TPTA were similar to herbicides targeting on the inhibition of fatty-acid biosynthesis in grasses, leading to death of the susceptible plants. In this point of view, the mode of action of TPTA might be correlated with the fatty-

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acid biosynthesis because the most important role of biotin is carboxylation of acetyl-CoA to give malonyl-CoA, which is the first step in fatty-acid biosynthesis. Biotin is an essential vitamin and acts as cofactor for a number of enzymes involved in facilitation of $CO_2$ transfer during carboxylation, decarboxylation, and transcarboxylation reactions that are related to fatty acid and carbohydrate metabolism. These biotin-dependent carboxylases in plants include cytosolic acetyl-CoA carboxylase, chloroplastic geranyl-CoA and acetyl-CoA carboxylases, and mitochondrial methylcrotonoyl-CoA carboxylase. This complex contribution of biotin and biotin-mediated reactions in the plant cell implies an intracellular trafficking of biotin and precursors, thus requiring transport mechanisms. These transport steps include transfer of an intermediate, KAPA, DAPA, or dethiobiotin, between the cytosol and mitochondria was demonstrated by Pinon *et al* (2005).

With these results, we report that SAM is an essential donor of amino groups for synthesis of the biotin precursor KAPA to 7,8-diaminopelargonic acid (DAPA) synthesis in plants, that KAPAS is a potential herbicidal target site in the biotin biosynthesis pathway, and that TPTA might be one of the potential KAPAS inhibitors.

## REFERENCES

Alexeev D., M. Alexeeva, R.L. Baxter, D.J. Campopiano, S.P. Webster, L. Sawyer (1998) The crystal structure of 8-amino-7-oxononanoate synthase: a bacterial PLP dependent, acyl-CoA-condensing enzyme. *J. Mol. Biol.* 284, 401–419.

Andrew C.E., J. Sandmark, G. Schneider, J.F. Kirsch (2002) The dual-specific active site of 7,8-diaminopelargonic acid synthase and the effect of the R391A mutation. *Biochemistry* 41, 12582–12589.

Arnon D.I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Betavulgaris*. *Plant Physiol*. 24, 1–5.

Eisenberg M.A. and C. Star (1968) Synthesis of 7-oxo-8-aminopelargonic acid, a biotin vitamer, in cell-free extracts of Escherichia coli biotin auxotrophs. *J. Bacteriol.* 96, 1291–1297.

Hiscox J.D. and G.F. Israelstam (1979) A method for the extraction of chlorophyll from leaf tissues without maceration. *Can. J. Bot.* 57, 1332–1334.

Hwang I.T., D.H. Lee, J.S. Choi, Y.K. Min, T.J. Kim, J.H. Ko, T.H. Kim, Y.S. Park, K.Y. Cho, S.W. Lee. (2003) Novel polypeptide having function of 7-keto-8-aminopelargonic acid synthase of plant and method for inducing growth inhibition and lethality by suppressing expression of the polypeptide, *Patent No. PCT/KR2003/001301*.

Kack H., J. Sandmark, K. Gibson, G. Schneider, Y. Lindqvist (1999) Crystal structure of diaminopelargonic acid synthase: evolutionary relationships between pyridoxal-5'-phosphate-dependent enzymes. *J. Mol. Biol.* 291, 857–876.

Lin T.-Y. and C.-H. Wu (2005) Activation of hydrogen peroxide in copper (II)/amino acid/$H_2O_2$systems: effects of pH and copper speciation. *J. Catal.* 232, 117–126.

Nakao Y., H. Nakamura, W. Mori, T. Sakurai, S. Suzuki, A. Nakahara (1986) Azide-, thiocyanate- or cyanate-bridged di- and trinuclear copper (II) complexes with N,N,N',N'-tetramethylethylenediamine, N,N-diethylethylenediamine, 2,20-bipyridyl or 1,10-phenanthroline. *Bull. Chem. Soc. Jpn.* 59, 2755–2760.

Nudelman A., D. Marcovici-Mizrahi, A. Nudelman, D. Flintd, V. Wittenbache. (2004) Inhibitors of biotin biosynthesis as potential herbicides. *Tetrahedron* 60, 1731–1748.

Pinon V., S. Ravanel, R. Douce, C. Alban (2005) Biotin synthesis in plants. The first committed step of the pathway is catalyzed by a cytosolic 7-keto-8-aminopelargonic acid synthase. *Plant Physiol.* 139, 1666–1676.

Ploux O. and A. Marquet (1992) The 8-amino-7-oxopelargonate synthase from *Bacillussphaericus*. Purification and preliminary characterization of the cloned enzyme overproduced in *Escherichia coli*. *Biochem. J*. 283, 327–331.

Ploux O., O. Breyne, S. Carillon, A. Marquet (1999) Slow-binding and competitive inhibition of 8-amino-7-oxopelargonate synthase, a pyridoxal-5-phosphatedependent enzyme involved in biotin biosynthesis, by substrate and intermediate analogs kinetic and binding studies. *Eur. J. Biochem.* 259, 63–70.

Stoner G.L. and M.A. Eisenberg (1975) Purification and properties of 7, 8-diamino pelargonic acid aminotransferase, *J. Biol. Chem.* 250. 4029–4036.

Vikrant M.B., D. Sagarika, G.R. Vasanthakumar, P. Kumar, S. Sinha, A. Surolia (2006) Broad substrate stereospecificity of the Mycobacterium tuberculosis 7-Keto-8-aminopelargonic acid synthase spectroscopic and kinetic studies. *J. Biol. Chem.* 281, 25076–25088.

Webster S.P., D. Alexeev, D.J. Campopiano, R.M. Watt, M. Alexeeva, L. Sawyer, R.L. Baxter. (2000) Mechanism of 8-Amino-7-oxononanoate synthase: spectroscopic, kinetic, and crystallographic studies. *Biochemistry* 39, 516–528.

Weinstein S. (1984) Resolution of optical isomers by thin layer chromatography. *Tetrahedron Lett.* 25, 985–986.

# ALLELOPATHIC POTENTIAL OF *PHORMIDIUM ANGUSTISSIMUM*

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## ABSTRACT

In a laboratory bioassay, the allelopathic effects of *Phormidium angustissimum* were investigated on seed germination, shoot length and root length of Chinese amaranth (*Amaranthus tricolor* L.) and barnyard grass (*Echinochloa crus-galli* [L] P. Beauv.).The seeds were germinated in Petri dishes with aqueous extract (1.25 to 10% concentrations) and crude organic extracts (500 to 4000 ppm concentrations) with distilled water used as control. The inhibitory effect on these test plants was directly proportional to the increasing concentration of aqueous extracts of *P. angustissimum*. At the highest concentration studied, the aqueous extract completely inhibited the germination of Chinese amaranth. In barnyard grass, the aqueous extract had a moderate effect on seed germination and seedling growth. To investigate the allelochemicals, *P. angustissimum* was extracted with organic solvents, hexane, ethyl acetate and methanol. The crude ethyl acetate was most inhibitory to Chinese amaranth, but all three crude organic extracts had low inhibitory effect on barnyard grass. Bioactivity guided extraction and chromatographic techniques were used to isolate and purify the allelochemicals from the crude ethyl acetate extract of *P. angustissimum*. The pure compounds were studied by extensive GC-MS and NMR spectroscopic methods in order to determine their structures. The crude ethyl acetate extract of *P. angustissimum* yielded $C_{11}$ norisoprenoid, dihydroactinidiolide and a mixture of long chain fatty acids. Dihydroactinidiolide displayed the highest inhibitory activity against seed germination and seedling growth of Chinese amaranth and barnyard grass.

**Keywords:** *Phormidium angustissimum*, allelopathic effects, aqueous extract, crude organic extract, *Amaranthus tricolor*, *Echinochloa crus-galli*

## INTRODUCTION

Plants are known to produce secondary metabolites that affect germination and the growth of other plants. Allelopathy is commonly defined as any direct or indirect, stimulatory or inhibitory, influence on plants due to chemicals released into the environment by plants (Rice, 1984). The chemical exudates from allelopathic plants play a major role in the allelopathy mode of action. These chemicals, called allelochemicals are natural products or phytogrowth inhibitors that help to regulate the structure of plant communities (Smith & Martin, 1994). Evidence shows that higher plants release various allelochemicals into the environment, which includes phenolics, alkaloids, long-chain fatty acids, terpenoids, and flavonoids (Rice, 1984; Chou, 1995). Allelochemic natural products offer excellent opportunities for new herbicidal solutions or lead compounds for new herbicides (Duke *et al*. 2000; Vyvyan 2002). Allelopathic phenomena are well recognized in the terrestrial plant kingdom, but very little is known about algae. Microalgae such as cyanobacteria (blue-green algae) from marine and freshwater habitats are known to produce a diverse array of toxic or bioactive metabolites (Cohen, 1999; Kreitlow *et al*. 1999; Legrand *et al*. 2003; Charoenying *et al*. 2010). A limited number of studies suggest that some of the compounds may have ecological roles as allelochemicals. *Phormidium angustissimum* is a photosynthetic cyanobacterium with known biological activity. Several studies have shown that cyanobacterias in the genus *Phormidium* produce a wide array of biologically active constituents with different bioactivities (Rodríguez-Meizoso *et al*. 2008). The objectives of this research were to determine the allelopathic potential of *P. angustissimum* and to isolate and identify the allelopathic constituents.

## MATERIALS AND METHODS

### Algal Culture and Test Plants

Dried *P. angustissimum* was obtained from the Division of Animal Production Technology and Fisheries, King Mongkut's Institute of Technology Ladkrabang, Bangkok. Algal cultures were grown photoautotrophically in a BG11 medium (Vonshak and Maske, 1982). Cultures were bubbled with air, at 25$^{o}$C, and the cultivar flasks were illuminated under 400 $\mu$molm$^{-2}$s$^{-1}$ light intensity. Biomass was harvested by filtration on a 40 $\mu$m sieve shaker, dried in an oven at 50$^{o}$C, and stored in an inert atmosphere until extraction. The seeds of Chinese amaranth were purchased from Thai Seed & Agriculture Co., Ltd., Bangkok, and barnyard grass seeds were collected (in August 2009) from paddy fields in the Minburi district of Bangkok, Thailand. Empty and undeveloped seeds were discarded by floating them in tap water. The remaining seeds were air-dried and hermetically stored at room temperature (28-32$^{o}$C). In germination tests, germination activity of these seeds was randomly checked and found to be > 80%.

### Effects of theAqueous Extracts on Seed Germination and Growth

This experiment was conducted in order to determine the water-soluble phytotoxic constituents in *P. angustissimum*. First, 10 g of dried algae were soaked in a 125 mL Erlenmeyer flask containing 90 mL distilled water, and agitated for 24 hours on a shake at room temperature. Next, the extract was strained through two layers of cheese cloth and then through a Whatman No. 1 filter paper. Then, the extract was refrigerated at 4$^{o}$C until use. Four concentrations of the aqueous extract were used in the experiment: 10, 5, 2.5 1.25% w/v. Five mL of each concentration was added to each Petri dishes (9 cm diameter) containing germination paper, and then 20 seeds of test plant were placed on the germination paper as per treatments. The control received only distilled water. The treatments were replicated four times in a completely randomized design. All Petri dishes were covered and placed at room temperature (32$^{o}$C day and 28$^{o}$C night). After seven days, germination percentage, shoot and root length were recorded in all treatments. Inhibition percentage relative to control, was calculated as:

$$\text{Inhibition (\% of control)} = [1-(\text{sample extracts/control})] \times 100$$

### Effects of Crude Organic Extracts

The dried *P. angustissimum* (300 g) was extracted by hexane-treatment for seven days at room temperature. The extract was then filtered through a Whatman No. 1 paper. The collected filtrate was evaporated to dryness under reducing pressure at 40$^{o}$C using a rotary evaporator to yield the crude hexane extract. The residue was then similarly extracted with ethyl acetate (EtOAc) and methanol (MeOH) to yield crude ethyl acetate and methanol extracts, respectively. The three dried samples concentrated from hexane, ethyl acetate and methanol were again dissolved in each solvent to compare their inhibitory effects. Next, 500 $\mu$L of each crude extract solution (5000, 10000, 20000 and 40000 ppm concenrtrations) were added to Petri dishes (9 cm diameter) lined with germination paper, the solutions were allowed to evaporate for three hours at room temperature. After evaporation, 5 mL of distilled water was added on the germination paper to obtain 500, 1000, 2000 and 4000 ppm concentrations. Then, 20 seeds of each test plant were placed on the treated germination paper for seven days. All germination tests were conducted under similar conditions as described above.

**Isolation and Identification of Allelopathic Substances**

Each crude extract obtained was submitted for a test of allelopathic activity with Chinese amaranth and barnyard grass. From this bioassay, the crude ethyl acetate was found to be the most active. Bioassay-guided fractionation of crude ethyl acetate (8 g) was subjected to silica gel column chromatography (Scharlau GE 0048), eluted stepwise with hexane with increasing amounts of ethyl acetate, yielding nine fractions ($F_1$-$F_9$) on the basis of TLC (Thin Layer Chromatography) analysis. These fractions were again evaluated in the allelopathic bioassay. The most interesting active principal was found in the fractions obtained by elution with 5% ethyl acetate in hexane ($F_6$). The semipure active fraction, $F_6$ (220 mg) was further purified on a silica column chromatography (MERCK silica gel 60) using hexane:ethyl acetate (95:5) as the eluting solvent, which resulted in the isolation of dihydroactinidiolide as the major active compound. The structure of this active principal was determined by $^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy and by comparison of the spectral data with the data reported in the literature.

**Analysis of Active Compounds**

Analytical TLC was performed on a MERCK silica gel 60 $F_{254}$ aluminum sheet. Spots were visualized by UV light (254 and 366 nm) or by spraying with anisaldehyde reagent. The plates were then heated for five minutes at 110$^{o}$C. Column chromatography was performed on Scharlau GE 0048 silica gel 60, 0.02-0.06 mm. Flash column chromatography was performed on MERCK silica gel (<0.063 mm). Nuclear magnetic resonance ($^{1}$H, $^{13}$C and distortionless enhancement by polarization transfer) spectra were recorded at 300 and 75.5 MHz, respectively, on a spectrometer (Bruker Avance 300 DPX) in deuterated chloroform using tetramethylsilane as an internal reference. The chemical composition of the fatty acids in crude ethyl acetate was analyzed by a gas chromatography-mass spectrometer (GC:Agilent Technologies Model 6890N, MS:Agilent Technologies Model 7683) using a DB-Wax column. The fractions were converted to fatty acid methyl esters by Yayli's (2001) method: the detector was FID. Each peak was identified by comparing the peak retention times with those of the authentic fatty acid methyl esters samples.

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### Seed Germination and Seedling Growth Bioassay

Seeds of Chinese amaranth and barnyard grass were used to assess the effects of dihydroactinidiolide. A stock solution of dihydroactinidiolide was prepared in ethyl acetate as the initial solvent. Next, 50 μL of solution at 10000, 5000, 2500, 1250 and 625 ppm concentrations was added in a vial (4.5 X 2 cm) lined with germination paper and evaporated to dryness for 3 hours at room temperature. After evaporation, 0.5 mL of distilled water was added to the germination paper to yield the final concentrations at 1000, 500, 250, 125 and 62.5 ppm, and then 10 seeds per species were placed on the germination paper. The vials were sealed with Parafilm to prevent loss of moisture and maintained at room temperature. All germination tests were conducted under similar conditions as described above.

### Statistical Analysis

Differences in the percentages of seed germination and root and shoot length were assessed by analysis of variance statistical methods. Comparisons between treatments were made at a 0.05 probability level using Duncan's Multiple Range Test (DMRT).

## RESULTS

### Effect of the Aqueous Extracts on Seed Germination and Growth

As shown in Table 1, the 1.25% and 2.5% concentrations had no allelopathic effects on seed germination and shoot length of Chinese amaranth. The 5% concentration had a moderate effect on the seed germination but a high inhibitory effect on seedling growth. Moreover, the highest applied concentration (10%) completely inhibited the germination and seedling growth of Chinese amaranth. In barnyard grass, the 1.25% and 2.5% aqueous extract did not influence the germination and shoot length but remarkably stimulated the root length. The 5% concentration significantly reduced the seed germination and seedling growth, while the highest concentration (10%) had a moderate effect on the seed germination and shoot length and completely inhibited the root length. Thus, the potent inhibitory activity of *P. angustissimum* extracts on seed germination and seedling growth of test plants depended on the extract concentration.

**Table 1.** Allelopathic effects of aqueous extract of *P. angustissimum* on seed germination and seedling growth of Chinese amaranth and barnyard grass

| Concentrations (% W/V) | Chinese amaranth (% Inhibition) | | | Barnyard grass (% Inhibition) | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0c | 0c | 0e | 0c | 0c | 0c |
| 1.25 | 0c | -46.92d | 4.02d | 0c | 0.11c | -35.03e |
| 2.5 | 0c | 1.65c | 51.75c | 2.5c | 0c | -20.41d |
| 5 | 77.5b | 90.23b | 95.12b | 12.5b | 18.65b | 62.51b |
| 10 | 100a | 100a | 100a | 35a | 77.28a | 100a |

Mean values in each column followed by the same letter are not significantly different at *P*=0.05 according to the Duncan's multiple range test.

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## Effects of Crude Organic Extracts

The effects of the crude organic extracts of *P. angustissimum* on Chinese amaranth became apparent when applied at high concentrations (Table 2). The crude hexane and ethyl acetate extracts at 4000 ppm completely inhibited the germination and seedling growth of Chinese amaranth, while crude methanol highly inhibited the germination and seedling growth. In barnyard grass, crude hexane and methanol extracts at 4000 ppm concentration slightly inhibited seed germination. All concentrations of crude hexane and methanol stimulated the root length of barnyard grass. The crude ethyl acetate at 2000 to 4000 ppm concentrations reduced seed germination and seedling growth.

**Table 2.** Allelopathic effects of crude organic extracts of *P. angustissimum* on seed germination and seedling growth of Chinese amaranth and barnyard grass

| Concentrations (ppm) | Chinese amaranth (% Inhibition) | | | Barnyard grass (% Inhibition) | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0f | 0ef | 0f | 0d | 0b | 0cd |
| Hexane 500 | 2.56f | -1.70ef | -20.72g | 3.03cd | -1.06bc | -14.26e |
| Hexane 1000 | 0f | 6.23e | 39.62d | 0d | -5.80c | -30.59f |
| Hexane 2000 | 69.23c | 54.67c | 55.51c | 3.03cd | 0.22b | -45.32g |
| Hexane 4000 | 100a | 100a | 100a | 15.15bc | 0.61b | -41.68g |
| EtOAc 500 | 0f | 4.82e | -22.05g | 0d | -0.33b | -1.90d |
| EtOAc 1000 | 12.82e | 31.73d | 19.58e | 0d | 2.45b | 3.49bc |
| EtOAc 2000 | 87.18b | 84.14b | 93.16b | 15.15bc | 12.88a | 5.55b |
| EtOAc 4000 | 100a | 100a | 100a | 30.30a | 16.06a | 53.57a |
| MeOH 500 | 2.56f | -7.93f | -39.92h | 0d | 0.613b | 42.95g |
| MeOH 1000 | 2.56f | 1.70e | -37.64h | 3.03cd | 1.17b | -51.1h |
| MeOH 2000 | 53.85d | 49.01c | 54.18c | 6.06b-d | 0.89b | 51.35h |
| MeOH 4000 | 94.87a | 88.67b | 96.20ab | 18.18ab | 0.06b | -3.65d |

Mean values in each column followed by the same letter are not significantly different at *P*=0.05 according to the Duncan's multiple range test.

## Isolation and Identification of Allelopathic Substances

All crude extracts obtained from solvent extraction were evaluated for their allelopathic potential (Table 2). The most noticeable inhibition was observed in the bioassay of crude ethyl acetate extract, which showed the highest activity on the germination and seedling growth in both test plants. Successive bioassay directed chromatography of the ethyl acetate extract on the silica gel column gave nine main fractions ($F_1$ to $F_9$), among which $F_6$ was found to be the most inhibitory at 1000 ppm (data not shown). Repeated column chromatography and preparative TLC led to the isolation of a plant growth inhibitor identified as a norisoprenoid compound, dihydroactinidiolide (9.6 mg). The structure was assigned using NMR spectroscopic techniques (Figure 1.)**.**

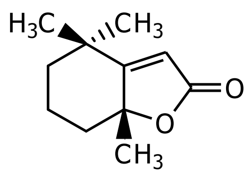


**Figure 1.** Chemical structure of dihydroactinidiolide

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The $^1$H NMR (300 MHz, $CDCl_3$) spectrum of the compound showed $\delta$ 1.22-1.34 (1H, m, H2-$\alpha$), 1.62-1.72 (1H, br.m, H-2$\beta$), 1.72-1.77 (2H, br.m, H-3), 1.46 (1H, m, H-4$\alpha$), 2.24 (1H, dq, $J$ = 12.47, 2.05 Hz, H-4$\beta$), 5.64 (1H, s, H-7) 1.22 (3H, s, H-9), 1.27 (3H, s, H-10) and 1.55 (3H, s, H-11). The $^{13}$C NMR (75.5 MHz, $CDCl_3$) spectrum of the compound showed $\delta$ 36.50, 41.66, 19.65, 40.09, 87.26, 171.99, 112.38, 182.51, 24.18, 29.83 and 24.3. From the comparison of these data with those reported in the literature (Mori and Khlebnikov 1993 ; Yao *et al* 1998 ; Eidman and MacDougall 2006). The GC-MS analysis of active constituents in other fractions (combination of $F_3$ and $F_4$) of crude ethyl acetate extract showed the presence of fatty acids. The results found palmitic acid (46.24%) to be a major constituent, oleic acid, 8-octadecenoic acid, linoleic acid, palmitoleic acid, stearic acid, 7,10-hexadecadienoic acid and 7-hexadecenoic acid, respectively.

## Effect of Dihydroactinidiolide on Seed Germination

The inhibitory activity of dihydroactinidiolide on the germination of Chinese amaranth and barnyard grass is shown in Table 3. The compound caused significant inhibition of seed germination on the two species. Germination of Chinese amaranth was inhibited at a concentration of 250 ppm by 54.55%. In particular, dihydroactinidiolide at concentrations of 500 and 1000 ppm exhibited complete inhibition on seed germination of Chinese amaranth. For barnyard grass, the concentrations of 250 and 500 ppm treatments, dihydroactinidiolide inhibited seed germination by 12.12% and 54.55%, respectively. Similar to the effect on barnyard grass, the 1000 ppm concentration completely inhibited the seed germination. It should be noted that dihydroactinidiolide had a greater effect on Chinese amaranth than on barnyard grass. In general, inhibition increased with greater concentrations of dihydroactinidiolide.

**Table 3.** Allelopathic effects of dihydroactinidiolide from *P. angustissimum* on seed germination and seedling growth of Chinese amaranth and barnyard grass

| Concentrations (ppm) | Chinese amaranth (% Inhibition) | | | Barnyard grass (% Inhibition) | | |
|---|---|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | Seed germination | Shoot length | Root length |
| Control | 0d | 0d | 0d | 0d | 0d | 0d |
| 62.5 | 0d | 3.96a | -0.46a | 0d | 13.02a | 38.20a |
| 125 | 6.06a | 9.35cd | 13.36b | 3.03d | 19.23a | 51.26a |
| 250 | 54.55a | 64.03a | 66.06a | 12.12a | 45.82a | 75.42a |
| 500 | 100a | 100a | 100a | 54.55a | 71.65a | 92.13a |
| 1000 | 100a | 100a | 100a | 100a | 100a | 100a |

Mean values in each column followed by the same letter are not significantly different at $P$=0.05 according to the Duncan's multiple range test.

## Effect of Dihydroactinidiolide on Seedling Growth

Dihydroactinidiolide showed significant shoot growth inhibition on Chinese amaranth (Table ). The 62.5 ppm concentration slightly inhibited the shoot length but stimulated the root length. In concentrations of 125 and 250 ppm, shoot growth was inhibited by 9.35% and 64.03%, respectively and completely inhibited in concentrations of 500 and 1000 ppm. Comparing barnyard grass with control, 62.5, 125, 250 and 500 ppm treatments significant reduced the shoot growth by 0, 13.02%, 19.23%, 45.82% and 71.65%, respectively. In the

1000 ppm concentration dihydroactinidiolide completely inhibited the shoot length of barnyard grass. This data indicate that dihydroactinidiolide had more shoot inhibition on Chinese amaranth than on barnyard grass. In both plants, significant reduction of root length was observed in the concentration of 62.5 ppm dihydroactinidiolide. In the 500 ppm concentration, Chinese amaranth was more sensitive to dihydroactinidiolide than barnyard grass, completely inhibiting on the root growth of Chinese amaranth.

## DISCUSSION

The aqueous extract of *P. angustissimum* showed allelopathic potential in terms of seed germination and seedling growth inhibition. Seed germination, shoot length and root length of test plants decreased proportionally with the increasing concentration of aqueous extracts. The results showed that root growth was slightly more sensitive than shoot growth to the presence of allelochemicals in aqueous extracts. In this study, we attempt to identify the bioactive substances associated with the toxicity observed in the crude ethyl acetate extract of *P. angustissimum*. Bioassay-guided fractionation, purification, and spectroscopic analysis led to the isolation of dihydroactinidiolide as one of the potential allelochemicals. The allelopathic effects of dihydroactinidiolide isolated from spikerush have been previously reported (Stevens and Merrill, 1980). This compound proved toxic at 50 ppm and completely inhibited raddish seed germination after five days. The active constituents in other fractions were identified as fatty acids. It might be possible that the activity observed in our initial experiment with the aqueous extract or crude organic extracts is related to a synergistic combination of dihydroactinidiolide, fatty acids and some other water soluble chemical constituents, which might also occur in substantial quantities that have not yet been identified. It is also possible that dihydroactinidiolide, fatty acids, or other unidentified components, might influence the overall allelopathic potential of *P. angustissimum* in an additive fashion.

## CONCLUSION

The *P. angustissimum* aqueous extract and crude organic extracts had moderate inhibitory activity. The biological activity of extracts increased at higher concentrations. All extracts of *P. angustissimum* were more inhibitory to the root length than shoot length. Using bioassay-guided fractionation, dihydroactinidiolide could be isolated from the ethyl acetate extract of *P. angustissimum* together with fatty acids. Chinese amaranth was more sensitive to the extracts and dihydroactinidiolide than barnyard grass. The results of this study provide evidence that *P. angustissimum* has allelopathic potential.

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## REFERENCES

Charoenying, P., Chotsaeng, P. and Laosinwattana, C. (2010). Effects of *Spirulina platensis* and C-Phycocyanin on Seed Germination and Seedling Growth of Two Monocot and Dicot Plants. *Allelopathy Journal* 25 (2): 453-460.

Chou, C.H. (1995). Allelopathy and sustainable agriculture. In : Inderjit, Dakshini, K.M.M., Einhrlling, F.A. (eds.). *Allelopathy: Organisms, Process and Applications*. ACS Symposium Series 582. American Chemical Society, Washington, DC. pp. 211- 233.

Cohen, Z. (1999). *Chemicals from Microalgae*. Taylor & Francis, London.
Duke, S.O., Romangi, J.G. and Dayan, F.E. (2000). Natural Products as Sources for New Mechanisms of Herbicidal Action. *Crop Protection* 19: 583-589.

Eidman, K.F. and MacDougall, B.S. (2006). Synthesis of Loliolide, Actinidiolide, Dihydroactinidiolide and Aeginetolide *via* Cerium Enolate Chemistry. *Journal of Organic Chemistry*. 71 (5): 9513-9516.

Mori, K. and Khlebnikov, V. (1993). Carotenoids and Degrade Carotenoids, VIII-Synthesis of (+)-Dihydroactinidiolide, (+)-and (-)-Actinidiolide, (+)- and (-)-Loliolide as well as (+)- and (-)-Epiloliolide. *Justus Liebigs Annalen der Chemie*. 1993 (1): 77-82.

Kreitlow, S., Mundt, S. and Lindequist. U. (1999). Cyanobacteria-a Potential Source of New Biologically Active Substances. *Journal of Biotechnology*. 70: 61-63.

Legrand, C., Rengefors, K., Fistarol, G.O. and Graneli, E. (2003). Allelopathy in Phytoplankton Biochemical, Ecological and Evalutionary Aspects. *Phycologia*. 42: 406-419.

Rice, E.L. (1984). *Allelopathy*. 2nd Ed. Acadamic Publishers. New York. USA. 424 pp.

Rodríguez-Meizoso, I., Jamie. L., Santoyo. S., Cifuentes, A., García-Blairsy Reina. G., Señoráns, F.J. and Ibáñez, E. (2008). Pressurized Fluid Extraction of Bioactive Compounds from Phormidium Species. *Journal of Agricultural and Food Chemistry*. 56 (10): 3517-3523.

Smith, A.E. and Martin, L.D. (1994). Allelopathic Characteristic of Three Cool-Season Grass Species in Forage Ecosystem. *Agronomy Journal* 86: 243-246.

Stevens, K.L. and Merrill, G.B. (1980). Growth Inhibitors from Spikerush. *Journal of Agricultural and Food Chemistry*. 28 (3): 644-646.

Vyvyan. J.R. (2002). Allelochemicals as Leads for New Herbicides and Agrochemicals. *Tetrahedron* 58: 1631-1636.

Vonshak, A. and Maske, H., (1982). Algae: Growth Techniques and Biomass Production. In: Coombs, J., Hall, D.O. (Eds.), *Techniques in Bioproductivity and Photosynthesis*. Pergamon Press, Oxford, pp. 66-77.

Yao, S., Johannsen. M, Hazell, R.G. and Jørgensen, K.A. (1998). Total Synthesis of (*R*)-Dihydroactinidiolide and (*R*)- Actinidiolide Using Asymmertic Catalytic Hetero-Diels-Alder Methodology. *Journal of Organic Chemistry*. 63 (1): 118-121.

Yayli, N., Kiran Z., Seymen, H. and Genc, H. (2001). Characterization of Lipids and Fatty Acids Methyl Ester Contents in Leaves and Roots of *Crocus vallicola*. *Turkish Journal of Chemistry*. 25: 391-395.

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# THE DISTRIBUTION AND SOCIO-ECONOMIC IMPACTS OF *MIKANIA MICRANTHA* (ASTERACEAE) IN PAPUA NEW GUINEA AND FIJI AND PROSPECTS FOR ITS BIOCONTROL

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## ABSTRACT

*Mikania micrantha* or mile-a-minute is a fast growing Neotropical vine found throughout much of Asia and the Pacific, invading small subsistence farms as well as plantations. In 2006, a biocontrol project, funded by the Australian Government and managed by the Queensland Government, commenced in Fiji and Papua New Guinea (PNG). To help plan activities and determine possible benefits from the project, the distribution, growth rate and socio-economic impacts of *M. micrantha* were determined before the importation of biocontrol agents. *Mikania micrantha* was recorded in all 15 lowland provinces in PNG and on all major islands in Fiji. Plants grew up to 1 m/month in PNG and about 0.5 m/month in Fiji. A socio-economic survey (of over 380 respondents in over 230 villages from 15 provinces in PNG) found that 79% of respondents considered *M. micrantha* to be a serious weed, with over 40% considering *M. micrantha* reduced their crop yield by more than 30%. About 44% of the respondents had over a third of their land infested with *M. micrantha*, which they spent 1-2 days per fortnight weeding. About 85% of respondents controlled *M. micrantha* by physical means, such as slashing and/or hand-pulling. In Fiji, *M. micrantha* infestations were less problematic than in PNG. There were 52 respondents from four islands, of which over 60% considered *M. micrantha* a serious weed, losing about 30% of potential crop yield due to the weed and 33% reported having more than 30% of their farm lands infested. Only 15% of respondents needed to weed fortnightly, with 56% using slashing and/or hand-pulling as the main means of control. Nearly 90% of respondents used *M. micrantha* as a medicinal plant to treat cuts and wounds. To help control the weed, the rust *Puccinia spegazzinii* was imported into both countries, following host specificity testing by CABI in the UK, and subsequently released widely. Initial laboratory trials and monitoring at a few sites, found that the rust can significantly reduce the growth of *M. micrantha* and offers great potential for the control of this weed in Fiji and PNG and other countries where *M. micrantha* is a problem.

**Keywords**: distribution, socio-economic impact, biocontrol

## INTRODUCTION

*Mikania micrantha* (Asteraceae) or mile-a-minute is an invasive plant originating from Central to South America and the Caribbean. The weed is widespread throughout Southeast Asia and the Pacific Islands (Waterhouse and Norris 1987), including Fiji and Papua New Guinea (PNG). At a regional workshop on invasive alien species, *M. micrantha* was ranked as one of the most important weeds of the Pacific (Dovey *et al.* 2004).

*Mikania micrantha* flowers prolifically and produces thousands of light-weight barbed seeds that are spread by wind or by people on clothing or possessions. The weed can also propagate vegetatively by producing roots and shoots from broken stems or leaves. *Mikania micrantha* grows rapidly and is one of the major weed invaders of subsistence gardens and trees grown in plantations in PNG and Fiji (Waterhouse and Norris 1987; Holm *et al*. 1991). It forms a thick ground cover, out-competing, smothering and causing the death of plants of many species, including food and cash crops such as sweet potato, taro, papaw, bananas and cassava. *Mikania micrantha* can also reduce flowering and yield of cocoa and interfere with the harvesting of coconut, oil palm and cocoa (Waterhouse and Norris 1987). Apart from a study by Teoh *et al*. (1985) in Malaysia and Muraleedharan and Anitha (2001) in India, there have been few studies quantifying the impacts of *M. micrantha* on agriculture and none have been undertaken in PNG and Fiji.

*Mikania micrantha* can be controlled by herbicides or manually through slashing and/or hand-weeding. However, these conventional methods of control are not practical as they are costly, time consuming and labour intensive. Biological control is seen as the only sustainable and cost-effective means to control this weed (Waterhouse and Norris 1987; Cock *et al*. 2000). A biological control program was first initiated against this weed in 1988, when the thrips *Liothrips mikaniae* was introduced into Solomon Islands and then Malaysia but it failed to establish in either country. The thrips was also introduced into PNG but died in quarantine before field releases could be conducted (Cock *et al*. 2000).

As part of a renewed effort against *M. micrantha*, an Australian Government-funded biocontrol program, aiming to reduce the impact of *M. micrantha* in PNG and Fiji, began in 2006 and involved the introduction of the rust fungus *Puccinia spegazzinii* (Pucciniales: Pucciniaceae) (Orapa *et al*. 2008). This paper reports on the distribution and growth of *M. micrantha* in PNG and Fiji and the physical and socio-economic impacts of the weed. Information gained in these studies will be used in the biocontrol agent release program and also to assess the benefits of the project.

## MATERIALS AND METHODS

### Distribution of *M. micrantha*

The distribution of *M. micrantha* in both PNG and Fiji was ascertained through field surveys undertaken by project staff travelling throughout both countries or from feedback from regional staff reporting on locations of the weed in their jurisdictions. Locations of the weed were recorded using a hand-held GPS unit and mapped using Arcview GIS. *Mikania micrantha* infestations closer than 5 km were deemed as a single infestation. Where locations could not be recorded using a GPS unit, the nearest village was used as the site descriptor.

## The Growth and Physical Impact of *M. micrantha*

The growth rate of *M. micrantha* was obtained by tagging 10 shoots at each of eight sites in PNG and four sites in Fiji and measuring the length from the node immediately above the tag to the tip of the shoot. As handling the plants will often damage the growing tips, recordings of the length of the shoot from the tag to the tip were only taken after two and four weeks.

The physical impact of *M. micrantha* on crops and plantation species was measured through visual observation and photographs. Descriptions of the weed infestations and crop health were also recorded.

## Socio-Economic Impact of *M. micrantha*

A questionnaire was developed to determine the social and economic impact of *M. micrantha* on crop production, including cost and time spent controlling the weed and farmer income, as well as the control methods used across different land uses. Project staff conducted surveys in their own provinces and in provinces to which they travelled. The questionnaire was also sent to other lowland provinces where *M. micrantha* was reported for completion by provincial staff. Results were tabulated and the proportion of each class for various questions graphed.

## Effectiveness of the Rust *P. spegazzinii*

Twenty three-week old cuttings infected with rust and 20 three-week old plants not infected with rust and used as a control, were placed in a quarantine glasshouse and monitored weekly. Their height and number of nodes present were recorded. The number of pustules present on the leaves, stems and petioles were also recorded. The experiment was terminated after five weeks due to the rapid growth of the plants.

At a field site near the research station at Kerevat, East New Britain Province, PNG, the percent cover of *M. micrantha* was estimated monthly and the number of pustules present on the leaves, stems and petioles recorded.

# RESULTS

## Distribution of *M. micrantha*

*Mikania micrantha* was found in all 15 lowland provinces in PNG, from sea level to 1200 m asl. It was particularly prevalent in the wetter provinces of East and West New Britain and New Ireland. Infestations were less prevalent in the drier areas, particularly in Central, Gulf, Madang and Morobe provinces (Figure 1).

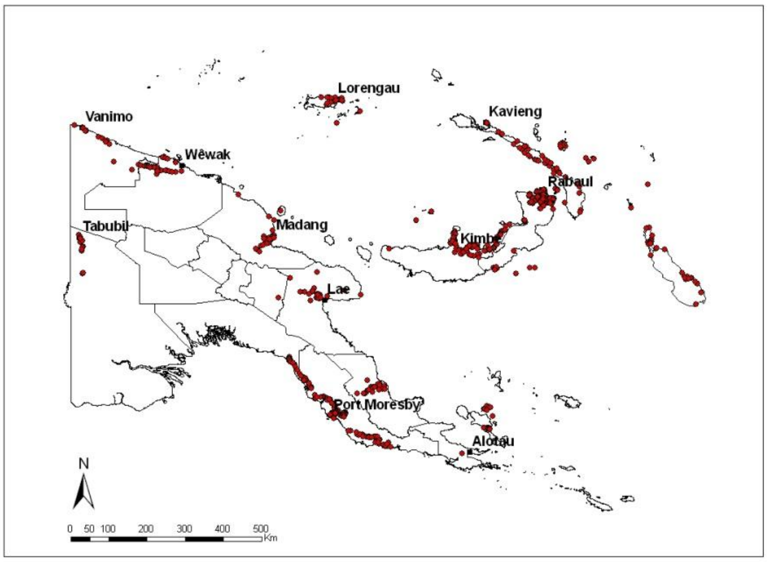


**Figure 1**. The distribution of *M. micrantha* in Papua New Guinea.

In Fiji, *M. micrantha* was found on all major islands, with the largest infestations being on Kadavu, Ovalau, Taveuni, Vanua Levu and Viti Levu (Figure 2). Infestations were less prevalent in the drier areas, particularly in the western parts of Viti Levu.

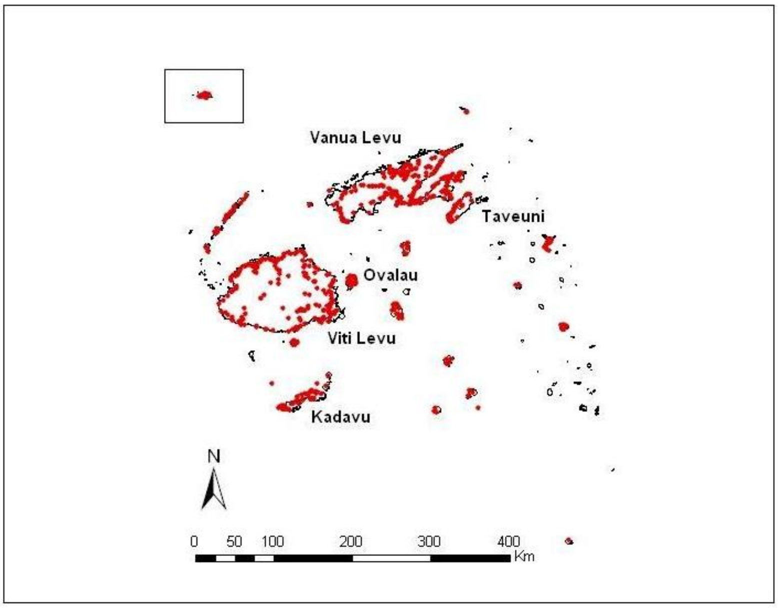


**Figure 2**. The distribution of *M. micrantha* in Fiji.

## The Growth and Physical Impact of *M. micrantha*

*Mikania micrantha* was found growing in dense infestations along roadsides and creeks where adequate moisture was available and in smallholdings and plantations in both PNG and Fiji. In PNG, *M. micrantha* was found to grow about 1 m/month, while in Fiji, it grew about 0.5 m/month. In small blocks, its rapid growth rate enabled it to grow over cash crops such as taro, cassava, papaw and banana, smothering the plants and often killing them. In plantations, *M. micrantha* can grow over cocoa, young oil palms and coconut palms, retarding growth. Plants covered with *M. micrantha* had reduced flowering and fruit

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compared to those free of *M. micrantha*, while harvesting fallen coconuts is difficult due to the smothering effect of *M. micrantha*.

**Socio-economic impact of *M. micrantha***

More than 380 respondents, covering all 15 provinces in PNG in which *M. micrantha* is present, completed the questionnaire. Over 70% of respondents were involved in mixed cropping or subsistence farming, while the remaining respondents were commercial or semi-commercial farmers. Approximately 79% of all respondents considered *M. micrantha* a serious weed, with over 40% considering it reduced their crop yield by more than 30% (Fig. 3). About 44% of the respondents had over a third of their property infested with *M. micrantha*, which they spent 1-2 days per fortnight weeding (Fig. 4). About 96% of respondents who practise mixed cropping, controlled *M. micrantha* by physical means such as slashing and/or hand-pulling, while only 68% of respondents involved in commercial or semi-commercial practices used physical means only. Approximately 32% of respondents used *M. micrantha*, with most utilizing the plant to treat cuts and wounds.

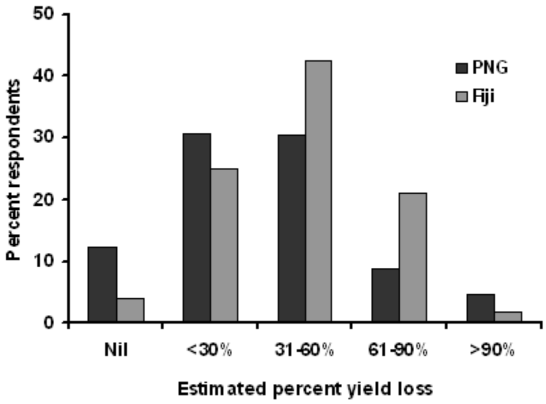


**Figure 3**. Yield losses due to *M. micrantha* as estimated by respondents in PNG and Fiji.

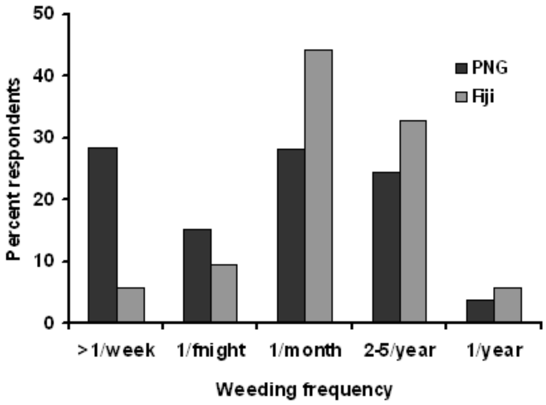


**Figure 4**. Weeding frequencies of *M. micrantha* as estimated by respondents in PNG and Fiji.

In Fiji, only 52 questionnaires were completed by farmers representing four islands. Approximately 60% of respondents considered *M. micrantha* a serious weed, losing about 30% of potential crop yield due to the weed (Fig. 3), while 33% had more than 30% of their farms infested. Only 15% of respondents needed to weed fortnightly (Fig. 4), with 56%

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using slashing and/or hand-pulling as the main means of control. Nearly 90% of respondents used *M. micrantha* as a medicinal plant to treat cuts and wounds.

**Effectiveness of the Rust *P. spegazzinii***

In laboratory trials, the growth rate of both single stem (2.14±0.3 S.E. cm/day) and multiple stem (1.62±0.13 S.E. cm/day) plants infected with *P. spegazzinii* was significantly lower than that of single stem (3.36±0.3 S.E. cm/day) and multiple stem (2.42±0.36 S.E. cm/day) plants not infected with rust ($F_{1,36}$=13.41, p<0.001) (Fig. 5).

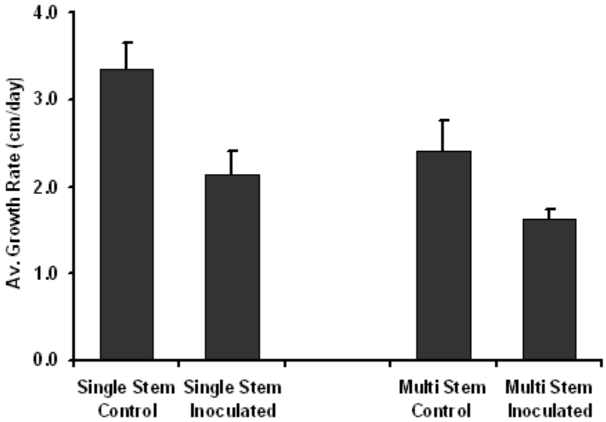


**Figure 5**. Growth rates of *M. micrantha* with and without rust under glasshouse conditions.

In field trials, as the number of leaves, petioles and stems of *M. micrantha* become infected with the rust, the percent plant cover decreased (Fig. 6). This was due in part to the number of dead stems and petioles found infected with *P. spegazzinii* but also to the presence of *Glycine wightii* (Fabaceae), which grew over the site and further suppressed *M. micrantha*.

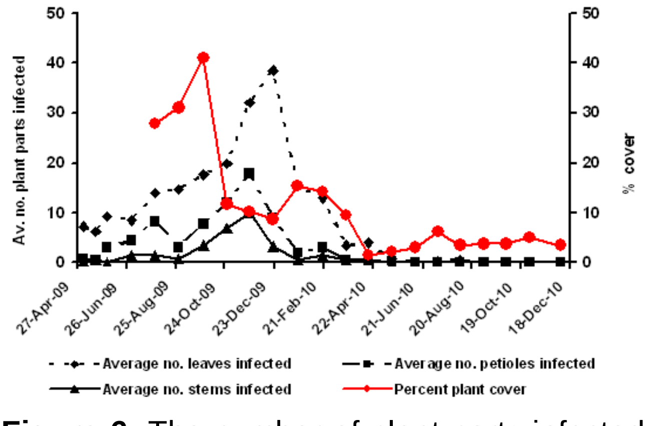


**Figure 6**. The number of plant parts infected with *P. spegazzinii* and the percent cover of *M. micrantha* at a field site at Kerevat, ENB, PNG.

## DISCUSSION

*Mikania micrantha* is becoming an increasing problem in many Pacific island countries and Southeast Asia (Dovey *et al*, 2004;Ellison *et al*. 2008). Its rapid growth and ability to climb and smother plants severely impacts on crop production and net income of farmers, who report that the weed can retard the growth of crops through direct competition for space,

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nutrients or light by smothering plants, thus reducing yield and income. Where *M. micrantha* is left untouched, it grows quickly over plants. In both countries, it kills bananas, taro and papaw, while in PNG it also reduces flowering and subsequent yield of cocoa.

Farmers in PNG slash and pull *M. micrantha* on a weekly basis, sometimes up to five days a week, while in Fiji, where infestations are not as large, farmers are weeding monthly. The amount of weeding required by farmers to keep *M. micrantha* out of their blocks reduces net income through increased labour costs or reduces the time available for other activities such as maintaining houses and fishing nets. As a consequence, landholders are resorting to farming smaller blocks, which are easier and less time-consuming to keep free of *M. micrantha,* but this also results in reduced production and income.

In Malaysia, *M. micrantha* has been reported to reduce yield of oil palm by 20%, particularly in the first five years, and the girth of rubber trees by 27% (Teoh *et al*. 1985). While the cost of *M. micrantha* in PNG and Fiji has not been estimated, in Malaysia the weed costs between US$ 8-10 million pa. In India, *M. micrantha* was identified as the number one problem faced by farmers in Kerala, with the presence of *M. micrantha* increasing production costs by about 10% (Muraleedharan and Anitha 2001).

Field surveys reveal that *M. micrantha* is widespread in both PNG and Fiji and it is likely that it is spreading to other climatically and edaphically suitable areas where it is not already present. The small seeds are easily dispersed, mainly by wind but also by people through attachment to clothing and possessions (Holm *et al*. 1991). *Mikania micrantha* will readily grow from stem fragments, which suggests that slashing may not be an effective control technique as first thought. The broken fragments can take root and, as a result, infestations may become thicker.

Herbicides have been used to control *M. micrantha* but their use has been mainly restricted to commercial plantations. Applying herbicides offers more effective control and reduces the frequency of control applications. However, the cost of herbicides is prohibitive for most smallholders.

As *M. micrantha* grows rapidly and conventional control measures are costly and time consuming, biological control is seen as a feasible, environmentally friendly and self-sustaining option to control large stands of *M. micrantha*. The rust *P. spegazzinii* has been introduced in several countries, including India, China, Taiwan, PNG and Fiji (C. Ellison CABI pers. comm.). In PNG and Fiji, it has been widely released and has established. In laboratory trials and preliminary field monitoring, it has reduced the growth of *M. micrantha* and it is expected to assist in the control of the weed in areas that are climatically suitable. Thus, it should reduce the costs and time that landholders spend in controlling the weed and increase food production and income.

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## REFERENCES

Cock, M.J.W., Ellison, C.A., Evans, H.C. and Ooi, P.A.C. (2000). Can failure be turned into success for biological control of mile-a-minute weed (*Mikania micrantha*)? In Spencer, N.R. (ed). *Proceedings of the X International Symposium on Biological Control of Weeds.* 4-14 July, 1999, Bozeman, Montana, USA. Montana State University, Bozeman. pp. 155-167.

Dovey, L., Orapa, W. and Randall, S. (2004). The need to build biological control capacity in the Pacific. In Cullen, J.M., Briese D.T, Kriticos, D.J, Lonsdale, W.M., Morin, L. and Scott, J.K. (eds). *Proceedings of the XI International Symposium on Biological Control of Weeds*. CSIRO Division of Entomology, Canberra, Australia. pp. 36-41

Ellison, C.A., Evans, H.C., Djeddour, D.H. and Thomas, S.E. (2008). Biology and host range of the rust fungus *Puccinia spegazzinii*: A new classical biological control agent for the invasive, alien weed *Mikania micrantha* in Asia. *Biological Control* 45: 133-145.

Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1991). *The World's Worst Weeds: Distribution and Biology*. Kreiger Publishing Company, Malabar, Florida.

Muraleedharan, P.K. and Anitha, V. (2001). The economic impact of *Mikania micrantha* on the Agroforestry Production System in the Western Ghats of Kerala. In Sankaran, K.V., Murphy, S.T. and Evans, H.C. (eds). *Workshop proceedings of alien weeds in moist tropical zones: banes and benefits*. Kerala Forest Research Institute, India and CABI Bioscience, United Kingdom. pp. 80-85.

Orapa, W., Day, M. and Ellison, C. (2008). New efforts at biological control of *Mikania micrantha* H.B.K. (Asteraceae) in Papua New Guinea and Fiji. In *Proceedings of the Australia and New Zealand Biocontrol Conference*. NSW Department of Primary Industries, Sydney. pg. 45.

Teoh, C.H., Chung, G.F., Liau, S.S., Ibrahim, G., Tan, A.M., Lee, S.A. and Mohammed, M. (1985). Prospects for biological control of *Mikania micrantha* HBK in Malaysia. *Planter Kuala Lumpur* 61: 515-530.

Waterhouse, D.F. and Norris, K.R. (1987). *Biological Control: Pacific Prospects*. Inkata Press, Melbourne.

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# ISOLATION AND IDENTIFICATION OF NOVEL ALLELOCHEMICALS AND UTILIZATION OF ALLELOPATHIC COVER PLANTS FOR SUSTAINABLE AGRICULTURE

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## ABSTRACT

We are now operating a National Project, entitled “Screening of innovative allelochemicals and their utilization” from 2008 to 2012. We have screened about 4000 plants all around the world, mainly from Japan and Asian countries, and evaluated these by specific bioassays for allelopathy, including “Plant Box Method”, “Sandwich Method”, “Dish-pack Method”, and “Rhizosphere Soil Method”.

Our final target is to isolate novel bioactive natural chemicals from allelochemicals. Strategies for screening new allelochemicals are based on “Total Activity Method”, carefully calculating the concentration of allelochemicals in plants. We have already isolated *cis*-cinnamic acid derivatives as potent allelochemicals, and obtained a patent and now are performing organic synthesis of derivatives to increase activity. Mode of action of new allelochemicals are evaluated by DNA microarray technique using *Arabidopsis* as the receiver plant. Volatile allelochemicals are another target of our screen for allelochemicals. Final goal of this study is the utilization of allelochemicals as new agrochemicals.

Another goal of our study is to use allelopathic plants as ground covers for agricultural and natural settings. Some promising domestic and alien plants with allelopathic activity were screened, and field tested to demonstrate their potential; utilization of these new ground cover plants will be discussed.

**Key words**: allelopathy, allelochemicals, cover plants, DNA microarray, organic synthesis

## INTRODUCTION

For the development of new herbicides and potential ground cover plants to suppress weeds, we have started a National Project, entitled "Screening of innovative allelochemicals and their utilization". We have developed specific bioassay methods for allelopathy, named "Plant Box Method", "Sandwich Method", "Dish-pack Method", and "Rhizosphere Soil Method". We have also developed a new strategy for the isolation of allelochemicals based on "Total Activity". By using these methods, trials for the isolation and identification of novel allelochemicals, and mode of action studies using DNA microarray will be performed for promising allelochemicals. Organic synthesis and organically synthesized derivatives were developed. Bioassay and field tests for the evaluation of chemicals and cover plants with allelopathic activity will also be performed.

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## RESULTS AND DISCUSSION

Research was directed to three sub-groups. Group A, National Institute for Agro-Environmental Sciences (NIAES), will select allelopathic plants and perform isolation and identification of allelochemicals. Up to now, potent allelopathic plants include *Cleome aculeate* and *Wendtia calycina* from Paraguai, *Trigonella foenum-graecum* from Egypt, and *Kelussia odoratissima* from Iran. We have isolated methyl isothiocyanate, (S)-(+)-carvone, *trans*-2-hexenal and other potent novel allelochemicals in action. We are also conducting transcriptome analysis for selected alleochemical candidates. We have also found from *Bletilla striata*, a Japanese ground cover, militarin and dactylorhin A as allelochemicals (Fig 1). *Geranium carolinianum*, a noxious invasive weed from North America now in Japan, produces ethyl gallate as an allelopathic and fungi-static chemical. The other candidates we are now focusing are *Enterolobium contortisiliquum*, *Pachysandra terminalis*, *Goniothalamus andersonii, Albizia saman, Albizia guachapele.* As for mode of action of these chemicals, specific genes responsible for their activity were checked by database system such as APASD-II. We have already reported on specific genes that are up or down regulated by L-DOPA and allelochemicals from buckwheat.

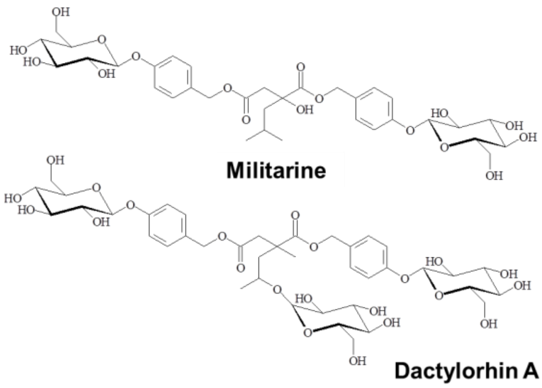


Figure 1 *Militarine and Dactylorhin A from Bletilla striata* (Shi-ran)

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Group B (University of Tokushima) is focusing on the organic synthesis of potent allelochemical candidates for novel herbicide or agrochemicals. Organic synthesis of sundifersifolide, a helianan type sesquiterpenoid, and brevion were established. Helibisabonol A and B were newly synthesized and absolute structure of these compounds were fixed (Fig 2). Xanthatine, a xanthanolide terpenoide, was also synthesized. Bioactivity of these synthesized compounds were sent to Group A at NIAES, and some potent candidates were re-evaluated. In this study, total organic synthesis of helibisabonol A and B were reported for the first time and their absolute configurations were specified. Total organic syntheses of zanthatin and sudiversifolide, both zanthanolide type terpenoides, were also accomplished. Bioassay of these compounds was performed by Group A and promising leads were selected for further consideration. A library of more than 60 derivatives of helianane type sesquiterpenenoids was established. As for brevione A and B, other allelochemicals with antimicrobial activities were synthesized by enanthio-regulated synthesis and coupling synthesis for hybrid compounds. Synthesized compounds were sent to NIAES and 37 of them with potent activity were set aside for further consideration as a precursor for herbicides. Total organic synthesis of ionone type terpenoids, 3-hydroxy- $\beta$ -ionone and 3-oxo- $\alpha$ -ionol, identified at NIAES, was also started.

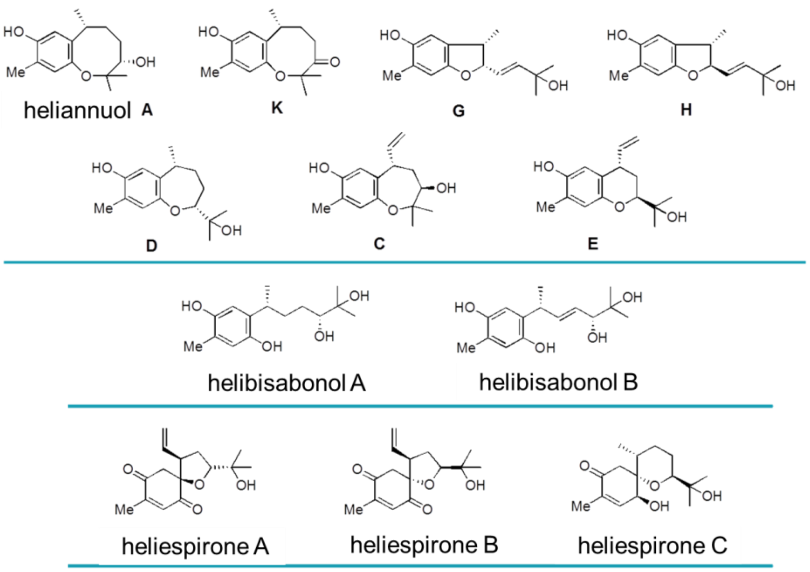


Figure 2 Organically synthesized allelochemicals

Group C (Kyusyu University, Research Institute for Innovative Chemicals), established total organic synthesis of xanthanoide type compounds in connection with Group B. Library construction of *cis*-cinnamic acid derivatives was started (Fig 3). *Cis*-cinnamic acid is a novel allelochemical with potent activity, identified by Group A from a Japanese plant, yukiyanagi, as first reported. More than 180 derivatives of *cis*-cinnamic acid were synthesized and herbicidal activity for plants were tested by Group A. Some derivatives showed stronger activity than original nonsynthetic compounds; therefore, we have submitted for a Japanese Patent. We are now performing structural activity studies, and studies of the mode of action of these cis-cinnamic acid derivatives are now on going.

With Group A, 21 allelochemicals were identified while 100 allelochemicals were synthesized by Group B and 180 derivatives of allelochemicals synthesized by group C are now undergoing evaluation as potential herbicides.

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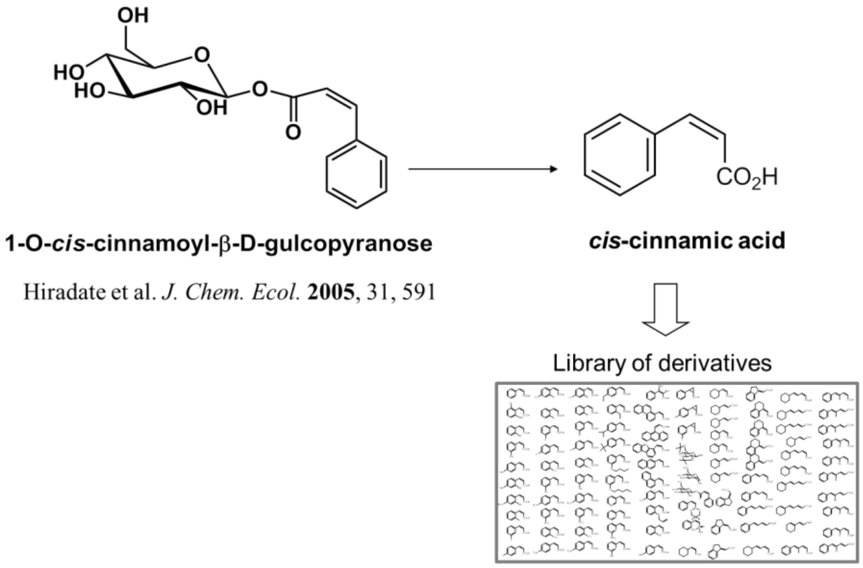


Figure 3 Organic synthesis of cis-cinnamic acid derivatives

## REFERENCES

Gilani, S.A., Fujii, Y, Sugano, M. and Watanabe, K. (2010). Chemotypic variations and phytotoxic studies of essential oils of endemic medicinal plant, *Seriphidium kurramense*, from Pakistan. *Journal of Medicinal Plant Research* 4: 309-315.

Gilani, S.A., Fujii, Y, Shinwari, Z. K., Adnan, S. M., Kikuchi, A., and Watanabe, K. (2010). Phytotoxic studies of medicinal plants species of Pakistan. *Pakistan Journal of Botany* 42: 987-996.

Golisz, A., Sugano, M., Hiradate, S. and Fujii, Y. (2011). Microarray analysis of *Arabidopsis* plants in response to allelochemical L-DOPA. *Planta* 233: 231-240.

Hiradate, S., Ohse, K., Furubayashi, A. and Fujii, Y. (2010). Quantitative evaluation of allelopathic potentials in soils: total activity approach. *Weed Science* 58: 258-264.

Jung, K., Fujii, Y., Yoshizaki, S., and Kobori, H. (2010). Evaluation of total allelopathic activity of heartseed walnut (*Juglans ailanthifolia* Carr.) and its potential to control black locust (*Robinia pseudo-acacia* L.). *Allelopathy Journal* 26: 243-253.

Kamo, T., Kasahara, R., Abe, S., Hirota, M., Sugano, M., Yamaya, H., Hiradate, S. and Fujii, Y. (2010). Carbon Sources of Natural Cyanamide in *Vicia villosa* subsp. *varia*. *Natural Product Research* 24: 1637–1642.

Kamo, T., Kato, K., Abe, S., Hirota, M., Yamaya, H., Hiradate, S. and Fujii, Y. (2009). Biosynthesis origin of the nitrogen atom in cyanamide in *Vicia villosa* subsp. *varia*. *Soil Science and Plant Nutrition* 55: 235-242.

Kato-Noguchi, H., Yamamoto, M., Teruya, T., Suenaga, K. and Fujii. Y. (2010). Isolation and identification of potent allelopathic substances in rat-tail fescue. *Plant Growth Regulation* 60: 127-131.

Matsuo, K., Ohtsuki, K., Yoshikawa, T., Shishido, K., Yokotani-Tomita, K. and Shindo, M. (2010). Total Synthesis of Xanthanolides, *Tetrahderon* 66: 8407-8419.

Ooshiro A., Hiradate, S., Kawano, S., Takushi, T., Fujii, Y., Natsume, M. and Abe, H. (2009). Identification and activity of ethyl gallate as an antimicrobial compound produced by *Geranium carolinianum*. *Weed Biology and Management* 9: 169-172.

Osaka, M., Kanematsu, M., Yoshida, M. and Shishido, K. (2011) Synthesis of (±)-heliannuol D based on platinum catalyzed regioselective addition of arylboronic acids to allene, *Heterocycles* (In Press)

Sakuno, E., Kamo, T., Takemura, T., Sugie, H., Hiradate, S. and Fujii , Y. (2010). Contribution of militarine and dactylorhin A to plant growth inhibitory activity of a weed-suppressing orchid, *Bletilla striata* (Thunb.) Reichb. fil. *Weed Biology and Management* 10: 202-207.

Shindo, M., Yoshikawa, T. and Yaji, K. (2009) Synthesis of 1,3-Dienes by Cycloaddition and Electrocyclic Reactions, in *Science of Synthesis,* V. H. Rawal and S. A. Kozmin (ed.), Vol. 46.7, Georg Thieme Verlag KG, 353-400.

Yoshida, M., Maeyama, Y. and Shishido, K. (2010) A synthetic study of bauhinoxepin J: Construction of a dibenzo[b,f]oxepin ring system by a DDQ-promoted oxidative dearomatization–cyclization approach, *Heterocycles* 80: 623-629.

# INVASIVE PLANT THREATS AND PREVENTION APPROACHES IN THE ASIA-PACIFIC REGION AND UNITED STATES

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## INTRODUCTION

Many countries have developed noxious or invasive species lists of plants that are economically or ecologically damaging to both agricultural and environmental areas. These lists, however, rarely include species not yet present in the region, but with the potential to cause significant harm. The challenge in many prevention programs is to anticipate what new weeds are likely to invade a region or country, how to assess their potential impact, what areas provide suitable habitat under current and predicted climate change scenarios, where is their most probably pathway of entry, and how to prevent their introduction and establish.

It is far easier for countries or regions to determine potentially harmful weeds by comparing and evaluating weedy and invasive species in other regions of the world with similar climates. This can include plant species that have been problematic for several years and are globally widespread, or plants that are newly established in only one region of the world. A far more difficult task, however, is to determine the potential weedy species that have yet to become problematic in any area of the world. In this paper, I initially compare the weedy and invasive species in Australia and California and then outline a process for determining new potentially invasive species and preventing their possible entry into a region.

### Comparison between Noxious and Invasive Weeds of Australia and California

Weedy and invasive plants in Australia and California were introduced from a variety of regions in the world. Using the *Aquatic and Riparian Weeds of the West* and *Weeds of California and Other Western States* (DiTomaso and Healy 2003, 2007) and the *Noxious Weeds of Australia* (Parsons and Cuthbertson 2001) to determine the origin of weedy species in the two regions, it is clear that harmful plants have been introduced from continents around the world (Table 1). The percentage of weedy or invasive plants originating from Africa, Asia, and Australia and New Zealand were very similar between California and Australia. However, more than twice as many noxious weeds of Australia originated from Central and South America compared to California. Conversely, far more weeds in California originated from Europe and North America compared to Australia.

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**Table 1.** Origin of weeds in Australia and California.

| Origin of weedy or invasive species | Australia | California |
|---|---|---|
| | Percent of total | |
| Africa | 16 | 13 |
| Asia | 9 | 6 |
| Australia and New Zealand | 7 | 6 |
| Central and South America | 26 | 11 |
| Europe | 29 | 43 |
| North America | 13 | 21 |

In comparing the most recent lists of weedy and invasive species between the two regions, there was a considerable number of species that were weedy or invasive in both Australia and California (Table 2). Of the 396 noxious or listed species in Australia (http://weeds.gov.au/), 41% of these plants are either naturalized or weedy in California. When considering the 305 species of noxious or invasive weeds in California (Cal-IPC 2006 and http://www.cdfa.ca.gov/phpps/ipc/weedinfo/winfo_list-synonyms.htm), 31% of these species are also listed as weedy in Australia.

**Table 2.** Total number of weedy or invasive species in Australia and California and the percent of species common to both countries.

| Region | Total species on noxious or invasive plant lists | Percent overlap |
|---|---|---|
| Australia | 396 | 41 |
| California | 305 | 31 |

The species considered noxious or on the invasive inventory in both regions are listed in Table 3.

**Table 3.** Australian noxious weeds (http://weeds.gov.au/) also listed on the California invasive plant inventory (Cal-IPC 2006) or the California Department of Food and Agriculture (CDFA) noxious plant list (http://www.cdfa.ca.gov/phpps/ipc/weedinfo). Species in bold are Australia Weeds of National Significance. Cal-IPC rankings include High, Moderate (Mod), and Limited invasiveness. CDFA lists include A (limited populations within state), B (regionally common), and C (widely dispersed in state).

| Species | Cal-IPC ranking | CDFA list | Species | Cal-IPC ranking | CDFA list |
|---|---|---|---|---|---|
| *Acacia paradoxa* | | B | *Foeniculum vulgare* | High | |
| *Acroptilon repens* (=*Rhoponticum repens*) | Mod | B | *Genista monspessulana* | High | C |
| *Aegilops cylindrica* | | B | *Hedera helix* | High | |
| *Ageratina adenophora* | Mod | | *Helianthus ciliaris* | | A |
| *Ailanthus altissima* | Mod | C | *Hirshfeldia incana* | Mod | |
| *Alhagi maurorum* | Mod | A | *Hydrilla verticillata* | High | A |
| *Allium vineale* | | B | *Hypericum perforatum* | Mod | C |
| ***Alternanthera philoxeroides*** | High | A | *Lepidium draba* (=*Cardaria draba*) | Mod | B |

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| | | |
|---|---|---|
| *Araujia sericifera* | | B |
| *Arundo donax* | High | B |
| ***Asparagus asparagoides*** | Mod | |
| *Asphodelus fistulosus* | Mod | B |
| *Bassia scoparia* (=*Kochia scoparius*) | Limited | |
| ***Cabomba caroliniana*** | | B |
| *Carduus nutans* | Mod | A |
| *Carduus pycnocephalus* | Mod | C |
| *Carduus tenuiflorus* | Limited | C |
| *Carthamus lanatus* | Mod | B |
| *Carthamus leucocaulos* | | A |
| *Cenchrus echinatus* | | C |
| *Cenchrus incertus* | | C |
| *Cenchrus longispinus* | | C |
| *Centaurea calcitrapa* | Mod | B |
| *Centaurea solstitialis* | High | C |
| *Centaurea stoebe* | High | A |
| *Chondrilla juncea* | Mod | B |
| *Cirsium arvense* | Mod | C |
| *Cirsium vulgare* | Mod | C |
| *Conium maculatum* | Mod | |
| *Convolvulus arvensis* | | C |
| *Cortaderia jubata* | High | B |
| *Cortaderia selloana* | High | |
| *Cotoneaster franchetii* | Mod | |
| *Cotoneaster pannosus* | Mod | |
| *Crataegus monogyna* | Limited | |
| *Leucanthemum vulgare* | Mod | |
| *Ludwigia peruviana* | | A |
| *Marrubium vulgare* | Limited | |
| *Myriophyllum aquaticum* | High | |
| *Myriophyllum spicatum* | High | C |
| *Olea europaea* | Limited | |
| *Onopordum acanthium* | High | A |
| *Onopordum tauricum* | | A |
| *Orobanche* spp. | | A |
| *Oryza rufipogon* | | B |
| *Oxalis pes-caprae* | Mod | |
| *Pennisetum setaceum* | Mod | |
| *Pistia stratiotes* | | B |
| ***Prosopis* spp.** | | A |
| *Pyracantha angustifolia* | Limited | |
| *Ricinus communis* | Limited | |
| *Robinia pseudoacacia* | Limited | |
| *Rorippa sylvestris* | | B |
| ***Rubus fruticosa* (complex)** | High | |
| *Rumex acetosella* (=*Acetosella vulgaris*) | Mod | |
| *Rumex crispus* | Limited | |
| ***Salvinia molesta*** | High | A |
| *Schinus terebinthifolius* | Limited | |
| *Scolymus hispanicus* | | A |
| *Senecio jacobaea* | Limited | B |
| *Silybum marianum* | Limited | |
| *Solanum elaeagnifolium* | | B |

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| | | | | | |
|---|---|---|---|---|---|
| *Crupina vulgaris* | Limited | A | *Solanum marginatum* | | B |
| *Cuscuta campestris* | | C | *Sonchus arvensis* | | A |
| *Cynara cardunculus* | Mod | B | *Sorghum halepense* | | C |
| *Cyperus esculentus* | | B | *Spartina anglica* | Mod | B |
| *Cytisus scoparius* | High | C | *Spartium junceum* | High | C |
| *Delairea odorata* | High | B | ***Tamarix aphylla*** | Limited | |
| *Dipsacus fullonum* | Mod | | *Tribulus terrestris* | | C |
| *Dittrichia graveolens* | Mod | | ***Ulex europaeus*** | High | B |
| *Egeria densa* | High | C | *Undaria pinnatifida* | Limited | |
| *Eichhornia crassipes* | High | C | *Verbascum thapsus* | Limited | |
| *Emex spinosa* | Mod | | *Vinca major* | Mod | |
| *Euphorbia terracina* | Mod | B | *Watsonia meriana* | Limited | |
| *Fallopia japonica* (=*Polygonum cuspidatum*) | Mod | B | *Zantedeschia aethiopica* | Limited | |

Despite the overlap in weedy and invasive species between the two regions, there are a number of species that are far more important either economically or environmentally in one region compared to the other. For example, *Asparagus asparagoides, Cabomba caroliniana, Carthamus lanatus, Echium plantagineum, Emex australis, Olea europaea*, and *Tamarix aphylla* are more widespread and problematic in Australia than in California or the United States (US). It is difficult to explain why *Echium plantagineum*, which is occasionally found naturalized in California, is not as widespread as in Australia. It is possible that this species is still in the lag phase in California and may eventually spread to become a more serious problem. *Olea europaea* is also very limited in its invasiveness in California compared to Australia. However, this may be due to the lack of appropriate bird feeders that facilitate dispersal (Aslan 2011).

Conversely, there are a few invasive species that, while present in Australia, are far more problematic and widely distributed in California and other western states. These include, among others, *Centaurea solstitialis, Cirsium arvense, Egeria densa, Euphorbia esula,* and *Linaria dalmatica.*

Interestingly, of the species considered native to Australia yet invasive in California (Table 4), none are listed as highly invasive. Conversely, of the species considered noxious in Australia, but native to California, nearly all of them are also weedy is agricultural, water use areas, or urban settings in California (Table 5). Thus, it is not surprising why they would become problematic in other countries, such as Australia, that share similar climates.

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**Table 4.** Species native to Australia and listed as invasive in California

| Species | Ranking of invasiveness (California Invasive Plant Council) |
|---|---|
| *Acacia dealbata* | Moderate |
| *Acacia melanoxylon* | Limited |
| *Acacia paradoxa* | State listed only |
| *Atriplex semibaccata* | Moderate |
| *Cordyline australis* | Limited |
| *Erechtites glomerata* | Moderate |
| *Erechtites minima* | Moderate |
| *Eucalyptus camaldulensis* | Limited |
| *Eucalyptus globulus* | Moderate |
| *Myoporum laetum* | Moderate |

**Table 5.** Species native to California and weedy or invasive in Australia.

| Species | Weediness in California |
|---|---|
| *Cuscuta* spp. | Very weedy |
| *Cyperus eragrostis* | Limited weediness |
| *Datura wrightii* | Limited weediness |
| *Elodea canadensis* | Moderate weediness |
| *Eremocarpus setigerus* | Moderate weediness |
| *Iva axillaris* | Limited weediness |
| *Parkinsonia aculeata** | Limited weediness |
| *Toxicodendron diversilobum* | Moderate weediness |
| *Typha latifolia* | Very weedy |
| *Verbesina encelioides* | Limited weediness |
| *Xanthium strumarium* | Very weedy |
| *Xanthium spinosum* | Moderate weediness |

*Weed of National Significance in Australia

## How to Determine the Next Invader

Although there are many different methods to anticipate new potential invasive or weedy species, perhaps the best initial approach is to conduct surveys of weedy species present in other countries with a similar climate. In California, through the efforts of the California Invasive Species Council, a survey was conducted of all invasive plants listed by other countries with a similar Mediterranean climate (Brusati *et al.,* unpublished data). This totaled 774 species. From this extensive list, 383 were already naturalized in California, of which 318 had been present before 1940 but had not spread or become problematic. As such, these species were eliminated from further consideration. The remaining 65 species that were more recent naturalized species in California (>1940) and the 391 species not yet naturalized in California were further evaluated. For these 456 species, 188 were sold by the nursery industry and 66 of these were currently being sold in California. Thus, the 774 initial species targeted by the survey was narrowed to 66 species that had the highest potential for introduction, establishment and invasion in the state. While there are other methods of developing such watch lists, this method was not extensively labor intensive and can serve as a good first screening of potential invasiveness for species already adapted to the climate in the new region.

## Climate Matching under Current and Future Climate Scenarios

Once a preliminary list of potentially invasive species had been determined, it is possible to utilize climate matching models to more accurately determine areas most suitable for possible invasion. This has been accomplished with numerous other studies both regionally and globally for potential invasiveness of introduced species (Thuiller *et al.* 2005) or proposed biofuel species (Barney and DiTomaso 2011). The California Invasive Species Council used climate matching models for 36 of their 200+ invasive plants to not only predict the potential suitable habitat within the state, but also to predict potential changes in climatic suitability under a proposed 3 C climate increase. The long-term goal, currently in progress, is to develop similar climate matching models for all known invasive plants within the state both with and without climate change parameters. This information will be used to develop a targeted Early Detection Rapid Response (EDRR) program directed to specific habitats throughout the state. A subset of potentially invasive species can be identified for each susceptible habitat in any region of the state. Similar EDRR programs can be established for plants that have the potential to be introduced, but are not currently present.

It is important to note that under a climate change scenario, not all invasive plants are predicted to increase in their distribution (Figure 1). In fact, our evaluation showed that an equal number of species were expected to decrease in their distribution as were expected to increase. Overall, the average Ecoclimatic Index of the 36 species evaluated showed virtually no change (~2% increase) compared with no climate change scenario.

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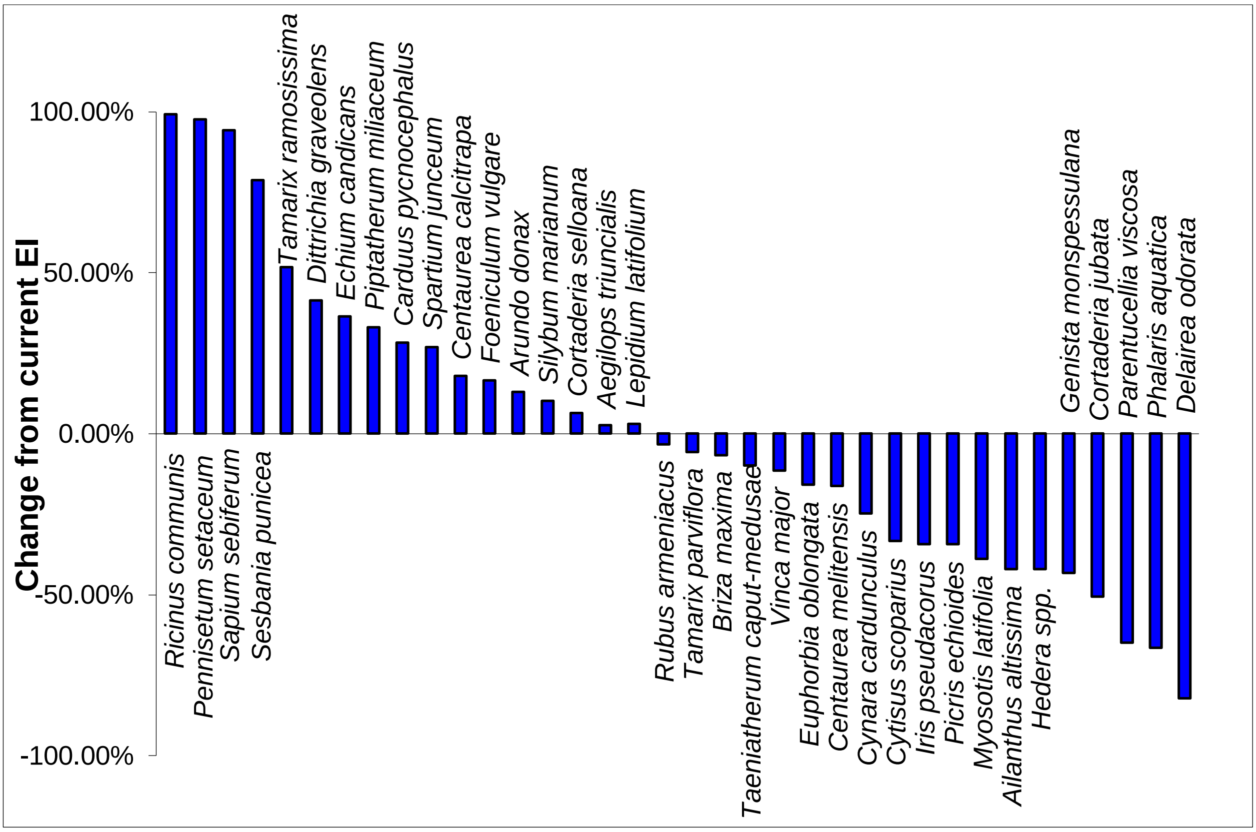
**Figure 1.** Predicted change in overall Ecoclimatic Index (EI) for 36 invasive plants of California. Bars above horizontal line indicate predicted increase in distribution, and values below horizontal line indicate predicted reduction in distribution with anticipated 3 C increase in temperature.




## Weed Risk Assessment Models

Several Weed Risk Assessment (WRA) models have been developed, the most common of which was developed by Paul Pheloung and his colleagues in Australia (Pheloung *et al.* 1999). The model has proved to have an overall accuracy of 90% when comparing known invasive and non-invasive ornamental plant species. Because of its high level of accuracy, it has been modified for use in New Zealand, Florida, Hawaii and other Pacific islands, and the Galapagos Islands (Pheloung 2005, Gordon *et al.* 2008). In addition, Barney and DiTomaso (2008) applied Pheloung's model as a pre-introduction screening of three biofuel candidate crops for specific target regions. In this case, the model was used to assess whether these economically beneficial crops would pose a risk of becoming invasive. This model has been instrumental as a prevention tool for reducing the potential of introducing new invasive or weedy species to a region.

In a collaboration with the nursery industry both in California and nationwide, the California Horticultural Invasive Prevention (Cal-HIP) partnership, in its "Plant Right" campaign, is attempting to develop a third party certification process to further prevent the sale and introduction of invasive ornamental species. This is critical, as 50% of the listed invasive plants in the state originated from the ornamental plant industry. In this effort, the Pheloung model has been shown to be too conservative in predicting invasiveness and is not likely to be accepted by the industry as it lists too many "non-invasive" ornamentals as invasive. Thus, Cal-HIP in collaboration with Elizabeth Seebacher, developed an abbreviated version of the Pheloung model (Plant Right WRA) that is more specific to the industry and the US (unpublished data). This model consists of 27 questions, compared to the 49 in the Pheloung model. In an independent evaluation using the same 170 invasive (horticultural and non-horticultural) or non-invasive species (horticultural), three biologists working in separate areas of the western US ranked the species and compared the two models. The accuracy in predicting both non-invasiveness and invasiveness averaged 99% among the three biologists. In contrast, the Pheloung model was highly accurate in predicting non-invasiveness (100%), but only 80% accurate in predicting invasiveness. As such, we are now in the process of working with the nursery industry to accept the Plant Right WRA as the most appropriate tool to evaluate current nursery stock, as well as newly introduced species, in a third party certification program.

**Pathways of introduction and prevention strategies**

After developing a reliable understanding of the species most likely to invade a new region, their potential for invasiveness using WRA models, as well as the climatic suitability of the species to the specific regions within the area, it is critical to recognize the potential pathways for their introduction. These pathways can include natural movement, as well as accidental or intentional introduction through human activity into a new region. In Table 6, I include potential methods of introduction of new species to a region, as well as pathways of moving weedy species within the region. Each of these pathways can be designated as having a high, moderate, low or no risk of movement.

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**Table 6.** Survey table of propagule dispersal methods and likelihood of an invasive species being introduced via that method.

| | Risk of dispersal | | | |
|---|---|---|---|---|
| **Means of propagule dispersal** | **High** | **Mod-erate** | **Low** | **None** |
| **Natural** | | | | |
| Water | | | | |
| Wind | | | | |
| Animal movement (dispersal of seed or vegetative propagules) | | | | |
| Hurricanes or cyclones | | | | |
| **Intentional** | | | | |
| Agricultural | | | | |
| Ornamental or horticultural | | | | |
| Biofuel | | | | |
| Legal human activities | | | | |
| Aquarium | | | | |
| Internet or mail order sales | | | | |
| Illegal human activities | | | | |
| Illegal entry (food or horticulture) | | | | |
| Ecoterrorism | | | | |
| **Accidental** | | | | |
| Agriculture or Commerce | | | | |
| Seed contaminant | | | | |
| Hay contaminant | | | | |
| Soil movement | | | | |
| Food products | | | | |
| Contaminants of nursery stock | | | | |
| Commercial watercraft | | | | |
| Travel or transportation activities | | | | |
| Vehicle, trailer or equipment contaminant | | | | |
| Ballast | | | | |
| Packing materials | | | | |
| Mail and packages | | | | |
| Legal import contaminant (including cargo containers) | | | | |
| Inadvertent human travel (internatl. & natl.) | | | | |
| Recreational activities | | | | |
| Recreational watercraft or vehicles | | | | |

An example of an invasive plant that is spreading rapidly around the world is *Mikania micrantha*, often referred to as Chinese creeper, South American climber, bittervine or mile-a-minute. It has a high potential for causing significant economic and ecological damage in agricultural and natural areas (Manrique *et al.* 2011). *Mikania* can be dispersed

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long distances by wind, as the seed contains a parachute-like pappus. Thus, the highest probability of introduction would be by wind and perhaps through hurricane activity. When populations occur in adjacent countries, states, provinces or regions special surveillance should be conducted following such natural climatic events, particularly in disturbed areas that show a high climatic suitability. This EDRR process as part of a prevention strategy would greatly reduce the probability of establishment and spread. As another example, when developing an EDRR program for species primarily dispersed long-distance through aquatic recreational vehicles, e.g., *Myriophyllum spicatum, Egeria densa*, or *Hydrilla verticillata*, inspection stations should be established at the docks of recreational areas to prevent both entry into an uninfested body of water or movement from a contaminated aquatic site.

In conclusion, by comparing the weedy and invasive flora of Australia and California it is clear that weedy plants can originate from around the globe, and though many species can be problematic in multiple regions, some species are more specific to one region of the world, while other species may still be in the lag phase in one region compared to another. Addressing the threats of invasive plant introductions is a multi-step process. It requires a complex program encompassing knowledge of plants likely to be introduced to a new region, their potential to establish, survive and spread, the predictive harmful impacts they may cause, and the most likely pathways of possible entry and movement. With this information, the probability of slowing the introduction and spread of new invasive plants to a region can be more efficient and cost-effective in the long-term.

## REFERENCES

Aslan, C.E. (2011). Implications of newly-formed seed-dispersal mutualisms between birds and introduced plants in northern California, USA. *Biological Invasions DOI 10.1007/s10530-011-9966-1*

Barney, J.N. and DiTomaso, J.M. (2008). *Nonnative species and bioenergy: are we cultivating the next invader? BioScience* 58:64-70.

Barney, J.N. and DiTomaso, J.M. (2011). Global climate niche estimates for bioenergy crops and invasive species of agronomic origin: Potential problems and opportunities. *PLoS ONE 6(3): e17222. doi:10.1371/journal.pone.0017222*

DiTomaso, J.M. and Healy, E.A. (2003). *Aquatic and Riparian Weeds of the West.* Univ. of California Ag. Nat. Res., Berkeley, California, Publ. #3421.

DiTomaso, J.M. and Healy, E.A. (2007). *Weeds of California and Other Western States.* Univ. of California Ag. Nat. Res., Berkeley, California, Publ. #3488.

Gordon, D.R., Onderdonk, D.A. Fox, A.M. Stocker, R.K. and Gantz, C. (2008). Predicting invasive plants in Florida using the Australian weed risk assessment. *Invasive Plant Science and Management* 1:178-195.

Manrique, V., Diaz, R., Cuda, J.P. and Overholt, W.A. (2011). Suitability of a new plant invader as a target for biological control in Florida. *Invasive Plant Science and Management* 4:1-10.

Parsons, W.T. and Cuthbertson, E.G. (2001). *Noxious Weeds of Australia,* 2nd Ed., CSIRO Publ., Collingwood, Australia.

Pheloung, P.C. (2005). Use of the weed risk assessment tool in Australia's approach to pest risk analysis. In *International Plant Protection Convention (IPPC) Secretariat. 2005. Identification of risks and management of invasive alien species using the IPPC framework.* Proceedings of the workshop on invasive alien species and the International Plant Protection Convention, Braunschweig, Germany, 22-26 September 2003. Rome, Italy: FAO. xii + 301 pp.

Pheloung, P.C., Williams, P.A. and Halloy, S.R. (1999). A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management* 57:239–251.

Thuiller, W., Richardson, D.M., Pysek, P., Midgley, G.F., Hughes, G.O. and Rouget, M. (2005). Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global Change Biology* 11:2234–2250

# HERBICIDAL CONTROL OF *LANTANA CAMARA*

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## ABSTRACT

Lantana is a perennial shrub that commonly infests pastures, roadsides, and natural areas. In Florida (USA), lantana flowers approximately 10 months of the year and is a prodigious seed producer. Though seed viability is reportedly low, lantana is most common in areas where birds roost: abandoned citrus groves, under power lines, along highway guard rails, etc. Many experiments have been conducted to manage this weed, but few successful herbicides have been found. In particular, foliar applications of triclopyr results in essentially no injury symptoms or reduction of lantana growth. However, little information is available for the effectiveness of fluroxypyr, aminopyralid, or aminocyclopyrachlor. Experiments were conducted in central Florida on a dense, natural infestation of lantana. Plots measured 8 meters by 16 meters and were replicated 4 times. Herbicides were applied in water at 230 L/ha. Aminopyralid (0.12 kg/ha), fluroxypyr (0.55 kg/ha), and aminocyclopyrachlor (0.2 kg/ha) were applied in the fall, approximately 2 months prior to frost. Half of the plots treated with each herbicide were re-treated the following spring (approximately 6 months later). Therefore, data consist of each herbicide applied in the fall alone as well as fall followed by spring. Aminopyralid was ineffective on lantana, either one or two applications resulted in <20% control one year after treatment (YAT). Fluroxypyr applied once resulted in 12% control at 1 YAT, but the two application treatment resulted in 80% control after one year. The combination of fluroxypyr + aminopyralid, applied twice, resulted in approximately 90% control 1 YAT. A single application of fluroxypyr + aminopyralid failed to provide greater than 20% control. Conversely, aminocyclopyrachlor applied once in the fall provided 98% lantana control at 1 YAT. Where aminocyclopyrachlor was applied twice, lantana control was 100%. From these data, lantana can be effectively controlled by two applications of fluroxypyr, two applications of fluroxypyr + aminopyralid, or a single application of aminocyclopyrachlor.

**KEYWORDS:** broadcast, basal, cut surface, aminocyclopyrachlor, fluroxypyr, aminopyralid

## INTRODUCTION

Lantana (*Lantana camara*) is an invasive exotic that is commonly found throughout the Southeast United States from Florida to Texas. Growing as either dense thickets or as individual plants, lantana can quickly dominate a landscape by out-competing native species (Day *et al.* 2003). This species has been documented to grow best in recently disturbed sites (Thaman 1974; Fensham *et al.* 1994). For this reason, lantana is reaching epidemic levels in central Florida's abandoned citrus groves that have been renovated to pasture. Statewide, lantana is one of the top 10 most troublesome weeds in Florida and has been documented in 58 of 67 counties (USDA, ARS 2004).

The competitive and invasive nature of lantana stems from two primary defenses: allelopathy and resistance to herbivory. First, allelochemicals produced in the roots and stems have been shown to negatively influence the growth and competitive ability of surrounding species and eventually to decrease biodiversity (Achhireddy and Singh 1984; Achhireddy *et al.* 1985; Foy and Inderjit 2001). This allows for monotypic stands to readily develop, and, once established, it is highly persistent in the environment. Persistence of lantana is due to many factors, but one in particular is its resistance to herbivory due to toxin accumulation in the leaves (Ghisalberti 2000). This, coupled with the fact that lantana can tolerate continual defoliation for 1 to 2 years (Winder 1980; Broughton 1999a), has greatly hampered the development of a biocontrol program (Broughton 2000; Baars 2003).

Lantana is considered one of the 10 most toxic weeds in the world (Sharma *et al.* 1988). Ingesting approximately 3 mg of dry leaves per kg of body weight is a toxic dose for ruminant animals (Ghisaberti 2000). Foraging of lantana by large animals can result in either acute (death within 12 to 24 hours) or chronic poisoning with the common symptoms of skin cracking and peeling (Knight and Walter 2001). Regardless of quantity eaten, cattle that show symptoms of lantana toxicity rarely recover and resume productive gains (Seawright 1963). The cattle industry is an important component of Southeast agriculture and it is critical to develop economically viable and sustainable control methods to prevent losses due to lantana.

Herbicidal control of lantana has been variable and difficult to achieve. The variability in control has been attributed to the fact that over 650 cultivars of lantana are known (Graaf 1986). Regardless of cultivar, most researchers have examined the efficacy of 2,4-D, glyphosate, and triclopyr. Glyphosate, though quite consistent (Toth and Smith 1984; Graaff 1986; Erasmus and Clayton 1992), is undesirable due to the non-target damage that is typical of its non-selective activity. In Florida, two commonly used pasture herbicides are 2,4-D and triclopyr. This is troubling since 2,4-D has frequently been shown to be inconsistent (Bartholomew and Anderson 1978; Singh *et al.* 1997), while triclopyr has little or no activity on lantana (Toth and Smith 1984; Graaff 1986). Therefore, it is important to test other herbicides that are safe on pasture grasses to determine if these possess activity on lantana. Aminopyralid is a herbicide with many structural and physical similarities to picloram (Fast *et al.* 2010). Considering the broad-spectrum activity that picloram possesses against woody brush, it is necessary to determine if aminopyralid would likewise be effective against lantana. Additionally, aminocyclopyrachlor is a new auxin-mimic herbicide that is being tested for use on woody brush (Turner *et al.* 2010). It is currently unknown if aminocyclopyrachlor possess activity against lantana. The objectives of this research were to 1) determine if foliar applied herbicides could effectively control lantana, and 2) determine if herbicides applied as a basal treatment to standing plants, or to cut surfaces, will result in plant death.

## MATERIALS AND METHODS

Experiments were initiated in September 2007 in Pasco County, FL and repeated in September 2009 in Lake County, FL. Both locations were livestock pastures with a naturally occurring lantana infestation of approximately one plant per 3 $m^2$.

Herbicides were applied using an all-terrain vehicle mounted sprayer calibrated to deliver 230 L/ha. Plots were 8 m wide by 16 m long with a 5 m non-treated buffer maintained between each set of sprayed plots. The non-treated buffer was used to allow accurate

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evaluation of lantana control to account for the variable density across the experimental area. Non-ionic surfactant was added to each herbicide treatment at 0.25% v/v. The experimental design was a randomized complete block with either three or four replications.

Foliar application of herbicides was initiated in the fall, approximately two months prior to the first frost. Lantana plants were not mowed prior to foliar applications. Herbicide treatments in 2007 consisted of aminopyralid (0.12 kg/ha), aminopyralid + 2,4-D ( 0.12 + 1 kg/ha), fluroxypyr (0.56 kg/ha), and fluroxypyr + aminopyralid (0.56 + 0.12 kg/ha). Each of these herbicides were applied once in the fall, or sequentially as fall followed by an additional application in the spring. In 2009, all these herbicides were applied in a similar fashion to that in 2007, with the inclusion of aminocyclopyrachlor (0.21 kg/ha).

In a separate trial at each location in 2007 and 2009, herbicides mixed with basal oil were applied to individual plants. Triclopyr[2] (20% solution), triclopyr + fluroxypyr[3] (50% solution), triclopyr + aminopyralid[4] (20 + 1%), and imazapyr[5] (3%) were applied at a volume sufficient to cover the base of the plant and the surrounding stems. Additionally, another set of plants were clipped to a height of 10 cm and the herbicide/oil mixture was applied immediately to the cut surfaces. For these experiments, each treated plant was considered a replication and ten replications per treatment were used. Aminocyclopyrachlor was not incorporated into this study because an oil miscible formulation was not available.

Visual estimates were used to evaluate percent weed control using a scale of 0 to 100 where 0 = no control and 100 = complete control. Evaluations were conducted at 2, 6, and 12 months after the final herbicide application. All data were tested for main effects and interactions using ANOVA. Treatment means were separated using Fisher's Protected LSD Test at P=0.05.

## RESULTS AND DISCUSSION

For experiments initiated in 2007, it was observed that neither aminopyralid nor aminopyralid + 2,4-D provided acceptable control at 12 months after treatment (MAT) (Table 1). At 2 and 6 MAT, the sequential treatments improved control by as much as 60%. However, both the single and sequential treatments provided less than 16% control at 12 MAT and no significant differences were observed. The application of fluroxypyr once in the fall resulted in control that was similar to the aminopyralid treatments. Though single fluroxypyr and fluroxypyr + aminopyralid application provided higher control initially, control did not differ from the single or sequential aminopyralid treatments by 12 MAT. Conversely, additional spring application of fluroxypyr or fluroxypyr + aminopyralid dramatically improved control over all other treatments. Fluroxypyr and fluroxypyr + aminopyralid provided 77 and 90% control, respectively at 12 MAT. No reports could be found that have documented the efficacy of aminopyralid or the impact of sequential applications on lantana control. Many reports have discussed the efficacy of 2,4-D (Singh *et al.* 1997; Graaff 1986), but at the rates tested in this trial, the low level of control was expected.

[2] Remedy Ultra: 480 g/L triclopyr ester. Dow Agrosciences, Indianapolis, IN 46268.
[3] Pasturegard: 180 g/L triclopyr ester + 60 g/L fluroxypyr. Dow Agrosciences, Indianapolis, IN 46268.
[4] Milestone: 240 g/L aminopyralid. Dow Agrosciences, Indianapolis, IN 46268.
[5] Chopper: 240 g/L imazapyr. BASF Corp., Research Triangle Park, NC 27709.

**Table 1.** Control of lantana from foliar broadcast applications. Applications were applied in Fall 2007 and Spring 2008.

| Herbicide | Timing | Rate (kg/ha) | 2 MAT[1] | 6 MAT | 12 MAT |
|---|---|---|---|---|---|
| | | | --------------% control[2]--------------- | | |
| Aminopyralid | Fall | 0.12 | 8 f | 6 e | 0 c |
| Aminopyralid | Fall + Spring | 0.12 | 36 e | 25 d | 16 bc |
| Aminopyralid + 2,4-D | Fall | 0.12 + 1 | 23 ef | 0 e | 6 bc |
| Aminopyralid + 2,4-D | Fall + Spring | 0.12 + 1 | 43 de | 60c | 0 c |
| Fluroxypyr | Fall | 0.56 | 68 bc | 65 bc | 12 bc |
| Fluroxypyr | Fall + Spring | 0.56 | 86 ab | 80 ab | 77 a |
| Fluroxypyr + aminopyralid | Fall | 0.56 + 0.12 | 63 cd | 53 c | 20 b |
| Fluroxypyr + aminopyralid | Fall + Spring | 0.56 + 0.12 | 97 a | 95a | 90 a |

[1] MAT – Months after treatment.
[2] Means followed by similar letters do not differ at P=0.05 level of significance.

The results in 2009 were similar to those in 2007. Aminopyralid (single and sequential), aminopyralid + 2,4-D (single and sequential), fluroxypyr (single), fluroxypyr + aminopyralid (single) all provided less than 35% control at 12 MAT (Table 2). Conversely, sequential applications of fluroxypyr and fluroxypyr + aminopyralid provided 80 and 93% control, respectively at 12 MAT. From these data it was observed that two applications of fluroxypyr are necessary if control is to approach acceptable levels. The addition of aminopyralid numerically improved control on both occasions, but was only statistically significant in 2009 at 12 MAT. Although fluroxypyr is effective, there are limitations to this program. First, two applications in close succession must be conducted. One application was shown to fail, while a fall followed by spring application was consistently successful. However, it is unknown if control from the sequential treatments will be adversely affected if the second application is delayed until summer or later. Secondly, the price of two applications of fluroxypyr + aminopyralid may likely be inhibitory for most ranchers. Currently, this treatment could cost as much as \$100/ha, not considering the cost of application (Ferrell and MacDonald 2010). Therefore, the efficacy of aminocyclopyrachlor was explored. It was observed that both single and sequential applications of aminocyclopyrachlor at rates of 0.21 kg/ha provided greater than 96% control at 12 MAT. Previous research has shown this same level of control (Toth and Smith 1984; Graaff 1986), but it was generally with glyphosate which cannot be applied broadcast to pastures due to unacceptable levels of grass injury. Conversely, aminocyclopyrachlor has been shown to be safe on numerous grass species (Enloe *et al.* 2010; Rhodes 2010). Although hundreds of lantana cultivars have been described and implicated as a reason for inconsistent herbicide control (Graaff 1986), the efficacy of aminocyclopyrachlor was striking. We cannot, from these data, draw sweeping conclusions about the efficacy of aminocyclopyrachlor on all lantana biotypes. However, aminocyclopyrachlor was greatly superior to all other treatments tested and additional research on geographically distinct populations is warranted.

**Table 2.** Control of lantana from foliar broadcast applications. Applications were applied in Fall 2009 and Spring 2010.

| Herbicide | Timing | Rate (kg/ha) | 2 MAT[1] | 6 MAT | 12 MAT |
|---|---|---|---|---|---|
| | | | --------------% control[2]----------- | | |
| Aminopyralid | Fall | 0.12 | 10 d | 0 c | 0 e |
| Aminopyralid | Fall + Spring | 0.12 | 15 c | 12 c | 20 d |
| Aminopyralid + 2,4-D | Fall | 0.12 + 1 | 5 d | 5 c | 9 de |
| Aminopyralid + 2,4-D | Fall + Spring | 0.12 + 1 | 70 b | 65 b | 35 c |
| Fluroxypyr | Fall | 0.56 | 72 b | 12 c | 12 de |
| Fluroxypyr | Fall + Spring | 0.56 | 92 a | 93 a | 80 b |
| Fluroxypyr + aminopyralid | Fall | 0.56 + 0.12 | 92 a | 5 c | 10 de |
| Fluroxypyr + aminopyralid | Fall + Spring | 0.56 + 0.12 | 95 a | 97 a | 93 a |
| Aminocyclopyrachlor | Fall | 0.21 | 47 c | 96 a | 96 a |
| Aminocyclopyrachlor | Fall + Spring | 0.21 | 98 a | 99 a | 100 a |

[1] MAT – Months after treatment.
[2] Means followed by similar letters do not differ at P=0.05 level of significance.

Experiments were also conducted to evaluate the efficacy of herbicides diluted in oil carrier applied directly to lantana stems, or freshly cut stem surfaces. Imazapyr was observed to provide excellent control at 6 MAT, but declined to 30% for basal applications and 70% for cut surface at 12 MAT (Table 3). Previous research has shown excellent control with imazapyr (Graaff 1986). Although plants in the current experiments were defoliated and stunted by the application, full regrowth was observed in many of the treated plants. Control with triclopyr alone was poor. Though triclopyr was applied at rates much higher than in previous reports, the level of control was similar (Graaff 1986). At 12 MAT, less than 27% control was observed when triclopyr was applied to stems or cut surfaces. However, the addition of fluroxypyr or aminopyralid greatly improved control over triclopyr alone. Therefore, if basal or cut surface applications are required, the addition of fluroxypyr or aminopyralid would be necessary to maximize control.

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**Table 3**. Efficacy of herbicides applied to individual lantana plants as a cut-surface or basal application.

| Herbicide[1] | Rate % v/v | Cut-surface 6 MAT[2] | 12 MAT | Basal 6 MAT | 12 MAT |
|---|---|---|---|---|---|
| | ---------------% control[3]----------- | | | | |
| Triclopyr | 20 | 53 b | 20 c | 50 c | 27 b |
| Triclopyr + fluroxypyr | 50 | 95 a | 95 a | 82 b | 80 a |
| Triclopyr + aminopyralid | 20 + 1 | 90 a | 95 a | 50c | 95 a |
| Imazapyr | 3% | 99 a | 70 b | 99 a | 30 b |

[1]Herbicides use rates were as follows: triclopyr (480 g /L), triclopyr + fluroxypyr (180 + 60 g /L), aminopyralid (240 /L), imazapyr (240 g /L).
[1] MAT – Months after treatment.

[2] Means followed by similar letters do not differ at P=0.05 level of significance.

## REFERENCES

Achhireddy, N.R., and Singh, M. (1984). Allelopathic effects of lantana on milkweed vine. *Weed Science*. 32:757-761.

Achhireddy, N.R., Singh, M., Achhireddy, L.L., Nigg, H.N. and Nagy, S. (1985). Isolation and partial characterization of phytotoxic compounds from lantana. *Journal of Chemical Ecology*. 11:979-988.

Baars, J. 2003. Geographic range, impact, and parasitism of lepidopteran species associated with the invasive weed *Lantana camara* in South Africa. *Biological Control*. 28:293-301.

Bartholomew, B.L., and Anderson, T.R. (1978). A new look at Lantana control. *Queensland Agriculture Journal*. July-Aug 339-344.

Broughton, S. (1999). Evaluation of some insect species for introduction for biological control of *Lantana camara* L. in South-east Queensland, Austriala. PhD dissertation, The Univ. of Queensland, Brisbane, Australia.

Broughton, S. (2000). Review and evaluation of lantana biocontrol programs. *Biological Control*. 17:272-286.

Day, M.D., Wiley, C.J., Playford, J., and Zalucki, M.P. (2003). *Lantana: current management status and future prospects.* ACIAR, Canberra, Australia. pp.2-105.

Enloe, S.F., Tredaway Ducar, J., and Dorough, H.D. (2010). Weed control with aminocyclopyrachlor in Alabama pastures. *Proceedings Southern Weed Science Society* 63:162.

Erasmus, D.J., and Clayton, J.N.G. (1992). Towards costing chemical control of *Lantana camara*. *South African Tydskr. Plant Grond*. 9:206-210.

Fast, B.J., Ferrell, J.A., MacDonald, G.E., Krutz, L.J., and Kline, W.N. (2010). Picloram and aminopyralid sorption to soil and clay minerals. *Weed Science.* 58:484-489.

Fensham, R.J., Fairfax, R.J., and Cannell, R.J. (1994). The invasion of *Lantana camara* in Forty Mile Scrub National Park, north Queensland. *Australian. Journal of Ecology*. 19:297-305.

Ferrell, J.A., and MacDonald, G.E. (2010). Approximate herbicide pricing. SS-AGR-16, Gainesville: University of Florida Institute of Food and Agricultural Sciences. http://edis.ifas.ufl.edu/wg056. Accessed May 15, 2011.

Foy, C.L., and Inderjit. (2001). Understanding the role of allelopathy in weed interference and declining plant diversity. *Weed Technology*. 15:873-878.

Ghisalberti, E.L. (2000). *Lantana camara* L. (Verbenaceae). *Fitoterapia* **71**:467–486.

Graaff, J. L. (1986). *Lantana camara*, the plant and some methods for its control. *South African Forestry Journal* 136:26-30.

Knight, A., and Walter. R. (2001). A guide to plant poisoning of animals in North America. Teton NewMedia, pp. 144.

Rhodes, G. N. (2010). Utility of DPX MAT-28 in cool-season grass pasture weed management systems. *Proceedings Southern Weed Science Society*. 63:164.

Seawright, A.A. (1963). Studies on experimental intoxication of sheep with *Lantana camara*. *Australian Veterinary Journal.* 39:340-344.

Sharma, O.P., Makkar, H.P.S., and Dawra, R.K. (1988). A review of the noxious plant *Lantana camara*. *Toxicon* 26:975-987.

Singh, R.D., Singh, B., Sud, R.K., Tamang, M.B., and Chakrabarty, D.N. (1997). Control of lantana in non-cropped area and in tea plantation. *International Journal Pest Management*. 43:145-147.

Thaman, R.R. (1974). *Lantana camara*: Its introduction, dispersal and impact on islands in the tropical Pacific Ocean. *Micronesica* 10:17-39.

Toth, J., and Smith, L.W. (1984). A low-volume, gas-powered spray gun for application of herbicides to blackberry and other woody perennials. *In* Proceedings of the 7th Australian Weed Conference (ed. R.W. Madin), Perth, Western Australia 53-63.

Turner, R.G., Pitts, J.R., Link, M.L., Hidalgo, E., and Claus, J.S. (2010). Aminocyclopyrachlor or blend products for brush and weed control on utility and roadside rights-of-ways. *Proceedings Southern Weed Science Society*. 63:181.

USDA, ARS. 2004. USDA, NRCS. 2004. The PLANTS Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Winder, J.A. (1980). Factors affecting the growth of lantana in Brazil. PhD Dissertation, Univ. of Reading, England.

# MANAGEMENT OF EMERGING WEEDS WITHIN WESTERN AUSTRALIAN WHEAT BELT

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## ABSTRACT

A recent comprehensive survey on the changes in weed spectrum within the Western Australian (WA) wheat belt shows that some emerging weeds species are becoming more problematic. A study was conducted at two locations within WA over three years to develop management practices for barley grass, brome grass (great brome) and silver grass within the WA wheat belt. In the rotation trial to control barley grass, a lupin-wheat rotation provided more effective suppression of barley grass than a wheat-wheat rotation. The herbicides (simazine, metribuzin, Select®) used in the lupin crop in 2009 provided excellent suppression of barley grass. Consequently, the herbicides used in the wheat crop in 2010 (Sakura 850 WG and Monza®) after the 2009 lupin crop also effectively suppressed barley grass where weed density was effectively reduced in the 2009 lupin crop, leading to yield increases. Sakura 850 WG and Monza® herbicides provided poor control of barley grass where its density was high in 2009, probably due to dry seasonal conditions. In the brome grass (Great brome) control trials, Sakura 850 WG, Boxer Gold® and Triflur Xcel® provided comparable suppression of brome grass plants in a wheat crop in 2009. However, Sakura 850 WG provided the greatest suppression of brome grass seed head production, followed by Boxer Gold® and Triflur Xcel®. Boxer Gold® was more toxic to the wheat crop than Sakura 850 WG or Triflur Xcel® in disc sowing systems when stubble was present in brome grass trials. However, the grain yield of wheat was not affected by early crop damage from herbicides. In silver grass trials, Boxer Gold® suppressed silver grass more effectively than Triflur Xcel® or tank mixes with other herbicides and increased wheat yield by 7 to 17% compared to Triflur Xcel®. Sakura 850 WG was not included in silver grass trials.

**Keywords:** barley grass, brome grass, silver grass, crop rotation, sowing methods, Sakura 850 WG, Boxer Gold®

## INTRODUCTION

Barley grass (*Hordeum spp)*, brome grass (great brome) (*Bromus diandrus)* and silver grass (*Vulpia myuros*) have traditionally been considered to be minor weeds, occurring in patches in certain areas within the Western Australian (WA) wheat belt. A field survey conducted in 2008 revealed that while barley grass numbers have remained consistent, brome grass and silver grass have decreased in infestation within the wheat belt (Michael et al. 2009). However, according to the growers' responses in a postal survey in 2008, the ranking of the top worst weeds has changed within the wheat belt in the last decade. Barley grass and brome grass have moved from fifth and sixth positions in 1997 up to third and fourth positions in 2008, suggesting that these two species have become increasingly problematic between 1997 and 2008 (Figure 1).

Prolific annual seed production by these weeds is the main reason for their persistence rather than carryover of dormant seeds in the soil. It is important therefore to reduce the seed production in these weed species. Some of these weeds have evolved resistance to ACCase herbicides and bipyridiliums in South Australia, New South Wales, Victoria and WA (Hashem et al. 2008, Heap 2011). Evolution of herbicide resistance may be part of the reason why growers find these species to be increasingly problematic. New herbicides from groups with alternative modes of action and new crop varieties that are tolerant to herbicides with different modes of action are now available. So, a study was undertaken to develop management packages for these three weeds, incorporating the new weed management tools that are now available.

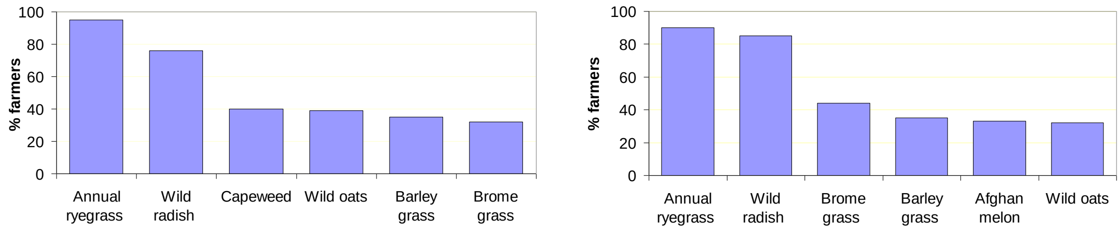


**Figure 1:** Changes in the worst weed species between 1997 (left) and 2008 (right) based on the observations of 168 growers within Western Australia wheat belt (Michael et al. 2009).

## MATERIALS AND METHODS

### Barley Grass Trials

A two-year rotation trial (2009 to 2010) was established in a Beverley WA field containing a naturally occurring infestation of barley grass. Crop rotations included lupin-wheat and wheat-wheat (wheat cv Eagle Rock (metribuzin-tolerant) in 2009 and Magenta in 2010, lupin cv Mandelup). Herbicide treatments are indicated in Table 1. Note that Sakura® is a registered product of Bayer. Sakura 850 WG is not currently registered in Australia but an application has been made for registration. The untreated control plots in 2009 remained as the untreated control plots in 2010. In both years, barley grass suppression was visually assessed at anthesis. Barley grass head number was counted in 2010. At harvest, grain yield of wheat and lupin was recorded in both years.

### Brome Grass Trial

A two-year trial (2009 to 2010) was established in a Northam WA field containing a naturally occurring infestation of brome grass (great brome). In 2009, herbicide treatments (Table 2) were applied and incorporated by sowing of wheat cv Wyalkatchem under three sowing methods (single disc, knife point or minimum tillage), in the presence or absence of wheat stubble. In 2010, Sakura 850 WG was incorporated by sowing wheat cv Magenta (using a disc sowing system) in all plots except the untreated control. Measurements in both years included brome grass plant number at five weeks after crop emergence, brome grass head numbers at the heading stage, crop phytotoxicity and crop grain yield.

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### Silver Grass Trials

Two separate trials (2008 and 2009) were conducted in a Beverley WA field with a dense infestation of silver grass. A range of pre-seeding herbicide treatments (Table 3) were applied in the wheat crop during each year. Untreated control plots were included in both trials. In each trial, silver grass head number was counted at anthesis and grain yield was recorded at harvest.

## RESULTS AND DISCUSSION

### Barley Grass Suppression

Barley grass suppression in the lupin-wheat rotation was greater than that in the wheat-wheat rotation in 2009. As a result, yields of both crops in the lupin-wheat rotation were greater than their yields in the other rotation (Table 1). The herbicides used in the lupin crop (Metribuzin® @ 150 g/ha or simazine @ 1.1 kg/ha) followed by Select® @ 250 mL provided 87-92% suppression of barley grass in the lupin crop in 2009. This also resulted in significant reduction in barley grass in the subsequent wheat crop treated with Sakura 850 WG @ 118 g/ha in 2010. Poor barley grass control in the wheat-wheat rotation by Metribuzin® @ 150 g/ha followed by Monza® @ 25 g/ha or Triflur Xcel® @ 2 L/ha followed by Monza® @ 25 g/ha in 2009 also resulted in poor barley grass control in subsequent wheat treated with Sakura 850 WG @ 118 g/ha followed by Monza® 25 g/ha in 2010.

It appears that where Sakura 850 WG @ 118 g/ha was used against a dense population of barley grass in wheat crops in the wheat-wheat rotation, weed suppression was poor. Sakura 850 WG @ 118 g/ha is usually highly effective against barley grass in normal seasonal conditions, and it is likely that Sakura 850 WG performance in 2010 was negatively affected by the dry conditions, making this herbicide less effective against dense populations of barley grass.

**Table 1.** Effects of herbicides in two crop rotations on initial barley grass suppression, barley grass head number and crop yield in 2009 and 2010 at Beverley, WA[1].

| Crops and herbicides | | Weed suppression (%) | | Barley grass (heads/m$^2$) | Wheat or lupin yield (kg/ha) | |
|---|---|---|---|---|---|---|
| 2009 | 2010 | 2009 | 2010 | 2010 | 2009 | 2010 |
| Lupin-wheat rotation | | | | | | |
| 1. Lupin (Metribuzin®/ Select®) | Wheat (Sakura 850 WG/ Monza®) | 87 | 88 | 118 | 1619 | 2226 |
| 2. Lupin (Simazine®/ Select®) | Wheat (Sakura 850 WG/ Monza®) | 92 | 83 | 90 | 1298 | 2084 |
| 3. Untreated | Untreated | 0 | 0 | 859 | 481 | 370 |
| LSD (5%) | | 20.6 | 17.6 | 377.4 | 219.5 | 206.8 |
| Wheat-wheat rotation | | | | | | |
| 1. Wheat (Metribuzin®/ Monza®) | Wheat (Sakura 850 WG/ Monza®) | 51 | 50 | 802 | 1376 | 1353 |
| 2. Wheat (Triflur Xcel®/ Monza®) | Wheat (Sakura 850 WG/ Monza®) | 39 | 53 | 663 | 1246 | 1258 |
| 3. Untreated | Untreated | 0 | 0 | 1352 | 468 | 196 |
| LSD (5%) | | 7.7 | 9.4 | 525.2 | 246.1 | 236.8 |

[1]Metribuzin® @ 150 g/ha (metribuzin 750 g/kg), Monza® @ 25 g/ha (sulfosulfuron 750 g/kg), Select® @ 250 mL/ha (clethodim 240 g/L), Sakura 850 WG @ 118 g/ha (pyroxasulfone 850 g/kg), Simazine® @ 1.1 kg/ha (simazine 900 g/kg), and Triflur Xcel® @ 2 L/ha (trifluralin 500 g/L). Metribuzin®, Simazine®, Triflur Xcel®, and Sakura 850 WG were incorporated by sowing. Monza® and Select® were applied at post-emergence.

## Brome Grass Suppression

In the untreated plots, brome grass density was 156 and 125 plants/m$^2$ and brome grass head number was 298 and 226 heads/m$^2$ in 2009 and 2010 respectively. In 2009, all herbicides provided a comparable reduction in brome grass numbers, but seed head reduction was the greatest in Sakura 850 WG @ 118 g/ha, and greater in Boxer Gold® @ 2.5 L/ha compared to Triflur Xcel® @ 2 L/ha. In 2010 (when Boxer Gold® was not included in the trial), Sakura 850 WG @ 118 g/ha again provided effective suppression of brome grass. Weed suppression by Sakura 850 WG @ 118 g/ha in 2010 was slightly less effective where weed density was high due to poor suppression by Triflur Xcel® @ 2 L/ha in 2009. Herbicide effectiveness was generally reduced in 2010, probably due to the dry seasonal conditions. Herbicide application increased wheat grain yield by 10-16% in 2009 and 23-31% in 2010, compared to the untreated control (Table 2). Despite the early

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greater crop phytotoxicity observed in the disc sowing system in 2009, final grain yield for Boxer Gold® @ 2.5 L/ha was not significantly lower than for Triflur Xcel® @ 2 L/ha.

In 2009, early crop phytotoxicity was apparent for all pre-seeding herbicide treatments in the retained stubble plots sown using a disc system, with 48% damage to the wheat crop from Boxer Gold® @ 2.5 L/ha, 18% from Triflur Xcel® @ 2 L/ha and 14% from Sakura 850 WG @ 118 g/ha. The knife point system did not cause a significant level of crop phytotoxicity (5% or less for all herbicides). Wyalkatchem wheat (grown in 2009) is a short coleoptile variety, and so is more prone to pre-seeding herbicide damage in years such as 2009, where rainfall causes movement of the herbicide into the seeding furrows, than a long coleoptile variety. This result highlights the need to maintain seeding depth selectivity of the herbicide. Phytotoxicity was not observed in 2010.

**Table 2.** Effect of pre-seeding herbicides on the suppression of brome grass density, brome grass seed head production and wheat yield in a wheat-wheat rotation in 2009 and 2010 at Northam, WA[1].

| Herbicide treatments | | Brome grass (plants/m$^2$) | | Brome grass (heads/m$^2$) | | Wheat yield (kg/ha) | |
|---|---|---|---|---|---|---|---|
| 2009 (wheat) | 2010 (wheat) | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 |
| Boxer Gold® | Sakura 850 WG | 81 | 31 | 111 | 64 | 2531 | 1848 |
| Sakura 850 WG | Sakura 850 WG | 85 | 29 | 48 | 63 | 2781 | 1920 |
| Triflur Xcel® | Sakura 850 WG | 101 | 83 | 169 | 104 | 2590 | 1723 |
| Untreated | Untreated | 156 | 125 | 298 | 226 | 2332 | 1318 |
| LSD (5%) | | 27.8 | 18.1 | 56.2 | 36.4 | 195.8 | 200.1 |

[1]Boxer Gold® @ 2.5 L/ha (120 g/L s-metolachlor + 800 g/L prosulfocarb), Sakura 850 WG @ 118 g/ha (pyroxasulfone 850 g/kg), Triflur Xcel® @ 2 L/ha (trifluralin 500 g/L). All the herbicides were incorporated by sowing.

## Silver Grass Suppression

Silver grass head numbers in the untreated control were 304/m$^2$ in 2008 and 651/m$^2$ in 2009. Boxer Gold® @ 2.5 L/ha suppressed 83% of silver grass heads in 2008 and 82% in 2009. Triflur Xcel® @ 1.45 L/ha suppressed 76% of silver grass heads in 2008 and 72% in 2009 (Table 3). The mixture of Triflur Xcel® @ 1.45 L/ha plus Avadex Xtra® did not improve silver grass head control compared to Triflur Xcel® @ 1.45 L/ha alone, in either year. Sakura 850 WG was not included in this study.

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**Table 3:** Effect of Boxer Gold® and other pre-seeding herbicides on the suppression of silver grass and yield of wheat in 2008 and 2009 at Beverley, WA.

| Herbicide treatments | Silver grass head suppression (%) | | Wheat yield (kg/ha) | |
|---|---|---|---|---|
| | 2008 | 2009 | 2008 | 2009 |
| Boxer Gold® | 83 | 82 | 3822 | 3502 |
| Triflur Xcel® | 76 | 72 | 3550 | 2891 |
| Boxer Gold® + Triflur Xcel® | 92 | 85 | 3125 | 3029 |
| Triflur Xcel® 1.45 L/ha + Avadex Xtra® | 79 | 67 | 3543 | 2658 |
| Untreated | 0 | 0 | 3140 | 2569 |
| LSD (5%) | 4.9 | 9.2 | 561 | 296 |

[1]boxer gold® @ 2.5 l/ha (120 g/l s-metolachlor + 800 g/l prosulfocarb), avadex xtra @ 1.6 l/ha (triallate 500 g/l), and triflur xcel® @ 1.45 l/ha (trifluralin 500 g/l). all herbicides were incorporated by sowing.

Weed control by Boxer Gold® @ 2.5 L/ha significantly increased wheat yield over the untreated control both in 2008 and 2009 (18-27%). Triflur Xcel® @ 1.45 L/ha or a mixture of Boxer Gold® @ 2.5 L/ha + Triflur Xcel® @1.45 L/ha increased wheat yield over the untreated control in 2009 only. The grain yield of wheat in the mixture of Boxer Gold® @ 2.5 L/ha + Triflur Xcel® @ 1.45 L/ha was significantly lower than the yield from Boxer Gold® @ 2.5 L/ha alone, even though this mixture provided better suppression of silver grass than Boxer Gold® @ 2.5 L/ha alone in 2008. Therefore, this mixture may have caused some crop damage, even though crop emergence and crop vigour appeared to be unaffected by this mixture in either year.

## CONCLUSION

Barley grass suppression was more effective in the lupin-wheat rotation, leading to greater yields of crops than in the wheat-wheat rotation. Initial suppression of brome grass by Boxer Gold®@ 2.5 L/ha, Sakura 850 WG @ 118 g/ha and Triflur Xcel® @ 2 L/ha was comparable in a wheat-wheat rotation. However, brome grass seed head reduction was the greatest in Sakura 850 WG @ 118 g/ha. Where stubble was present, crop damage occurred if Boxer Gold® @ 2.5 L/ha, Sakura 850 WG @ 118 g/ha or Triflur Xcel® were applied using a disc sowing system (due to heavy rainfall after sowing and use of a short coleoptile wheat variety) but not in a knife point sowing system. Silver grass head suppression was significantly greater in Boxer Gold® @ 2.5 L/ha, leading to 7-17% greater wheat yield than Triflur Xcel® @ 2.5 L/ha. A mixture of Boxer Gold® @ 2.5 L/ha and Triflur Xcel® @ 1.45 L/ha improved silver grass suppression but did not increase wheat grain yield, presumably due to some degree of crop damage. The results clearly demonstrate that the new pre-seeding herbicides such as Boxer Gold® and Sakura 850 WG were effective on the emerging weeds in this study.

## REFRENCES

Hashem A., Borger C. and Pathan S. (2008). Brome grass has developed multiple resistance to Group B and C herbicides. In Douglas, A. (ed). *Agribusiness Crop Updates – Weed Updates*. Department of Agriculture and Food Western Australia, Perth. pp 41-44.

Heap, I. (2011). International survey of herbicide resistant weeds. http//www.weedscience.org/in.asp. (Weed Science Society of America, USA).

Michael P.J., Mandel R., Hashem A., D'Antuono M.F., Bowran D. and Keally J. (2009). Changes in weed occurrence in the agricultural region of south-west Western Australia over the past decade. In Scott, J. (ed). *Western Australian Weeds Conference 2009: Fostering Solutions for the Future*. Weed Science Society of Western Australia, Perth. pp 10-10.

# CHARACTERIZATION OF *KRICT PX2* XYLANASE FROM THE *PAENIBACILLUS* SP. HPL-002 FOR UTILIZATION OF PLANTS AS BIO-RESOURCES

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## ABSTRACT

A new alkalophyllic endo-1, 4-beta-xylanase gene, *KRICT PX2* (GU967374) isolated from *Paenibacillus* sp. HPL-002 (KCTC11410BP) was expressed in *E. coli* and the biochemical properties of the purified enzyme was investigated. The specific activity of the purified xylanase was 51.26 µmol/min/mg proteins. And also, $K_m$ and $V_{max}$ values of the protein for birch wood xylan were verified to have 0.061 µM and 55.3 µmol/min/mg proteins, respectively. The optimum pH and temperature for the activity of the enzyme were pH 8~9 and 50°C, respectively, and also the activity were stably maintained at 40°C. Most metallic salts, ethylenediamine tetra-acetic acid, 2-mercaptoethanol, phenylmethane sulphonyl fluoride, and furfural have no impact on the enzyme activity at 1 mM. The simulated 3-D structure of this xylanase is similar to *Xyn*10B from *Paenibacillus barcinonensis*. Further research on the degradation of different-origin xylans and enzyme production will be necessary for the practical application.

**Keywords**: alkalophyllic xylanase, cloning, expression, *Paenibacillus* sp. HPL-002

## INTRODUCTION

Xylan is a complex polysaccharide comprising a backbone of xylose residues linked by β-1, 4-glycosidic bonds with the main chain composed of β-xylopyranose residues. It is the most common hemicellulosic polysaccharide in cell walls of land plants, representing up to 15-30% and 7-12% of the total dry weight in hardwood from angiosperms and softwood from gymnosperms, respectively (Saha, 2003). Chemical hydrolysis of lignocelluloses results in hazardous byproducts such as phenolic compounds from lignin degradation, furan derivatives (furfural and HMF) from sugar degradation, and aliphatic acids (acetic acid, formic acid, and levulinic acid). They are considered to be fermentation inhibitors generated from chemical conversion of lignocellulosic biomass (Palmqvist and Hahn-Hagerdal, 2000). Consequently, the use of microbial enzymes which are specific in action for xylan hydrolysis has been accepted as an environmentally friendly option (Wong et al, 1988, Biely et al, 1997). Bioconversion of xylan has received much attention because of its practical applications in various agro-industrial processes: delignification of paper pulp, digestibility enhancement of animal feedstock, clarification of juices, and improvement in the consistency of beer. Currently, there is great interest in the utilization of xylose essential for cost-reductive conversion of lignocellulosic materials to fuel ethanol and other value-added fermentation products (Ragauskas et al, 2006). Due to structural heterogeneity of xylan, complete degradation of this biopolymer requires synergistic action of different xylanolytic enzymes such as endo-xylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase, and esterase. Among them, endo-xylanase (1, 4-β-D-xylan xylohydrolase, EC 3.2.1.8) is considered as the most important one and it initiates the degradation of xylan into xylose and xylooligosaccharides of different sizes (Collins et al, 2005). There are different types of xylanases varying in substrate specificities, primary sequences, folds and physicochemical properties and these are produced by a number of bacteria and fungi (Wong et al, 1988; Howard, 2003). Owing to the increasing biotechnological importance of xylanases, much attention has been paid to discover new xylan-degrading enzymes, which can be applicable in various industrial processes (operating) at high temperature and broad range of pH condition. Thus, many attempts are being made to isolate new strains and to discover more relevant xylanases (Collins, 2005; Li et al, 2009). Through the consecutive collection of microorganisms and screening for biomass degrading activity, we have recently isolated a strain of *Paenibacillus* sp. HPL-002 (Korean Collection for Type Culture: KCTC11410BP) from the old discarded mushroom farm located in Gara Mt., Geoje City, Gyeongsangnam-do, Korea (ROK), showing excellent xylanase activity.

In the present study, we cloned and expressed the *KRICT PX2* gene (GenBank accession code: GU967374) in *Escherichia coli* and examined some biochemical properties of the purified enzyme for applying to agronomical residues and weeds as a biomass resources.

## MATERIALS AND METHODS

### Selection and Identification of Bacterial strain

The xylan-overlaid plates were prepared by overlaying 0.7% molten agar containing 1% birchwood xylan (Fluka) over the solidified 1% agar containing 1X M9 minimal salts (Sigma) in a plastic Petri dishes with a diameter of 87 mm. The inoculated Petri dishes were incubated for 24 h at 37ºC, and stained with 0.1 % (wt/vol) Congo red (Aldrich) for 30 min and repeatedly washed with 1 M NaCl until the transparent halo was appeared around colonies because of xylanase activity. The active bacterial isolate was identified by the 16S rRNA analysis with the two bacterial universal primers, 518F 5(5'-CCAGCAGCCGCGGTAATACG-3') and 800R (5'- TACCAG GGTATCTAATCC-3') (Lane, 1991), and the full sequence of 16S rRNA gene was analyzed and aligned with data in Ribosomal Database Project (http://rdp.cme.msu.edu) for the species identification. Image of this bacterium was taken by a scanning electron microscope (SEM 515, Philips). The identified bacterial strain was deposited to the Korean Collection for Type Culture (KCTC11410BP), KRIBB (Korean Research Institute of Bioscience and Biotechnology, Yuseong, Daejon 305-806, Korea).

### Construction and Screening of a *Paenibacillus* sp. HPL-002 Gene Library

DNA fragments (around 5 kb) were collected and purified for library construction, and blunt-end repaired and dephosphorylated, then ligated into pCB31 plasmid vector (MACROGEN Co., Korea). The packaged library was electroporated into *Escherichia coli* DH10B cells according to the manufacturer's instructions. The total 1,152 clones of transformants were collected into twelve 96-well plates containing 200 µl LB broth (Difco) in each well. After incubation for 24 h at 37°C, the xylanase activity of each clone was screened by overlaid-xylan staining (Hwang, 2010) and DNS (3,5-dinitrosalicylic acid) assay (Miller, 1959), simultaneously. The xylanase active clone in each well appeared as dark brownish color due to the reaction between DNS and the reducing sugar ends of hydrolyzed product.

### DNA Sequencing and Expression of Xylanase in *E. coli*

The most xylanase-active clone (01B3 clone, arbitrary named) was selected from the library screening, and the nucleotide sequence of the insert was determined by automated sequencing under BigDye™ terminator cycling condition. The reacted product was purified using ethanol precipitation and run with Automatic Sequencer 3730xl (Applied Biosystems, Weiterstadt, Germany). The each PCR product was inserted into pSTV28 plasmid vector and transformed into *E. coli* JM109 (Takara Bio Inc.), and the xylanase activity of each transformant was examined with xylan-overlaid plate and DNS assay in liquid. The gene was inserted into the pIVEX GST fusion vector for the transformation into *E. coli* BL21 (Roche Applied Science) to produce the recombinant fusion protein.

## Purification of Recombinant Xylanase

The transformed *E. coli* with GST-fused xylanase were grown overnight in 10 ml LB medium containing ampicillin (100 µg/ml) at $37^{o}C$ and 200 rpm in a shaking incubator. The culture was induced with 1 mM IPTG and incubated under the same conditions for 3 h longer. The cells collected from the final washing process were treated with sonic disruptor (CosmoBio Co., LTD). After cell disruption, the lysate was centrifuged at 10,000xg for 20 min at $4^{o}C$, the supernatants was eluted through the GST binding resin column (Novagen, Madison WI, USA). All fractions were examined with Bradford's protein determination, PAGE analysis, and DNS assay. Active fractions were pooled and treated with Restriction Protease Factor Xa (Roche Applied Science), and eluted through p-aminobenzamidine-agarose column (Sigma-Aldrich) according to the manufacturer's instruction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% polyacrylamide was performed to separate each protein, and the protein fractions mixed with denaturing agent were boiled for 3 min and applied to the gel. Proteins were visualized by Coomassie brilliant blue R 250 staining. The protein concentration was determined with Bradford reagent (Sigma-Aldrich) assay using bovine serum albumin as a standard.

## Properties of Recombinant Xylanase

Xylanase activity was measured according to the method as previously reported (Hwang, 2010), using 50 µl of 1% (w/v) solution of birchwood xylan (Fluka) and 200 mM of each pH buffer incubated with 30 µl of an appropriately diluted enzyme (3.3 mg/ml) for 20 min at different temperatures. The released reducing sugars were assayed using the DNS method. One unit of xylanase activity was defined as the amount of the enzyme that liberated reducing sugar ends equivalent to 1 µmol of xylose per minute under the assay conditions. The optimal temperature and pH condition for the xylanase activity of recombinant *KRICT PX2* protein were examined in 96-well micro plates with DNS assay at various temperatures (ranging from 10 to 80ºC) and pH conditions (ranging from pH 2 to 12). The effect of birchwood xylan concentration on xylanase activity was evaluated under optimal assay conditions. Xylanase activity was measured with DNS assay at 540 nm as described above. Effect of metallic ions and other chemicals on the xylanase activity of *KRICT PX2* protein was studied as described above at pH 9 with addition of 1 mM NaCl, LiCl, KCl, $NH_4Cl$, $CaCl_2$, $MgCl_2$, $MnCl_2$, $CuSO_4$, $ZnSO_4$, $FeCl_3$, $CsCl_2$, ethylenediamine tetra-acetic acid (EDTA), 2-mercaptoethanol (2-ME), dithiothreitol (DTT), phenylmethane sulphonyl fluoride (PMSF), acetate, and furfural, respectively.

## TLC Analysis of Hydrolytic Products

The hydrolyzed products of xylan was analyzed by the thin-layer chromatography (TLC) using silica gel plates 60 F 254 (Merck KGaA, Germany) as reported previously (Hwang, 2010). Aliquots (0.2 ml) of the samples were collected at 0, 15, 30, 60, 120, 240, and 480 min of the incubation period. After immediate boiling of each sample, 10 μl of each aliquot with xylo-oligomer standard and enzyme blank was spotted on a TLC plates. The plates were subsequently developed with acetonitrile:water (80:20, v/v). After elution for 2 h, the resultant plate was sprayed with a staining solution (1% diphenylamine and aniline in acetone, and 10% phosphoric acid), and heated for 10 minutes at 120°C in an oven to visualize the xylo-oligomers prior to take photographs. A xylo-oligosaccharide mixture was consisted of xylose, xylobiose, xylotriose, xylotetrose, and xylopentose (Wako Chemical and Megazyme).

### Nucleotide Sequence Analysis and Simulation of 3D Structure

Nucleotide and deduced amino acid sequences were analyzed with CLC Free Workbench, Ver. 3.2.1 (CLC bio A/S, www.clcbio.com). Related sequences were obtained from database searches (SwissPort and GenBank). The genome sequence of *KRICT PX2* was submitted to GenBank, and assigned as Accession Number GU967374. The biomolecular 3D structure of *KRICT PX2* xylanase was predicted with a deduced amino acid sequence as homology model structure compared to Xyn10B (PDB ID: 3emz) from *Paenibacillus barcinonensis* with Discovery Studio 2.5 (accelrys®).

## RESULTS

### Screening and Selection of The Bacterial Strain Degrading Xylan

From the screening of the natural bacteria, the strain of HPL-002 exhibited the highest xylanolytic activity (Figure 1A and B). Therefore, this strain was selected for further identification at the molecular level. Its full sequence of 16S rRNA gene was amplified and analyzed through a RDP tool, SeqMatch with data set involving both type and non-type strains, both environmental (uncultured) sequences and isolates, and near-full-length sequences (>1,200 bases) showing good quality sequences against total RDP data. Alignment of the 1,234 bases of 16S rRNA gene showed that it was very close to 16S rRNA genes of bacteria from genus *Paenibacillus* with similarity score range of 0.904~0.963. From this result, HPL-002 was identified as *Paenibacillus* sp., and the deposit number of KCTC 11365BP was obtained from Korean Collection for Type Culture, KRIBB. The bacterial cells were Gram-positive (data not shown) with rod shape of 1.1~1.5 x 2.5~3.0 μm in size without any flagella, and ellipsoidal spores were formed in swollen sporangia (Figure 1C). The photographs were taken after Congo red (0.1%) staining and repetitive washing with 1 M NaCl. Scanning Electron Microscope (SEM) photograph (C) of the isolated *Paenibacillus* sp. HPL-002

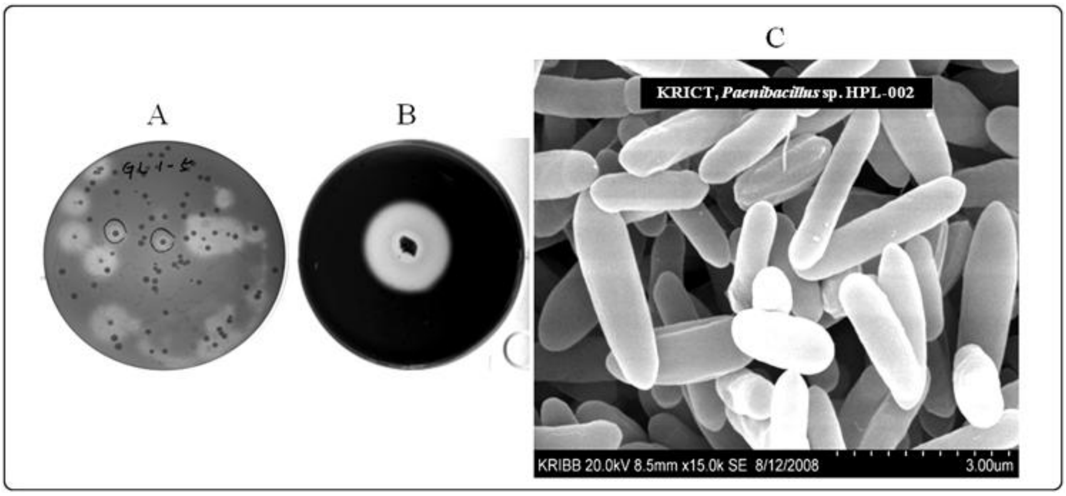


**Figure 1.** Screening of xylan degrading bacteria (A), and isolation of *Paenibacillus* sp. HPL-002 (B) in the xylan-overlaid plates.

**Purification and Characterization of Recombinant Xylanase**

DNS assay and overlaid xylan analysis of the *Paenibacillus* sp. strain HPL-002 genomic DNA library led to the isolation of single clone, arbitrary named as 01B3 (the first plate, well number of B3) with great xylanase activity. The sequence analysis of the insert in 01B3 clone exhibited that this insert has total insert size of 4,180 bps, and further ORF Finder analysis revealed that it is constructed with 7 ORFs. The final preparation of *KRICT PX2* xylanase gave a single band on SDS-PAGE (Figure 2-A). The molecular weight (MW) of the protein was estimated to be 38.4 kDa with the deduced amino acid sequence of the gene. The purified xylanase was appeared slightly over the 36 kDa MW markers. And also, the GST-fused *KRICT PX2* protein calculated as MW 64.4 kDa was observed just below the 66 kDa MW markers.

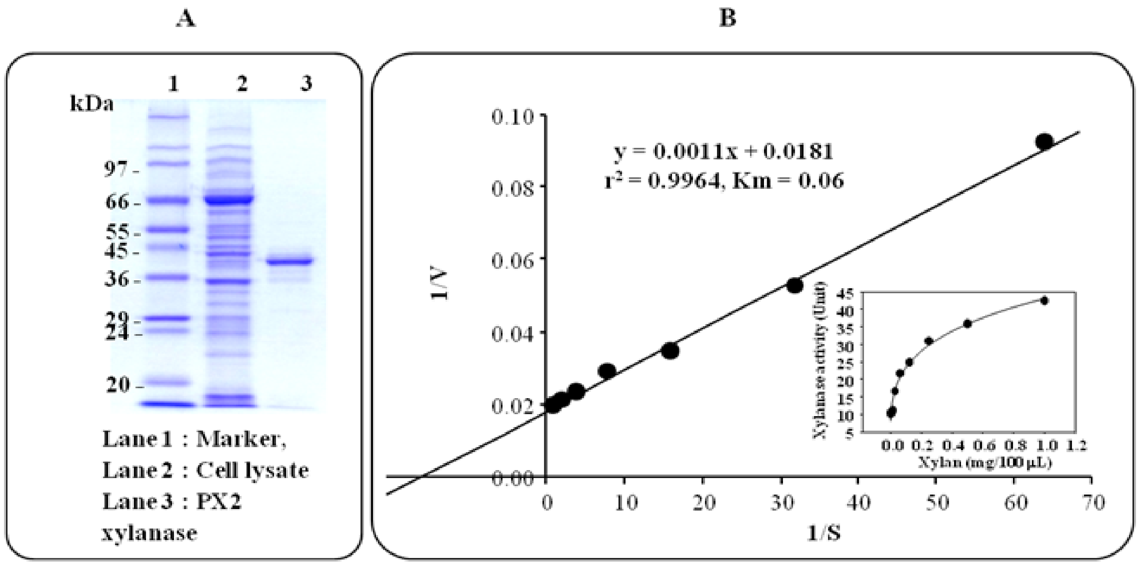


**Figure 2.** SDS PAGE analysis of the purified *KRICT PX2* xylanase (A, MW 38.4, lane 3) from cell lysate (lane 2) over-expressed with GST-fused xylanase in *E. coli* BL21 (64.4 kDa, fused with GST 26 kDa and *KRICT PX2* 38.4 kDa), and molecular weight markers (lane 1), and Lineweaver-Burk double reciprocal analysis of *KRICT PX2* xylanase with birchwood xylan as substrate.

The enzyme activity was measured with various concentrations of birchwood xylan in 50 mM citric buffer (pH 9.0) at 50°C. The optimal pH was the pH in which the enzyme displayed its maximal activity, which was considered 100% activity. The optimal pH for *KRICT PX2* xylanase activity was determined at pH 9.0, which was considered 100% activity, and retaining about 98% of its activity at pH 8.0, and about 90% of its activity at pH 7 and pH 10, respectively (Figure 3A). The optimal temperature for *KRICT PX2* xylanase activity was determined at 50°C, which was considered 100% activity, and retaining about 75% of its activity at 40 and 60$^{o}$C (Figure 3B). The *KRICT PX2* xylanase was very stable for one hour at 40°C, however, the activity of *KRICT PX2* xylanase was sharply decreased at 50°C conditions after incubation for 10 min (data not shown). Kinetic analysis of this xylanase with birchwood xylan as substrate (Figure 2) was performed at 50°C in pH 9.0. The specific activity of the purified *KRICT PX2* xylanase was 51.26 µmoles/min/mg proteins. And also, $K_m$ and $V_{max}$ values of the protein for birch wood xylan were determined as 0.061 µM and 55.3 µmol/min/mg proteins, respectively. Most salts, such as NaCl, LiCl, KCl, $NH_4Cl$, $CaCl_2$, $MgCl_2$, $MnCl_2$, $CuSO_4$, $ZnSO_4$, $FeCl_3$, $CsCl_2$ did not significantly change the enzyme activity at 1 mM. And also, 1 mM of ethylenediamine tetra-acetic acid, 2-mercaptoethanol, phenylmethanesulphonyl fluoride, and furfural were not effective on the enzyme activity (Table 1). The hydrolysis products of birchwood xylan were analyzed by TLC. As shown in Figure 4, the products hydrolyzed by the *KRICT PX2* xylanase were mainly xylobiose and much smaller amount of xylose and xylopentose. Especially, large amount of xylobiose was continuously produced from the start of the reaction.

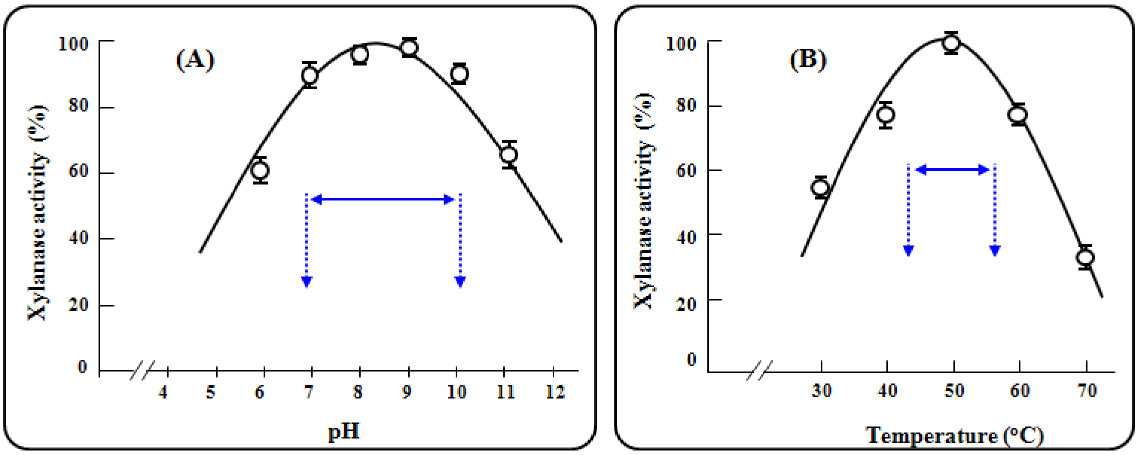


**Figure 3.** Xylanase activity of *KRICT PX2* at various pH (A) and temperature (B) conditions. The enzyme activity was assayed at 50$^{o}$C in 50 mM citric buffer (pH 2~6.5), phosphate buffer (pH 7~9), and glycine buffer (pH 9.5~12), and also assayed at different temperature in 50 mM citric buffer (pH 5.5) and glycine buffer (pH 9.5), respectively.

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**Table 1.** Effect of metallic ions and other chemicals on the xylanase activity of *KRICT PX2* protein

| Additives (1 mM) | Relative activity (%) | Additives (1 mM) | Relative activity (%) |
|---|---|---|---|
| None | 100 | $CuSO_4$ | 101 |
| NaCl | 95 | $ZnSO_4$ | 99 |
| LiCl | 101 | $FeCl_3$ | 103 |
| KCl | 104 | EDTA | 98 |
| $NH_4Cl$ | 110 | 2-ME | 115 |
| $CaCl_2$ | 116 | DTT | 115 |
| $MgCl_2$ | 108 | PMSF | 107 |
| $MnCl_2$ | 84 | Acetate | 82 |
| $CsCl_2$ | 81 | Furfural | 97 |

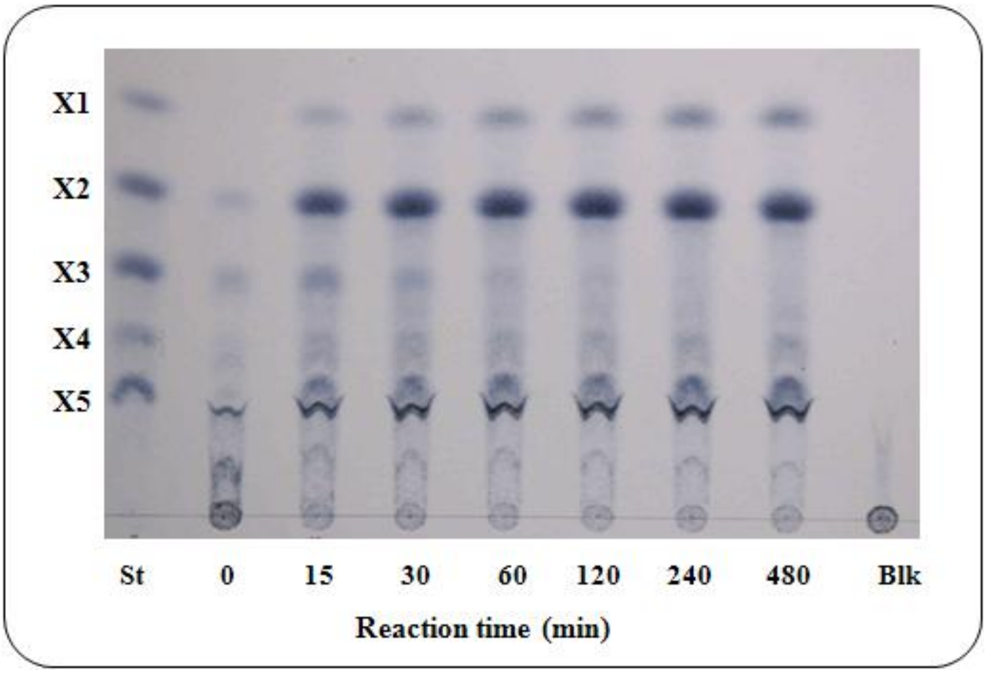


**Figure 4.** TLC analysis of the products after hydrolysis of birch wood xylan (0.5%) by *KRICT PX2* xylanase (160 ug/ml) for each reaction time of 0, 15, 30, 60, 120, 240, and 480 min in 50mM glycine buffer (pH 9.0) at 40°C. St: xylo-oligomer standards, X1: xylose, X2: xylobiose, X3: xylotriose, X4: xylotetrose, X5: xylopentose, respectively. Blk: blank without xylanase.

### Sequence Analysis and 3D Model Structure

The complete nucleotide sequence of the plasmid harboring 999bp insert of ORF5 isolated from 01B3 clone was compared with related amino acid sequences obtained from database (SwissPort and GenBank). The deduced amino acid sequence of *KRICT PX2* (GU967374) was aligned with closely-related xylanases, and turned out to be very much

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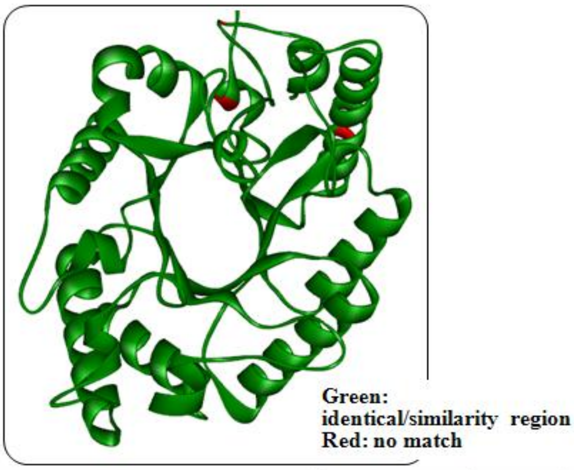
correlated with family 10 glycosyl hydralase (GH10) with many common conserved regions and similar essential amino acids for the catalytic activity as xylanase. And also, the simulated 3D structure of *KRICT PX2* appeared to have the typical (α/β) 8 barrel fold structure of family GH 10 xylanase (Figure 5).




| Template structure | Resolution | description 1 | description 2 | length | Identity | Similarity |
|---|---|---|---|---|---|---|
| 3emz | 2.08 | Crystal structure of xylanase Xyn10B from *Paenibacillus barcinonensis* | intracellular xylanase | 331 | 90% | 96% |

**Figure 5.** Xylanase *KRICT PX2* homology model structure.

## DISCUSSION

Biomass is originally photosynthesized organic materials from inorganic compounds such as $CO_2$, minerals, water and solar energy and is continuously produced by plants and microorganisms in any places on the earth surface. Cellulosic materials, such as grass and woods, are not easily digested by human enzymes (Gilbert and Hazelwood, 1993). Therefore, human cannot degrade such recalcitrant biomass but some microorganisms can do by their enzymes, cellulases, xylanases, and chitinases (Kunio et al, 2005). Xylan is the second most abundant biopolymer after cellulose and the major hemicellulosic polysaccharide found in the plant cell wall.

Xylanases have attracted considerable research interest because of their potential industrial applications to improve the quality and texture of bakery products, reduce the amount of chlorine required for bleaching of paper pulp, and increase the quality of poultry diet (Beg et al, 2001). Recently, xylanase has been refocused in fuel alcohol industry to convert xylan-rich agricultural residues such as corn stover, wheat straw, and sugar cane bagasse and energy crops such as switch grass into fermentable sugars (Dhiman et al, 2008).

Characterization experiments with the purified *KRICT PX2* xylanase revealed a great deal of information regarding the biochemical nature of this xylanase presenting a possibility for practical application of this xylanase. One of the interesting properties of this enzyme is that the optimal temperature of 45~60$^oC$ and alkalophillic pH range of 7~10 with maximal peaks at pH 9.0. The optimal temperature for *KRICT PX2* xylanase activity was 50$^oC$, and very stable for more than one hour at 40$^oC$. Gallardo et al (2003) described the pH dependence of xylanase (CAA07074) from *Paenibacillus barcinonensis*. Its optimum pH is 5.5 and shows more than 75% of maximum activity from pH 5 to 10, and still active at pH

12 with optimum temperature of 50°C. However, the activity was lost completely after incubation for 15 minutes at 50°C as like *KRICT PX2*.

From the amino acid sequence analysis and alignment with other microbial xylanase, it was confirmed that *KRICT PX2* xylanase belongs to the GH10 family with many highly conserved regions and almost similar essential amino acids for the catalytic activity (Marchler-Bauer, 2009). Xylanase *KRICT PX2* model structure is highly homologous to Xyn10B from *Paenibacillus barcinonensis*. Xyn10B is highly homologous to six xylanases of the GH10 family (XynX from A. caviae, XynA2 from *Bacillus stearothermophilus* T-6, XyaA from *Bacillus* sp. N137, Xyn2 from *B. stearothermophilus* 21, XynA from *Thermobacillus xylanilyticus*, and XynA from *Caldicellulosiruptor saccharolyticus*), and also they do not exhibit a signal peptide sequence similar to Xyn10B (Gallardo et al, 2010). This signal peptide-less xylanases form a distinctive group of enzymes that cluster separately from the rest of GH10 xylanases and seem to constitute a new type of xylanases.

The degradation profile of birchwood xylan by *KRICT PX2* xylanase monitored by the TLC analysis revealed that the major product is xylobiose with smaller amount of xylose, xylopentose, and some longer xylo-oligomers. Interestingly, the amount of xylose and xylopentose is consistently increasing in proportion to reaction time. This indicates that *KRICT PX2* is an endo-type xylanase with similar catalytic property with most GH10 xylanases (Ducros et al, 2000; Cheng et al, 2009). Furthermore, the TLC separation of product from the birchwood xylan degradation by *KRICT PX2* xylanase revealed that the produced xylo-oligomers have somewhat smaller numbers of xylose residues than other GH10 xylanases previously reported.

Considering many advantages of moderate optimum temperature, thermostability, alkalophillic pH range, good xylanolytic efficiency. *KRICT PX2* xylanase may provide a candidate for future biocatalyst development. To maximize the efficient utilization of xylan by the xylanase, *KRICT PX2* isolated from *Paenibacillus* sp. strain HPL-002 supports its further development and genetic exploitation for long-term goal to convert lignocellulosic weeds or agricultural residue biomass to alternative fuels and bio-based products.

**REFERENCES**

Beg QK, Kapoor M, Mahajan L, Hoondal GS. (2001) Microbial xylanases and their industrial applications: A review. *Appl Microbiol Biotechnol* 56:326-38.

Biely P, Vrsanska M, Tenkanen M, Kluepfel D. (1997) Endo-β-xylanases families: differences in catalytic properties. *J Biotechnol* 57:151-66.

Cheng HL, Tsai CY, Chen HJ, Yang SS, Chen YC. (2009) The identification, purification, and characterization of STXF10 expressed in Streptomyces thermonitrificans NTU-88. *Appl Microbiol Biotechnol.* 82:681–9.

Collins T, Gerday C, Feller G. (2005) Xylanases, xylanase families and extremophilic xylanases. *Fed Eur Microbiol Soc Microbiol Rev* 29: 3-23.

Dhiman SS, Sharma J, Battan B. (2008) Industrial applications and future prospects of microbial xylanases: A review. *BioResources* 3:1377-402.

Ducros V, Charnock SJ, Derewenda U, Derewenda ZS, Dauter Z, Dupont C, Shareck F, Morosoli R, Kluepfel D, Davies GJ. (2000) Substrate specificity in glycoside hydrolase family 10. Structural and kinetic analysis of the Streptomyces lividans xylanase 10A. *J Biol Chem.* 275:23020–6.

Gallardo O, Pastor FI, Polaina J, Diaz P, Lysek R, Vogel P, Isorna P, Gonzalez B, Sanz-Aparicio J. (2010) Structural insights into the specificity of Xyn10B from Paenibacillus barcinonensis and its improved stability by forced protein evolution. *J Biol Chem* 285:2721-33.

Gallardo O, Diaz P, Pastor FIJ. (2003) Characterization of a Paenibacillus cell-associated xylanase with high activity on aryl-xylosides: a new subclass of family 10 xylanases. *Appl Microbiol Biotechnol* 61:226-33.

Gilbert HJ, Hazelwood GP. (1993) Bacterial cellulases and xylanases. *J Gen Microbiol* 139:187-94.

Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. *Afr J Biotechnol* 2:602-19.

Hwang IT, Lim HK, Song HY, Cho SJ, Chang JS, Park NJ. (2010) Cloning and characterization of a xylanase, KRICT PX1 from the strain Paenibacillus sp. HPL-001. *Biotechnol Adv*. 28:594-601.

Kunio O, Kazuo S, Tetsuya K. (2005) Anaerobic bacteria degradation for the effective utilization of biomass. *Biotechnol Bioprocess Eng* 10:482-93.
Lane DJ. (1991) 16S/23S rRNA sequencing. *In*: Stackebrandt E, Goodfellow M. editors. Nucleic acid techniques in bacterial systematics. *New York: Wiley*; p. 115-175.

Li LL, McCorkle SR, Monchy S, Taghavi S, van der Lelie D. (2009) Bioprospecting metagenomes: glycosyl hydrolases for converting Biomass. *Biotechnol. for Biofuels.* 2:2-10.

Marchler-Bauer A, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, He S, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Liebert CA, Liu C, Lu F, Lu S, Marchler GH, Mullokandov M, Song JS, Tasneem A, Thanki N, Yamashita RA, Zhang D, Zhang N, Bryant SH. CDD: (2009) Specific functional annotation with the Conserved Domain Database. *Nucleic Acids Res* 37:D205-10 (Database issue).

Miller GL. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. 31:426-8.

Palmqvist E, Hahn-Hagerdal B. (2000) Fermentation of lignocellulosic hydrolyzates. II. Inhibitors and mechanism of inhibition. *Biores Technol* 74:25-33.

Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick Jr. WJ, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T. (2006) The path forward for biofuels and biomaterials. *Science* 311:484-9.

Saha BC (2003) Hemicellulose bioconversion. *J Ind Microbiol Biotechnol.* 30:279-91.

Wong KKY, Tan LUL, Saddler JN. (1988) Multiplicity of beta-1, 4-xylanases in microorganisms: functions and applications. *Microbiology and Molecul Biol Rev.* 52:305-17.

# PROPYRISULFURON, A NEW SULFONYLUREA HERBICIDE FOR RICE

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**Abstract**

Propyrisulfuron, 1 - (2 - chloro - 6 - propylimidazo [1, 2 - *b*] pyridazin - 3 - ylsulfonyl) - 3 - (4, 6 - dimethoxypyrimidin - 2 - yl) urea is a new herbicide for use in paddy rice, which has been developed by Sumitomo Chemical Co., Ltd. Results of pot tests in greenhouse studies indicated that propyrisulfuron at 25 - 100 g a.i./ha afforded excellent control of annual and perennial paddy weeds, including *Echinochloa* spp., sedges and broadleaf weeds.

Application of propyrisulfuron at 27 - 55 g a.i./ha by spraying, followed by flooding, reduced the shoot biomass of *Echinochloa oryzicola* at 3 - 5-leaf stage to under 10% of the untreated control. Propyrisulfuron was absorbed by both shoots and roots of *Echinochloa oryzicola*, however, the contribution of the herbicidal activity of root absorbed Propyrisulfuron was estimated be more important than shoot absorbed herbicide. Rice showed good tolerance to propyrisulfuron at 200 g a.i./ha.

**Keywords**: Propyrisulfuron, herbicide, paddy rice, paddy weed

## INTRODUCTION

Propyrisulfuron (Figure 1 and Table 1) is a new sulfonylurea herbicide that has a fused heterocyclic moiety (Yamato *et al*. 2010, Ikeda *et al.* 2010, Ikeda *et al.* 2011), and was commercialized in Japan, 2010. The granular formulation, suspension concentrate formulation and 'Jumbo' formulation (granules packed in water-soluble film) are available for paddy rice as a brand name of Zeta-One®.

This paper reports the properties of Propyrisulfuron, such as herbicidal activity and safety to rice.

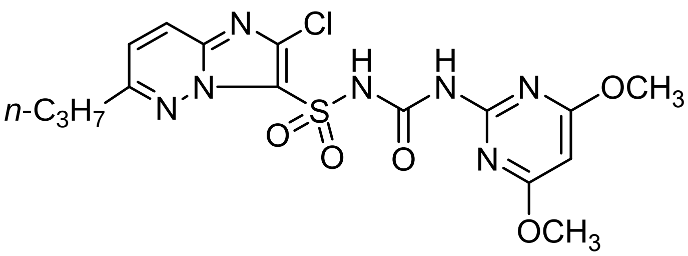

**Figure 1.** The chemical structure of Propyrisulfuron

**Table 1.** Properties of Propyrisulfuron.

| | |
|---|---|
| **Chemical and Physicochemical** | |
| Appearance | : White solid |
| Melting point | : >193.5 ºC |
| Water solubility | : 0.98 mg/L (20 ºC) |
| Partition coefficient | : Log Pow 2.9 (25 ºC) |
| Acid dissociation constant | : pKa 4.89 (20 ºC) |
| **Toxicological** | |
| Acute oral | : $LD_{50}$ > 2000 mg/kg, rat ♂ |
| Acute dermal | : $LD_{50}$ > 2000 mg/kg, rat ♂♀ |
| Acute inhalation | : $LC_{50}$ > 4300 mg/m$^3$, rat ♂♀ |
| Skin irritation | : Not irritant, rabbit |
| Eye irritation | : Slightly irritant, rabbit |
| Mutagenicity | : Negative (Ames test) |
| Common carp | : NOEC (96 hrs) >10 mg/L |

## Materials and Methods

### Herbicide

The herbicides used for testing were 0.5 g/L provisional emulsions or 100 g/L suspension concentrate. The provisional emulsions were prepared by dissolving the technical grade of propyrisulfuron in acetone including 20 g/L sorbitan monolaurate (TWEEN™ 20; Sigma, St Louis, USA), or in N, N-dimethylformamide including the 20 g/L sorbitan monolaurate.

### Weed control

Pot tests were conducted in a greenhouse using 200 cm$^2$ × 19 cm plastic pots or 100 cm$^2$ × 14 cm plastic pots filled with light clay soil (pH 6.1 - 6.5, organic matter 2.0 - 2.3 %) or sandy soil (pH 5.5, organic matter 1.9 %). After the soil was puddled and flooded, the seeds or tubers of weeds shown in Figure 3, Figure 4 and Figure 5 were planted in the pots. The weeds were grown in a greenhouse up to the leaf stages indicated in Figure 3, Figure 4 and Figure 5.

For submerged application tests, propyrisulfuron at 25 - 100 g a.i./ha was applied directly into standing water. The flooding depth was kept in 3 - 5 cm during experiments. The weed control efficacy was evaluated at 3 - 5 weeks after the application, using a visual rating scale of 0 (no effect) to 100 (completely killed) compared to an untreated control. Each test was conducted with three replications.

For spray application tests, propyrisulfuron at 50 and 100 g a.i./ha was sprayed over the top of plants. The volume rate was 500 L/ha. There was no flooding water at the application. Pots were flooded to 2 - 5 cm above the soil surface at 2 - 4 days after the application. The weed control efficacy was evaluated at three weeks after the application, using a visual rating scale of 0 (no effect) to 100 (completely killed) compared to an untreated control. Each test was conducted with two replications.

Dose responses of *Echinochloa oryzicola* at different growth stages to propyrisulfuron by spray application were investigated. Propyrisulfuron at 7 - 55 g a.i./ha was sprayed over the top of plants. The volume rate was 154 L/ha. There was no flooding water at the application. Pots were flooded to 5 cm above the soil surface at a day after the application. The shoot biomass (dry weight) was evaluated at four weeks after the application. The test was conducted with two replication.

$ED_{90}$ value, the dose giving 90 % reduction of shoot biomass compared to an untreated control, was calculated by non-linear regression using the model:

$f(x)=c+(d-c)/\{1+9\times(x/ED_{90})^b\}$ ,

where f(x) = shoot biomass, x = dose, c = lower limit of response, d = upper limit of response, b = slope (Scabenberger *et al*. 1999).

**Absorption site study**

The seedlings of *Echinochloa oryzicola* at 2.5 - 2.7-leaf stage were prepared using a seedling box for rice transplanters. The plants were transplanted in 100 $cm^2$ × 14 cm plastic pots filled with light clay soil (pH 5.5, organic matter 2.0 %). The soil was flooded at 1 cm, without making the water level reach the shoots.

The activity of propyrisulfuron at 40 and 90 g a.i./ha was evaluated under two different application practices as shown in Figure 2: (1) Practice-1. Propyrisulfuron was applied directly into standing water; (2) Practice-2. After the surface of soil was covered with polyethylene sheet, propyrisulfuron was sprayed on the foliage of the plants with a volume rate of 1000 L/ha. The sheet was removed immediately after the application.

The shoot biomass (dry weight) was investigated at four weeks after the application. The test was conducted with three replications.

The data of the herbicide-treated plants were analyzed by the two-way ANOVA with the factors of ‘dose’ and ‘practice’ to determine the effect of the application practices on the weed control efficacy.

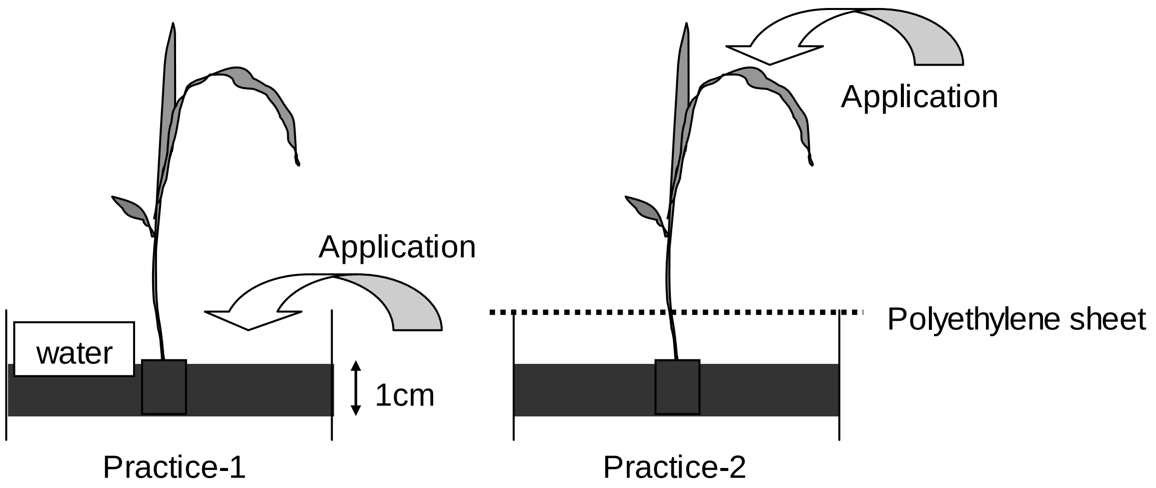


**Figure 2.** The application practices to investigate the absorption site of Propyrisulfuron.

**Safety to rice**

Pot tests were conducted in a greenhouse using 100 $cm^2$ × 14 cm plastic pots filled with light clay soil (pH 6.1, organic matter 2.2 %). After the soil was puddled and flooded, the seeds of rice (cv. Nipponbare and Basmati 370) were planted in the pots. The rice plants were grown in a greenhouse up to 3 - 4-leaf stage.

Propyrisulfuron was sprayed over the top of plants with a volume rate of 200 L/ha. The spray solution included 1 % Hasten™, a crop oil concentrate. There is no flooding water at the application. Pots were flooded to 4 cm above the soil surface at three days after the application.

The influence of propyrisulfuron on rice growth was evaluated by comparing shoot biomass (dry weight) with an untreated control at three weeks after the application.

**Statistical analysis**

The statistical analysis mentioned above (non-linear regression and ANOVA) was carried out with the software package, JMP™ ver. 4 (SAS Institute Japan Ltd.).

**Weed control**

In the pot experiment, propyrisulfuron controlled troublesome weeds of paddy fields, including *Echinochloa*, annual and perennial sedges, and annual and perennial broad leaf weeds at 25 - 100 g a.i./ha by submerged application (Figure 3).

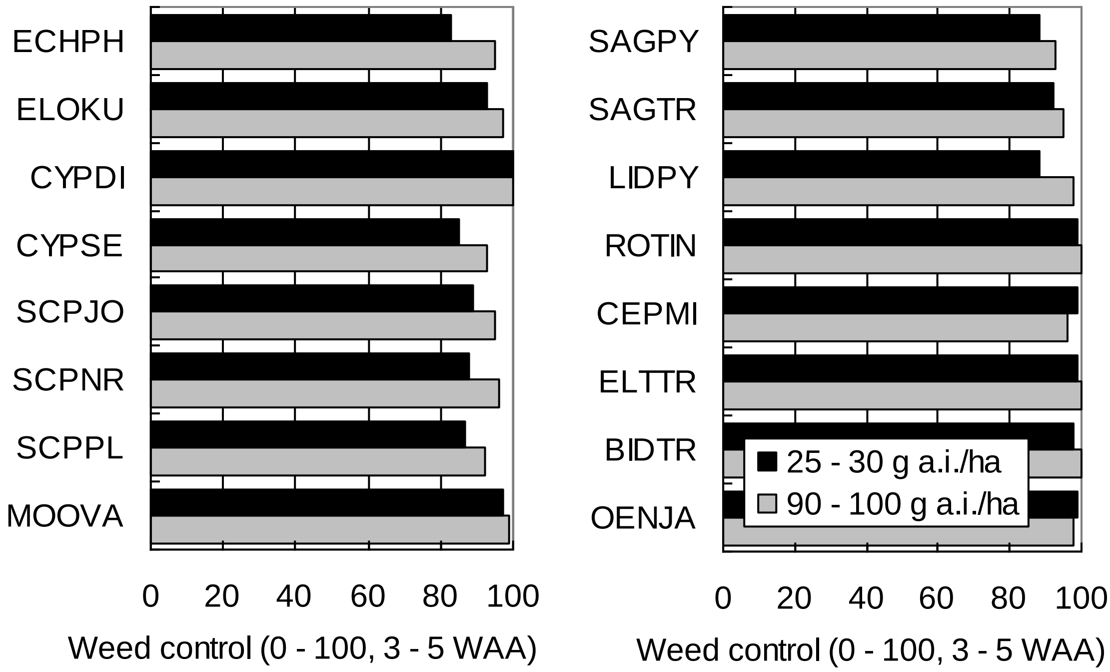


**Figure 3.** Herbicidal activity of Propyrisulfuron by submerged application. Tested plants and their growth stages at the application were: ECHPH, *Echinochloa oryzicola* (2.5-leaf stage); ELOKU: *Eleocharis kuroguwai* (9 - 10 cm plant height); CYPDI, *Cyperus difformis* (2-leaf stage); CYPSE, *Cyperus serotinus* (2 - 3-leaf stage); SCPJO, *Schoenoplectus juncoides* (2.5-leaf stage); SCPNR, *Schoenoplectus nipponicus* (2-leaf stage), SCPPL: *Bolboschoenus koshevnikovii* (3 - 4-leaf stage); MOOVA, *Monochoria vaginalis* (2-leaf stage); SAGPY, *Sagittaria pygmaea* (1 - 2-leaf stage); SAGTR, *Sagittaria trifolia* (2-leaf stage); LIDPY, *Lindernia procumbens* (1-leaf stage); ROTIN, *Rotala indica* (1 - 2-leaf stage); CEPMI, *Centipeda minima* (1-leaf stage); ELTTR, *Elatine triandra* (1 - 2-leaf stage); BIDTR, *Bidens tripartita* (1-leaf stage); OENJA, *Oenanthe javanica* (at emergence). Medians of averaged data of several tests were presented.

Propyrisulfuron also controlled *Echinochloa,* sedges and broad leaf weeds at 50 - 100 g a.i./ha by spray application followed by flooding 2 - 4 days after the application (Figure 4).

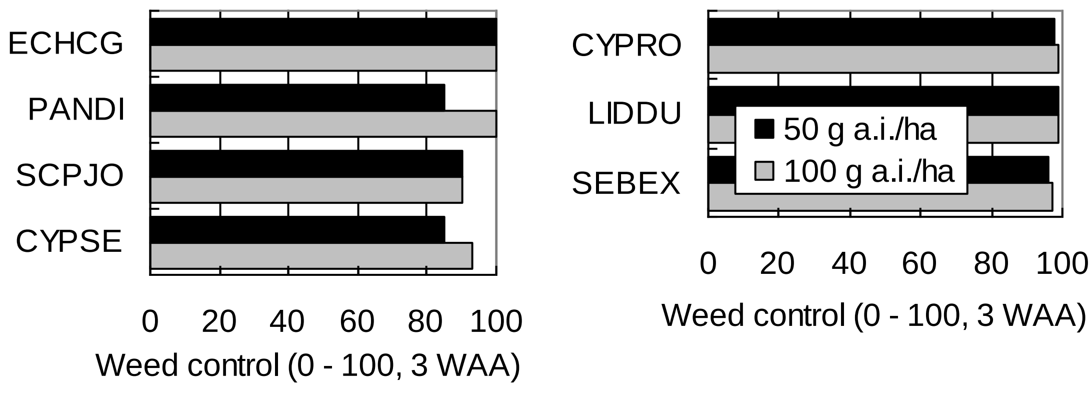


**Figure 4.** Herbicidal activity of Propyrisulfuron by spray application followed by flooding. Tested plants and their growth stages at the application were: ECHCG, *Echinochloa crus-*

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*galli* (4 - 5-leaf stage); PANDI, *Panicum dichotomiflorum* (4 - 5-leaf stage); SCPJO, *Schoenoplectus juncoides* (13 - 19 cm plant height); CYPSE, *Cyperus serotinus* (35 - 44 cm plant height); CYPRO, *Cyperus rotundus* (21 - 38 cm plant height); LIDDU, *Lindernia dubia* (6 - 8-leaf stage); SEBEX, *Sesbania exaltata* (4-5-leaf stage). Averaged data were presented.

Propyrisulfuron at 27 - 55 g a.i./ha reduced the shoot biomass of *Echinochloa oryzicola* at 3 - 5-leaf stage to under 10 % of the untreated control by the spray application followed by flooding a day after application (Figure 5).

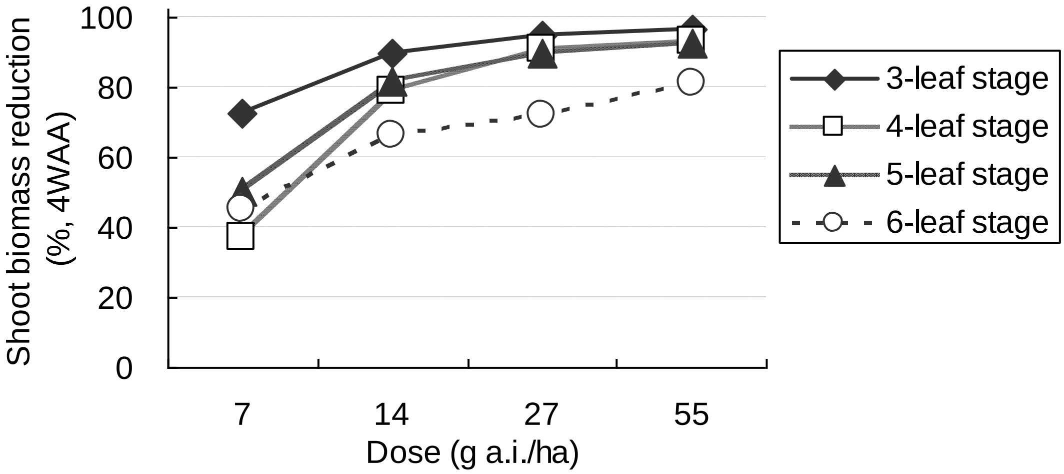


**Figure 5.** The activity of Propyrisulfuron against *Echinochloa oryzicola* at different growth stage (leaf stage) by spray application followed by flooding. Averaged data were presented.

$ED_{90}$ values, the doses of propyrisulfuron giving 90 % reduction of shoot biomass, were calculated by non-linear regression analysis as: 3 - leaf stage, 12.0 g a.i./ha; 4 - leaf stage, 16.0 g a.i./ha; and 5 - leaf stage, 14.8 g a.i./ha.

Propyrisulfuron at 25 - 100 g a.i./ha showed a wide spectrum by both submerged application, which is a common practice in Japan and Korea, and spray application followed by flooding, which is a common practice in most rice cultivating area in the world.

## Absorption site study

By both application practices, mentioned above (Figure 2), propyrisulfuron reduced the shoot biomass of *Echinochloa oryzicola* (Figure 6). This result suggested that the herbicide was absorbed by both shoot and root systems of plants. However, the herbicidal activity of propyrisulfuron absorbed by roots was higher than that absorbed by shoots.

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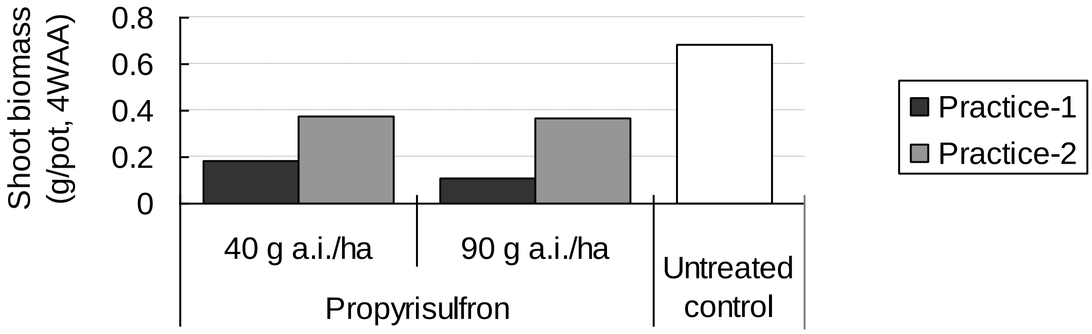


**Figure 6.** The activity of Propyrisulfuron against *Echinochloa oryzicola* by two different application practices. Application methods were described in MATERIALS AND METHODS and shown in Figure 2. The herbicide was absorbed from roots in Practice-1 and from shoots in Practice-2. Averaged data were presented.

The result of ANOVA on the shoot biomass of the plants treated with propyrisulfuron, showed that the factor ‘practice’ had the significant effect under the probably level of 0.005. There were no significant effect of ‘dose’ and no significant interaction between factors ‘dose’ and ‘practice’ (Table 2).

**Table 2.** Two-way ANOVA of the effects of the dose of Propyrisulfuron (40 - 90 g a.i./ha) and the application practice (described in MATERIALS AND METHODS and shown in Figure 2) on the biomass of *Echinochloa oryzicola* treated with Propyrisulfuron.

| Factor | Degrees of freedom | Sum of squares | F | p-value |
|---|---|---|---|---|
| Dose | 1 | 0.0050 | 0.4833 | 0.5066 |
| Practice | 1 | 0.1539 | 14.8699 | 0.0048 |
| Dose x Practice | 1 | 0.0037 | 0.3585 | 0.5659 |
| Error | 8 | 0.0828 | | |
| Total | 11 | 0.2454 | | |

From this result, even in spray application over the top of plants, the contribution of the herbicide absorbed from roots was estimated be more important. It follows from this that water condition of soil after application may affect the herbicidal activity. The appropriate water management after the spray application of propyrisulfuron remains to be investigated.

**Safety to rice**

Propyrisulfuron caused no practical damage against two cultivars of direct seeded rice at 50 and 200 g a.i./ha (Figure 7). Regarding transplanted rice, propyrisulfuron at 90 g a.i./ha has been used in Japan and its safety was confirmed in public trials conducted by the Japan Association for Advancement of Phyto-Regulators.

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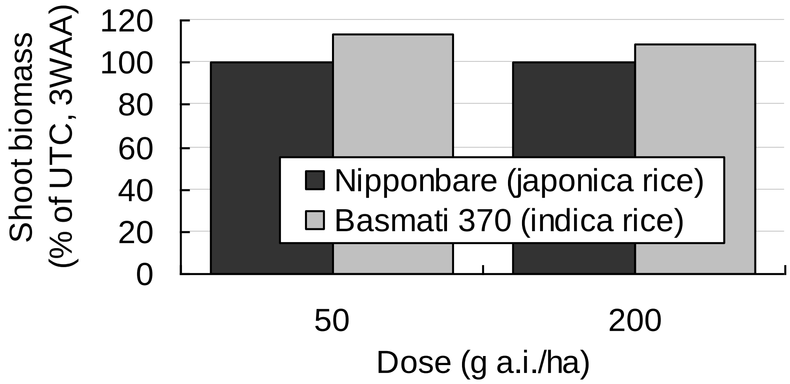


**Figure 7.** Influence of Propysulfuron on rice growth by spray application followed by flooding. Averaged data were presented. The growth stage of rice at the application: Nipponbare, 3 - 4-leaf stage, 27 - 32 cm plant height; Basmati 370, 3 - 4-leaf stage, 20 - 24 cm plant height.

## Conclusion

The results of the pot experiments showed that propyrisulfuron was a promising rice herbicide offering a wide spectrum of weed control in rice, including grasses such as *Echinochloa*, and several sedges and broad leaf weeds.

## References

Ikeda, H., Nishiyama, T., Tsukamoto, O., Kajiwara, Y., Yamato, S. and Tanaka, Y. (2010) Characterization of propyrisulfuron as a new herbicide for paddy rice – 1. Herbicidal activity. *J. Weed Sci. Technol.* 55(suppl.):15.

Ikeda, H., Yamato, S., Kajiwara, Y., Nishiyama, T., Tabuchi, T. and Tanaka, Y. (2011) Evaluation of novel sulfonylurea derivatives with a fused heterocyclic moiety as paddy herbicides that control sulfonylurea-resistant weeds. *Weed Biol. Manag.* in press.

Schabenberger, O., Tharp, B.E., Kells, J.J. and Penner, D. (1999) Statistical tests for hormesis and effective dosages in herbicide dose response. *Agron. J.* 91:713-721.

Yamato, S., Kajiwara, Y., Ikeda, H., Nishiyama, T., Tabuchi, T., Tanaka, Y. (2010) Study on a new paddy rice herbicide propyrisulfuron - synthesis and herbicidal activity of propyrisulfuron and its related derivatives. P*roceedings the 35th Annual Meeting of Pesticide Science Society of Japan.* 89.

# INTRODUCTION OF A NONDESTRUCTIVE METHOD FOR THE INVESTIGATION OF HERBICIDE EFFICACY IN GREENHOUSE BIOASSAYS BASED ON IMAGE ANALYSIS

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## ABSTRACT

The North China Plain (NCP) is the main winter wheat growing region in China. In winter wheat, *Bromus japonicus* is an important annual monocotyledonous weed, which is difficult to control and causes high yield losses as a consequence for lack of effective herbicides. For this reason dose-response studies were conducted in a greenhouse to test the efficacy of several herbicides on *B. japonicus*. Two ACCase inhibitors, five ALS inhibitors and one PS(II) inhibitor were tested at six different application rates. Efficacy on *B. japonicus* was measured by dry weight assessment as well as non-destructively by leaf coverage measurements with a bi-spectral camera. The efficacy of the tested ACCase and PS(II) inhibitors was not satisfactory for weed management, even at high dosages. However the tested ALS inhibitors showed high efficacies. Coverage measurements with the bi-spectral camera correlated very well with the dry matter weight of *B. japonicus*. Therefore it can serve as an easy, rapid and non-destructive method to determine herbicide efficacy over time and also under field conditions. It is necessary to introduce new herbicides to the NCP for efficient weed control, to ensure the security of the national food supply. Therefore comprehensive dose-response studies are necessary to evaluate herbicides of interest with different modes of action on all important weeds of the NCP.

**Keywords:** North China Plain, Japanese brome, *Bromus japonicus,* herbicide, bi-spectral camera, coverage

## INTRODUCTION

The North China Plain is the most important winter wheat growing region of China. With about 50 % of the national yield, production of wheat in the NCP makes a substantial contribution to the national food security (Wu et al., 2006, Zhang et al., 1999). Besides other limiting factors such as water supply, weed infestation can also strongly reduce winter wheat yield. During two surveys performed in 2009 and 2010, we noted that *B.japonicus* was an abundant and widespread grass weed in winter wheat, and was difficult to control with the available herbicides. Infestation can result in large yield losses. Furthermore, we noticed that herbicide application rates required to achieve control were often excessive, which results in increased selection pressure and potential for resistance development to progress more rapidly.

For these reasons, dose-response studies were conducted in a glasshouse with the aims (1) to evaluate the efficacy of different herbicides, which are not available in the NCP for control of *B.japonicus*, (2) to screen different herbicides with respect to resistance of

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*B.japonicus* and in addition (3) to test coverage measurement with a bi-spectral camera for assessment of herbicide activity.

## MATERIALS AND METHODS

### Plant Material and Growth Conditions

Seeds of the Chinese biotype of *B.japonicus* were derived from agricultural sites from the Hebei province in the NCP. The sensitive biotype was derived from Herbiseed, UK.

Experiments were conducted in a greenhouse with day/night length of 12 h and temperature regime of 15 °C/5 °C. Seedlings were transplanted in the 1-leaf-stage (BBCH 11).

### Herbicides

Eight herbicides were selected for dose-response studies; these included fenoxaprop-p-ethyl, pinoxaden + clodinafop, iodosulfuron + mesosulfuron, flucarbazone and isoproturon which are common in the NCP, and pyroxsulam + florasulam, propoxycarbazone and sulfosulfuron which are not currently available in the NCP. Each herbicide was applied at six application rates, with the highest rate consisting of the recommended field rate (Table 1).

**Table 1.** Herbicides and their application rates used in the experiment.

| HRAC-class | Commercial name | Active ingredient | Application rates used [g a.i./ha] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 |
| A | Ralon Super® | fenoxaprop-P-ethyl | 63.60 | 31.80 | 15.90 | 7.95 | 3.98 | 1.99 |
| A | Axial + Topik 100® | pinoxaden + clopdinafop | 56.73 | 28.37 | 14.18 | 7.09 | 3.55 | 1.77 |
| B | Broadway® | pyroxsulam + florasulam | 20.04 | 10.02 | 5.01 | 2.51 | 1.25 | 0.63 |
| B | Atlantis WG® | iodosulfuron + mesosulfuron | 18.00 | 9.00 | 4.50 | 2.25 | 1.13 | 0.56 |
| B | Attribut® | propoxycarbazone | 66.34 | 33.17 | 16.59 | 8.29 | 4.15 | 2.07 |
| B | Monitor® | sulfosulfuron | 20.00 | 10.00 | 5.00 | 2.50 | 1.25 | 0.63 |
| B | Everest 70 WG® | flucarbazone | 29.40 | 14.70 | 7.35 | 3.68 | 1.84 | 0.92 |
| C2 | Arelon flüssig® | isoproturon | 1500 | 750 | 375 | 187.5 | 93.75 | 46.88 |

### Application

Herbicides were applied to *B. japonicus* in the 3-leaf stage (BBCH 13) with a laboratory sprayer (Aro, Langenthal, Switzerland) equipped with a broadcast nozzle (8004 EVS, TeeJet Spraying Systems Co., Wheaton, IL, USA) using a velocity of 800 mm/s, at a height over plants of 500 mm, a water volume of 400 l/ha and a pressure of 300 kPa.

### Assessment of Herbicide Activity

Leaf-coverage of *B.japonicus* was measured three weeks after herbicide application with a bi-spectral camera. Herbicide activity on *B.japonicus* was visually rated and afterwards aboveground biomass was harvested and dried for 48 h at 80 °C before dry weight was obtained.

## Bi-spectral Camera

The bi-spectral camera takes pictures simultaneously in the infrared (> 700 nm) and red (620-660 nm) wave lengths. Both images were overlaid and normalized to a similar grey level intensity before creating a differential image (infrared-red).The differential image enhances the contrast between green leaf matter and background. This facilitates the calculation of a binary image by setting a grey level threshold (Figure 1).
From this binary image, plant coverage was calculated by counting number of white pixels and dividing by the total number of pixels.

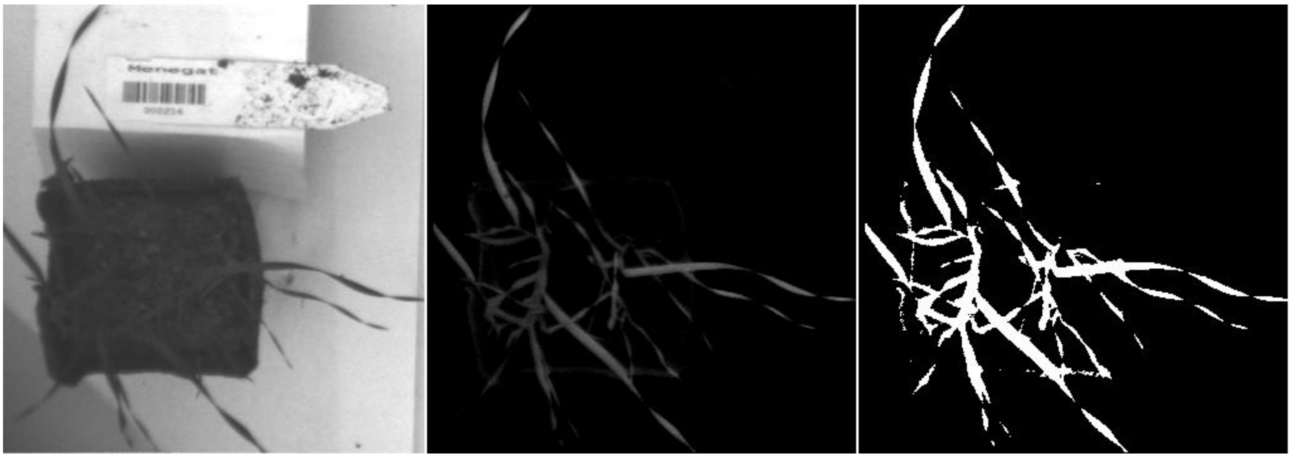

**Figure 1.** Infrared (left), differential (middle) and binary (right) image of *B. japonicus.*

## Experimental Design and Statistical Analysis

Experimental layout consisted of a completely randomized design with 4 replications for each treatment. One replication consisted of four *B.japonicus* plants per pot.

Statistical analysis was performed using the statistical software *R (*R Development Core Team 2011). Dose-response analyses were performed with the *R* extension package drc (Ritz and Streibig, 2005). For comparison of biotypes, data were normalized according to Streibig (1995), the Streibig-function was fitted using the following equation $y = C + ((D – C) / (1 + \exp(b \ln(x / ED50))))$ (Streibig, 1988) and ED50-values were tested on mean differences (F-test, $\alpha$=0.05).

For comparison of coverage measurements and dry weight measurements, Pearson's product-moment correlations were done with the procedure cor.

## RESULTS

## Herbicide Efficacy and Resistance

The ACCase-inhibitors fenoxaprop-p and pinoxaden + clodinafop did not control *B.japonicus,* even at the highest dosage. The PS(II)-inhibitor isoproturon as well as the ALS-inhibitor flucarbazone, with an activity of 63.75 % and 66.25 %, respectively, did not show satisfactory inhibitory activity as well (Table 2). The tested ALS-inhibitors pyroxsulam + florasulam, iodosulfuron + mesosulfuron, propoxycarbazone and sulfosulfuron all showed high efficacies, however none of these herbicides was able to completely control *B.japonicus.* The herbicides sulfosulfuron and pyroxsulam + florasulam with 83.75 % and

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82.5 % control, respectively, showed the highest efficacies of the herbicides under evaluation.

**Table 2.** Efficacy of herbicides on the Chinese biotype of *B. japonicus* at the recommended field rate.

| herbicide | Mean herbicide efficacy on *B.japonicus* [% activity] |
|---|---|
| Fenoxaprop-p-ethyl | 7.5 |
| Pinoxaden + Clodinafop | 0 |
| Pyroxsulam + Florasulam | 82.5 |
| Iodosulfuron + Mesosulfuron | 71.25 |
| Propoxycarbazone | 78.75 |
| Sulfosulfuron | 83.75 |
| Flucarbazone | 66.25 |
| Isoproturon | 63.75 |

Comparison of ED50-values of the NCP-population with the "sensitive" standard showed no indication of evolved resistance of *B. japonicus* against the herbicides commonly used in the NCP, as observed in Figure 2 and Figure 3 for the herbicides flucarbazone and iodosulfuron + mesosulfuron.

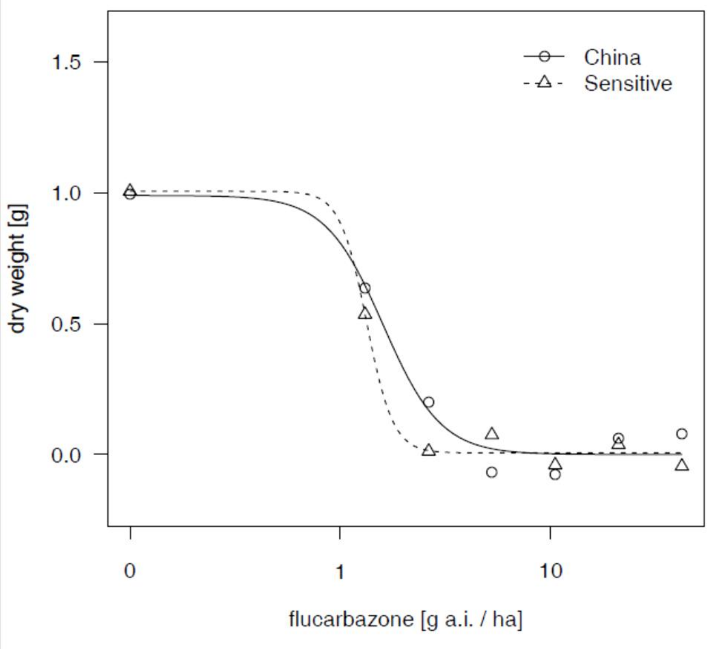


**Figure 2**. Dose-response relationship for the herbicide flucarbazone upon dry weight [g] of the Chinese and "sensitive" biotypes of *B. japonicus*.

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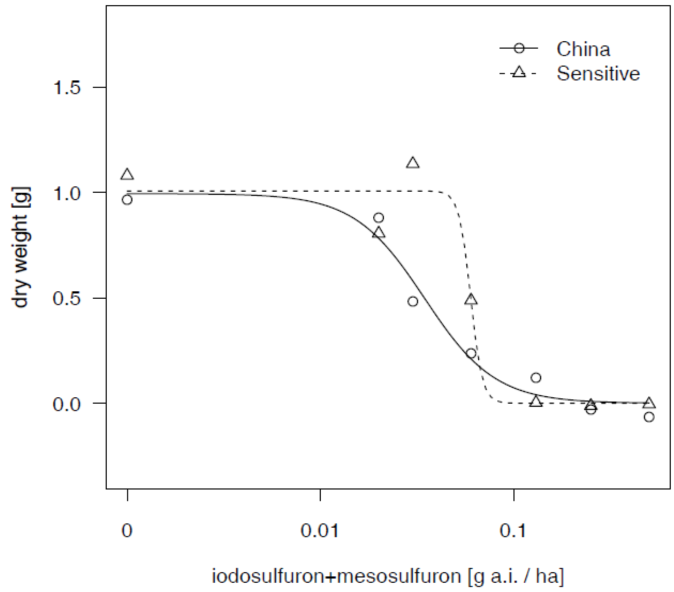


**Figure 3.** Dose-response relationship for the herbicide iodosulfuron + mesosulfuron upon dry weight [g] of the Chinese and "sensitive" biotypes of *B. japonicus*.

## Comparison of Methods

Correlation analyses showed that coverage measurements with the bi-spectral camera correlated very well with dry weight data. Each correlation was significant and correlation coefficients ranged between 0.62 and 0.93 (Table 3). Furthermore it was possible to fit the Streibig-function to the coverage data (Figure 4) so that identification of herbicide resistance would be potentially possible using the method of coverage measurement with bispectral camera as well as for method using dry weight measurement.

**Table 3.** Correlation coefficients of different treatments and biotypes.

| herbicide | biotype | Correlation coefficient r |
|---|---|---|
| Fenoxaprop-p-ethyl | China | 0.62 |
| | Sensitive | 0.81 |
| flucarbazone | China | 0.77 |
| | Sensitive | 0.80 |
| Iodosulfuron + mesosulfuron | China | 0.85 |
| | Sensitive | 0.83 |
| isoproturon | China | 0.82 |
| | Sensitive | 0.82 |
| pinoxaden + clodinafop | China | 0.68 |
| | Sensitive | 0.78 |
| propoxycarbazone | China | 0.92 |
| pyroxsulam + florasulam | China | 0.92 |
| | Sensitive | 0.87 |
| sulfosulfuron | China | 0.93 |
| | Sensitive | 0.86 |

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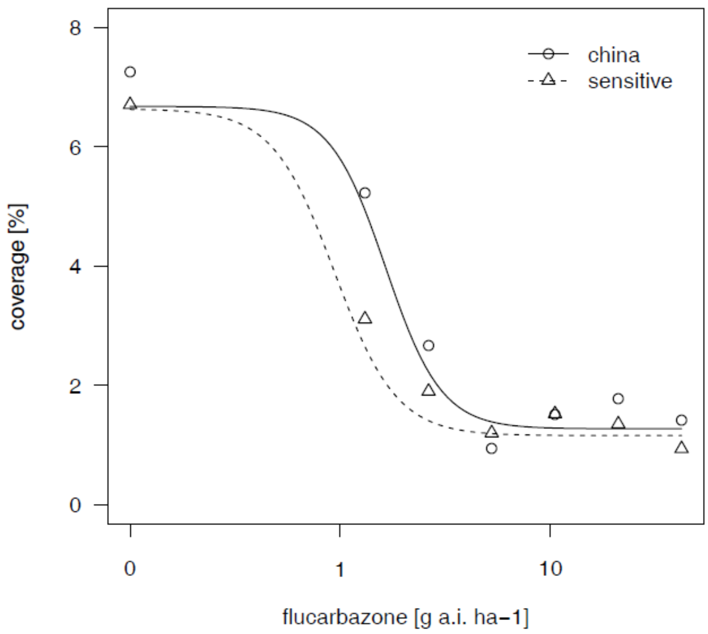


**Figure 4.** Dose-response relationship for the herbicide flucarbazone upon coverage [%] of the Chinese and “sensitive” biotypes of *B. japonicus*.

## DISCUSSION

The dose-response study showed that none of the herbicides available in the NCP have sufficient inhibitory activity on *B. japonicus.* The additionally tested herbicides propoxycarbazone, pyroxsulam + florasulam and sulfosulfuron, which are not currently available in the NCP, had the highest activities on *B. japonicus,* but did not result in complete control. These results reveal the necessity of introducing more effective herbicides to the NCP for control of *B. japonicus* in winter wheat together with additional methods for cultural weed control.
Herbicide resistance of *B. japonicus* against herbicides common in the NCP was not detected in this screening trial. Obviously, the herbicides commonly used in the NCP had a much lower efficacy than the ALS-inhibitors, which have not yet been introduced to the NCP. This could be an indication that there is an evolved tolerance of *B. japonicus* to these herbicides and that the “sensitive” standard used in this study was not really sensitive. This could be seen in tendencies in the dose-response studies, for example, of iodosulfuron + mesosulfuron where the sensitive standard was less susceptible to the herbicide than the biotype from the NCP. The only group of herbicides which showed satisfactory control was the group of ALS-inhibitors.

However, many weeds have evolved rapid resistance to ALS inhibitors; in the USA, resistant populations exist in Kansas to propoxycarbazone, pyroxsulam and sulfosulfuron (Peterson 2007). Those herbicides showed the highest activity in the dose-response-studies, but are not currently available in the NCP. Due to issues associated with known resistance to herbicides, a resistance management program is indispensable when introducing new herbicides to the NCP in which other weed control strategies than herbicides are utilized for sustainable control of *B. japonicus.*

Comparison of dry weight and coverage methods for assessment of herbicide efficacy and resistance showed that evaluation of coverage is a suitable method for detecting herbicide

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efficacy as well as herbicide resistance. For assessment of herbicide activity it is was more useful than even dry weight assessment. Herbicide activity is better modeled in this system because an activity of 100 % equals green leaf surface coverage of 0 %, whereas with dry weight measurements a residual biomass will persist and it is not possible to accurately predict herbicide activity from this data. That is to say, use of a bispectral camera to assess coverage provides equivalent data to visual assessment of herbicide activity, with the difference that the coverage assessment method is more precise. It is an easy method to employ, especially with high throughput. This technique, in addition, is non-destructive so that assessment of the dynamics of herbicide activity is possible and the optimal timing of herbicide activity assessment can be met.

Future research should be conducted to develop comprehensive dose-response studies to evaluate more effective herbicides for the most abundant and serious weeds of the NCP.

**ACKNOWLEDGEMENT**

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**REFERENCES**

Peterson, D. (2007): http://www.weedscience.org/Case/Case.asp?ResistID=5447

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ritz, C., Streibig, J.C. (2005): Bioassay analysis using R. *Journal of Statistical Software* 12: 1-22.

Streibig, J.C. (1988): Herbicide bioassay. *Weed research* 28: 479-484.

Streibig, J.C., Walker, A., Blair, A.M., Anderson-Taylor, G., Eagle, D.J., Firedlander, H., Hacker, E., Iwanzik, W., Kudsk, P., Labhart, C., Luscombe, B.M., Madafiglio, G., Nel, P.C., Pestemer, W., Rahman, A., Retzlaff, G., Rola, J., Stefanovic, L., Straathof, H.J., Thies, E.P. (1995): Variability of bioassays with metsulfuron-methyl in soil. *Weed Research* 35 (4): 215-224.

Wu, D., Yu, Q., Lu, C., Hengsdijk, H. (2006). Quantifying production potentials of winter wheat in the North China Plain. *European Journal of Agronomy* 24: 226-235.

Zhang, H., Wang, X., You, M., Liu, C. (1999): Water-yield relations and water-use efficiency of winter wheat in the North China Plain. *Irrigation Science* 19: 37-45.

# WEED BIOSECURITY BREACH THROUGH COCO PEAT IMPORTS

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## ABSTRACT

Coco peat (coir or coconut fibre) is widely used in the nursery industry. During 2008/09 some 44 plant species were identified in imports of the coco peat, with 31 species being new to New Zealand. These weeds comprised a multitude of growth forms from semi-aquatic to dryland, prostrate to small trees and included grasses, sedges and broadleaf species. Several plant nurseries around New Zealand were found to have been affected by this incursion. The biosecurity breach was managed at these sites through a combination of methods, including hand removal and the use of a pre-emergence herbicide. Risk assessments concluded that these weeds pose a low risk to New Zealand, primarily because they are tropical and not well suited to New Zealand's temperate climate.

**Keywords:** Coco peat, biosecurity, weed seeds, weed incursion, risk assessment.

## INTRODUCTION

Since humans started domesticating plants for their own use, they have been responsible for moving species around the globe. By 8000 BC all the major land masses except Antarctica were occupied by humans. Prior to the agricultural revolution plants had to be collected by hand so only the most important crop species would have been moved with these human migrations and there was less chance of inadvertently spreading unwanted species (McNeill 1984). However, with mechanisation and the period of great colonisation from the 1400s onwards there was an increase in the movement of plant species around the world, including ornamental garden plants. As Oceania was the last large area to be colonised and that only relatively recently, the impacts of introduced species is more evident and better documented (Atkinson and Cameron 1993). Although some introductions were strongly opposed, such as heather (*Calluna vulgaris*) 100 years ago, it was not until relatively recently (1970s) that strong measures have been put in place to limit the introduction of unwanted organisms into Australia and New Zealand. Fortunately this was in time to help curb the flow associated with the third mass invasion of new species, that originating from the increase in global travel and relative ease of importing goods by an increasingly affluent population. However, many of the common weeds of agriculture and horticulture were not deliberately introduced but were contaminants associated with something desirable. During the initial colonisation period weed seeds were often contaminants of pasture and crops seeds. Today's strict border biosecurity is designed to minimise such accidental entry pathways, but every so often a breach does occur. This paper reports on one such breach that occurred recently.

**Weed Seeds in Coco Peat**

Coco peat is processed coir (the fibrous material found between the hard, internal shell and the outer coat of a coconut) and is imported in the form of compressed briquettes which are a Group A product, MAF Import Health Standard for coco peat (Anon.). It is used extensively, either alone or in potting mix, by the nursery trade for growing potted plants and by the vegetable industry as a hydroponic substrate (Anon. 2008a). The importation of coco peat is a relatively recent event, as a result of the high cost, decreasing availability, and environmental concerns about the use of locally mined natural peat. Pure coco peat has proven to be an ideal growing medium for many New Zealand native species, especially monocots such as flax (*Phormium tenax*) and cabbage tree (*Cordyline australis*). It is also made into long briquettes for the hydroponics industry, where typically four plants of a crop such as tomatoes (*Solanum esculentum*) or cucumber (*Cucumis sativus*), are grown in each briquette for about 12 months, after which the briquettes are dumped. Diluted with bark, pumice and other materials, coco peat is included in potting mixtures and is distributed throughout New Zealand for commercial and domestic use (Anon. 2008a). Normally, nurseries control weeds in potted plants with Rout® (20 g/kg oxyfluorfen and 10 g/kg oryzalin) but this herbicide cannot safely be used on many native monocots. Potted native plants have three main markets, export, home gardens and commercial replanting.

In February 2008, an astute nurseryman discovered strange looking plants growing from the potting mix he used (Popay et al. 2008). After an initial enquiry, the suspect plants were delivered to Ruakura Research Centre, where they were acknowledged as not being presently found in New Zealand and held for further verification in a containment glasshouse. The Ministry of Agriculture and Forestry (MAF) was immediately informed and began an investigation. Inspections of other nurseries found more foreign plant species, while the source of the infestations was identified as coco peat imported from Sri Lanka. Plants from five other nurseries were taken to Ruakura for identification. They were generally grown for up to 9 months for identification, sampled for herbarium records and then destroyed. The following summer the pots were emptied and the coco peat from the bottom half of the pots collected. This was broken up and laid out in trays to a depth of 20 mm to see if any more foreign species would emerge.

Many new seedlings germinated in the trays, including several species that had not previously been found in the nurseries. In total 44 species were identified (Tables 1 and 2), all being listed in the Flora of Ceylon (Dassanayake and Fosberg 1990-2006). Of these, 31 species were deemed to be new to New Zealand (Table 1) while 13 were already present (Table 2). The seedling emergences in the trays also gave an estimate of the number of seeds in the contaminated coco peat. Assuming that when the coco peat was placed in the pots the contaminant seeds were evenly distributed and that no seeds germinated from the lower half of the 300 mm deep pots due to depth constraints (James et al 2002), the seedlings that emerged subsequently in the trays provide a good estimation of the original seed population in the coco peat. From these numbers it is estimated that the seed contamination was up to 18,000 seeds/$m^3$. The majority of the seeds were from quick maturing species with a prostrate plant form, including *Eleutheranthera ruderalis*, the two *Mollugo* spp., the two *Phyllanthus* spp., *Oldenlandia corymbosa* and *Richardia brasiliensis*. These species are first colonisers of bare ground and would possibly be first to grow on coco peat stored outside, thus explaining their presence in such high numbers.

Weed risk assessments were carried out on 22 of the early identifications (Tables 1 and 2). These generally concluded that the species would pose little or no threat to New Zealand because they were adapted to a warmer climate. Taken individually this is a reasonable assessment. However, taken collectively the risk is statistically much greater. Two of the species (*Amaranthus spinosus* and *Leucaena leucocephala*) and several of the genera are in Daehler's (1998) list of 381 global natural area invaders. Both these species have been reported in New Zealand previously but have not become established, possibly only due to low propagule pressure. Propagule pressure is regarded as the most significant factor in trying to predict why weed species become established in a new country (Kolar and Lodge 2001). The volume of imported risk goods is a good indicator of both the propagule pressure and probability that an introduction is likely to result (Reaser et al. 2008; Simberloff 2009). Daehler (1998) also found that species in the Poaceae and Fabaceae families were more likely to become invasive than members of other families. Of the introduced weeds found in coco peat, only two were Fabaceae but seven were Poaceae.

**Table 1.** List of species found in Cocopeat which are new to New Zealand.

| Species | Family (Mabberly 2008) | Plant form |
|---|---|---|
| *Brachiaria subquadripara*[1] (Trin.) A. Hitchc. | Poaceae | grass |
| *Cleome rutidosperma*[1] DC. | Cleomaceae | herb |
| *Cleome viscosa*[1] L. | Cleomaceae | herb |
| *Cyanotis axillaris* (L.) Sweet | Commelinaceae | herb |
| *Cyperus distans*[1] L. | Cyperaceae | sedge |
| *Cyperus haspan* L. | Cyperaceae | sedge |
| *Cyperus iria* L. | Cyperaceae | sedge |
| *Dactyloctenium aegyptium*[1] (L.) Willd. | Poaceae | grass |
| *Digitaria longiflora* (Retz.) Pers. | Poaceae | grass |
| *Echinochloa glabrescens* Munro ex Hook | Poaceae | grass |
| *Eclipta prostrata*[1] (L.) L. | Asteraceae | herb |
| *Eleutheranthera ruderalis*[1] (Swartz) Sch.Bip. | Asteraceae | herb |
| *Evolvulus nummularis* (L.) L. | Convolvulaceae | climber |
| *Fimbristylis complanata* (Retz.) Link | Cyperaceae | sedge |
| *Fimbristylis cinnamometorum*[1] (Vahl) Kunth | Cyperaceae | sedge |
| *Kyllinga polyphylla* Willd. Ex Kunth | Cyperaceae | sedge |
| *Lindernia ciliata*[1] (Colsmann) Pennell | Linderniaceae | herb |
| *Lindernia crustacea*[1] (L.) F. Muell. | Linderniaceae | herb |
| *Melochia corchorifolia* L. | Sterculiaceae | herb |
| *Mollugo nudicaulis*[1] Lam. | Molluginaceae | herb |
| *Mollugo oppositifolia*[1] L. | Molluginaceae | herb |
| *Muntingia calabura* L. | Tiliaceae | tree |
| *Oldenlandia corymbosa*[1] L. | Rubiaceae | herb |
| *Passiflora foetida* L. | Passifloraceae | climber |
| *Perotis indica*[1] (L.) Kuntze | Poaceae | grass |
| *Phyllanthus debilis*[1] Klein ex Willd. | Euphorbiaceae | herb |

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| | | |
|---|---|---|
| *Physalis angulata* L. | Solanaceae | herb |
| *Richardia brasiliensis*[1] Gomés. | Rubiaceae | herb |
| *Scoparia dulcis*[1] L. | Plantaginaceae | herb |
| *Spermacoce assurgens*[1] Ruiz & Pavon. | Rubiaceae | herb |
| *Trianthema portulacastrum*[1] L. | Aizoaceae | herb |

[1] Species for which a weed risk assessment was carried out.

There are other areas of concern which made this incursion a higher threat. One is the wide range of plant forms, from sedges (which generally grow best in damp environments) to grasses, herbs, shrubs, climbers and trees (Tables 1 and 2). Thus they are likely to threaten a range of environments. It also indicates that they came from a variety of sources. For example *Carica papaya* (cultivated for its fruit) and *Ficus religiosa* (cultivated for its religious association) were both found in the coco peat. Contamination with multiple species of variable types, e.g. arable and aquatic, is likely to be explained by the traditional method of sun drying husks on the ground and then soaking them in water-filled open troughs or ponds, a process called retting. Normally, if a commodity was contaminated with multiple species they would be expected to be from a similar habitat.

Another area of concern is that the growth form of some introduced species was different from that already present here. This was most noticeable with *Amaranthus deflexus*. In New Zealand it has a procumbent to decumbent growth habit and a brown flower/seedhead, while the introduced species has an upright growth habit and a more purplish flower/seedhead. *A. deflexus* is currently not as problematic in New Zealand as other *Amaranthus* spp. but a more upright form could become so due to it being a better competitor for light in crops. Hybridisation of the two growth forms of this species is also a potential risk. Furthermore, at least two of the introduced weeds (*A. deflexus* and *Erigeron karvinskianus)* are already well established here, with the latter being a serious weed of the natural environment and banned under the National Pest Plant Accord (Anon. 2008b). So although the weed risk assessments for individual species concluded they pose only a small risk, due to their origin being a much warmer climate, at least two of the introduced species are already growing well in New Zealand. The risk of one or more of the new species becoming established and problematic will increase if further entry of their seeds into New Zealand is not prevented. Many of the native monocots, grown in pure coco peat and not treated with herbicides, were destined for planting along roadsides and revegetation of construction and old industrial sites such as mines etc. This practice could have directly introduced these weeds into natural ecosystems.

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**Table 2.** List of species found in Cocopeat which are already present in New Zealand.

| Species | Family (Mabberly 2008) | Plant form |
|---|---|---|
| *Amaranthus spinosus* L. | Amaranthaceae | herb |
| *Amaranthus deflexus* L. | Amaranthaceae | herb |
| *Axonopus compressus* (Sw.) P. Beauv. | Poaceae | grass |
| *Carica papaya* L. | Caricaceae | tree |
| *Eragrostis tenella*[1] (L.) P. Beauv. Ex Roem. & Schult. | Poaceae | grass |
| *Erigeron karvinskianus* DC. | Asteraceae | herb |
| *Euphorbia hirta* L. | Euphorbiaceae | herb |
| *Ficus religiosa* L. | Moraceae | tree |
| *Kyllinga nemoralis*[1] L. | Cyperaceae | sedge |
| *Leucaena leucocephala* (Lam.) De wit | Fabaceae | tree |
| *Ludwigia hyssopifolia*[1] (G. Don.) Exell | Onagraceae | herb |
| *Mimosa pudica* L. | Fabaceae | shrub |
| *Phyllanthus amarus* Schum. | Euphorbiaceae | herb |

[1] Species for which a weed risk assessment was carried out.

**Post-Incursion Actions**

The immediate response to the incursion was to hold all current stocks of coco peat and fumigate them before they could be used. Further, no plant pots were allowed to leave the nurseries with any weeds in them. To alert nurseries regarding this issue, MAF produced two booklets containing comprehensive identification photographs of all the weeds found in the nurseries (Anon. 2008c). These were circulated to all the nurseries in New Zealand. A training folder “The Complete Story on Weeds Imported from Sri Lanka with Coco Peat” was also produced and training workshops were held for Pest Plant Officers from the most vulnerable regions (Northland, Auckland, Waikato and Bay of Plenty Regional Councils). These officers were then able to carry out nursery inspections and identify and destroy the unwanted plants.

The MAF Import Health Standard (IHS), “Importation of Coco peat and Coir fibre Products” (Anon.), was updated after a visit to Sri Lanka to conduct an audit of the processing facilities. The revised IHS now details five Options for Entry Conditions, including fumigation with ethylene oxide (Bullians et al. 2009) in the country of origin or heat treatment in New Zealand, or approved grow-out tests, either in the country of origin or in New Zealand, which show the absence of contaminants. A number of pre-border general conditions were also included in the standard to mitigate the risk of contaminated seed in coco peat, including: coco peat must be kept clean and free of seeds, pests, soil, animal material and any other contamination; no imported coco peat is to be produced from the traditional method of retting husks in open troughs or ponds; washing or rinsing of coco peat, chips or crush must be carried out with bore water, not pond or dam water; coco peat drying areas must be buffered underneath from contact with soil; and a plant free buffer zone of 3 m must be maintained around the coco peat drying areas unless concrete walls are built on the perimeter of concrete pads.

The updated IHS also details the monitoring protocols presently in place. If the goods arrive from an approved facility in the country of origin, or with approved certification of compliance (fumigation), then no inspection of the consignment is required. However, if the goods are not accompanied by the appropriate paper work, the coco peat must be held at a transition facility and inspected (grow-out testing required) before clearance is given. The Audit Team, as part of the Border Standards Directorate, oversees and audits the above for compliance and can revise the IHS as required.

It is difficult to determine the effectiveness of the above procedures in the short term. However, various Pest Plant Officers from the regional councils carry out regular inspections of nurseries and no new species were found in 2009 or 2010, which would indicate that the instigated procedures are working well.

## ACKNOWLEDGEMENTS

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## REFERENCES

Anon. *MAF Biosecurity New Zealand (Plants) Standard – Importation of Coco peat and Coir fibre Products*. Biosecurity New Zealand, P.O. Box 2526, Wellington, New Zealand. Pp. 15.

Anon. (2008a). Risk discussion document for the importation of coco peat. *MAF Biosecurity Discussion Paper No: 2008/02*. Biosecurity New Zealand, P.O. Box 2526, Wellington, New Zealand. 11 pp.

Anon. (2008b). *National Pest Plant Accord*. Ministry of Agriculture and Forestry Biosecurity New Zealand. Pp. 132.

Anon. (2008c). *New to New Zealand Weeds – A Guide to Weeds Found in Imported Coco Peat*. Biosecurity New Zealand, P.O. Box 2526, Wellington, New Zealand. Pp. 36.

Atkinson, I.A.E. and Cameron, E.K. (1993). Human influence on the terrestrial biota and biotic communities of New Zealand. *Trends in Ecology & Evolution*. 8: 447-451.

Bullians, M.S., Allison, V., Sarty, M. and Glassy, K. (2009). Methyl bromide fumigation of weed seeds in compressed coco peat. *New Zealand Plant Protection* 62: 416.

Dassanayake, M.D. and Fosberg, F.R. (1990-2006). *A Revised Handbook to the Flora of Ceylon*. 16 volumes + Index. CRC Press.

Daehler, C.C. (1998). The taxanomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. *Biological Conservation* 84: 167-180.

James, T.K.; Rahman, A., Webster, T. and Waller, J. (2002). Emergence of weeds as affected by vertical seed distribution in arable soils. *New Zealand Plant Protection* 55: 213-217.

Kolar, C.S. and Lodge, D.L. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199–204.

Mabberley, D.J. (2008). Mabberley's Plant-Book. Cambridge University Press. ISBN 978-0-521-82071-4. Pp. 1020.

McNeill, W.H. (1984). Human migration in historical perspective. *Population and Development Review*. 10(1): 1-18.

Popay, A.I., James, T.K., Sarty, M., Dickson, M. and Bullians, M.S. (2008). Pineapple leaves and coconut husks: closing biosecurity pathways to prevent further infiltration. In eds Froud, K.J., Popay, A.I. and Zydenbos, S.M. *Surveillance for Biosecurity: pre-border to pest management*. New Zealand Plant Protection Society: pp45-49.

Reaser, L., Meyerson, A. and Von Holle, B. (2008). Saving camels from straws: how propagule pressure-based prevention policies can reduce the risk of biological invasion. *Biological Invasions* 10: 1085-1098.

Simberloff, D. (2009). The Role of Propagule Pressure in Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics* 40: 81-102

# THE INFLUENCE OF SEED PRIMING ON WEED SUPPRESSION IN AEROBIC RICE

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## Abstract

Aerobic rice refers to growing dry direct seeded rice in non-saturated soils without any standing water layer. Aerobic rice cultivation is a promising approach to cope with water scarcity. However, this method of cultivation has suffered from increased problems due to weeds and from poor stand establishment. Seed priming (controlled moisture addition technique allowing seeds to be hydrated partially without radicle emergence) reduces emergence time, boosts germination percentage and favors synchronized emergence, this might have a great influence on weed suppression, seedling stand and yield. The present study was, therefore, designed to explore the possibility of adopting seed priming technique for suppressing weeds in aerobic rice. The experiment was conducted at Universiti Putra Malaysia, Malaysia with aerobic rice germplasm AERON 1 with four priming techniques: hydropriming, hardening, Zappa® priming and untreated control; and two levels of weed control: weed free and weedy. Seed priming significantly improved germination attributes, weed suppressive ability and yield of rice, whilst unprimed control exhibited inconsistent germination, poor seedling establishment and less weed competitiveness resulting lower yield. Priming increased germination percentage by 6% and reduced mean germination time by 2 days. Priming enhanced the germination index and seedling vigor index to a great extent. Reduction in weed dry matter due to priming ranged from 22 to 27 % compared to control. A positive influence of priming was also reflected in rice yield. Weed inflicted relative yield loss was curtailed by 10% as a consequence of seed priming. Among different priming techniques, priming with Zappa® solution was the best and thus may be considered as a pragmatic tool for sustainable weed management in aerobic rice.

**Key words:** Seed invigoration, Zappa®, seedling vigor and weed competitiveness

## INTRODUCTION

Rice is the largest user of fresh water consuming one third of world freshwater utilization (Barker *et al.* 1999). Traditionally, rice has been cultivated in flooded conditions mostly for higher yield and better weed management (Bouman 2003). In the last few decades, sustainability of global water resources has been a major concern (Juraimi *et al.* 2010), and declining water availability has endangered flood-irrigated rice system (Anwar *et al.*

2010). It is, therefore, crucial to find alternate ways of rice cultivation with limited water input. Among different water wise technologies, aerobic rice is considered to be the most promising one. Aerobic rice refers to growing dry direct seeded rice in non-saturated soils (aerobic soil) without any standing water layer (Bouman 2003), which minimizes water use and boosts up water productivity compared to lowland rice. Poor emergence along with uneven crop stand and high weed infestation are amongst the major constraints to aerobic rice cultivation (Balasubramanian and Hill 2002). Lack of a 'head start' and the absence of standing water layer to suppress weeds make aerobic rice highly vulnerable to weeds, causing severe yield loss (Zhao *et al.* 2007). Therefore, developing a sustainable weed management approach has been a challenge for widespread adoption of aerobic rice technology.

Seed priming refers to a carefully-controlled moisture addition technique, where seeds are allowed to be hydrated partially to that point where germination-related metabolic activities occur, but seeds do not reach the irreversible point of radicle emergence (Bradford 1986). Beneficial effects of seed priming includes increased germination rate, higher germination uniformity, better allometric (changes in growth of plant parts over time) attributes and faster emergence of seedlings (Farooq *et al*. 2007); as well as early germination and better stand establishment, priming led to crops growing faster, flowering earlier and yielding higher (Du and Tuong 2002).

Seed priming can improve the traits closely associated with weed competitiveness of rice include early height, growth rate, early crop biomass and early vigor. Various priming techniques have been employed to improve speed and synchrony of seed germination. Common techniques include pre-soaking, hardening, hormonal priming, hydropriming, halopriming, osmoconditioning, and ascorbate priming (Farooq *et al*. 2009).

Harris *et al.* (2002) reported that due to seed priming rice seedlings could compete more successfully with weeds. Clark *et al.* (2001) revealed that seed priming improved the competitive ability of maize against weed, and faster emergence along with increased vigor of a primed stand are the key factors for tolerating weeds. A robust seedling stand obtained from primed seeds enhanced weed competitiveness of wheat against weed and improved tolerance to environmental stress (Ghiyasi *et al*. 2008). Du and Tuong (2002) also observed a positive influence of seed priming on weed suppression in direct seeded rice at low seeding rate. Zhao *et al*. (2007), on the other hand, did not find any effect of seed priming on weed suppression and grain yield in aerobic rice. Therefore, it may be hypothesized that seed priming might influence the weed suppressive ability of crop.

Several studies have reported the influence of seed priming on the weed competitiveness of different crops. So far, however, there has been a little discussion on aerobic rice. Therefore, the objectives of the present study were to explore the possibility of adopting seed priming technique as a tool for weed management and to assess effects of seed priming on germination behavior, seedling vigor, phenology and yield performance of rice under aerobic soil conditions.

## Materials and Methods

A trough experiment was conducted at the Plant House, Universiti Putra Malaysia, Malaysia ($3^{o}$ 02' N, $101^{o}$ 42' E; elevation 31 m) during April to June 2010. The local climate is hot-humid-tropic with plentiful rainfall. The troughs were filled with soil having clayey texture with pH 4.8. The experiment was organized in a randomized complete block design with four replicates comprising four seed invigoration techniques: (a) unprimed seed (control), (b) hydropriming (soaking seeds in tap water at room temperature for 48 h and redrying back to original moisture content), (c) hardening (soaking seeds in tap water at room temperature for 24 h and re-drying back to initial moisture content and cycle was repeated one more) and (d) Zappa® priming (soaking seeds in Zappa® solution for 24 h followed by air drying for 12 h); and two levels of weed control: (a) weed free and (b) weedy.

Aerobic rice germplasm AERON 1 was used as the plant material in the study. Rice seeds were dry seeded following 25 cm × 15 cm spacing with 7seeds/hill in troughs containing non-saturated soil. Troughs were irrigated as necessary to maintain around field capacity throughout the growing period. Different intercultural operations and plant protection measures were conducted following standard practices.

Data were collected on germination attributes, yield, relative yield loss and phenology of rice. Weed rating was done; weed density and weed dry matter were recorded.

Data were subjected to Analysis of Variance (ANOVA) conducted by Statistical Analysis System (SAS 9). Significant differences among means were adjudged using Fisher's protected Least Significant Differences (LSD) test at $P \geq 0.05$.

## Results

### Germination behavior and seedling vigor

Seed priming treatments exhibited significant effect on germination of AERON1 (Table 1). Germination percentage and germination index were higher, while mean germination time (MGT) and days to 50% germination ($T_{50}$) were lower with primed seeds compared to control. Maximum germination percentage and germination index were obtained from the seeds subjected to Zappa® priming, followed by hydropriming. Lowest MGT and $T_{50}$ also were noted with Zappa® priming.

Seed priming with Zappa® increased germination percentage by 6 % and reduced emergence time by more than 2 days. Germination index was three times higher in Zappa® primed seeds compared with unprimed seeds. All the priming treatments increased seedling vigor (Table 1). These treatments also produced the most vigorous seedlings with 50% more vigor index compared to unprimed seeds.

In terms of seedling shoot and root lengths, seeds treated with Zappa® performed the best. Seedling dry weight was greatly enhanced by priming treatment; compared to unprimed control, seedling dry weight was increased by almost 110, 60 and 35% due to Zappa® priming, hardening and hydropriming, respectively.

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**Table 1.** Germination behavior and seedling vigor of AERON1 as influenced by seed priming.

| Priming technique | Germination behavior | | | | | Seedling vigor | | |
|---|---|---|---|---|---|---|---|---|
| | Germination percentage | Mean germina-tion time (days) | Days to 50% Germina-tion | Germina-tion index | Seedling vigor index | Shoot length (cm) | Root length (cm) | Dry matter (g) |
| Unprimed | 85.88c | 4.37a | 3.59a | 18.67c | 15.56c | 17.9c | 1.77c | 0.14d |
| Hydropriming | 88.75b | 2.59b | 2.06b | 37.14b | 23.13a | 25.7a | 2.20b | 0.19c |
| Hardening | 86.01c | 2.41c | 1.79c | 38.27b | 18.41b | 21.8b | 2.26b | 0.22b |
| Zappa® priming | 91.80a | 1.95d | 1.55d | 50.73a | 23.08a | 25.7a | 2.83a | 0.29a |
| LSD | 1.222 | 0.046 | 0.168 | 1.511 | 2.151 | 2.529 | 0.167 | 0.005 |

Within a column means sharing same alphabets are not significantly different at P≥0.05.

## Weed suppression

Seed priming showed a positive influence on weed suppression (Table 2). Weed ratings were lower in priming treatments compared with unprimed control. In rice stand obtained from unprimed seeds, weed infestation was high and was rated >7, while weed ratings were < 6 in the unprimed stand, though there were no significant differences among priming treatments. Weed dry matter also was reduced due to seed priming.

On average, priming treatments reduced 25% weed dry matter compared with unprimed control. In contrast, seed priming effect on weed density was not significant. Weed inflicted relative yield loss was lower with primed stand compared to that of unprimed one (Table 2). Relative yield loss was reduced by around 10% due to priming, but reduction was not significant among priming treatments.

**Table 2.** Seed priming effect on weed suppressive ability and phenology of AERON1

| Priming techniques | Weed suppressive ability | | | | Phenology | |
|---|---|---|---|---|---|---|
| | Weed rating (1 to 9 scale) | Weed dry weight (g/m$^2$) | Weed density (no./m$^2$) | Relative yield loss over weed free control (%) | Days to flowering | Days to maturity |
| Unprimed | 7.25a | 185.23a | 226.00 | 30.88a | 51.00a | 80.50a |
| Hydropriming | 5.50b | 143.29b | 210.50 | 20.87b | 48.75c | 77.13c |
| Hardening | 5.25b | 143.67b | 208.75 | 19.68b | 50.12b | 78.50b |
| Zappa®priming | 5.25b | 135.45b | 205.75 | 19.95b | 48.25c | 76.13d |
| LSD | 0.854 | 34.69 | 30.78 | 6.56 | 0.627 | 0.918 |

Within a column means sharing same alphabets are not significantly different at P≥0.05.

## Phenology and yield performance

Earlier flowering and maturity occurred in primed stands, compared with the unprimed one (Table 2). Days to flowering and maturity were delayed in the control treatments. Among the priming treatments, Zappa® priming required the least time to mature.

All the priming treatments were better than unprimed control in terms of yield and biomass production (Figure 1). Zappa® priming topped the list followed by hardening and hydropriming in terms of yield. The total dry matter production also was highest with Zappa® priming followed by hardening.

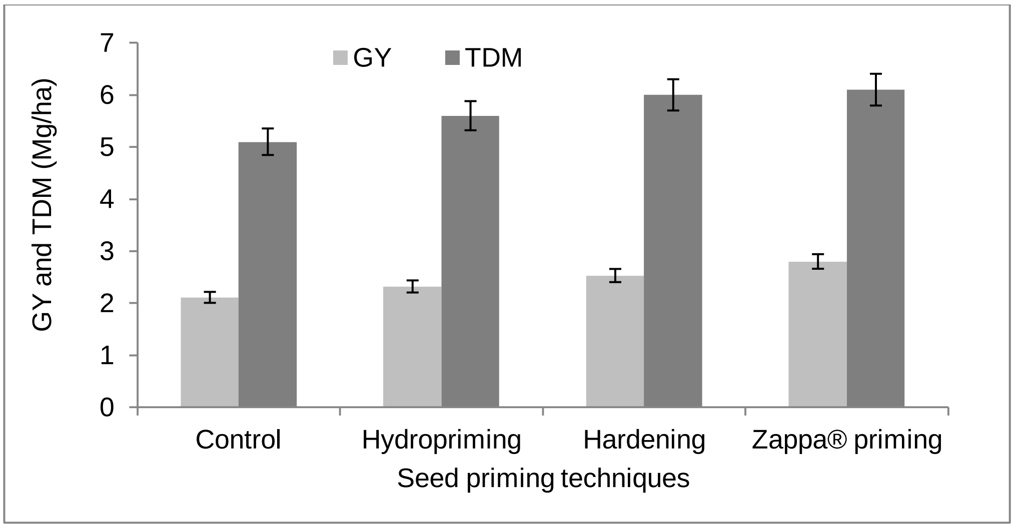


**Figure 1**. Seed priming effect on grain yield (GY) and total dry matter (TDM) of AERON 1.

## DISCUSSION

Our study demonstrated that seed priming promoted the growth of the rice plant through early ontogeny and increased its competitive ability against weeds. Seed priming enhanced seed vigor, which improved the speed and rate of seedling emergence, as indicated by higher germination percentage and germination index, and lower MGT and $T_{50}$. Faster emergence of primed seeds might be due to the completion of pre-germination metabolic activities, while higher and synchronized emergence was the consequence of reduced physiological non-uniformity in the seed bulk due to priming.

In our study, primed stand of rice was more competitive against weed than unprimed stand as reflected by lower weed rating and weed dry matter. Faster and higher emergence rate, along with vigorous stand, resulting from priming might offer rice plants a preliminary advantage to outcompete weeds (Clark *et al*. 2001; Ghiyasi *et al*. 2008). In contrast, less vigorous and poor stands of unprimed seed encouraged weed growth resulting higher weed rating and weed dry matter. There are several reports that seed priming can increase weed competitiveness of crop (Du and Tuong 2002; Harris *et al*. 2002).

However, at the same time conflicting findings have also been reported by Zhao *et al.* (2007). Seed priming led to improved phenological features of rice. Primed plants enjoyed a vigorous start, grew faster and matured earlier than unprimed counterparts.

The enhanced phenology due to priming might be attributed to rapid emergence and reduced imbibitions period. Higher number of panicle bearing tillers due to low mortality of seedlings (data not shown) resulted in increased grain yield in primed stand compared to unprimed stand. Increased rice yield due to priming has also been reported by many researchers (Harris *et al.* 1999; Farooq *et al.* 2009).

In conclusion, this study substantiates our hypothesis that seed priming can increase the weed suppressive ability of rice, and consequently, employing seed priming may help increase grain yield of direct seeded aerobic rice by reducing the risks of poor stand establishment and crop losses due to weeds.

## REFERENCES

Anwar, M. P., Juraimi, A. S., Man, A., Puteh, A., Selamat, A. and Begum, M. (2010). Weed suppressive ability of rice (*Oryza sativa* L.) germplasm under aerobic soil conditions. *Australian Journal of Crop Science* 4(9): 706-717.

Balasubramanian, V. and Hill, J. E. (2002). Direct seeding of rice in Asia: Emerging issues and strategic research needs for the 21st century, pp 15-39. In: S. Pandey et al (Eds) *Direct Seeding: Research strategies and opportunities.* IRRI, Los Banos, Philippines

Barker, R., Dawe, D., Tuong, T. P., Bhuiyan, S. I, and Guerra, L. C. (1999). The outlook for water resources in the year 2020: challenges for research on water management in rice production. *In: Assessment and Orientation towards the 21st Century*. Proceedings of the 19th session of the International Rice Commission. 7-9 September 1998, Cairo, Egypt. Food and Agriculture Organization, pp 96-109.

Bouman, B. A. M. (2003). Examining the water-shortage problem in rice systems: water saving irrigation technologies. In: Mew T.W. et al (Eds) *Proceedings of the international Rice Research Conference*, Beijing, China, 16-19 September 2002.

Bradford, K. J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Horticultural Science* 21:1105-1112.

Clark, L. J., Whalley, W. R., Ellis-Jones, J., Dent, K., Rowse, H. R., Finch-Savage, W. E., Gatsai, T., Jasi, L., Kaseke, N. E., Murungu, F. S., Riches, C. R. and Chiduza. C. (2001). On farm seed priming in maize: a physiological evaluation. Seventh Eastern and Southern Africa Regional Maize Conference, 11-15 February. pp. 268-273.

Du, L. V. and Tuong, T. P. (2002). Enhancing the performance of dry-seeded rice: effects of seed priming, seedling rate and time of seeding, In: Pandey, S., Mortimer, M., Wade, L., Tuong, T. P., Lopes, K. and Hardy, B. (Eds.), *Direct seeding:* research strategies and opportunities, international Rice Research Institute, Manilla, Philippines, pp.241-256.

Farooq, M., Basra, S. M. A. and Ahmad, N. (2007). Improving the performance of transplanted rice by seed priming. *Plant Growth Regulator* 51: 129-137.

Farooq, M., Basra, S. M. A., Ahmad, N. and Murtaza, G. (2009). Enhancing the performance of transplanted course rice by seed priming. *Paddy Water Environment* 7: 55-63.

Ghiyasi, M., Abbasi, A. M., Tajbakhsh, A. and Sallehzade, R. (2008). Effect of osmopriming withpoly ethylene glycol 8000 (PEG8000) on germination and seedling growthof wheat (*Triticum aestivum* L.) seeds under salt stress. *Research Journal of Biological Sciences* 3(9):1249-1251.

Harris, D., Joshi, A., Khan, P. A., Gothkar, P. and Sodhi, P. S. (1999). On-farm seed priming in semi-arid agriculture development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture* 35:15-29.

Harris, D., Tripathi, R. S. and Joshi, A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice, pp. 231-240. In: Pandey, S., Mortimer, M., Wade, L., Tuong, T.P., Lopes, K. and Hardy, B. (Eds.).Proceedings of the International workshop on Direct Seeding in Asian Rice systems : Strategic Research Issues and

Opportunities, 25-28 January 2000, Bangkok, Thailand. international Rice Research Institute, Los Banos, Philippines, 383 pp.

Juraimi, A. S., Begum, M., Najib, M. M. Y. and Azmi, M. (2010). Efficacy of herbicides on the control weeds and productivity of direct seeded rice under minimal water conditions. *Plant Protection Quarterly* 25(1): 19-25.

Zhao, D. L., Bastiaans, L., Atlin, G. N. and Spiertz, J. H. J. (2007). Interaction of genotype × management on vegetative growth and weed suppression of aerobic rice. *Field Crops Research* 100: 327-340.

# INDAZIFLAM – A NEW HERBICIDE FOR PRE-EMERGENT CONTROL OF WEEDS IN TURF, FORESTRY, INDUSTRIAL VEGETATION AND ORNAMENTALS

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## ABSTRACT

Indaziflam is a new pre-emergent herbicide under development for the control of weeds in turf, forestry, industrial vegetation and ornamentals. Indaziflam inhibits cellulose biosynthesis in plants. It belongs to the alkylazine group of herbicides. In turf, C4 warm-season turfgrass species generally have good tolerance to indaziflam. In forestry plantations, indaziflam can be applied pre- or post-planting to *Pinus* species and post-planting to *Eucalyptus* species. Indaziflam has been in field development in Australia since 2005. Indaziflam is used at a low rate ranging between 50 and 150g a.i./ha. Indaziflam has favourable human health and ecological profiles.

**Keywords**: Indaziflam, forestry, turf, industrial, ornamental

## INTRODUCTION

Indaziflam is a new herbicide which potentially provides pre- and post emergence control of broadleaf and grass weeds. It was first registered in 2010 for use on turf by the U.S. Environmental Protection Agency.

Indaziflam represents a new chemistry from the alkylazine chemical class. The alkylazines work by inhibiting cell wall biosynthesis. Indaziflam is the most potent inhibitor of cellulose biosynthesis so far discovered. Compared to many other pre-emergence herbicides indaziflam has a longer half-life in the soil (>150 days) which may allow for greater flexibility with application timing. Indaziflam also inhibits crystalline cellulose deposition in the plant cell wall, severely affecting cell wall formation, cell division as well as cell elongation. This means that fully developed leaves, tissues and organs are not or hardly affected by the compound since cell wall formation is already completed and no new cellulose synthesis occurs. Indaziflam is also used as a soil herbicide. It inhibits seed germination of weeds. Hence, it must be applied prior to weed emergence. Post-emergence efficacy has been seen, given right conditions, up to a weed stage of 2 leaves. With indaziflam being a lipophilic compound, adequate soil moisture is required on application.

Indaziflam has the potential to control a broad spectrum of weeds and provides long-lasting efficacy at low application rates. It controls annual grasses at rates of 25 to 100 g a.i./ha by inhibiting cellulose biosynthesis in susceptible species (Myers *et al*. 2009). Indaziflam presents a new mode of action for pre-emergence annual grass control which may be of benefit to turfgrass managers particularly where dinitroaniline resistance has been observed in wintergrass (*Poa annua)* and crowsfootgrass *(Eleusine indica)* in the southeastern United States (Cutulle *et al*. 2009; Isgrigg111 *et al*. 2002, Mudge *et al*. 1984 and Vaughn *et al*. 1990). Compared to some other pre-emergent herbicides, indaziflam is more water soluble, mobile, and persistent in aerobic soil environments. The water solubility of indaziflam is 2.8 mg/L (at 20C$^{o}$), the Koc value is <1000 mL/g and the soil half-life is > 150 (Senesman 2007; Tompkins 2010). In turf the control of summer and winter weeds historically includes separate control programs in spring (for summer weeds) and autumn (for winter weeds). Perry *et al*. (2011) showed that indaziflam could provide control of some summer and winter weeds from one application with residual pre-emergence activity extending 29 weeks after treatment.

## TRIAL RESULTS

### Turf

Annual grass weeds in sport and recreational turf are a major problem in Australia. A variety of selective pre and post-emergent herbicides are used for the management of these weeds. The most significant species are *P. annua*, *E. indica* and *Digitaria* sp.
In Australia in 2005 a trial program commenced to evaluate indaziflam as a pre-emergent herbicide for the control of these grass weeds in established warm season (C4 grass species) turf swards. Overseas research had established that significant phytotoxicity occurs with indaziflam when applied to established cool season species such as *Agrostis* sp., *Lolium perenne*, *Poa* sp. and *Festuca* sp.

From 2005 to 2009 nine trials were conducted with indaziflam in turf situations to examine control of the three previously mentioned weeds. A summary of the results is shown in Table 1. Based on these trials and overseas research an effective rate for the control of *Digitaria* is turf situations is 75g a.i./ha. For *P. annua* and *E. indica* 50 g a.i./ha is an effective rate.

**Table 1**: The range of weed control (%) when compared to untreated control treatments across warm season turf trials done in Australia between 2005 and 2009 with indaziflam 200SC.

| | | indaziflam g/ha | | | | | |
|---|---|---|---|---|---|---|---|
| | | 37.5g | 40g | 50g | 75g | 80g | 100g |
| Weed Species | *Poa annua* | 94-96% | 99% | | 93-100% | 99% | 100% |
| | *Digitaria* sp. | 69-98% | 94-97% | 89% | 97-100% | 100% | 94-100% |
| | *Eleusine indica* | | 100% | 100% | | 100% | 100% |

Indaziflam turf safety has been tested in Queensland at the facilities of the Department of Employment, Economic Development and Innovation and in NSW at the turf research plots of the University of Sydney. These trials have shown good turf safety on varieties including couchgrass (*Cynodon dactylon*), hybrid couchgrass (*C.dactylon* x *C. transvaalensis*), kikuyu (*Pennisetum clandestinum*), buffalograss (*Stenotaphrum secandatum*), zoysia (*Zoysia* sp.) and Queensland blue couch (*Digitaria didactyla*).

### Forestry

Indaziflam evaluation in *Pinus* and *Eucalyptus* forestry plantation commenced in Australia in 2008. Over 2008 and 2009 over 20 forestry trials were conducted in the major forestry growing areas on mainland Australia. These trials covered both pre and post planting on *Pinus* and *Eucalyptus* species.

The trial results (Table 2) have generally shown weed control to be dose related and stronger with monocot species rather than dicots.

**Table 2**: The mean weed control (%) when compared to untreated control treatments across *Pinus* and *Eucalyptus* forestry trials in Australia in 2008 and 2009.

| | Indaziflam g/ha | | |
|---|---|---|---|
| | 75g | 150g | 300g |
| Total | 37% | 46% | 61% |
| Monocots | 50% | 64% | 75% |
| Dicots | 42% | 36% | 47% |

While the trials have shown indaziflam to give good weed control in some situations, it has become evident that to expand the weeds spectrum in many cases indaziflam will require tank mixing with another residual herbicide. To date the mixing partners which have been tested have been hexazinone in *Pinus* and simazine and sulfometuron in *Eucalyptus*. The trials with these mixing partners have demonstrated that it has been possible maintain good efficacy while reducing the rate of the older chemicals.

The trials have shown indaziflam to be safe pre and post-planting on *Pinus* species, but only safe pre-planting on *Eucalyptus* species. Phytotoxicity is experienced on Eucalyptus species when indaziflam is applied post planting.

## DISCUSSION

Bayer Environmental Science will market indaziflam in turf under the trade name Specticle®. This Australian registration into the turf market will be followed by planned entries into the ornamental, forestry and industrial vegetation management segments. The rates used will be from 50 g a.i./ha (turf uses) to 150g a.i./ha (industrial vegetation management). Indaziflam will be registered in these segments in the U.S.A, Latin America and Asia Pacific. Australia will have the first registration of this product in turf in 2012.

## REFERENCES

Cutulle, M.A., McElroy J.S., Millwood R.W., Sorochan J.C., and Stewart C.N. (2009). Selection of bioassay method influences detection of annual bluegrass resistance to mitotic-inhibiting herbicides. *Journal of Crop Science*. 49: 1088-1095.

Isgrigg III, J., Yelverton F.H., Brownie C., and Warren L.S. (2002). Dinitroaniline resistant annual bluegrass in North Carolina. *Weed Science*. 50:86-90.

Mudge, L.C., Gossett B.J., and Murphy T.R. (1984). Resistance of goosegrass to dinitroaniline herbicides. *Weed Science*. 32:591-594.

Myers, D.F., Hanrahan R., Michel J., Monke B., Mudge L., Olsen C., Parker A., Smith J., and Spak D. (2009). Indaziflam/BCS AA170717- A new herbicide for preemergent control of grasses and broadleaves in turf and ornamentals. *Proceedings Southern Weed Science Society*. 62:393.

Perry, D. H., McElroy, J. S., Doroh, M. C., and Walker, R. H. (2011). Indaziflam utilization for controlling problematic turfgrass weeds. Online. *Applied Turfgrass Science* doi:10.1094/ATS-2011-0428-01-RS.

Senseman, S.A. (2007). *Herbicide Handbook*. Weed Science Society America. Lawrence, KS. pp. 265-266, 286-288.

Tompkins, J. (2010). Environmental Protection Agency Pesticide Fact Sheet: Indaziflam. Available at: http://www.epa.gov/opprd001/factsheets/indaziflam.pdf.

Vaughn, K.C., Vaughn M.A., and Gossett B.J. (1990). A biotype of goosegrass with an intermediate level of dinitroaniline herbicide resistance. *Weed Technology*. 4:157-162.

# UTILITY TAG, FARMING ELEMENTS AND ITK FOR SUSTAINABLE MANAGEMENT OF WEEDS IN CHANGING CLIMATE

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## ABSTRACT

A predominance of small holder farms in Asia offers scope for using component elements in a farming system for sustainable management of weeds that behave invasive in a changing climate. Altered precipitation, evaporation and temperature patterns due to climate change have resulted in weed flora shifts in northern coastal districts of Tamilnadu state, India. In particular, there has been a preponderance of invasive alien species such as *Leptochloa chinensis* (L.) and *Marsilea quadrifolia* L. in wetlands, *Trianthema portulacastrum* L. in uplands and *Eichhornia crassipes Mart. Solms* in aquatic systems. Research undertaken at Annamalai University in India is providing some alternative solutions to manage these problematic weeds. Innovative use of fish culture and poultry rearing in rice fields was shown to compliment weed control through 400 on-farm experiments, with biomass reductions of invasive alien species ranging from 31 to 38 per cent, in these districts. Similarly, using goats for off-season grazing reduced the biomass of weeds in upland crops. For example, biomass of the dominant *T. portulacastrum* declined by 23 to 29 per cent in 500 on-farm participatory experiments. Involving pigs for burrowing the puddled fields and addition of Tamarind husk complimented control of rice weeds especially nut sedge, which was reduced by 61 per cent. The invasive weed *E. crassipes* in aquatic systems was controlled in seasonal waterbodies within a season, by innovative and integrated use of insect agent (*Neochetina eichhorniae*) and plant product of *Coleus amboinicus* L. Utility modes for consuming the extensive biomass of *E. crassipes* have also been compared (*viz.,* manure, cattle feed and nanofiber extraction). Results indicate that manurial use and tempo mediated extraction of nanofibers offers an innovative tag of utility for management of this weed.

**Keywords:** Climate change, Invasive alien weeds, Weed utility, Farming elements, Sustainable management.

## INTRODUCTION

The options for integration in a weed management programme are wide, as several elements such as pattern of cropping, land management practices, agricultural inputs and component enterprises offer ancillary benefits of managing weeds and these could well be integrated with the weed control options such as mechanical, biological and chemical measures. Swaminathan (1987) reported an integrated farming system approach to address not only a reliable way of obtaining fairly high productivity with substantial fertilizer economy, but also a concept of ecological soundness leading to sustainable agriculture. A judicious combination of any one or more of the enterprises with a cropping component was observed to result in better utilization of available resources through effective recycling of residues or wastes. It also offered employment for family labour during the off-season, making the farm a viable unit (Behera and Mehapatra, 1998). Sustainability in managing weeds under varied farming conditions such as wetland rice farming and rainfed or irrigated upland farming with these integrations and the consequences of such integration on crop pest complexes are discussed in this paper. Aquatic systems are more delicate considering the weed management options as their impact is reflected on multiple resources like water, soil, crops and associated flora and fauna. Further, the invasive spread of the weeds in the system is much faster. However, the absence of soil interface, unlike in terrestrial systems could either hasten or impede the efficacy of management strategies depending upon the nature of such options. The absence of soil interface in the aquatic system that largely contributes to rapid degradation of allelochemicals, triggers the scope for integrating allelopathic interference as one of the tools in managing weeds. Though allelopathic suppression of weeds could not be construed as an alternate to replace synthetic herbicides, the same can fit in an integrated weed management programme very well as a prime component.

Research undertaken in these areas of alternative solutions or weed problems at Annamalai University, India are reviewed.

## Weeds and Changing Climate

Global warming directly reflects on rising sea levels due to melting of ice caps and natural expansion of sea water as it becomes warmer. Consequently, areas adjoining the coast and wetlands could be frequently flooded and the distribution pattern of monsoon rains may alter, through more intense downpours, storms and hurricanes. The meteorological data available at the Annamalai University, for the tail end of the Cauveri river delta region of Tamil Nadu State, India, shows that the average annual rainfall during the last 20 years has increased by 233 mm compared to the average of the previous 10 years (1588 and 1355, respectively). In contrast, annual evaporation has reduced by 453 mm (2153 and 1700, respectively) (Table 1.).

A phytosociological survey of floristic composition of weeds in this region reveals the recent invasion of the wetland rice fields by the alien invasive weeds *L. chinensis* and *M. quadrifolia* (Table 2.). These two weed species dominated over the native weeds such as *Echinochloa sp.* by virtue of their amphibious adaptation to alternating flooded and residual soil moisture conditions prevalent during recent years in this region (Yaduraju and Kathiresan, 2003; Kathiresan, 2005).

**Table 1.** Rainfall and evaporation pattern in the Cauvery river delta region of India.

| Period | Annual Rainfall (mm) | Annual Evaporation (mm) |
|---|---|---|
| 1980-1990 | 1355 | 2153 |
| 1990-2000 | 1483 | 1898 |
| 2000-2010 | 1588 | 1700 |

*L. chinensis* owes it's invasive behaviour to a longer life span that extends in to the relay crop of mung bean after transplanted rice. These two crops differ widely in the soil conditions that they prefer, with transplanted rice surviving in inundated water, where as mung bean thrives in residual soil moisture below 30 per cent. *L. chinensis* shows adaptation to both the extremes of climate, with in the same generation. *M. quadrifolia* is tolerant to most of the grass killer herbicides used like butachlor (Machete). Further, frequent floods do favour it's perpetuation.

**Table 2.** Floristic composition of weeds in rice fields irrigated by channels in Cauvery river delta (Importance Value Index %), India.

| Weed species | Channel I | | Channel II | | Channel III | |
|---|---|---|---|---|---|---|
| | 1990 | 2010 | 1990 | 2010 | 1990 | 2010 |
| *Echinochloa sp.* | 25.56 | 7.93 | 28.48 | 8.01 | 27.52 | 4.02 |
| *L. chinensis* | 22.74 | 30.41 | 24.81 | 29.85 | 23.64 | 32.17 |
| *Cyperus rotundus* L. | 17.23 | 12.50 | 22.28 | 17.25 | 17.01 | 4.80 |
| *Sphenoclea zeylanica* | 2.02 | 6.28 | 0.68 | 2.17 | 1.68 | 7.24 |
| *M. quadrifolia* | 1.46 | 39.61 | 0.63 | 41.84 | 0.46 | 40.32 |

A major weed that has been invading irrigated upland agroecosystems in several tropical Asian countries is *T. portulacastrum.* This weed is reported to have originated from tropical Africa and has invaded several continents *viz*., Australia, Africa and Asia (Rawson and Bath, 1984). A survey conducted in different irrigated upland crops of Veeranum Ayacut in Tamilnadu, India indicated that *T. portulacastrum* predominates as the dominant species in three crops *viz*., sugarcane, sunflower and gingelly with important value index percentages of 28.73, 26.83 and 25.99 respectively. This weed tops the list of 15 weed species recorded in all these crops in different locations (Kathiresan, 2004). One of the most important characteristics responsible for it's invasiveness is thermal induction of seed germination once soil temperature reach around 35º C. This results in synchronized and mass germination of seeds, producing a green carpet of seedlings. In a field study conducted at Annamalai University, it was observed that, increasing soil temperature with the summer months of June and July triggered the mass germination of seed of this weed, which led to suppression of the native species. The seeds of this weed undergo dormancy during winter and thermo-induction to break the dormancy requires soil temperatures above 35º C which normally coincides with the June, July months (Sundari and Kathiresan 2001).

Surveys in the distributary channels of lake Veeranum during 1990 and 2010 (Table 3.) indicate that the invasive alien species *E. crassipes* has invaded the watersheds in north Tamilnadu. This is because, the distribution from lake Veeranum during the period before 1990 was mainly from the river Cauvery, which received water from the adjoining state of Karnataka through Mettur Dam. Accordingly, the water was flowing with higher velocity during the monsoon periods commencing from June extending upto December. However, after 1990, following a dispute between the two states of Karnataka and Tamilnadu, these channels primarily served the purpose of drainage outlets following flash floods. Such events were frequent during this last 20 year segment. A comparatively lesser quantity of river water received during August and September was also distributed through the channels. The flood waters from inland wetlands have served as infestation sources of invasive species such as *E. crassipes*.

**Table 3.** Survey of Aquatic weeds in five of the distributary channels of Lake Veeranum in Tamilnadu (Importance Value Index %).

| Weed species | Channel I | | Channel II | | Channel III | | Channel IV | | Channel V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1990 | 2010 | 1990 | 2010 | 1990 | 2010 | 1990 | 2010 | 1990 | 2010 |
| *Ipomea reptans Per* | 10.3 | 6.4 | 21.3 | 4.8 | 14.6 | 3.1 | 19.6 | 6.0 | 27.2 | 2.9 |
| *Typha angustata L* | 1.3 | 3.2 | - | - | 2.7 | - | 7.2 | 2.0 | - | - |
| *L. chinensis* | 24.30 | - | 31.0 | 4.2 | 19.8 | 4.9 | 12.6 | - | 7.4 | 1.7 |
| *E. crassipes* | - | 39.42 | - | 46.4 | - | 42.6 | 7.8 | 58.6 | - | 63.4 |

**Farming Elements Offering Weed Solutions**

Fish Culture and Poultry Rearing in Rice

Annamalai University has evolved an innovative integrated rice farming system to manage weeds. Through 12 years of rigorous institutional field experimentation with statistically replicated experimental design, the best suited component elements fish culture and poultry rearing were selected from among rabbit, duck, fish and poultry birds for integration. The optimum mode of integration was also determined, including stocking density of fish fingerlings and poultry birds, size of fish trenches, size of poultry cages and nature and quantity of agro inputs (Kathiresan, RM., 2007a). In the NAIP – Comp III – SRLS 36 project, the system that has been optimized and perfected is being disseminated for adoption over 430 farmers holdings (Figure 1.). In each of the 430 development partners' rice fields, inputs including poultry cages, concrete posts, birds, feed and fish fingerlings were given free of cost for practicing integrated rice + fish + poultry farming in 200m$^2$ of rice area. Unlike other rice farming systems demonstrated elsewhere, wherein the component enterprises would remain as separate entities, in this IFS approach, fish poly culture with Catla, Roghu, Mrigal, common carp and grass carp in equal proportions of a stocking density of 2000 fingerlings / ha is taken up in trenches running along the border of one side of rice fields (20 x 10 m). The trenches are one metre deep and with a top width of 0.75 m and bottom width of 0.5 m so that the total dimension is 20 m x 0.75 / 0.5 m, occupying 15 m that contributes for 7.5 per cent of rice area. Broiler birds @ 1 bird / 10 m$^2$ of rice area, are housed in cages (6 x 4' of floor space and a height of 3' so as to accommodate a maximum of 20 birds) that are installed any where in the field using four concrete posts of height 8', 4' buried inside the field and 4' protruding above lifting the cages above crop canopy. The bottom of the cages are made of wire mesh (0.5 sq. inch) so that the broiler waste falls straight into the 10 cm deep water of the rice field. The poultry waste then dissolves and is able to serve both as rice manure and fish feed. This excludes the need for collecting the poultry waste and applying it to the rice field, the task of which is laborious and sometimes wasteful.

The herbivorus feeding habits of fish fingerlings contributes to weed suppression while the acidic pH and allelomediatry principles of poultry waste interferes with weed seed germination (Kathiresan, 2007b). These positive contributions from these two component farming elements are responsible for the suppression of both the invasive alien species in the rice ecosystems in three districts shown in Table 4. Each of these three districts consisted of three villages where 100 farmers with their small or marginal holdings participated in the research as a development partner. Pest incidence in rice as shown in Table 5, is also reduced due to integration of the fish culture and poultry components, because of the feeding habits of fishes that suppresses the egg masses, larvae and alternate weed hosts of pests.

**Figure 1.** Integrated rice + fish + poultry farming system.

**Table 4.** Weed Suppression due to Fish & Poultry Components in Rice.

| Location | Weed count /$m^2$ | | | | Weed biomass g/ $m^2$ | | | |
|---|---|---|---|---|---|---|---|---|
| Districts | *L. chinensis* | | *M. quadrifolia* | | *L. chinensis* | | *M. quadrifolia* | |
| | Rice alone | R+F+P | Rice alone | R+F+P | Rice alone | R+F+P | Rice alone | R+F+P |
| Cuddalore | 16 | 11 | 38 | 22 | 56 | 38 | 42 | 26 |
| Villupuram | 9 | 7 | 26 | 19 | 42 | 31 | 46 | 32 |
| Nagapattinam | 21 | 13 | 42 | 27 | 62 | 34 | 32 | 21 |

**Table 5.** Rice + Fish + Poultry and Pest Incidence in Rice.

| Districts | Leaf Damage in % after 40 Days | | *Nilaparvata lugens* Population after 7 Days | |
|---|---|---|---|---|
| | Rice Alone | Rice+Fish+Poultry | Rice Alone | Rice+Fish+Poultry |
| Cuddalore | 23.0 | 18.0 | 11.0 | 8.0 |
| Villupuram | 21.0 | 17.0 | 14.0 | 10.0 |
| Nagapattinam | 17.0 | 14.0 | 15.0 | 11.0 |

## Goats in Upland Crops

This technology involves rearing goats and using them for manuring as well as plant protection in crops that are grown during the succeeding cropping season. Under existing goat rearing modes, farmers rear goats, exclusively on herbs and vegetation available on social and ranching sites. In this intervention, farmers are trained to rear the goats, allowing them to graze on the weed vegetation (mostly perennial grasses like *Cynodon dactylon* (L.) and sedges like *C. rotundus*) that predominate the cropped lands during the off-season. Simultaneously, collecting the goat manure during the off-season and incorporating them for the crops (millet / vegetable / flower crop) during the rainfed seasons greatly compliments the crop by virtue of improved organic matter, soil nutritional status, pest, disease and weed control (through depletion of soil weed seed bank and suppression of alternate weed hosts for the pest and diseases). This greatly helps control of perennial weeds. However, these goats (reared @ 4/acre or 10/ha) need to be fed with tree loppings and other freely and easily available forages during the cropping season. The development partners of five clusters, each of three villages, were given two goats each (1000 goats in total) free of cost. In return they were asked to integrate them into half an acre of their holding.

The reduction in weed biomass in the farmers fields because of grazing by goats in the off-season (Figure 2.) was higher in Cuddalore and Nagapattinam districts compared to Villupuram. This is attributed to closer grazing of goats for want of excessive or adequate flushes of weed vegetation in the off-season in these two districts compared to Villupuram.

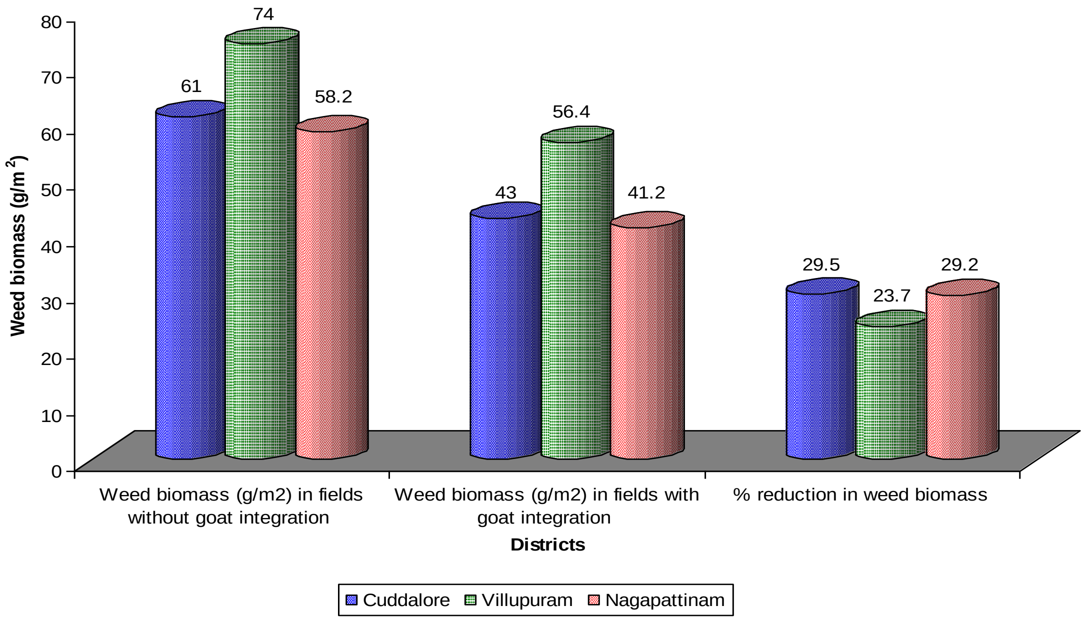


**Figure 2.** Weed suppression due to goat grazing in upland clusters.

## Use of Pigs for Weed Control in Rice

Experiments during consecutive rice seasons at Annamalai University revealed that the use of pigs for burrowing the puddle fields before transplanting of rice compared better to other off-season land management techniques *viz*. summer ploughing and glyphosate (Roundup) spray @ 1.0 kg a.i ha$^{-1}$ 45 days before transplanting, in reducing nut sedge population (Figure 3.). This treatment in combination with incorporation of tamarind husk @ 10 t ha$^{-1}$ and hand weeding recorded the highest bio-mass weed control indices (Table 6.).

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**Table 6.** Weed Control Index (%).

| Main treatments | Mean Value | |
|---|---|---|
| | Kuruvai | Late Samba |
| Control | - | - |
| Use of pigs during land preparation | 62.93 (47.86) | 59.91 (51.42) |
| Off-season ploughing | 49.85 (35.05) | 51.47 (40.84) |
| Glyphosate spraying | 58.46 (41.22) | 55.30 (47.37) |
| SEd | 1.04 | 1.09 |
| CD | 2.08 | 2.18 |
| Sub Treatments | | |
| Un weeded control | - | - |
| Twice Hand weeding | 58.03 (58.14) | 56.82 (53.93) |
| Tamarind husk @ 10 t ha$^{-1}$ | 52.53 (29.57) | 50.34 (36.76) |
| Oxyfluorfen (Goal) @ 0.25 ai ha$^{-1}$ | 51.72 (32.30) | 49.41 (34.35) |
| Tamarind husk + Hand weeding | 59.39 (51.19) | 57.34 (56.49) |
| Oxyfluorfen + Hand weeding | 55.64 (36.28) | 53.51 (42.19) |
| SEd | 1.12 | 1.13 |
| CD | 2.24 | 2.25 |

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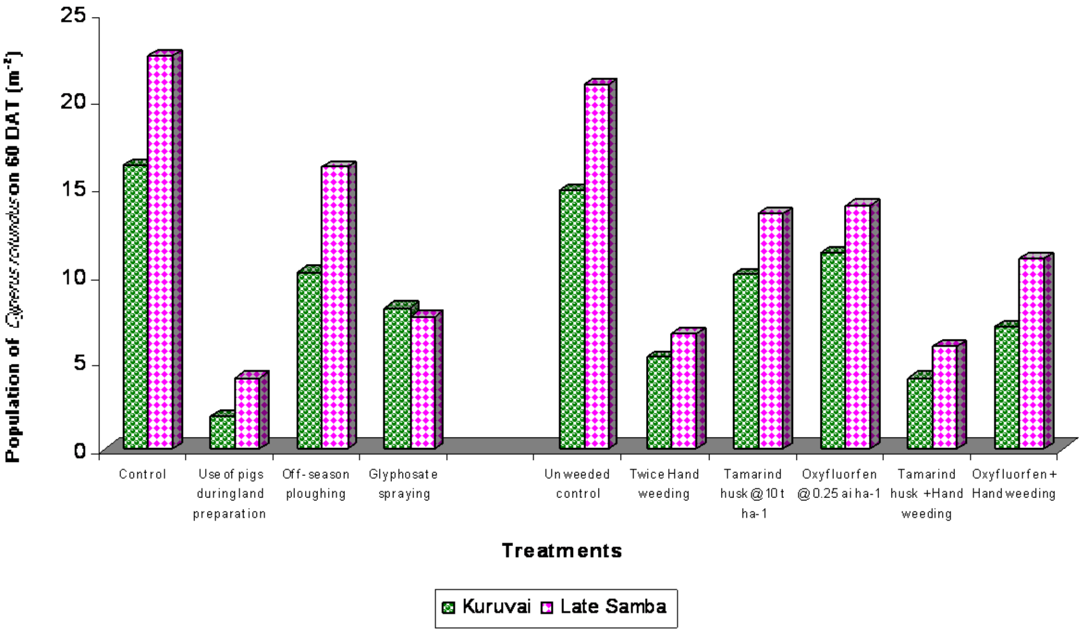


**Figure 3.** Population of *C. rotundus* on 60 DAT ($m^{-2}$).

This is because, the burrowing of the puddled field by pigs before transplanting of rice, brought all the underground tubers of *C. rotundus* to the surface, many of which were eaten by the pigs, whilst others were skimmed away before final land preparation and levelling. Thus, the treatment was very effective in depleting the soil reserve of tubers of *C. rotundus* which were chiefly responsible for the perennation of the world's worst weed.

## Integrated control of invasive *E. crassipes*

Training in integrated management for the floating aquatic weed water hyacinth in water bodies such as farm or village ponds, irrigation channels and aquaculture habitats, is being given to farmers in several districts. It includes the use of insects, allelopathic plant products, fishes and weed utility. However, the prime strategy for managing the aquatic weed water hyacinth is through the integration of the insect bio-control agent *N. eichhorniae* / *bruchii* with the use of dried plant material of the medicinal herb *C. amboinicus*. This herb is allelopathic on water hyacinth through the mechanism of membrane disruption and electrolyte leakage and the dried plant powder easily gets absorbed into the weed through the leaf scrapings made by the insects (Kathiresan 2000; Kathiresan 2007b). Farmers are encouraged to cultivate *C. amboinicus* in small areas of their fields and the harvested leaves are made in to dry powder. The insects are being reared in the lead centre. Subsequently, they were released in 24 watersheds spread throughout the district @ 1000 per water shed at the first instance in October 2009. The aqueous extract of dry leaf powder of *C. amboinicus* was sprayed in January 2010. Observations made on the weed population at quarterly intervals are furnished in Table 7.

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**Table 7.** Weed population of *E. crassipes.*

| Location | Weed population / $m^2$ | | | | |
|---|---|---|---|---|---|
| | January 2010 | April 2010 | July 2010 | October 2010 | January 2011 |
| Cuddalore | 34 | 4 | - | 11 | 20 |
| Villupuram | 22 | 2 | 4 | 7 | 15 |
| Nagapattinam | 31 | 6 | - | 14 | 17 |
| Thiruvannamalai | 27 | 9 | 4 | 12 | 14 |

Among various modes tried for using this weed in order to affix the tag of utility for speeding up public participation in controlling this weed, mixing dried and powdered water hyacinth upto 15 per cent in the daily ration for cattle, continuously for 15 days, did not cause any adverse change in the health of animals (Table 8.). However, fresh weed vegetation was rejected by the cattle. For utilizing water hyacinth as a manure, various treatments *viz*., water hyacinth compost @ 6.25 t $ha^{-1}$, dried water hyacinth @ 6.25 t $ha^{-1}$, fresh water hyacinth @ 6.25 t $ha^{-1}$ were compared with other organic manures like green leaf manure @ 6.25 t $ha^{-1}$ and Farm Yard Manure 12.5 t $ha^{-1}$ in transplanted rice. Using water hyacinth as compost @ 6.25 t $ha^{-1}$ recorded the highest growth and yield attributes resulting in the highest grain yield of 3850 kg $ha^{-1}$ (Table 9.).

**Table 8.** Clinical parameters in calves after 180 days of feeding water hyacinth powder mixed feed.

| Treatments | Mean | | | | |
|---|---|---|---|---|---|
| | Body weight (kg) | RBC ($x10^6/\mu l$) | RBC ($x10^3/\mu l$) | Hb (g/dl) | PCV (g/dl) |
| Water hyacinth fed animals | 56 | 6.93 | 9.5 | 11.2 | 31.4 |
| Control group | 58 | 7.01 | 9.17 | 11.4 | 31.6 |
| SEd | NS | NS | NS | NS | NS |
| CD(p=0.05) | NS | NS | NS | NS | NS |

NS - Non-significant

RBC – Red Blood Corpuscles

Hb – Haemoglobin

Packed Cell Volume

**Table 9.** Rice grain yield (Kg $ha^{-1}$).

| Treatments | Mean |
|---|---|
| Water hyacinth compost @ 6.25 t $ha^{-1}$ | 3850 |

| | |
|---|---|
| Water hyacinth fresh @ 6.25 t $ha^{-1}$ | 3330 |
| Water hyacinth dried @ 6.25 t $ha^{-1}$ | 3625 |
| Green leaf manure @ 6.25 t $ha^{-1}$ | 3215 |
| FYM @ 12.5 t $ha^{-1}$ | 2898 |
| Control | 2670 |
| SEd | 96.96 |
| CD (p=0.05) | 194.88 |

Another mode of utility for the aquatic weed *E. crassipes viz*. has been the successful extraction of nanofibers using three methods; chemical (alkali and peroxide) and mechanical treatments (TEMPO mediated oxidation treatment). The obtained nanofibers from the weeds (Figure 4.) using the above three treatments was estimated to be about 5 - 100 nm in diameter of the fibers and lengths in several micron meters. From the nanofibers, the transparent thin film, transparent sheet, paper and then the transparent biodegradable nanocomposites were prepared. The biodegradability test conducted following OECD Guidelines for the Testing of Chemicals OECD 30IB clearly indicates that the compound is readily biodegradable. (Patent Application No-1877/DEL/2010 filed on 11/08/2010 in Intellectual Property of Rights. New Delhi on TEMPO (2, 2, 6, 6 - Tetramethylpiperidinyl-1-oxyl radical) mediated catalytic oxidative synthesis of cellulose nanofibers 5-50 nm size from the aquatic weed water hyacinth").

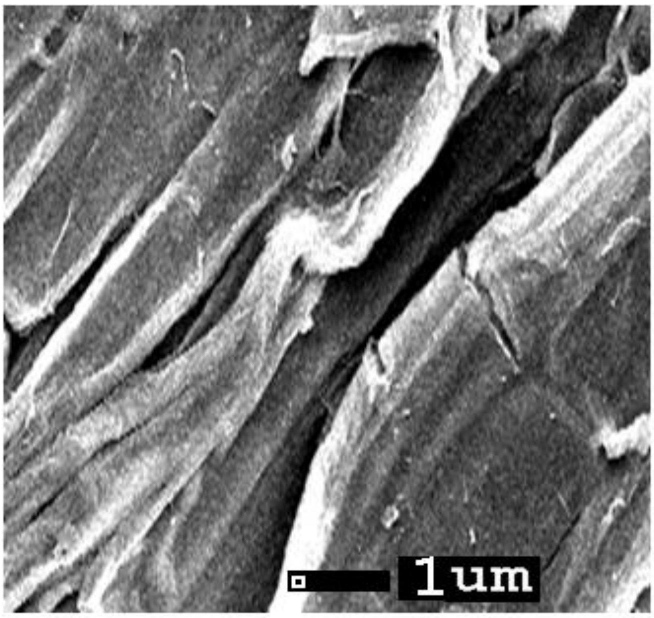


**Figure 4.** Sem picture for cellulose nanofibers from waterhyacinth.

## CONCLUSION

The role of changing climate in triggering the invasive behaviour of certain weed species resulting in a shift in the floristic composition of weeds is becoming obvious. Such a scenario warrants the need for multiple options to address a particular weed problem rather than relying upon unified approach. Accordingly, exploring the feasibility of engaging a systems approach of integrated farming, indigenous knowledge base and weed utility offers good weed solutions that reinforces sustainability.

## REFERENCES

Behera, U.K and Mehapatra, I.C. (1998). Income and employment generation for small and marginal farmers through integrated farming. *Indian Farming*. 51(3): 17-19.

Kathiresan, RM. (2000). Allelopathy Potential of Native plants against water hyacinth. *Crop Protection.* 19(8-10): 705-708.

Kathiresan, RM. (2004). Invasive weeds in agro-ecosystem of South India. In: Abstracts National workshop on Invasive alien species and biodiversity in India. Banarus Hindu University. Varanasi. India. p.149.

Kathiresan, RM. (2005). Case study on Habitat management for the control of Alien in invasive weed (*Prosopis juliflora*), Report submitted to water resource Organization, Public works Department, Tamilnadu, India.

Kathiresan, RM. (2007a). "Linking Environment and weed management through integrated farm management". In: Proceedings of the *21st Asian Pacific Weed Science Society Conference*. Colombo. Sri Lanka. p. 21-26.

Kathiresan, RM. (2007b). Integration of elements of farming system for sustainable weed and pest management in the tropics. *Crop protection*. 26. 424-429.

Rawson, J.E and Bath. (1984). Chemical control of giant pigweed, sesbania pea and fierce thorn apple in sorghum. *Queensland Journal of Agriculture & Animal Science.* 38(1): 13-19.

Sundari A. and Kathiresan, RM. (2001). Weed biology - Periodicity of germination of *Trianthema portulacastrum*. In: Abstracts of Biennial Conference on Eco – Friendly weed management options for sustainable Agriculture. *Indian Society of Weed Science.* Bangalore. India. pp. 61.

Swaminathan, M.S. (1987). Inaugural address at the Intern. Symposium of sustainable agriculture. The rate of decomposition of green manure crops in rice farming system. Int. Rice Res. Inst. Los Banos. Philippines.

Yaduraju, N.T. and Kathiresan, RM. (2003). Invasive Weeds in the tropics. In Proceedings: 19th *Asian Pacific Weed Science Society Conference*, Manila. Philippines. vol. I. P. 59-68.

# WEED SEED SPREAD BY VEHICLES: A CASE STUDY FROM SOUTHEAST QUEENSLAND, AUSTRALIA

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## ABSTRACT

Weed seed spread, from infested to uninfested areas, is by a number of biotic and abiotic mechanisms, and this spread of seed aids the invasion process across the landscape. Currently in Queensland there are approximately 3.2 million motorized vehicles, each capable of carrying, and therefore spreading, weed seeds. Studies were conducted in 2009/10 to investigate the role of the utility vehicle in the spread of weed seeds in south east Queensland. A large number (209) of viable seed were found on vehicles and in each of the four seasons of the year. The largest number seeds per vehicle were collected in the autumn (48%) and the lowest number in the winter (14%). These viable seeds were found on a number of parts of the vehicles and were contained within mud or dust that had presumable transferred on to the vehicle as it undertook its routine activities. The highest percentages of seed were collected from the underside of the vehicle (36%), followed by back mudguards (24%), front mudguard (16%) and cabin (12%). Lower percentages were found on engine, radiator (3%) and tyres and rims(9%). The seeds found on the vehicles belonged to 90 species, coming from 26 families. The majority of these species were alien to Australia (66%) and Queensland (73%).The early implications from this present study are that utility vehicles are capable of collecting, carrying and presumably distributing large numbers of viable weed seeds, that seed is carried on many parts of the vehicle and that this occurs in all seasons of the year. Thus, any washing or cleaning procedure used to remove weed seeds from vehicles will need to concentrate on all parts of the vehicle and that this should be done in all seasons. Cleaning vehicles at appropriate places should be seen as a possible way to reduce weed seed spread by utility vehicles.

**Key words:** Weed seeds, vehicle, southeast Queensland

## INTRODUCTION

The majority of plants considered to be weeds in Australia have come from many regions of the world. So far a total of 429 weeds have been declared to be noxious or are under some form of legal control within Australia (AWS, 2006). In today's world people are

[6] This study is a part of my PhD dissertation

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travelling more and more, both locally and internationally. With this increased movement, the invasion of weeds is also increasing. Spread of weed seeds by human-induced mechanisms is now more important than movement by natural mechanisms of spread (e.g. by water, wind or animals) and is now considered to be the main source of weed seed spread globally (Mack and Lonsdale, 2001). Among these human-induced mechanisms, weed seed spread by vehicles is one of the most important. As far back as 1930 Ridley (1930) has suggested that weed seed spread along roadsides was mainly by weed seed attached to vehicles in mud. This conclusion was based on the observations only that many weeds first appear along roadsides before anywhere else in the landscape. A later study by Clifford (1955) also indicated the importance of vehicle transfer of weed seed, who estimated that every kg of mud attached to vehicles contained 100 to 180 weed seeds. More recently Wace (1977) has also confirmed that the motor vehicle is a very important vector for weed seed spread in the modern landscape.

Due to the increasing number of vehicles on Australian roads, the opportunities for weed seed spread is increasing. More recently Moerkerk (2006) has shown that an average of 16.5 weed species is carried by every tractor, slasher, mower, truck, grader, backhoe, trailer, excavator and dozer travelling along Australian roads. Another study has shown that many viable weed seeds (18,566 from 259 species) were to be found at the bottom of an automatic carwash (Wace, 1977) indicating that motor vehicles do indeed carry great numbers of viable weed seed. In another study, Lonsdale and Lane (1994) assessed tourist vehicles that entered Kakadu National Park (Australia) and found 1,960 viable weed seeds (from 88 species) on 304 tourist's vehicles (Lonsdale and Lane, 1994). In this study they found a significant variation in number of seed per vehicle and majority of the vehicle carried one or no seeds. However some carried very high number of seed (789). Moreover, season did not show any significant effect on the number of seed per vehicle. Thus, the objectives of the present study were: (1) to determine how many weed seeds are to be found on utility vehicles that have undertaken field work close to an urban centre, Brisbane, and in different seasons of the year and (2) to determine which parts of the vehicle these weed seeds collected on.

## MATERIALS AND METHODS

In each of the four season of the year (2009/10), six utility vehicles were identified and cleaned, and the samples collected assessed for their viable weed seed content. These vehicles had been cleaned then exposed to a week of routine business for Powerlink Ltd. This business included visits to field sites for power cable repair and maintenance in southeast Queensland. Prior to vehicle cleaning, each vehicle was driven over a large black plastic sheet. In the cleaning process, mud was scraped off the exposed surfaces of the vehicle by hand using a plastic spatula, and a brush and then recleaned using the house-hold vacuum cleaner. For collecting samples, the following order of cleaning of vehicle was followed and number of small components were considered as one part in the following order: (1) Mud-guards, wheel arches, flaps (front) and around the break lines, (2) Mud-guards, wheel arches, rims and flaps (back) and around the break lines, (3) Tyres and rim (front and back added together) (4) Underside including the chassis rail, recess and holes, around struts and stabilizers, steering components, the axel, the spare tyres, fuel tank and silencer), (5) Engine including front grill, radiator and other cooling cores, the grill and recess under the wiper, blades, the engine mounts, top of the gearbox and battery recess/tray and (6) The cabin including foot well, the carpets and mats, the seats and toolbox. If mud fell off the vehicle it was swept from the plastic sheet and added to the sample, so all final samples contained the dry mud scrapings the vacuum samples and the plastic sheet sweepings.

Air-dried samples were then weighed and the contents broken up and spread thinly over a 2 cm layer of potting compost contained within a germination tray (30 x 20 cm). Thirty six trays (one per vehicle part for six vehicles) were placed in a glasshouse for 16 weeks and watered daily to field capacity level. An additional tray was placed in the glasshouse with potting compost alone to act as a control to detect any weed seeds that may be in the compost or in the glasshouse atmosphere. Upon emergence all seedlings were counted and identified and then removed from the germination trays. Weed seed germination was recorded weekly and all data sets for six vehicles in all four seasons of the year were analysed and compared. After germinations, number of seeds germinated data per each part of the vehicle was transformed into percentage of the total number of seeds per vehicle. Then percent data was analysed through analysis of variance (ANOVA) and general linear model (GLM) techniques, using the Minitab computer software 15 (Minitab Inc., 2007). Means were separated through Tukey's simultaneous test at $P \leq 0.05$.

## RESULTS AND DISCUSSION

### Season

An average of 209 viable seeds was found on each vehicle and in each season. These seed represented 90 species, coming from 26 families (Table 1) of which 66 % were alien to Australia and 80 % were alien to Queensland. An analysis of variance revealed that the total number of viable weed seeds found each season were significantly different (at $P \leq 0.05$p-value = 0.000) with the greatest number of seeds to be found in the autumn (47%) and followed by summer (23%), and lowest number of seeds were found in winter (14%); (Figure 1). Number of seeds associated with the amount of mud was different in different season of the year. For example, in autumn these seeds were collected in a total of 5.1 kg of dry mud, with an average of 0.85 kg of mud per vehicle. However, in summer these seeds were collected in a total of 5.6 kg of dry mud, with an average of 0.93 kg of mud per vehicle. These results for number of seeds per kg of mud in different season of the year are comparable to those of Clifford (1959), who found 100 seed per kg dry mud in one season and 180 weed seeds per kg of dry mud in other season. There was variation among the species found and their attachment position on the vehicle. Among these seeds found on vehicles in all seasons, the maximum number of species came from the Asteraceae or Poaceae (Table 1). This could be due to the nature of the dispersal unit of these families which are either small in size or have appendages that enable them to stick into mud. Our results are similar to those Moerkerk (2005), who also showed that the most common species found on vehicles were from the Poaceae, asteraceae and fabaceae.

**Table 1.**The families and number of species from those families that were found on utility vehicles used for routine field work for 1 week around southeast Queensland.

| **No** | **Family** | **No. of species in each season in of the year** | | | |
|---|---|---|---|---|---|
| | | **Autumn** | **Winter** | **Spring** | **Summer** |
| 1 | Asteraceae | 14 | 7 | 3 | 8 |
| 2 | Poaceae | 14 | 11 | 7 | 8 |
| 3 | Cyperaceae | 3 | 2 | 1 | 1 |
| 4 | Apiaceae | 2 | | | |
| 5 | Brassicaceae | 2 | 1 | | 2 |
| 6 | Caryophyllaceae | 2 | | | |
| 7 | Chenopodiaceae | 2 | 2 | | |
| 8 | Fabaceae | 2 | 2 | 2 | 1 |
| 9 | Polygonaceae | 2 | 1 | | |
| 10 | Portulacaceae | 2 | 2 | 2 | 1 |
| 11 | Verbenaceae | 3 | | | |
| 12 | Amaranthaceae | 1 | 1 | 1 | 3 |
| 13 | Campanulaceae | 1 | | | |
| 14 | Clusiaceae | 1 | | | |
| 15 | Crassulaceae | 1 | | | |
| 16 | Euphorbiaceae | 1 | 2 | 5 | 2 |
| 17 | Gentianaceae | 1 | | | |
| 18 | Lamiaceae | 1 | | | |
| 19 | Onagraceae | 1 | | | |
| 20 | Oxalidaceae | 1 | 1 | 1 | 1 |
| 21 | Plantaginaceae | 1 | | | 1 |
| 22 | Solanaceae | 1 | 1 | | |
| 23 | Aizoaceae | | | | 1 |
| 24 | Moraceae | | | | 1 |
| 25 | Rubiaceae | | | | 1 |
| 26 | Verbenaceae | | 1 | 1 | 2 |
| | **Total** | **59** | **34** | **23** | **33** |

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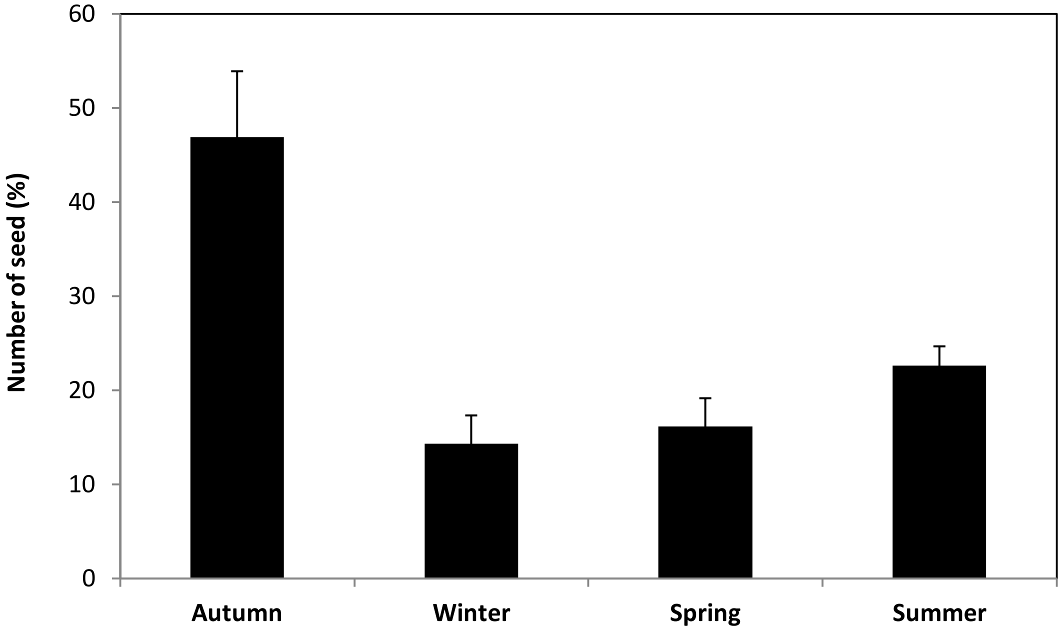


**Figure 1.** Number of viable weed seed (%) attached to six different parts of the utility vehicles in different season of the years. In each season, the amount of mud was collected by a single utility vehicle was different, such as in autumn (0.85 kg), in winter (0.70 kg), spring (0.63 kg) and Summer (0.93 kg), where number of seed and association mud is different in different seasons**.**

### Parts of vehicle

All vehicle parts analysed carried weed seed but the proportion on each part differed possibly due to their ability to have mud and dust stick to them and also due to the amount of air movement each part is exposed to. The association of mud and number of seeds is different in different season of the year. For example in autumn, the maximum amounts of mud were collected from the underside (0.32 kg)of the vehicle and the back (0.20 kg) and front mud guards (0.15 kg) of the vehicle and these parts carried the highest number of weed seeds (Figure 2). However, other parts of the vehicles such as the cabin (18.3%), the engine (0.4%) and the tyres (3.2%) also carried weed seeds, where less amounts of mud were collected. However, front mudguard carried the less number of seeds as compared to the back mudguard. As front tyres disturbed the soil and subsequently soil and seeds were taken by either back mud guard or underside of the utility vehicle (Figure 2).

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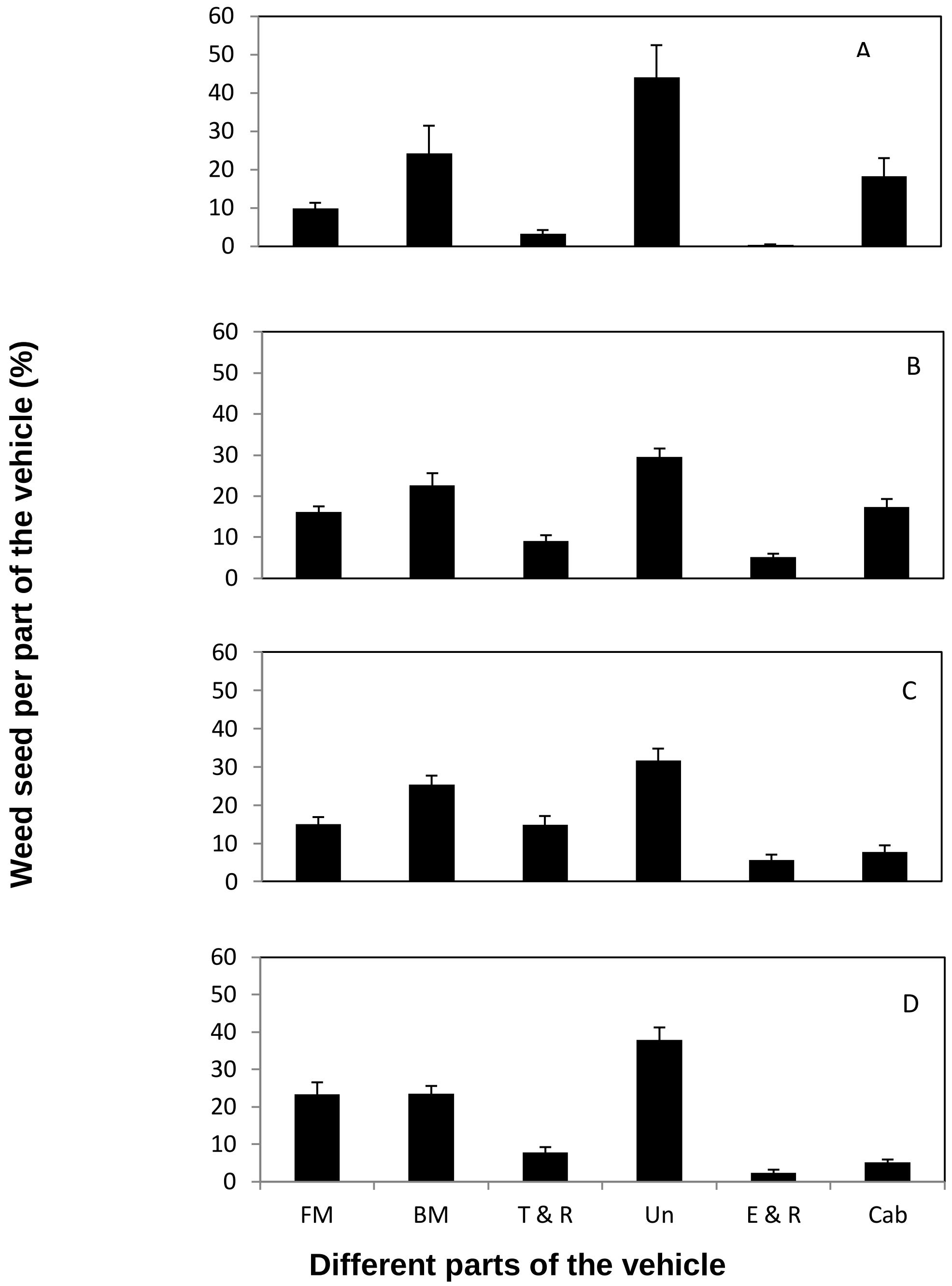


**Figure 2.** Number of viable weed seed (percent of the total viable seed on a single vehicle) attached to six different parts of the utility vehicles in the four seasons of the year (A) autumn (B) winter, (C) spring (D) summer (2009/10). The part of the vehicle cleaned was BM = Back mudguard, Ca = Cabin, E & R = Engine and Radiator, FM = Front Mudguard, T & R = Four Tyres and Rims and Un = Underside.

## Species

There was an association between parts of the vehicle seed stuck to and the plant species. Even though there was less mud in cabin, a large number of weed seeds were found within fruits with spines or hooks. Examples of such species include Khaki Weed (*Alternanthera repens* L.) or Cobbler's pegs (*Bidens pilosa* L.) and were found only in the cabin and nowhere else on the vehicles. The study also showed that the engine (including front grill, radiator and other cooling cores etc.), although trapping and carrying many

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seeds, only had a few viable seeds per vehicle. Presumably the heat and desiccation received by the seeds in these areas of the vehicle were enough to kill them.

The conclusions are that utility vehicles can pick up and carry a large number of weed seeds. These weed seeds can attach to almost all parts of the vehicle, often in mud from the ground. Therefore much of this seed load is to be found on the underside, on the back and front mudguards while smaller and important collection were made from the cabin and the radiator, the engine, and the tyres. To prevent weed seed spread by vehicles, cleaning procedures, including washing and vacuuming, can be used to remove weed seeds. This will need to be applied to all parts of the vehicle and in all four seasons of the year.

## REFERENCES CITED

AWS 2006. *Australian Weed Strategy- A national strategy for weed management in Australia.* National Resource Management Ministerial Council (Australia) Canberra: Australian Government Department of the Environment and Water Resources.

Clifford, H. T. 1959. Seed dispersal by motor vehicles. *Journal of Ecology,* 47**,** 311-315.

Lonsdale, W. M. & Lane, A. M. 1994. Tourist vehicles as vectors of weed seeds in Kakadu National Park, Northern Australia. *Biological Conservation,* 69**,** 277-283.

Mack, R. N. & Lonsdale, W. M. 2001. Humans as global plant disperser: getting more than we bargained for. *Bioscience,* 51**,** 95-102.

Moerkerk, M. 2006. Risk of weed movement through vehicles, plant and equipment: results from a Victorian study. *15th Australian Weeds Conference, Papers and Proceedings, Adelaide, South Australia, 24-28 September 2006: Managing weeds in a changing climate***,** 458-461.

Moerkerk, M. 2005. Machinery Hygeine**-** What is on our vehicles? *Proc. 2nd Victorian Weed Conf., pp 99-101. (Weed Society of Victoria, Melbourne).*

Ridley, H. N. 1930. The dispersal of plants throughout the world. *Ashford, (Kent), Reeve.*

WACE, N. Year. Assessment of dispersal of plant species - The car born flora in Canberra. *In: Proceeding of the Ecological society of Australia, 1977. 167-186.*

# RICE ALLELOPATHY AND MOMILACTONE

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## ABSTRACT

Rice has been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management options. All available information indicates that rice plants possibly release unknown allelochemicals into the neighbouring environments. A large number of compounds, such as phenolic acids, fatty acids, indoles and terpenes, were identified in rice root exudates and decomposing rice residues as putative allelochemicals. Among them, momilactone B may play an important role in rice defence mechanism in the rhizosphere for the competition with invading root systems of neighbouring plants.

**Keywords:** Allelopathy, Allelochemical, Momilactone, Root exudates, Sustainable weed management.

## INTRODUCTION

Weeds cause reductions in rice yield and quality and remain one of the biggest problems in rice production. The negative impacts of commercial herbicide use on the environment make it desirable to diversity weed management options. Allelopathy is one of the options (Rimando and Duke 2003, Macías et al. 2007, Kong 2008, Tesio and Ferrero 2010).

Allelopathy is the direct influence of an organic chemical released from one living plant on the growth and development of other plants (Inderjit and Duke 2003, Belz 2007, Macías et al. 2007). Allelochemicals are such organic chemicals involved in the allelopathy (Rice 1984, Putnam and Tang 1986, Inderjit 1996). Allelochemicals can provide a competitive advantage for host-plants through suppression of soil microorganism and inhibition of the growth of competing plant species because of their antibacterial, antifungal, and growth inhibitory activities (McCully 1999, Hawes et al. 2000, Bais et al. 2004).

Rice has also been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management options. A large number of rice varieties were found to inhibit the growth of several plant species when these rice varieties were grown together with these plants under the field or/and laboratory conditions (Dilday et al. 1994, 1998, Kim et al. 1999, Olofsdotter et al. 1999; Azmi et al. 2000, Gealy et al. 2003, Seal et al. 2004a, Kim et al. 2005). These findings suggest that rice may produce and release allelochemicals into neighbouring environment, thus encouraging the exploration of allelochemicals in rice.

Many secondary compounds, such as phenolic acids, fatty acids, indoles and terpenes, were identified in rice root exudates and decomposing rice residues as putative allelochemicals (Takeuchi et al. 2001, Rimando and Duke 2003, Khanh et al. 2007).

However, these compounds are almost ubiquitous in plants and rice allelopathy can not be explained by these compounds (Olofsdotter et al. 2002b, Seal et al. 2004b). Diterpen, known as momilactone B, which are unique to rice, have been isolated (Kato-Noguchi et al. 2002). Momilactone B inhibits the growth of typical rice weeds like *Echinochloa crus-galli* and *E. colonum* at concentrations greater than 1 μM (Kato-Noguchi et al. 2008a). Rice plants secrete momilactone B from the roots into the rhizosphere over their entire life cycle (Kato-Noguchi et al. 2003b). These observations suggest that rice plants may inhibit the growth of the neighbouring plants through the secretion of momilactone B into their rhizosphere.

## Rediscovery of Momilactone

About 5,000 rice seedlings, cv. Koshihikari, were hydroponically grown for 14 days in order to screen for any allelochemicals in rice root exudates. Keeping track of the biological activity, the culture solution was purified by several chromatographic fractionations and finally 2.1 mg of putative compound causing the inhibitory effect of the rice seedlings was isolated (Kato-Noguchi et al. 2002, Kato-Noguchi and Ino 2003a). The chemical structure of the inhibitor was determined from high-resolution MS, and $^{1}$H- and $^{13}$C-NMR spectral data as momilactone B. Momilactone B was later found in root exudes of other allelopathic rice cultivars, PI312777, with 5,7,4'-trihydroxy-3',5'-dimethoxyflavone and 3-isopropyl-5-acetoxycyclohexene-2-one-1 (Kong et al. 2004). In addition, another potential allelochemical momilactone A was found in rice root exudates of cv. Koshihikari (Kato-Noguchi et al. 2008b).

Momilactone A and B were first isolated from rice husks as growth inhibitors (Kato et al. 1973, Takahashi et al. 1976). Momilactone A and B were later found in rice leaves and straw as phytoalexins (Cartwright et al. 1977, 1981, Kodama et al. 1988, Lee et al. 1999). Thereafter, the function of momilactone A as a phytoalexin has been extensively studied and several lines of evidence indicate that momilactone A has an important role in rice defense system against pathogen attacks (Nojiri et al. 1996, Araki and Kurahashi 1999, Takahashi et al. 1999, Tamogami and Kodama 2000, Agrawal et al. 2002). Although the growth inhibitory activity of momilactone B was much greater than that of momilactone A (Takahashi et al. 1976, Kato et al. 1977), the efforts to find the function of momilactone B were limited.

## Biological Activity of Momilactone

Momilactone A and B, respectively, inhibited the growth of *Amaranthus lividus, Digitaria sanginalis* and *Poa annua* at concentrations greater than 20 ppm (ca. 60 μM) and 4 ppm (ca. 12 μM) (Chung et al. 2005). Momilactone A and B were also reported to inhibit the growth of *Echinochloa crus-galli* and *E. colonum,* which are the most noxious weeds in rice fields, at concentrations greater than 10 and 1 μM, respectively. Thus, effectiveness of momilatone B on growth inhibition is much greater than that of momilactone A. The growth inhibitory activities of momilactome B are also greater than those of momilactone A under other bioassay systems, (Takahashi et al. 1976, Kato et al. 1977, Fukuta et al. 2007, Toyomasu et al. 2008).

Momilactone A and B, respectively, inhibited root and shoot growth of rice seedlings at concentrations greater than 100 and 300 μM. $IC_{50}$ values of momilactone A and B on rice root and shoot were not obtained because of their weak inhibitory activities against rice. The inhibitory activities of momilactone A and B, respectively, on the root and shoot growth of rice seedlings were 1 - 2 % and 0.6 - 2 % of those on the root and shoot growth of *E. crus-galli* and *E. colonum*. Thus, the effectiveness of momilactone A and B on the growth of rice seedlings was much less than that on the growth of *E. crus-galli* and *E. colonum.* These results suggest that the toxicities of momilactone A and B to rice seedlings are much less than those to the two weed species (Kato-Noguchi et al. 2008a).

## Momilactone in Rice Life Cycle

Momilactone A and B were secreted from rice plants into the rhizopsphere throughout all life cycle stage of rice (Kato-Noguchi et al. 2003b, 2008a). The secretion level of momilactone A and B increased until flowering initiation, and decreased thereafter. The level of momilactone A and B at day 80 (around flowering) was 1.1 and 2.3 µg/plant/day, which was 55- and 58-fold greater than that at day 30. Although concentration of momilactone A in rice was greater than that of momilactone B, secretion level of momilactone B was greater than that of momilactone A, which suggests that momilactone B may be selectively secreted into the rhizophere than momilactone A.

Considering the growth inhibitory activity and concentrations found in the bioassay medium, momilactone A may cause only 0.8 - 2.2% of the observed growth inhibition of *E. crus-galli* roots and shoots by rice. However, momilactone B in the medium was estimated to cause 59 - 82% of the observed growth inhibition of *E. crus-galli* roots and shoots by the rice seedlings. In addition, the concentrations of momilactone B in the medium reflected the observed differences in the growth inhibition of *E. crus-galli* by the eight rice cultivars investigated (Kato-Noguchi et al. 2010). This suggests that the allelopathic activity of rice may be primarily depend on the secretion level of momilactone B. Therefore, momilactone B may play a very important role in rice allelopathy.

## Rice Allelopathy and Allelochemicals

Since the first observation of allelopathy in rice by Dilday et al. (1989), more than 16,000 rice accessions from 99 countries in the USDA-ARS germplasm collection have been screened. Of these, 412 accessions inhibited the growth of *Heteranthera limosa* and 145 accessions inhibited the growth of *Ammannia coccinea* (Dilday et al. 1994, 1998). Similar attempts have been conducted in some other countries, and a large number of rice varieties were found to inhibit the growth of several plant species when these rice varieties were grown together with these plants under field and/or laboratory conditions (Kim et al. 1999, Olofsdotter et al. 1999, Azmi et al. 2000, Gealy et al. 2003, Seal et al. 2004a, Kim et al. 2005). These findings suggest that rice may produce and secrete allelochemicals into its neighboring environments.

Although mechanisms of the exudation are not well understood, it is suggested that plants are able to secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic-derived exudation, and proton-pumping mechanisms (Hawes et al. 2000, Bais et al. 2004). Through the root exudation of compounds, plants are able to regulate the soil microbial community in their immediate vicinity, change the chemical and physical properties of the soil, and inhibit the growth of competing plant species (MuCully 1999, Hawes et al. 2000, Bais et al. 2004). Momilactone B was secreted from rice plants into the rhizopsphere throughout all life cycle stage of rice (Kato-Noguchi et al. 2003b, 2008a). Considering the inhibitory activity and the secretion level, momilactone B may play a very important role in rice defense mechanism in the rhizosphere as an allelochemical.

The use of allelopathic rice cultivars and allelochemicals can definitely reduce the ecological impact, particularly by reducing the amount of herbicide used. Allelopathic rice cultivars combined with cultural management options is, therefore, an interesting and potential technique, contributing to alternative chemical control of weeds in paddy ecosystems (Weston 1996, Olofsdotter 2001, Olofsdotter et al. 2002a). Such an allelopathy-based technique for paddy weed control is the most easily transferable to the low-input management systems prevailing in most Asian rice farming systems (Kong 2008). Therefore, the rice allelopathy may be one of the options in the sustainable weed management strategies.

## REFERENCES

Agrawal, G.K., Rakwal, R., Tamogami, S., Yonekura, M., Kubo, A. and Saji, H. (2002) Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. *Plant Physiology and Biochemistry* 40: 1061-1069.

Araki, Y. and Kurahashi, Y. (1999) Enhancement of phytoalexin synthesis during rice blast infection of leaves by pre-treatment with carpropamid. *Journal of Pesticide Science* 24; 369-374.

Azmi, M., Abdullah, M.Z. and Fujii, Y. (2000) Exploratory study on allelopathic effect of selected Malaysian rice varieties and rice field weed species. *Journal of Tropical Agricultural Food Science* 28; 39-54.

Bais, H.P., Park, S.-W., Weir, T.L., Callaway, R.M. and Vivanco, J.M. (2004) How plants communicate using the underground information superhighway. Trends in Plant Science 9; 26-32.

Belz, R.G. (2007) Allelopathy in crop/weed interactions - an update. *Pest Management Science* 63; 308-326.

Cartwright, D., Langcake, P., Pryce, R.J., Leworthy, D.P. and Ride, J.P. (1977) Chemical activation of host defence mechanisms as a basis for crop protection. *Nature* 267; 511-513.

Cartwright, D.W., Langcake, P., Pryce, R.J., Leworthy, D.P. and Ride, J.P. (1981) Isolation and characterization of two phytoalexins from rice as momilactones A and B. *Phytochemistry* 20; 535-537.

Chung, I.-M., Hahn, S.-J. and Ahmad, A. (2005) Confirmation of potential herbicidal agents in hulls of rice, *Oryza sativa*. *Journal of Chemical Ecology* 31; 1339-1352.

Dilday, R.H., Nastasi, P. and Smith, R.J. Jr. (1989) Allelopathic observations in rice (*Oryza sativa* L.) to ducksalad (*Heteranthera limosa*). *Proceedings of Arkansas Academic Science* 43; 21-22.

Dilday, R.H., Lin, J. and Yan, W. (1994) Identification of allelopathy in the USDA-ARS rice germplasm collection. *Australian Journal of Experimental Agriculture* 34; 907-910.

Dilday, R.H., Yan, W.G., Moldenhauer, K.A.K. and Gravois, K.A. (1998) Allelopathic activity in rice for controlling major aquatic weeds. In: Olofsdotter M. (Ed.) *Allelopathy in Rice,* Manila, Philippines: International Rice Research Institute, pp 7-26.

Fukuta, M., Xuan, T.D., Deba, F., Tawata, S., Khanh, T.D., and Chung, I.M. (2007) Comparative efficacies in vitro of antibacterial, fungicidal, antioxidant, and herbicidal activities of momilatones A and B. *Journal of Plant Interaction* 2; 245-251.

Gealy, D.R., Wailes, E.J., Estorninos, L.E. and Chavez, R.S.C. (2003) Rice cultivar differences in suppression of barnyardgrass (*Echinochloa crus-galli*) and economics of reduced propanil rates. *Weed Science* 51; 601-609.

Hawes, M.C., Gunawardena, U., Miyasaka, S. and Zhao, X. (2000) The role of root border cells in plant defense. *Trends in Plant Science* 5; 128-133.

Inderjit (1996) Plant phenolics in allelopathy. *Botanical Review* 62; 186-202.

Inderjit, and Duke, S.O. (2003) Ecophysiological aspects of allelopathy. *Planta* 217; 529-539.

Kato, T., Kabuto, C., Sasaki, N., Tsunagawa, M., Aizawa, H., Fujita, K., Kato, Y. and Kitahara, Y. (1973) Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedron Letters* 39; 3861-3864

Kato, T., Tsunakawa, M., Sasaki, N., Aizawa, H., Fujita, K., Kitahara, Y. and Takahashi, N. (1977) Growth and germination inhibitors in rice husks. *Phytochemistry* 16; 45-48.

Kato-Noguchi, H., Ino, T., Sata, N. and Yamamura, S. (2002) Isolation and identification of a potent allelopathic substance in rice root exudates. *Physiologia Plantrum* 115; 401-405.

Kato-Noguchi, H. and Ino, T. (2003a) Rice seedlings release momilactone B into the environment. *Phytochemistry* 63; 551-554.

Kato-Noguchi, H., Ino, T. and Ichii, M. (2003b) Changes in release level of momilactone B into the environment from rice throughout its life cycle. *Functional Plant Biology* 30; 995 - 997.

Kato-Noguchi, H., Ota, K. and Ino, T. (2008a) Release of momilactone A and B from rice plants into the rhizosphere and its bioactivities. *Allelopathy Journal* 22; 321-328.

Kato-Noguchi, H., Ino, T. and Ota, K. (2008b) Secretion of momilactone A from rice roots to the rhizosphere. *Journal of Plant Physiology* 165; 691-696.

Kato-Noguchi, H,, Hasegawa, M., Ino, T., Ota, K. and Kujime, H. (2010) Contribution of momilactone A and B to rice allelopathy. *Journal of Plant Physiology* 167; 787-791.

Khanh, T.D., Xuan, T.D., and Chung, I.M. (2007) Rice alleloapthy and the possibility for weeds management. *Annals of Applied Biology* 151; 325-339.

Kim, K.U., Shin, D.H., Kim, H.Y., Lee, Z.L. and Olofsdotter, M. (1999) Evaluation of allelopathic potential in rice germplasm. *Korean Journal of Weed Science* 19; 1-9.

Kim, S.Y., Madrid, A.V., Park, S.T., Yang, S.J. and Olofsdotter, M. (2005) Evaluation of rice allelopathy in hydroponics. *Weed Research* 45; 74-79.

Kodama, O., Suzuki, T., Miyakawa, J. and Akatsuka, T. (1988) Ultraviolet-induced accumulation of phytoalexins in rice leaves. *Agricultural and Biological Chemistry* 52; 2469-2473.

Kong, C., Liang, W., Xu, X. and Hu, F. (2004) Release and activity of allelochemicals from allelopathic rice seedlings. Journal of Agricultural and Food Chemistry 52; 2861-2865.

Kong, C. (2008). Rice allelopathy. *Allelopathy Journal* 22, 267-273.

Lee, C.W., Yoneyama, K., Takeuchi, Y., Konnai, M., Tamogami, S. and Kodama, O. (1999) Momilactones A and B in rice straw harvested at different growth stages. *Bioscience, Biotechnology and Biochemistry* 63; 1318-1320.

Macías, F.A., Molinillo, J.M.G., Varela, R.M. and Galindo, J.G.G. (2007). Allelopathy –a natural alternative for weed control. *Pest Management Science* 63; 327-348.

McCully, E. (1999) Roots in soil: unearthing the complexities of roots and their rhizospheres. *Annual Review of Plant Physiology and Plant Molecular Biology* 50; 695-718.

Nojiri, H., Sugimori, M., Yamane, H., Nishimura, Y., Yamada, A., Shibuya, N., Kodama, O., Murofushi, N. and Omori, T. (1996) Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. *Plant Physiology* 110; 387-392.

Olofsdotter, M., Navarez, D., Rebulanan, M. and Streibig, J.C. (1999) Weed-suppressing rice cultivars: Does allelopathy play a role? *Weed Research* 39; 441-454.

Olofsdotter, M. (2001) Rice: A step toward use of allelopathy. *Agronomy Journal* 93; 3-8.

Olofsdotter, M., Jensen, L.B. and Courtois, B. (2002a) Improving crop competitive ability using allelopathy: An example from rice. *Plant Breeding* 121; 1-9.

Olofsdotter, M., Rebulanan, M., Madrid, A., Dali, W., Navarez, D. and Olk, D.C. (2002b) Why phenolic acids are unlikely primary allelochemicals in rice. *Journal of Chemical Ecology* 28; 229-242.

Putnam, A.R. and Tang, C.-S. (1986) Allelopathy: State of the science. In: Putnam A.R. & Tang C.-S. (Eds.) *The Science of Allelopathy*, New York: John Wiley & Sons, pp 1-19.

Rice, E.L. (1984) *Allelopathy*, Ed. 2. Orlando: Academic Press:

Rimando, A.M. and Duke, S.O. (2003) Studies on rice allelochemicals. In: Smith C.W. & Dilday, R.H. (Eds.) *Rice; Origin, History, Technology and Production*, Hoboken, New Jersey: John Wiley & Sons, pp 221-244.

Seal, A.N., Pratley, J.E., Haig, T. and An, M. (2004a) Identification and quantitation of compounds in a series of allelopathic and non-allelopathic rice root exudates. *Journal of Chemical Ecology* 30; 1647-1662.

Seal, A.N., Haig, T. and Pratley, J.E. (2004b) Evaluation of putative allelochemicals in rice roots exudates for their role in the suppression of arrowhead root growth. *Journal of Chemical Ecology* 30; 1663-1678.

Takahashi, N., Kato, T., Tsunagawa, M., Sasaki, N. and Kitahara, Y. (1976) Mechanisms of dormancy in rice seeds. II. New growth inhibitors, momilactone-A and -B isolated from the hulls of rice seeds. *Japanese Journal of Breeding* 26; 91-98.

Takahashi, A., Kawasaki, T., Henmi, K., Shii, K., Kodama, O., Satoh, H. and Shimamoto, K. (1999) Lesion mimic mutants of rice with alterations in early signaling events of defense. *Plant Journal* 17; 535-545.

Takeuchi, Y., Kawaguchi, S. and Yoneyama, K. (2001) Inhibitory and promotive allelopathy in rice (*Oryza sativa* L.). *Weed Biology and Management* 1; 147-156.

ISBN Number: 978-0-9871961-0-1

Tamogami, S. and Kodama, O. (2000) Coronatine elicits phytoalexin production in rice leaves (*Oryza sativa* L.) in the same manner as jasmonic acid. *Phytochemistry* 54; 689-694.

Tesio, F. and Ferrero, A. (2010) Allelopthy, a chance for sustainable weed management. *International Journal of Sustainable Development & World Ecology* 17; 377-389.

Toyomasu, T., Kagahara, T., Okada, K., Koga, J., Hasegawa, M., Mitsuhashi, W., Sassa, T. and Yamane, H. (2008) Diterpene phytoalexins are biosynthesized in and exuded from the roots of rice seedlings. *Bioscience, Biotechnology and Biochemistry* 72; 562-567.

Weston, L.A. (1996) Utilization of allelopathy for weed management in agroecosystems. *Agronomy Journal* 88; 860-866.

# UTILIZATION OF WEEDS AS BIOLOGICAL RESOURCES AND AS A MANAGEMENT TOOL

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## ABSTRACT

Although weeds are considered undesirable or troublesome plants, many weeds and their relatives, occurring on mountains, disturbed habitat and on arable lands, are very important for us to use as good natural resources. In Korea, native plants, and/or weeds were found to belong 185 plant families, and included 1,065 genera, and 4,596 species in total. Some of these weeds have medicinal uses; others are edible, and still others possess dyeing properties. Colonizing plants can be used to remove environmentalcontaminants, raw materials for ethanol bio-fuel, and as sources for phyto-chemicals and allelopathic chemicals. Allelopathic phenomena may also be increasingly used in the field, for weed suppression.

Recent reviews indicate extensive and similar uses of weeds, which have been reported from many Asian countries. Of the major avenues of utilization, the use of colonizing plants to remove environmental pollutants, their use as bio-fuel sources and the exploitation of their allelopathic potential are all receiving significant attention in the Asia-Pacific Region. Weeds or their relatives have a variety of genetic diversity from which useful genetic resources, such as a gene(s) for functional substances can be derived and introduced into cultivating crops. Some plants, such as wild oat (*Avena fatua* L.) and spotted knapweed (*Centaurea maculosa* Lam.) have a strong allelopathic potential or parasitic ability by which neighboring plants are completely controlled.

Strategies for isolating and characterizing functional substances could be employed for economic exploitation of weeds. Genes coding for the functional substances should be cloned and introduced into cultivating crops for developing a new genotype. Future efforts in Weed Science should not ignore the various possibilities of utilization of weeds or their relatives as biological resourcesIncreased exploitation of colonizing species will be a useful management tool for some species, which are currently considered problematic.

**Key words:** functional substances, biological resources, exploitation of weeds.

## INTRODUCTION

A weed is commonly defined as a plant growing where it is not desired. Weed species interfere with our endeavors, such as agriculture or animal farming, recreational pursuits, including gardening, transport, bush walking and water sports etc. There are a large number of publications including books and review papers that have shown the loss of agricultural production caused by ‘weeds’ or ‘invasive species’, and the threats posed by such species to biodiversity.

Globally, the utilization of weeds has been patchy over the past five decades. Nevertheless, there is a renewed interest in focusing on utilization of weeds in productive ways, so that people may benefit from an aspect that has been largely ignored (Chandrasena, 2007). Weeds have been used for long time as sources of food, fiber, dye, medicines, animal fodder, a remover of water pollutants, green manure, materials for slope management, mulches, ornamental plants, handicraft, broomstick, feeding honey bee, roof thatching and allelopathic plant. Among these, the most significant uses have been found on weeds as sources of edible and medicinal plants, and aquatic weeds to remove pollutants, with plant residues largely utilized as mulches etc. Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) causes a risk in agricultural production, while this weed is used for food and therapeutic purpose. This means that most of weed species have a negative effect on crop production, but they have also beneficial aspects.

In this regard, there is a relevant review publication on weed utilization in the Asian-Pacific region edited by Kim *et al.* (2007), covering ten different countries. An article contributed by Chandrasena (2007) in this publication seems to be a lead review essay, which helps us understand the utilization of weeds.

The present paper present a brief overview of the uses of weeds as bio-resources in the Asian-Pacific Region, based on contributions from the above ten countries in the publication on 'Utility of Weeds and their Relatives as Resources'.

## Weeds as Biological Resources

### Edible Weeds

Edible weed species reported in different countries in the Asian-Pacific region were as follows: 59 weed species in 45 families in Thailand (Maneechote, 2007), about 34 weed species in Malaysia (Bakar, 2007), about 37 weed species in Sri Lanka (Abeysekera and Herath, 2007), about 131 weed species in Japan (Morita 2007) and about 150 weed species in China (Li and Qiang, 2007). Those who are interested in the use of weed species as edible plants may refer to the assay of Morita (2007), in which detailed plant parts for their utilization were presented.

Many of those weed species listed overlapped in different countries. There are numerous accounts on the use of weeds and their relatives as vegetables among Asians. When shortage of food becomes severe from this day forward, weeds and their relatives will serve as supplement of the staple foods. Li and Qiang (2007) reminded of 59 common weed species which have been popular as wild vegetables in China. For instance, a pigweed, *Amaranthus* spp. in the Amaranthacea family occurs in upland at both temperate and tropical region and is a very well known edible plant for all the Asians. This plant species is commonly considered as a weed, but is directly harvested in the wild for vegetable or is cultivated as a vegetable crop. There are many other examples of the use of common noxious weed species, such as common purslane (*Portulaca oleracea* L.) and common lambsquarters (*Chenopodium album* L.), and prostrate knotweed (*Polygonum aviculare* L.) etc. which are all used as vegetable sources. Parts of weeds which are utilized are mostly young leaves, shoots and whole plants, but flowers, roots, bulbs and tubers are rarely used.

### Medicinal Plants

There are numerous accounts on weed species used as medicinal purpose in the Asian-Pacific region. There were, as weeds being used for medicinal purpose, about 40 species in Malaysia (Bakar, 2007), 200 weed species in Sri Lanka (Joseph 2001), about 120 weed species in India (Naidu *et al.* 2005), 43 weed species in 29 families in Thailand

(Maneechote, 2007), 127 weed species for ethno-medicinal use in Pakistan (Hamayun and Lee, 2007), 400 weed species in China (Qiang, 2002) and 132 grasses, including weeds in Korea (Lee *et al*., 2007). Just like edible weeds, many of them from different countries, belong to the same genus.

Weeds are highly valued in traditional medicine systems and have been used by indigenous communities for curing different ailments for thousands of years. Most of weeds have been known to posses therapeutic properties and the pertinent traditional knowledge was transferred orally through generations. It is clear that new pharmaceuticals are like to be found in colonizing plants, and as Stepp (2004) suggested, weeds need to be given more attention as potential sources of phytomedicines. This is important because 80% of the world population continue to rely mainly on traditional medicines for their health care (Gurib-Fakim, 2006).

Purple Nutsedge (*Cyperus rotundus* L.) which is one of world worst weeds listed by Holm *et al.* (1977), is used as medicinal plant in Asia. Tubers of this weed are used to cure body ailment such as fever, headache, sores, vomiting, eye inflammation and skin itching etc. (Hamayun & Lee, 2007; Maneechote, 2007). Seeds of cocklebur (*Xanthium strumarium* L.), roots of curly dock (*Rumex crispus* L.) and whole plants of common dayflower (*Commelina communis* L.) are also used for medicinal purpose.

There are numerous uses of weed species as herbal medicines to cure a host of body ailments and diseases in the Asian-Pacific region. However, more research is needed to verify the active chemicals and how herbal medicines, based on weeds, can cure human diseases. Thus, investigating therapeutically or allelopathically active compounds in colonizing plants presents a scientific challengeElucidating the chemistry of these bio-active compounds will led to identifying opportunities for future development of medicines (Schűtz *et al*, 2006).

Restoration Of Polluted Environments

Many studies have shown that weed species had high accumulating abilities of heavy metals and so were used to remove heavy metals from polluted environment. Weed species such as black nightshade (*Solanum nigrum* L.) and horse weed (*Conyza canadensis* L.) have high endurance to Cd and Cd-Pb-Cu-Zn complex contamination, and also have high accumulating ability of Cd (Wei *et al*. 2003). Another species, giant ragweed (*Ambrosia trifida* L.) accumulates Cd and Zn in its tissue at levels that are two-to-three times greater than other plant species, suggesting the use of this plant for remediation of heavy metal-polluted soils (Peles *et al*., 1998).

Some other species *Pteris vita* L. (Chen *et al.*, 2002), *Sedium alfredii* (Yang *et al*. 2002) and *Viola baoshanensis* (Liu *et al.,* 2002) have high ability to accumulate Zn, Cd and arsenich. Another weed species, *Xanthium sibiricum* is highly capable of bio-accumulating mineral substance such as Mn, Cu, B, P. and Pb (Li *et al*., 2005). An exotic weed, water hyacinth absorbed a large amount of $Cl^-$ and $PO_4^{3-}$ and heavy metals such as Cr, Pb, Hg, Ti, Ag, Co and Sr from polluted water (Wu, 2003).

Despite water hyacinth causing major problems in some parts of the world, the risk of using it in closed and controlled treatment ponds in areas where the plant is already present might be acceptable (Ebel *et al*., 2007). For further information, please refer to the articles presented by Li and Qiang (2007) and Ebel *et al*. (2007).

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In Korea, common reed [*Phragimites australis* (Cav.) Trin ex Steud], narrowleaf cattail (*Typha angustifolia* L.), duck weed (*Lemna aequinoctialis* Welw.) and eared watermoss (*Salvinia auriculata* Aublet) etc. are used for cleaning polluted water.

Weeds Used As Dye

Plant species used for dyeing are not numerous as the edible plant and medicinal herbs. In Korea, the following species such as Japanese mugwort (*Artemesia princes* Pampan.), safflower (*Carthamus tinctouris* L.), American false daisy [*Eclipta prostrata* (L.) L.], annual fleabane [*Erigeron annuus* (L.) Pers.], and indigo (*Polygonum tinctorium* L.) etc. are known to produce natural dyes (Lee *et al.* 2007).

Natural dyeing materials have received a great attention in Korea because people prefer a natural dye than artificial one. Indigo plant is a good example, which is commercially used as a natural source of dyes for staining clothes. This is why this plant species is regarded as one of industrial crops.

## OTHER USES OF WEEDS

Weeds as fodders and animal feeds

Due to increasing demand of livestock production, it needs an increase of animal feeds. Graminaceous weed species are by and large used as herbage or fodders for cattle, goats and ducks etc. Species from the genera *Axonopus, Brachiaria, Digitaria, Leersia, Leptochloa, Paspalum* and *Pennisetum* are fairly commonly used by farmers as fodders for their animals in Malaysia.

Sedges are not favored as animal fodders. Some broadleaf weeds like *Asystasia gangetica*, water hyacinth and *Limnocharis flava* (L.) Buch. etc. are served as animal fodders in Malaysia (Bakar 2007). The latter two species are normally fed to pigs. The comparative nutritive values of some of these species were presented in the assay of Bakar (2007). There are many other similar uses of weeds as fodders or animal feeds in various countries in Asia.

Weeds used as green manure and compost

Weeds can be used as composting materials, without wasting them. Conversion of green waste-leaves, branches and grass-clippings from parks, garden, roadsides and home to composts is common in Asian-pacific farmers. Colonizing species make up a substantial part of this material.

Although composting is a valuable practice, ineffective commercial composting has been implicated in spread of certain weeds through seeds and propagules, which are not dead (Chandrasena, 2007). Thus, it needs very careful preparation for compost or green manure not to disseminate undesirable seeds or propagules.

Farmers have used water hyacinth, *Sanvinta molesta* D.S. Mitchell and *Parenthium hysterophorus* L.for composting material in India and various weed species, such as water hyacinth, *Ipomoea cornea* Jacq., *Chromolaena odorata* (L.) H.M. King & B.L. Robinson (Kathiresan, 2007) and *Crotalaria juncea, Sesbania rostrata and Croton laccifera* (Abeysekera *et al.*, 2001) have used for green manure materials. Many research data show that combinations of green manure with moderate amount of chemical fertilizer are more effective than sole chemical fertilizer application.

Weeds used as shelter

Some weeds such as cogongrass [*Imperata cylindrica* (L.) P. Beauv.] and *Vetiveria zizanoids* (L.) Nash can be used as materials for covering roof of shelter for human and animals (Abeysekera and Herath, 2007). Some other grasses are also used for the same purpose for shelter.

<u>Weeds used as making handicraft</u>
Many weeds of Cyperaceae family such as *Cyperus pilosus* Vahl, *Scirpus articulatus* L. and *Scirpus grossus* L.f. are used for making mats, hats, carpet and souvenirs in Sri Lanka (Abeysekera and Herath 2007). These handicrafts can give an additional income to farmers of Sri Lanka. Utilization of these weeds is an indirect method of weed control while providing additional income to Asian farmers.

<u>Bio-energy</u>

The dry stem and leaves of many weeds can be directly burned as fuel. The alien weed species *Eupatorium adenophorum* L. and water hyacinth (Zhang 1996) can be used to produce methane, and *Helianthus tuberosus* (Jiang and Zhang 2003) for ethanol. In addition, silvergrass (*Miscanthus sinensis* Anderss) and common reed can be used to produce bio-energy.

<u>Genetic resources and gene pool</u>
Wild soybean (*Glycine soja* Siebold & Zucc.) and wild rice (*Oryza rufipogon* Griff.) are excellent genetic resources which can be used for varietal improvement tolerant to adverse environment and insect-diseases. Wild soybean has been used for improvement on seed quality (functional substances: isoflavon content) of the recommended variety. Parasitic plant, *Tryphysaria versicolor* can be used for determining allelopathic potential through haustorium formation.

**Weeds Used as Mananagement Tools**

<u>Allelopathy</u>
Allelopathy is defined as ‘any direct or indirect harmful or beneficial effect by one plant to another through the production of chemical compounds that escape into the environment’ (Rice 1984). It is known that many weeds exhibit or assumed to exhibit allelopathy. Most allelopathic weeds are economically destructive to crop production and attempts to control them have met with limited success.

However, a few allelopathic weeds and their allelochemicals can be used for pest management and control in agricultural ecosystems or employed for biorational pesticides (Duke *et al*. 2000; Kong *et a*l. 2006; Macias *et al.* 2007). Despite this, relatively little attention has been paid to how allelopathic weeds and their allelochemicals could be potentially utilized as an important part of pest management and control in agricultural systems (Kong 2010).

The use of allelopathic plant mulches for ecological pest management and control has received a great attention (Hong *et al*. 2004). Allelopathic weeds may be used as covering chips or intercropping species for pathogen and weed reduction (Xuan *et al* 2005).

The mulches of a number of allelopathic weeds such as *Ageratum conyzoides*, *Bidens pilosa*, *Euphorbia hirta*, *Tephrosia candida*, *Lencaena glauca, Morus alba*, wild oat and spotted knapweed might be useful as an alternative materials for biological control and for the reduction of herbicide dose that are used in paddy fields. The use of these allelopathic

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weeds as mulches promoted rice growth and yield, and greatly reduced paddy weed growth at dose of 2 t $ha^{-1}$. Particularly, two species such as *B. pilosa* and *T. candida* were the most effective candidates, attaining over 80% weed control and increasing rice yield by 20% (Hong *et al*. 2004; Khanh *et al*. 2005).

However, utilization of allelopathic weed mulches in practice might be effective under the careful integration of cultural management and herbicides. The use of dry powder from allelopathic weeds such as *Parthenium hysterophorus*, *Coleus amboinicus*, and *Tragia biflora* was also effective to control an aquatic weed, water hyacinth (Kathiresan, 2000).

Allelopathic weeds can biosynthesize a wide variety of phytochemicals that have relatively broad-spectrum activity and some are known to provide defense mechanisms against other plant competitors and to attack microbes or insects and animal predators. Thus, the research and development of allelochemicals that have been derived from allelopathic weeds as sources of natural herbicides have been carried out throughout world (Duke *et al.* 2000; Macias *et al*. 2007).

A recent study on the cases of *Ageratum conyzoides* L., *Ambrosia trifida* L. and *Lantana camera* L., provides examples of allelopathic weeds and use of their allelochemicals that have been incorporated into ecological pest management and control in China (Kong, 2010).

Many studies verified the mechanisms of a self-defense system, including allelopathy in plants. Most plant chemicals associated with allelopathic activity are secondary metabolites from shikimic acid or acetate pathway and the terpenoid pathway. Plants respond to environmental stress through a variety of biochemical reactions, which may provide protection against casual agents. It has been well documented that allelopathic phenolics and terpenoid compounds are increased under stress environment, for example, enhanced UV-B light induces the accumulation of phenylpropanoids and flavonoids in various plant species.

What are allelopathic traits? Morphological characteristics such as early seedling emergence, seedling vigor, fast growth rates that produce a dense canopy, greater plant height, greater root volume and longer growth duration are known to increase the ability of crop competiveness. However, it is not easy to identify morphological traits directly related to allelopathy. If morphological traits or allelochemicals or genes responsible for allelopathic effects are identified, allelopathic traits can be easily incorporated into improved cultivars through breeding techniques available at the present time.

A strategy for isolating and characterizing functional substances could be employed for economic exploitation of weeds. Genes coding for the functional substances should be cloned and introduced into cultivating crops for developing a new genotype. Such efforts will give some fruits of success for utilization of weeds or their relatives as biological resources and as management tool. Allelopathic plants or weeds, can also be used as strongly allelopathic mulches in intercropping as a weed management tool.

## CONCLUSION

Weeds are clearly highly successful plants, largely due to characteristics that confer superior colonizing ability and competitiveness. These attributes can be useful in many situation, such as in repairing damaged ecosystems.

It is accepted that in some situations in agricultural fields, there are huge crop losses due to excessive and unmanaged weed growth.. However, as discussed in this essay, Asia-Pacific countries should look at the positive aspects of weeds as well; i.e. their utilization as resources. As indicatedabove, proper utilization of weeds can contribute significantly to enhance the income of the poor farmers, besides giving various other benefits in various ecosystems.

Many weed species have been used for edible and medicinal plant sources. In a use of edible purpose, investigation on nutrition value of such weeds will help their utilization. Weed species which are used for medicinal plants will receive more attention because 80% of the world population continues to rely mainly on traditional medicines for their health care. Studies on verification of chemical component in specific medicinal weeds will give a clue of synthesizing a new medicine.

Many weed species can be used for phytoremediation which uses plants and plant process to remove, degrade, or render harmless hazardous materials, such as nonvolatile hydrocarbons and immobile inorganic matter, including heavy metals present in the soil and ground water. Weed species which help eliminate pollutants from soil and water, can be recommended in required ecosystems.

It needs to utilize abundantly available weed biomass for some useful purpose. Making compost from weeds has a great potential which can be utilized by the poor farmers at very low cost. Efforts should be made to popularize the compost preparing techniques among the farmers, and thus farmers can save money for purchasing inorganic fertilizers.

Some weeds can also be an alternative source of bio-energy. This is an area which should be developed because petroleum is gradually exhausting.

Many woody weeds may be converted into compacted fuel in the form of briquettes (Gunasena and Puspakumara 2004). Some weeds can be sources of essential oil, gum and dye, and materials for furniture, hand-made paper and thatching etc.

On the other hand, allelopathy can be applicable for management tools in crop production through breeding of allelopathic crops, mulches of allelopathic weeds and making powder from allelopathic weeds. Allelopathy alone is not likely to replace totally other weed control practices because its effectiveness is influenced by many factors. However, marginally reduced use of herbicide over time will be a significant economical benefit to farmers and will also reduce the ecological impact on the environment.

This essay introduces various accounts of weed utilizations. This information can be usable for the poor farmers in the Asian-pacific region. Further studies on weed utilization including wild plants and allelopathy will broaden the horizon of weed science and shed more light on beneficial effects of weeds.

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## REFERNCES

Abeysekera, A. S. K., Sirisena, D. N. and Wickrama, U. B. (2001). Green manure application and its effect on weed management and grain yield of rice. *Proceedings 18th Asian Pacific Weed Science Conference*, Beijing, China. May 28th – June 2nd, 2001. pp. 239-245.

Abeysekera, A. and Herath, S. (2007). Utilization of weeds in Sri Lanka. In Kim, K. U. *et al.* (Eds). *Utility of weeds and their relatives as resources*. Kyungpook National University. Daegu, Korea. pp.123-132.

Bakar, B. H. (2007). Utilization of weeds and their relatives: Malaysian perspectives. In Kim, K. U. *et al*. (Eds). *Utility of weeds and their relatives as resources*. Kyungpook National University. Daegu, Korea. pp. 57-106.

Chandrasena, N. R. (2007). Liabilities or assets? Some Australian perspectives on weeds. In Kim, K. U. *et al*. (Eds). *Utility of weeds and their relatives as resources.* Kyungpook National University. Daegu, Korea. pp. 9-56.

Chen, T., Wei, C., and Huang, Z.. (2002). Arsenc hyper-accumulator *Pteris vittata* L. *Chinese Science Bulletin,* 47(11): 902-905.

Duke, S. O., Dayan, F. E., Romangi, J. G. and Rimanda, A. M. (2000). Natural products as sources of herbicides: current status and future trends. *Weed Research,* 40: 99-111.

Ebel, M., Evangelou, M. W. H. and Schaeffer, A. (2007). Cyanide phytoremediation by water hyacinth (*Eichhornia crassipes*). *Chemosphere,* 66: 816-823.

Gunasena, H. P. M. and Puspakumura, D. K. N. G. (2004). Publication of the national multipurpose tree species in Sri Lanka. Published by *the Council for Agricultural Research Policy,* Sri Lanka. pp. 10-42.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspect of Medicine,* 27:1-93.

Hamayun, M. and Lee, I. J. (2007). Weeds as source of traditional medicine in Pakistan. In Kim, K. U. *et al.* (Eds). *Utility of weeds and their relatives as resources.* Kyungpook National University, Daegu, Korea. pp. 145-158.

Hong, N. H., Xuan, T. D., Eiji, T. and Khanh, T. D. (2004). Paddy weed control by higher plants from Southeast Asia. *Crop Protection,* 23: 255-161.

Holm, L. G., Pancho, J. V., Herberger, J. P.and Plucknet, D. L. (1977). *The world's worst weeds. Distribution and Biology*, University of Hawaii Press, Honolulu, USA.

Jing, Y. and Zhang, H. (2003). A review of controlling common water hyacinth at home and abroad. *Review of China Agricultural Science and Technology,* 5(3): 72-75.

Joseph, K. D. S. M. (2001). Conservation and sustainable use of medicinal plants. *Proceedings of the workshop on multipurpose in Kandy*, Sri Lanka. Pp. 5-30.

Kathiresan, R. M. (2000). Allelopathic potential of native plants against water hyacinth. *Crop Protection,* 19(8): 705-708.

Kathiresan, R. M. (2007). Utility of weeds and their relatives in India. In Kim, K.U. *et al.* (Eds). *Utility of Weeds and their relatives as resources*. Kyungpook National University, Daegu, Korea. pp. 133-144.

Khanh, T. D., Hong, N. H., Xuan, T. D. and Chung, I. M. (2005). Paddy weed control by medicinal and leguminous plants from Southeast Asia. *Crop Protection,* 24: 421-431.

Kim, K. U., Shin, D. H. and Lee, I. J. (2007). *Utility of weeds and their relatives as resources.* Ililsa Press. Daegu. Korea. p. 222.

Kong, C. H., Wang, P., Zhang, C. X., Zhang, M. X. and Hu, F. (2006). Herbicidal potential of allelochemicals form *Lantana camara* against *Eichhornia crassipes* and the alga *Microsystis aeruginosa*. *Weed Research,* 46: 290-295.

Kong, C. H. (2010). Ecological pest management and control by using allelopathic weeds (*Ageratum conyzoides*, *Ambrosia trifida*, and *Lantana camara*) and their allelochemicals in China. *Weed Biology and Management,* 10: 73-80.

Lee, I. Y., Park, J. E. Shin, D. H., Lee, I. J. and Kim, K. U. (2007). Utility of weeds and their relatives as resources in Korea. In Kim, K. U. *et al*. (Eds). *Utility of Weeds and their relatives as resources.* Kyungpook National University, Daegu, Korea. pp. 213-222.

Li, Q., Xiang, W. and Hao, W. (2005). The studying and utilization of *Xanthium sibiricum* Patr. *Chinese Agricultural Science Bulletin,* 21(9):116-120.

Li, R. H. and Qiang, S. 2007. Utilization of weeds in China. In Kim, K. U. *et al.* (Eds). *Utility of weeds and their relatives as resources*. Kyungpook National University, Daegu, Korea. pp. 181-196.

Liu, W., Shu, W. and Lan, C. (2003). *Viola baoshanensis*-a plant that hyperaccumulates cadium. *Chinese Science Bulletin,* 49(1): 29-33.

Macias, E. A., Molinillo, J. M. G., Varela, R. M. and Glindo, J. C. G. (2007). Allelopathy-a natural alternative for weed control. *Pest Management Science,* 63: 327-348.

Maneechote, C. (2007). Utilization of weeds and their relatives as resources in ThailandIn Kim, K. U. *et al.* (Eds). *Utility of Weeds and their relatives as resources*. Kyungpook National University, Daegu, Korea. pp. 107-121.

Morita, H. (2007). Edible wild plants including weed species, mainly used as vegetables in Japan. In Kim, K. U. *et al.* (Eds). *Utility of Weeds and their relatives as resources*. Kyungpook National University, Daegu, Korea. pp. 169-180.

Naidu, V. S. G. R., Yaduraju, N. T. and Gogoi, A. K. (2005). Weeds that heal. National Research Center for Weed Science. Maharajpur, Jabalpur (M.P.). p. 120.

Peles, J. D., Brewer, S. R. and Barrett, G. W. (1998). Heavy metal accumulation by old-field plant species during recovery of sludge-treated ecosystems. *American Midlands Naturalist*. 140: 245-251.

Qiang, S. (2002). Weed diversity of arable land in China. *Journal of Korean Weed Science,* 22(3): 187-198.

Rice, E. L. (1984). Allelopathy, 2nd Edition., Academic Press Inc. New York. pp. 130-188.

Schűtz, K., Carle, R. and Schieber, A. (2006). *Taraxacum*-A review on its phytchemical and pharmacological profile. *Journal of Ethnopharmacology,* 107: 313-323.

Stepp, J. R. (2004). The role of weeds as sources pharmaceuticals. *J. of Ethnopharmacology,* 92: 163-166.

Wei S., Zhou, Q. and Wang, X.. (2003). Identification of weed species with hyperaccumulative characteristics of heavy metals. *Progress in Natural Science* 13(12):1259-1265.

Wu, W. (2003). Primary study on management techniques on *Eichhornia crassipes*. *Shanghai Envvironment Science* (*suppl*.) pp. 146-149.

Xuan, T. D., Shinkichi, T., Khanh, T. D. and Chung, I. M. (2005). Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview. *Crop Protection,* 24: 197-206.

Yang, X., Long, X., Ni, W. *et al*. (2002). *Sedum alfredii,* A new Zn hyperaccumulating plant first found in China. *Chienese Science Bulletin,* 47(19): 1634-1637.

Zhang, W. (1996). The utilization of worst poisonous weed *Eupatorium adenophorum*. *Yunnan Forestry Science and Technology,* 1: 78-81.

# CONTROL OF HERBICIDE RESISTANT *ECHINOCHLOA ORYZOIDES* WITH PRE AND POST EMERGENT HERBICIDES BASED ON THE LEAF STAGES

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## ABSTRACT

The effect of weed control was investigated, based on the leaf growth stages and/or several different herbicide treatments, for an integrated weed management of herbicide resistant *Echinochloa oryzoides* in a rice field. The effectiveness of soil-applied herbicide treatments for pre-emergent control of *E. oryzoides* resistant to herbicides was very high with oxadiargyl 1.7% EC, oxadizon 12% EC, and fentrazamide·oxadiargal 3.3% EC.

Pentaxazon 5% SC achieved over 98% of weed control, although some *E. oryzoides* emerged 31 days after the treatment. Up to the 2nd leaf growth stage of *E. oryzoides*, six herbicides, azimsulfuron·carfenstole 1.05% GR, bensulfuron-methyl·benzobicyclone·mefenacet 24.52% SC, bensulfuron-methyl·fentrazamide 7% SC, bensulfuron-methyl·mefenacet·oxadiargyl 21.6% SC, benzobicyclon·mefenacet·penoxulam 21.5% SC and mefenacet·pyrazosulfuron -ethyl 3.57% GR achieved 100% control. However, only two herbicides, benzobicyclone·mefenacet·penoxulam 21.5% SC and mefenacet·pyrazosulfuron-ethyl 3.5% GR could control E. oryzoides up to the 3rd leaf growth stage. The study indicates that it is very important to select the right herbicides for treatment and apply them at the correct timing to achieve a high level of control of *E. oryzoides* resistant to ACCase- and ALS-herbicides.

**Key words**: ACCase; cyhalofop-butyl; *Echinochloa oryzoides*; herbicide resistance.

## INTRODUCTION

Barnyard grass [*Echinochla oryzoides* (Ard.) Fritsch], widely distributed in the world, reduces rice grain production greatly and is one of the most problematic weeds in paddy fields. For example, only four to eight barnyard grass plants/ $m^2$ may decrease rice yield by seven to 13%. It is very difficult to obtain good rice grain production when barnyard grass occurs in high abundance (Kwon *et al*., 2002).

It is anticipated that occurrence of barnyard grass will be significantly increased when large-scale rice cultivation is undertaken on the reclaimed land at Seosan in Korea, and the area used for direct seeding is increased. This will increase the use of acetyl Co a carboxylase (ACCase) foliar-applied herbicides such as cyhalofop-butyl and fenoxaprop-P-ethyl. It will also be increasingly difficult to control weeds using these kinds of herbicides. Therefore, it is very important to detect at the early stages whether or not weeds are herbicide resistant. Subsequent treatments of foliar-applied herbicides should be different depending on whether herbicide resistant weeds are present.

Park *et al*. (2010) reported that barnyard grasses resistant to ACCase foliar-applied herbicides also show resistance to acetolactate synthase (ALS) herbicides. To effectively control weeds showing cross resistance, an integrated weed management strategy would be required, based on the selection of appropriate herbicides and the treatment timing at the correct leaf growth stages. Lim *et al*. (2010) reported the occurrence of barnyard grasses resistant to ACCase herbicides such as cyhalofop-butyl and fenoxaprop-p-ethyl on the reclaimed land at Seosan of Choongnam in Korea.

This study, therefore, was conducted to evaluate the response to available herbicides, and to develop effective control strategies for barnyard grasses resistant to herbicides, based on herbicides with different modes of action.

## MATERIALS AND METHODS

Chemical response testing to develop the integrated weed management strategies against herbicide resistant barnyard grasses was conducted with *E. oryzoides* resistant to herbicides. Seeds were collected on October, 2009 from resistant population from Jooksanmyeon, Gimjaesi of Jeonbuk, Korea and were conserved at $4^0$C until April, 2010.

The barnyard grass plants were raised from these seeds and planted in 1/2000a Wagner pots filled with paddy soil. Chemical responses were observed on pre-emergence and post-emergence treatments respectively. Ten different soil applied herbicides were tested for pre-emergence treatment as given in Table 1.

In the second study, 14 different herbicides, including butachlor 5% GR, azimsulfuron·thiobencarb 7.05% GR, mefenacet·pyrazosulfuron-ethyl 3.57% GR, and bensullfuron-methyl· fentrazamide 7% SC were used as post-emergence herbicides, based on the leaf stages of barnyard grass with soil-applied herbicides. Herbicides for post-emergence were applied at different leaf stages of barnyard grass: i.e. 1, 2, and 3 leaf stages, at the recommended field rates of the herbicides.

All experiments were conducted in a glasshouse and had untreated controls. Experimental treatments were replicated three times and treatments were randomized. The weed control effect of each herbicide was investigated by measuring the dry weight of barnyard grasses in each pot and comparing with the untreated control at 45 days after pre-emergence treatment and 34~38 days after post-emergence treatment.

The data were subjected to an ANOVA and presented as a mean± standard deviation of at least three replicates. The mean values were separated by using the Least Significant Difference test at $P<0.05$.

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**Table 1.** Soil-applied herbicides applied as pre-emergence treatments to control barnyard grasses resistant to ACCase and/or ALS inhibitors herbicides

| Herbicide[1)] | Dosage (g a.i./ha) | Treatment time[2)] |
|---|---|---|
| benzobicyclon 3.5% SC | 140 | IAP~2DBT |
| butachlor 33% EW | 1,320 | IAP~2DBT |
| oxadiargyl 1.7% EC | 68 | IAP~3DBT |
| oxadizon 12% EC | 480 | IAP~2DBT |
| pentoxazon 5% SC | 200 | IAP |
| pretilachlor 37% EW | 1,480 | IAP~2DBT |
| benzobicyclone·pretilachlor 12% SE | 480 | IAP |
| benzobicyclone·thiobencarb 32.5% SE | 1,300 | IAP |
| pentrazamide·oxadiagil 3.3% EC | 132 | IAP~2DBT |
| Untreated | - | - |

[1)] SC: suspension concentrate; EW: oil emulsion in water; EC: emulsifiable concentrate; SE: suspension emulsion; [2)] IAP: immediately after puddling; 2DBT: 2 days before transplanting.

**Table 2.** Soil-applied herbicides applied as post-emergence treatments to control barnyard grasses resistant to ACCase and/or ALS inhibitors herbicides

| Herbicide[1)] | Dosage (g a.i./ha) | Treatment time (Leaf stage) | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| butachlor 5% GR | 1,500 | ○ | - | - |
| pretichlor 37% EW | 555 | ○ | - | - |
| esprocarb·pyrazosulfuron-ethyl 5.07% GR | 1,521 | ○ | ○ | - |
| azimsulfuron·carfenstole 1.05% GR | 315 | ○ | ○ | - |
| azimsulfuron·thiobencarb 7.05% GR | 2,115 | ○ | ○ | - |
| bensullfuron-methyl·benzobicyclon·mefenacet 24.52% SC | 1,226 | ○ | ○ | - |
| bensullfuron-methyl·fentrazamide 7% SC | 350 | ○ | ○ | - |
| bensullfuron-methyl·indanofan 3.4% SC | 170 | ○ | ○ | - |
| bensullfuron-methyl·mefenacet·oxadiargyl 21.6% SC | 1,080 | ○ | ○ | - |
| benzobicyclon·penoxulam 3.48% SC | 174 | ○ | ○ | - |
| dymuron·imazosulfuron·oxaziclomefone 11.5% SC | 575 | ○ | ○ | - |
| benzobicyclon·mefenacet·penoxulam 21.5% SC | 1,075 | ○ | ○ | ○ |
| pyrazosulfuron-ethyl·pyriftalid 0.67% GR | 201 | ○ | ○ | ○ |
| mefenacet·pyrazosulfuron-ethyl 3.57% GR | 1,071 | ○ | ○ | ○ |
| Untreated | - | - | - | - |

[1)] GR: granule; EW: oil emulsion in water; SC: suspension concentrate

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## RESULTS AND DISCUSSION

### Weed control using pre-emergence treatments

Ten different soil-applied pre-emergence herbicides were applied at the time of harrowing or two days before transplanting, and the weed control effects measured at 45 DAT. All herbicides applied achieved over 90% weed control by 10 days after treatment (data not shown). However, only five of them, butachlor 33% EW, oxadizon 12% EC, pentoxazon 5% SC, oxadiargyl 1.7% EC, and pentrazamide· oxadiagil 3.3% EC, could control over 95% of barnyard grasses (Table 3).

Plant height and dry weight are shown in Table 3. The results show that butachlor 33% EW, oxadizon 12% EC and the others will provide effective control of barnyard grasses resistant to herbicides in the rice field, if the treatments are applied at the time of harrowing.

**Table 3.** Growth status and weed control effect of barnyard grasses resistant to ACCase after treatment of soil-applied pre-emergence herbicides

| Herbicide | Plant height (cm) | Dry weight (g/pot) | Weed control (%) at 45 DAT |
|---|---|---|---|
| benzobicyclon 3.5% SC | 47.6 | 8.9 c | 66.8 |
| butachlor 33% EW | 26.3 | 1.5 d | 94.4 |
| oxadiargyl 1.7% EC | 0.0 | 0.0 d | 100.0 |
| oxadizon 12% EC | 0.0 | 0.0 d | 100.0 |
| pentoxazon 5% SC | 28.5 | 0.4 d | 98.5 |
| pretilachlor 37% EW | 53.0 | 7.1 c | 73.5 |
| benzobicyclone·pretilachlor 12% EW | 40.8 | 2.9 d | 89.2 |
| benzobicyclone·thiobencarb 32.5% EW | 50.8 | 8.5 c | 68.3 |
| pentrazamide·oxadiargyl 3.3% EC | 0.0 | 0.0 d | 100.0 |
| Untreated | 49.4 | 26.8 a | - |
| C.V(%)[1] | 27.1 | | |

[1] Means with the same letters in a row did not significantly differ at 5% by DMRT.

### Weed control using post-emergence treatments

### Effect at 1st leaf growth stage

Fourteen different soil-applied herbicides were applied at the 1st leaf growth stage and weed control effects measured 38 days after treatment. The results (Table 4) indicate 100% control of barnyard grass by treatment with bensullfuron·methyl· benzobicyclon·mefenacet 24.52% SC, bensullfuron-methyl·fentrazamide 7% SC, dymuron·imazosulfuron·oxaziclomefone 11.5% SC, benzobicyclon·mefenacet·penoxulam 21.5% SC and mefenacet·pyrazosulfuron-ethyl 3.57% GR. On the other hand, azimsulfuron·carfenstole 1.05% GR, bensullfuron-methyl·indanofan 3.4% SC,

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bensullfuron-methyl·mefenacet·oxadiargyl 21.6% SC and pyrazosulfuron-ethyl·pyriftalid 0.67% GR provided 96-99% weed control.

A few herbicides achieved 80~90% weed control, while esprocarb· pyrazosulfuron-ethyl 5.07% GR and azimsulfuron ·thiobencarb 7.05% GR could control less than 50% of the barnyard grasses resistant to herbicides (Table 4).

**Effect at 2nd leaf growth stage**
The weed control effects of the fourteen different soil-applied post-emergence herbicides on the 2nd leaf growth stage of *E. oryzoides* are provided in Table 4. One hundred percent control of barnyard grass was obtained by six herbicides, i.e., azimsulfuron·carfenstole 1.05% GR, bensullfuron- methyl· benzobicyclon· mefenacet 24.52% SC, bensullfuron·methyl· fentrazamide 7% SC, bensullfuron·methyl·mefenacet·oxadiargyl 21.6% SC, benzobicyclon· mefenacet· penoxulam 21.5% SC, and mefenacet·pyrazosulfuron-ethyl 3.57% GR at the 2nd leaf growth stage.

**Effect at 3rd leaf growth stage**
Treatment timing is very important for controlling barnyard grasses, because the tillers are formed right after the 3rd leaf growth stage. Herbicide treatments must be applied by this time, or weed control becomes very labor-intensive. One hundred percent control of barnyard grass was obtained 34 days after treatment with the treatments of benzobicyclon·mefenacet·penoxulam 21.5% SC and mefenacet· pyrazosulfuron-ethyl 3.57% GR (Table 4).

**Table 4.** Weed control effect of soil-applied post-emergence herbicide treatments at the 1 to 3 leaf growth stage of barnyard grasses resistant to ACCase inhibitors herbicides

| Herbicide | Weed control (%)[1] | | |
|---|---|---|---|
| | 1 | 2 | 3[2] |
| butachlor 5% GR | 78.0 | - | - |
| pretichlor 37% EW | 63.1 | - | - |
| esprocarb·pyrazosulfuron-ethyl 5.07% GR | 82.8 | 66.8 | - |
| azimsulfuron·carfenstole 1.05% GR | 98.5 | 100.0 | - |
| azimsulfuron·thiobencarb 7.05% GR | 90.3 | 32.1 | - |
| bensullfuron-methyl·benzobicyclon·mefenacet 24.52% SC | 100.0 | 100.0 | - |
| bensullfuron-methyl·fentrazamide 7% SC | 100.0 | 100.0 | - |
| bensullfuron-methyl·indanofan 3.4% SC | 98.5 | 89.2 | - |
| bensullfuron-methyl·mefenacet·oxadiargyl 21.6% SC | 98.9 | 100.0 | - |
| benzobicyclon·penoxulam 3.48% SC | 48.5 | 32.8 | - |
| dymuron·imazosulfuron·oxaziclomefone 11.5% SC | 100.0 | 94.0 | - |
| benzobicyclon·mefenacet·penoxulam 21.5% SC | 100.0 | 100.0 | 100.0 |
| pyrazosulfuron-ethyl·pyriftalid 0.67% GR | 96.3 | 96.6 | 91.8 |
| mefenacet·pyrazosulfuron-ethyl 3.57% GR | 100.0 | 100.0 | 100.0 |

1) Weed control effects at 34~38 days after treatment; 2) Leaf growth stages.

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Weed control achieved by the soil-applied post-emergence treatment at each leaf growth stage are summarized in Table 5. Four of them, i.e., azimsulfuron·carfenstole 1.05% GR, bensullfuron- methyl ·benzobicyclon· mefenacet 24.52% SC, bensullfuron-methyl·fentrazamide 7% SC, and bensulfuron- methyl·mefenacet·oxadiagyl 21.6% SC could control the barnyard grasses resistant to herbicides until the 2nd leaf growth stage.

Benzobicyclon· mefenacet· penoxulam 21.5% SC, pyrazosulfuron- ethyl· pyriftalid 0.67% GR and mefenacet· pyrazosulfuron-ethyl 3.57% GR showed great weed control effect from leaf stages 1 to 3. A few could achieve over 90% of weed control effect, but not 100%.

**Table 5**. Summary of effective soil-applied post-emergence herbicides at the 1 to 3 leaf growth stage of barnyard grasses resistant to ACCase inhibitors herbicides

| Herbicide | Treatment time[1)] | Weed control effects[2)] | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| azimsulfuron·carfenstole 1.05% GR | 15 DAT | ○ | ◎ | - |
| bensulfuron-methyl·benzobicyclon·mefenacet 24.52% SC | 15 DAT | ◎ | ◎ | - |
| bensulfuron-methyl·fentrazamide 7% SC | 15 DAT | ◎ | ◎ | - |
| bensulfuron-methyl·indanofan 3.4% SC | 15 DAT | ○ | △ | - |
| bensulfuron-methyl·mefenacet·oxadiagyl 21.6% SC | 15 DAT | ○ | ◎ | - |
| dymuron·imazosulfuron·oxaziclomefone 11.5% SC | 15 DAT | ◎ | ○ | - |
| benzobicyclon·mefenacet·penoxulam 21.5% SC | 10~12 DAT | ◎ | ◎ | ◎ |
| pyrazosulfuron-ethyl·pyriftalid 0.67% GR | 15 DAT | ○ | ○ | ○ |
| mefenacet·pyrazosulfuron-ethyl 3.57% GR | 5~15 DAT | ◎ | ◎ | ◎ |

1) DAT: days after treatment

2) Weed control effects: ◎ 100%, ○ 91~99%, △ 89% (at 34~38 days after treatment)

## CONCLUSIONS

The effectiveness of soil-applied herbicide treatments for pre-emergent control of *E. oryzoides* resistant to herbicides was very high with oxadiargyl 1.7% EC, oxadizon 12% EC, and fentrazamide·oxadiargal 3.3% EC. Six soil-applied post-emergence herbicides achieved perfect control of *E. oryzoides* up to the 2nd leaf growth stage, while only two herbicides, benzobicyclone· mefenacet· penoxulam 21.5% SC and mefenacet· pyrazosulfuron-ethyl 3.5% GR could control the weed up to its 3rd leaf growth stage.

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## REFERENCES

Kwon, O.D., Yong, I.K., Lee, D.L., Shin, H.R., Park, I.J., Kim, E.B. and Guh, J.O.. (2002). Growth and yield of rice as affected by competitive period of resistant *Monochoria vaginalis* biotypes to Sulfonylurea herbicides. *Kor. J. Weed Sci.* 22(2):147-153.

Lim, S.H., Song, J.S., Zhang, C. and Kim, DS. (2010). ACCase inhibitor cyhalofop-butyl resistance in *Echinochloa oryzicola* collected in Chungnam and Jeonbuk province, Korea. *Kor. J. Weed Sci.* 30(Supp. 1):45-46.

Park, T.S., Ku, B.I., Kang, D.K. Choi, M.K., Park, H.K., Lee, K.B. and Ko, J.K.. (2010). Response of the resistant biotype of *Echinochloa oryzoides* to ACCase and ALS inhibitors, and effect of alternative herbicides. *Kor. J. Weed Sci*. 30(3):291-299.

# INFLUENCE OF ENVIRONMENTAL FACTORS ON SEED GERMINATION AND SEEDLING EMERGENCE OF YELLOWTOP (*FLAVERIA BIDENTIS*)

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## ABSTRACT

Laboratory experiments were conducted in Beijing from 2008-2010 to determine the effects of light, temperature and planting depth on *Flaveria bidentis* germination and emergence. *F. bidentis* had positively photoblastic seeds which required light in germination. Seed germination had a wide temperature range, 15 C to 40 C, with the optimum temperatures from 22.5 C to 35 C. The threshold temperature for germination was 14.7 C and the effective accumulated temperature required to reach 90% germination was 40.4 C. Germination was increased by 12% to 100% when the temperature from 12.5 C to 20 C. Seedling emergence was highest on the soil surface, and no seedlings emerged from a soil depth of 1 cm or deeper. The information gained from the study could help predict its potential distribution area and facilitate the development of effective weed control strategies.

**Key words:** *F. bidentis* , light, temperature, burial depth.

## INTRODUCTION

Yellowtop (*Flaveria bidentis* (L.) Kuntze), a member of the Asteraceae family, is an invasive weed native to North America (Powell 1978), which was first observed in China in 2003 (Gao *et al*. 2004; Liu 2005). At a surprising rate, the species has now spread to 84 counties of the middle south of Hebei province, five districts of Tianjin and three counties in Henan and Shangdong province. Invasion of diversified habitats in these areas, such as roadsides, transit points, construction waste grounds, wastelands, urban green spaces and ditch edges, has been attributed to its ecological adaptations, growth plasticity and competitiveness. It is worrying that *F. bidentis* has invaded farmlands adjacent to roads, and its rapid growth and high reproduction coefficient allow the weed to be a good competitor against crops. In addition, aqueous extracts of *F. bidentis* were documented to reduce germination of more than 20 plant speices, so the presence of the weed may therefore influence the growth of certain plants other than competition (Li *et al*. 2007). Consequently, invasion and colonization of *F. bidentis* in North China should be highly monitored for its widespread and distribution may pose a potential threat to ecosystem and agricultural production.

Seed germination is one of the most critical events for the success of any weed because it represents the first stage at which the weed can compete for an ecological niche (Forcella *et al*. 2000), and is mediated by various environmental variables such as temperature, light, pH, and soil salinity, and moisture (Chachalis and Reddy 2000; Chauhan *et al*. 2006).

Acquiring the germination characteristics provides a biological basis for the spread and establishment of *F. bidentis* to allow better timing of weed control treatments and facilitate development of agronomic practices that discourage weed establishment in crops.

The present study was designed to examine the effects of constant and alternating temperature regimes, light and burial depth on seed germination and seedling emergence of *F. bidentis*. The information gained from the study could help understand current distribution, predict its potential spread area and facilitate the development of effective weed control programs.

## MATERIAL AND METHODS

### Seed collection

Experiments were conducted in 2008 and 2009 at Institute of Plant Protection, Chinese Academy of Agricultural Sciences. Seeds used in the experiments were collected in late October 2007 from wastelands in Handan City, Hebei Province, China. After air-drying, seeds were stored at room temperature (20 to 25 C) until experiment initiation. The 10,000-seed weight of *F. bidentis* was 1.50 to 1.97 g.

### General germination tests

*F. bidentis* germination was evaluated by placing 100 seeds evenly in a 7.5-cm- diameter petri dish containing two layers of Whatman No.1 filter paper, moistened with either 3 ml of deionized water or a treatment solution. Dishes were sealed with Parafilm and placed in a growth chamber set at 30 C (temperature determined to be optimum for germination), except for the temperature experiment. The photoperiod was set at 12 h with fluorescent lamps used to produce a light intensity of 12000 Lux. A seed was characterized as germinated when the radicle was the same as seed length, while the cotyledon was equal to one half of seed length. Germination percentage was calculated as the total number of seeds germinated divided by the total number of seeds in each replication.

### Effect of light

Seed germination was studied under 12/12, 0/24, and 24/0 h light/dark regimes at 30 C. Petri dishes assigned to the dark treatment were wrapped in two layers of aluminum foil to ensure no light penetration; other treatments were left uncovered to allow continuous light exposure (12000 Lux light intensity). Germinated seeds were counted after 5 d of incubation.

### Effect of temperature

*F. bidentis* seeds were placed in petri dishes and incubated under constant (12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 35, 40, 45 C) or fluctuating temperatures (15/10, 20/5, 20/10, 20/15, 25/5, 25/10, 25/15, 30/10, 30/15, 30/20, 35/10, 35/15 C day/night). Photoperiod was set at 12 h to coincide at high temperature. Seed germination was assessed every 12 h by counting and removing germinated seeds. The seed were kept hydrated by adding distilled water as needed to avoid a moisture effect.

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Data about time to onset of germination and final germination percentages were used to compute the days required to reach 90% germination as overall germination(t90) (Ramon *et al*. 2004) and germination rates (V). The intercept (C, germination threshold temperature) and slope (K, effective accumulated temperature required to reach 90% germination) were calculated by the method of least squares (Cai *et al*. 2001) based on the effective accumulated temperature principle. Equations are as follows:

$$t_{90} = (H\,p - L\,p)^{-1} + L$$
$$V = 1 / t_{90}$$
$$K = (n\sum VT - \sum V \sum T) / [n\sum V^2 - (\sum V)^2]$$
$$C = (\sum V^2 \sum T - \sum V \sum VT) / [n\sum V^2 - (\sum V)^2]$$

where L is the last day before 90% germination was reached. Lp is the observed germination percentage on day L, Hp is the observed germination percentage on the day when germination reached or exceeded 90%, T is the tested temperatures, and n is the value of temperature.

## Effect of seed burial depth

Twenty seeds were planted in soil passed through a 2-mm sieve in 15-cm-diameter by 20-cm-deep plastic pots at depths of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1,2,3,4 and 5 cm in each of four replications. For all treatments, the uppermost layer of soil was leveled and then pressured with the same force before and after seed placement to standardize depths. Pots were irrigated from the bottom as needed to maintain adequate soil moisture. Seedling emergence was counted every 5 d for 25 d.

## Statistical analyses

All experiments were conducted in a randomized complete-block design, and each experiment was repeated at least twice. Effect of temperature on seed germination was replicated six times, and the other experiments were four replicates. Significant differences among treatments were identified with the use of Duncan's multiple-range test ($P = 0.05$). Regression analysis was used to determine the effect of salinity on germination. Data met all normality conditions; therefore, data transformation was not required. Means were separated using standard error of mean. All statistical analyses were performed using SPSS.

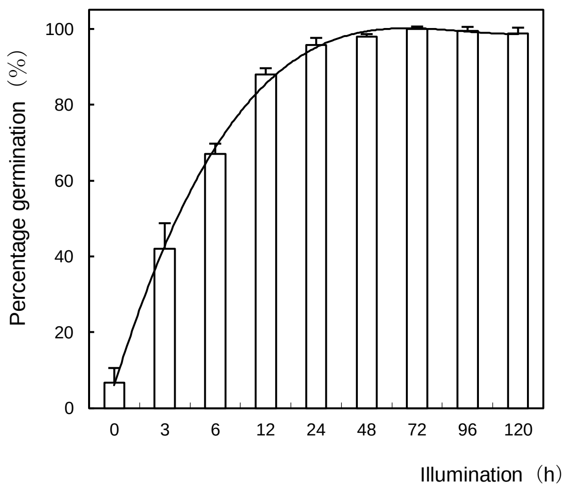


Figure 1. Germination of *F.bidentis* in illumination and darkness

# RESULTS AND DISCUSSION

## Effect of light

Light had an impact on germination. There were no differences ($P=0.05$) in germination percentage between seed placed in a 12-h photoperiod and seed exposed to continuous light, which was significantly higher than the germination of complete darkness (Figure 1).

It is reported that seed germination response to light is species-specific. Seeds of some species germinate equally in light and dark (Norsworthy and Oliveira 2005; Zhou *et al*. 2005; Wei *et al*. 2009); some require light to stimulate germination (Lu *et al*. 2006; Chauhan and Johnson 2008); others are favored by darkness (Chauhan *et al*. 2006). Our results demonstrated that seeds of *F. bidentis* were positively photoblastic and light stimulation was necessary for seed germination, whereas no or few seeds germinated in the dark.

**Effect of temperature**

Germination percentages differed significantly among constant temperature regimes (P=0.05) (Table 1). In general, germination increased from 2.7 to 99.3% between 15 and 22.5 C; it was greater than 97% over the temperature range of 22.5 C to 35 C with no significant differences (P=0.05), and then dropped with increased temperature to 19% at 40 C with obvious radical length reduction. No germination occurred at either 12.5 C or 45 C (data not shown).

Time to onset of germination gradually decreased as the temperature rose from 15 C to 35 C. For example, when exposed to constant temperature regimes of 22.5, 25, 27.5, 30, 35 C, seed germination began and final germination percentage was greater than 90% after 36 to 60 h of incubation and 2 to 6 d of incubation, respectively. Together, the optimum germination temperature was between 22.5 and 35 C. The threshold temperature for germination was 14.7 C and the effective accumulated temperature required to reach 90% germination was 40.4 C.

The final germination percentages and germination rates of all the constant temperature treatments was compared with the germination of all the alternating temperature treatments (Table 1). In comparison, germination of alternating temperatures increased significantly (P=0.05) in response to mean temperatures of 12.5 to 20 C; constant temperature treatments and alternating temperature treatments with 22.5 C and 25 C mean temperatures had no significant effect on seed germination (P=0.05); germination was greater than 94.0% at mean temperatures ranging from 15 to 25 C, which indicated that *F. bidentis* seeds had a broad temperature range for germination.

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**Table 1.** *F. bidentis* seed germination for 30 d in alternating and constant temperatures (C)

| Temperatures | Time to onset of germination (d) | Germination (%) | $t_{90}$(d) |
|---|---|---|---|
| 12.5 | / | 0.0 f | NE |
| 15/10 | 13 | 19.8±6.9 e | NE |
| 20/5 | 6 | 93.0±2.1 b | 29.11 |
| 15 | 7 | 2.7±1.1 f | NE |
| 20/10 | 6 | 97.0±1.0 ab | 26.15 |
| 25/5 | 4 | 94.0±1.4 ab | 13.43 |
| 17.5 | 4 | 38.8±3.0 d | NE |
| 20/15 | 4 | 98.0±0.5 ab | 24.20 |
| 25/10 | 4 | 98.2±0.5 ab | 6.11 |
| 20 | 3 | 85.5±1.9 c | NE |
| 25/15 | 3 | 99.0±0.5 ab | 5.06 |
| 30/10 | 2.5 | 97.2±0.7 ab | 4.64 |
| 22.5 | 2.5 | 99.3±0.3 a | 5.95 |
| 30/15 | 2.5 | 99.5±0.3 a | 4.04 |
| 35/10 | 2.5 | 98.5±0.7 ab | 3.65 |
| 25 | 2 | 99.7±0.2 a | 3.57 |
| 30/20 | 2 | 99.2±0.3 ab | 3.58 |
| 35/15 | 2.5 | 98.7±0.5 ab | 3.02 |

Notes: "/" indicated no seed germinated.

Increasing the mean temperature from 12.5 C to 25 C reduced time to onset of germination and time to 90% germination ($t_{90}$). Temperature alternation not only stimulated *F. bidentis* germination but also reduced the mean temperature required to promote the same germination as the maximum germination shown by treatments with constant temperature. Increased amplitude of the diurnal temperature alternation increased percent germination as well as germination rate, and this was more evident at the lower temperature of 12.5 C.

Temperature is an important factor in seed germination. *F. bidentis* seed germination had a wide optimum temperature range and the germination threshold temperature was 14.7 C, partially explaining why the invasive weed may emerge throughout the season from the first ten days of April through mid-October provided soil moisture is favorable in North China. Consequently, it is vital to establish a season-long control system. The low but insignificant germination, particularly at the low temperatures (15 C), may be biologically significant. Even an individual plant, if uncontrolled, is capable of producing approximately 360, 000 seeds to the soil seed bank (Li *et al*. 2006).

The stimulation in germination with alternating temperatures had been also reported in other studies (Ramon *et al*., 2004; Steckel *et al*., 2004). Moreover, the ability of *F. bidentis* seeds to germinate quickly on account of temperature alternation would be of benefit for rapid seedling establishment in the field following summer rainfall events prior to soils becoming dry. So the greater response to warm fluctuating temperatures may suggest greater emergence of these species on bare ground, where the greatest diurnal fluctuations would be expected.

**Effect of seed burial depth**

Seedling emergence decreased dramatically with increasing planting depth. Emergence was at its maximum for seeds planted on the soil surface with the emergence rate of 96%; emergence fell sharply even with shallow burial (data not shown). No seedlings emerged from seed buried at 1 cm. These results agree with that of the light study, in which *F. bidentis* seeds had a light requirement for germination. Benvenuti (1995) reported that light penetration fell below 0.01% at a depth of no more than 4 mm and that with increasing soil depth, light permeability was proportional to wavelength, leading to progressive decline in the red-far red ratio. The reason of no seedlings emerged from seeds placed at a depth of 1 cm was in part that very little light was reached at the depth or that shortwave lights required for emergence were filtrated. Further research will need to focus on effect of light quality on seed germination. In addition, emergence from different soil depths has been found to be positively proportional to seed energy reserves (Benvenuti *et al*. 2001; Li *et al*. 2004; Zheng *et al*. 2006). The 10,000-seed weight of *F. bidentis* was only 1.50 to 1.97 g, so the species might not have enough energy to emerge from deeper soil layers. Furthermore, physical obstacles from soils and decreasing thermal fluctuation (Kegode *et al*. 1998) are responsible for depth inhibition. The observed preference for seed germination from the soil surface could result in greater abundance of *F. bidentis* under no-till farming systems. In these systems, the weed seed bank would be concentrated on or near the soil surface (Clements et al. 1996). In our study, seedling emergence on the soil surface was slightly lower than germination observed in petri dishes in the light. This difference could be due to lower soil-to-seed contact and water availability on the soil surface than on the filter paper (Ghorbani *et al*. 1999).

Inferences drawn from the results of this study should be limited to the weed population sampled because weed ecotypes often vary in germination requirements. *F*. *bidentis* seeds were positively photoblastic; thus, shallow tillage will help reduce the weed populations and seedling emergence was the greatest for seeds present on the soil surface. These results suggest that *F*. *bidentis* has the potential of becoming a problematic weed under no-till systems. *F*. *bidentis* was able to germinate over a broad range of temperatures, and the germination threshold temperature was low, which contribute to in part explain why the invasive weed may emerge throughout the season from the first ten days of April through mid-October provided soil moisture is favorable in North China.

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## References

Benvenuti, S., and M. Macchia. (1995). Effect of hypoxia on buried weed seed germination. *Weed Res*. 35: 343-351.

Benvenuti, S., M. Macchia, and S. Miele. (2001). Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Sci*. 49: 528-535.

Cai, W. Z., X. F. Pang, and B. Z. Hua, et al. (2001). *General Entomology [M]. Beijing: China Agricultural University Press.* 7: 396-398. (In Chinese)

Chachalis, D., and K. N. Reddy. (2000). Factors affecting Campsis radicans seed germination and seedling emergence. *Weed Sci.* 48: 212-216.

Chauhan, B. S., and D. E. Johnson. (2008). Influence of Environmental Factors on Seed Germination and Seedling Emergence of Eclipta (*Eclipta prostrata*) in a Tropical Environment. *Weed Sci*. 56: 383-388.

Chauhan, B. S., G. Gill, and C. Preston. (2006). Factors affecting seed germination of threehorn bedstraw (*Galium tricornutum*) in Australia. *Weed Sci*. 54: 471-477.

Clements, D. R., D. L. Benoit, and S. D. Murphy, et al. (1996). Tillage effects on weed seed return and seedbank composition. *Weed Sci.* 44: 314-322.

Forcella, F., R. L. Benech-Arnold, and R. Sanchez, et al. (2000). Modeling seedling emergence. *Field Crops Res.* 67: 123-139.

Gao, X. M., T. G. Tang, and Y. Liang, et al. (2004). An Alert regarding biological invasion by a new exotic plant, *Flaveria bidentis*, and strategies for its control. *Biodiversity Science.* 12(2): 274-279. (In Chinese).

Ghorbani, R., W. Seel, and C. Leifert. (1999). Effects of environmental factors on germination and emergence of *Amaranthus retroflexus*. *Weed Sci.* 47: 505-510.

Kegode, G. O., R. B. Pearce, and T. B. Bailey. (1998). Influence of fluctuating temperatures on emergence of shattercane (*Sorghum bicolor*) and giant foxtail (*Setaria faberi*). *Weed Sci.* 46: 330-335.

Li, R. P., D. M. Jiang, and Z. M. Liu, et al. (2004). Effects of sand-burying on seed germination and seedling emergence of six psammophytes species. *Chinese Journal of Applied Ecology*. 15(10): 1865-1868. (In Chinese).

Li, X. J., G. Q. Wang, and C. X. Zhang, et al. (2006). Distribution, characteristics and chemical controls of *Flaveria bidentis* (L.) Kuntze, a New Exotic Plant. *Chinese Weed Science.* 12(12): 274-279.

Li, X. J., M. R. Zhang, and Y. J. Li, et al. (2007). Effect of aqueous extracts of *Flaveria bidentis* (L.) Kuntze on seed germination and radical length. *Chinese Weed Science.* 4: 15-19.

Liu, Q. R. (2005). *Flaveria* Juss . (Compositae), a newly naturalized genus in China. *Acta Phytotaxonomica Sinica*. 43(2): 178-180. (In Chinese)

Lu, P., W. G. Sang, and K. P. Ma. (2006). Effects of environmental factors on germination and emergence of Crofton weed (*Eupatorium adenophorum*). *Weed Sci*. 54: 452-457.

Norsworthy, J. K., and M. J. Oliveira. (2005). Coffee senna (*Cassia occidentalis*) germination and emergence is affected by environmental factors and seeding depth. *Weed Sci*. 53: 657-662.

Powell A M. (1978). Systematics of *Flavaria*. *Annuals of the Missouri Botanical Garden*. 65 (2): 590-636.

Ramon, G. L., A. D. Knapp, and M.D.K. Owen. (2004). Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*). *Weed Sci*. 52: 67-73.

Steckel, L. E., C. L. Sprague, and E. W. Stoller, et al. (2004). Temperature effects on germination of nine Amaranthus species. *Weed Sci*. 52: 217-221.

Wei, S. H., C. X. Zhang, X. J. Li, H. L. Cui, H. J. Huang, B. F. Sui, Q. H. Meng, and H. J. Zhang.(2009). Factors affecting buffalobur (*Solanum rostratum*) seed germination and seedling emergence. *Weed Sci.* 57: 521–525.

Zheng, M. Q., Y. R. Zheng, and L. H. Jiang. 2006. Effects of one-time water supply and sand burial on seed germination and seedling emergence of four popular psammophyte in Mu Us sandy land. *Acta Ecologica Sinica*. 8: 2474-2484. (In Chinese).

Zhou, J. K., E. L. Deckard, and W. H. Ahrens. (2005). Factors affecting germination of hairy nightshade (*Solanum sarrachoides*) seeds. *Weed Sci.* 53: 41-45.

# CANOLA (*BRASSICA NAPUS*) COMPETITION FOR WEED MANAGEMENT

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## ABSTRACT

Canola (*Brassica napus*) is an important break crop in the temperate cropping zone of southern Australia. Although a wide range of herbicide-tolerant cultivars are now available, weeds can still significantly reduce grain yield and quality. Crop competition is a useful tool for reducing weed impacts and suppressing weed growth and spread. A field experiment in 2009 studied the impact of two canola genotypes and four crop densities on the competitive ability of canola with wheat, an important volunteer weed in canola. Significant effects of competition on the suppression of the weed and grain yield were recorded. Manipulation of crop agronomy by choice of genotype and increasing crop density to reduce weed impacts is a cost-effective and simple way for famers to improve weed control, increase herbicide efficacy and prolong the life of useful chemicals by reducing the rate of development of herbicide resistance.

**Keywords**: weed competition, cultivars, herbicide resistance, weed interference, hybrid, seed rate

## INTRODUCTION

In recent years weed control options for canola in Australia have improved considerably with the development of a wide range of herbicide–tolerant cultivars with resistance to glyphosate, triazine, or imidazinolinone herbicides. However, the widespread incidence of weed resistance to many herbicide groups requires the inclusion of non-chemical control tactics in management strategies to reduce weed costs and dependence on herbicides. Volunteer wheat can be a significant weed in canola even in herbicide-tolerant crops (Lemerle *et al*. 2001). Crop competition is an important tactic to facilitate herbicide performance and integrated weed management.

Previous studies in Canada indicate that more competitive canola cultivars and higher seeding rates increased the ability of crops to compete with weeds (Harker *et al*. 2003, Beckie *et al*. 2008). $F_1$ hybrids are taller, more vigorous, and establish a denser canopy than the open-pollinated types (Zand and Beckie 2002). In Australia, triazine–tolerant cultivars were poorly competitive against weeds compared to the vigorous hybrids (Lemerle *et al.* 2010). We hypothesise that a combination of cultivar and seeding rate will increase weed suppression and optimise grain yield and quality in the presence of weeds. This study compared the effects of canola genotype and seeding rate on crop grain yield in the presence and absence of volunteer wheat.

## MATERIALS AND METHODS

The experiment was conducted at Wagga Wagga, New South Wales, Australia (Latitude $35^0$ 05 S, Longitude$147^0$ 35 E) in 2009 in a silty clay loam with pH 4.6 and organic matter of 1.8%. Treatments were arranged in a split-split plot design with three replicates. Main plots were two genotypes, split-plots were four crop densities and sub-plots were weedy and weed-free conditions. Genotypes were a low vigour, conventional triazine-tolerant cultivar (ATR-409) and a vigorous commercial $F_1$ hybrid (46Y78). Four canola seeding rates (15, 30, 60, 120 seeds/m$^2$) were used to target crop densities of 10, 20, 40 and 80 plants/m$^2$ (assuming emergence rate at about 60%) as recommended by McCaffery *et al*. (2009). Plot size was 1.8 m (eight 22 cm rows) wide by 10 m long. The experimental area was cultivated and harrowed on 4 June and treated with Trifluralin applied at 1.8 L/ha in 100 L water/ha) and incorporated on 15$^{th}$ June to control broadleaf weeds. The canola treatments densities were sown on 16th June, plus and minus cv. Ellison at 15 kg/ha as the surrogate weed. DAP fertiliser was applied at sowing, equivalent to 20 kg/ha P and 18 kg/ha N.

Crop establishment was estimated by counting plant numbers using a 0.5 m ruler between 2 crop rows at five random locations per plot. Weed numbers were recorded using 50 cm X 50 cm quadrats in six random locations per plot. In-crop growing-season (1 April – 31 October) rainfall was only 230 mm about half the annual average of 460 mm.

Destructive hand harvests for biomass were taken at flowering on 16$^{th}$ September using one randomly placed 1m$^2$ quadrat per plot. Crop and weeds were separated and weighed. After drying at 70 $^0$C for three days, dry weights were recorded. Plots were harvested for grain yield using a Kingaroy small-plot harvester on 20$^{th}$ November. The samples were cleaned and sub-samples taken for quality assessment by NIR (moisture, oil, protein and glucosinolate contents – data not reported here).

Data were analysed using AS-REML in R version 2.12.2.

## RESULTS AND DISCUSSION

### Plant densities

For the three lower seeding rates, the achieved crop (canola) plant density was very close to the targeted number. However, for the highest rate (80 plants/m$^2$) the results varied from 67 to 88 plants/m$^2$ on average for the two cultivars. In this environment the farmers' target is around 40-60 plants/m$^2$ for all genotypes (McCaffery *et al*. 2009), while lower densities are recommended in drier areas. Poor establishment is a common problem in canola due to dry conditions at sowing, where residues from the previous crop are present, and from insect, bird or mouse damage.

The weed (volunteer wheat) plant density was much more variable and ranged from 49 to 100 plants/m$^2$, due to sowing difficulties and poor establishment. Weed density varied from 61 to 87 plants/m$^2$ (averaged across the seeding rate by genotype combinations).

### Grain Yield

Grain yield was affected by a significant interaction between canola genotype, canola density and the presence or absence of volunteer wheat. Yields were low due to drought and ranged from 0.05 to 0.25 t/ha depending on the treatment combination (Figure 1). There was no indication in these low-yielding conditions that the hybrid 46Y78 was higher yielding than ATR-409, in fact it was lower yielding at higher densities possible due to greater early vigour and water use. Weed-free plots yielded much more than weedy ones (Figures 1 A and B).

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The weed-free yield response to canola density reached a plateau at about 40 plants/m$^2$ in the hybrid 46Y78 but continued to increase with crop density in ATR-409 (Figure 1A).

However, the reduction in yield in the face of weed competition was somewhat smaller for the hybrid than ATR-409 (Figure 1C), that is about 25% of the weed-free controls for the hybrid and 40-45% for ATR-409, especially at the higher densities. This is expected and is a similar result to the Canadian experience (Harker *et al*. 2003, Beckie *et al*. 2008). Further studies will confirm this in another contrasting season.

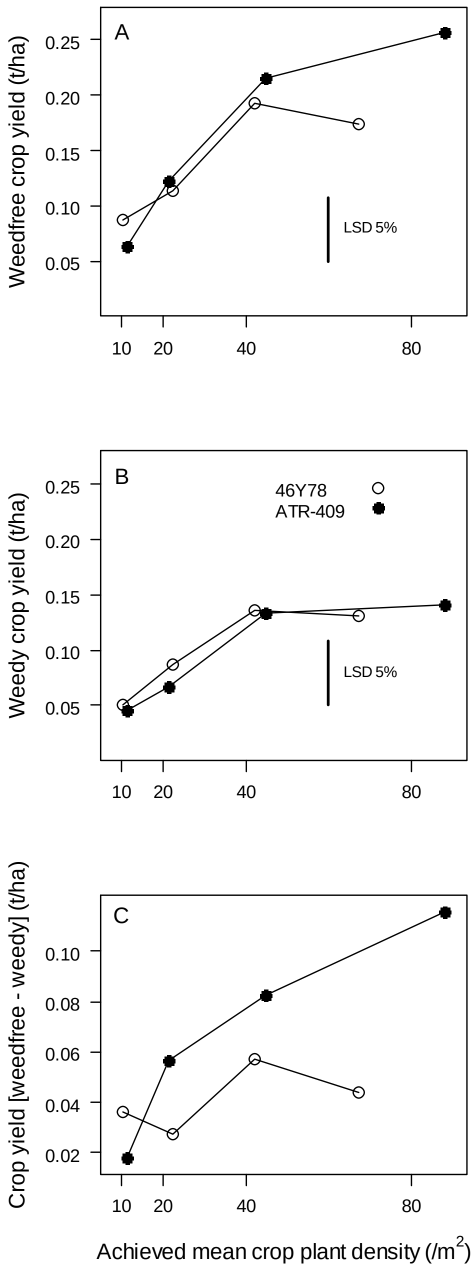


**Figure 1**. Grain yield (t/ha) for two canola cultivars, low vigour, conventional triazine-tolerant cultivar ATR-409 (closed symbol), and vigorous $F_1$ hybrid - 46Y78 (open symbol) in A) weed-free plots, and B) in weedy plots. Panel C) shows the reduction in yield (t/ha).

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X-axis tick marks show the targeted crop plant densities (plants/m$^2$), while the data is plotted at the achieved densities.

**Dry Matter (Biomass)**

Crop and weed dry matter (biomass) at the end of flowering were negatively correlated (Figure 2) as was found with 15 canola hybrids and annual ryegrass (Lemerle *et al.* 2010), confirming the importance of crop vigour and high biomass accumulation for weed suppression. The impact of canola allelopathy in suppression of weeds also requires further investigation.

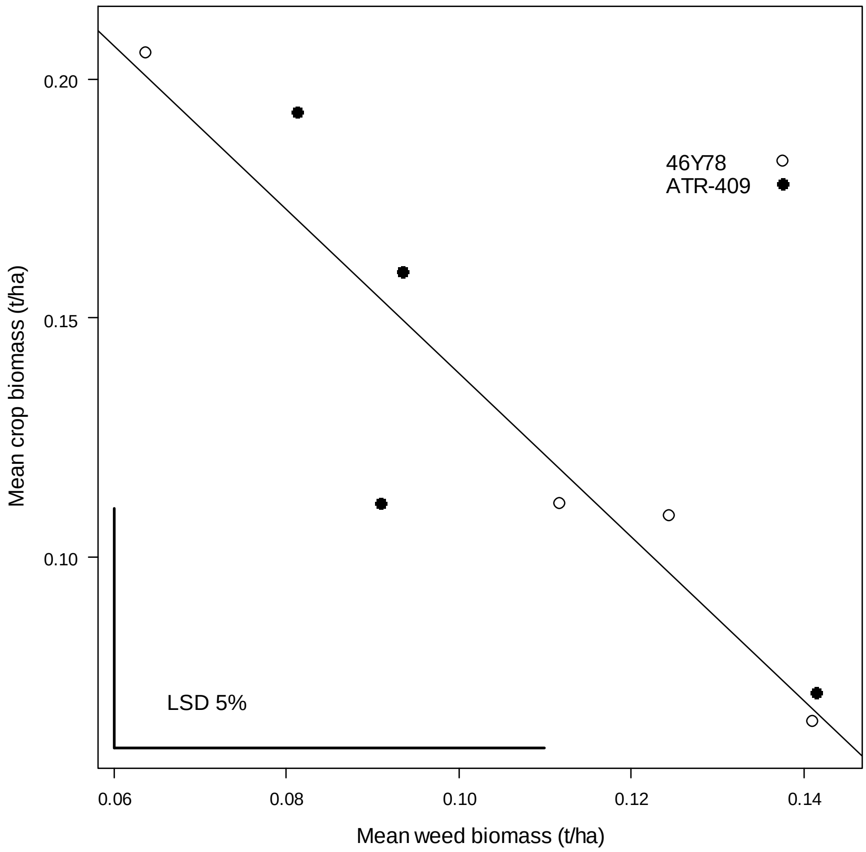


**Figure 2.** Relationship between crop and weed dry matter (t/ha) at late flowering for two canola cultivars grown in weedy plots in competition with volunteer wheat. The LSDs (5%) are large and show the inherent variability in this type of data. The plotted regression line illustrates the overall negative relationship.

In conclusion, these preliminary results show an important interaction between canola genotype and seeding rate on crop grain yield in the presence and absence of volunteer wheat. Higher seeding rates will be useful low-cost technique for reducing weed impact.

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## REFERENCES

Beckie, H.J., Johnson, E.N., Blackshaw, R.E. and Gan, Y. (2008). Weed suppression by canola and mustard cultivars. *Weed Technology* 22: 182-185.

McCaffery, D., Potter, T., Marcroft, S. and Pritchard, F. (2009). Canola: Best Practice management Guide for South east Australia. (Grains Research and Development Corporation, Canberra).

Harker, K.N., Clayton, G.W., Blackshaw, R.E., O'Donovan, J.T. and Stevenson, F.C. (2003). Seeding rate, herbicide timing and competitive hybrids contribute to integrated weed management in canola (*Brassica napus*). *Canadian Journal of Plant Science* 83: 433-440.

Lemerle, D., Blackshaw, R.E., Smith, A.B., Potter, T.D. and Marcroft, S.J. (2001). Comparative Survey of weeds surviving in triazine-tolerant and conventional canola crops in south-eastern Australia. *Plant Protection Quarterly* 16: 37-40.

Lemerle, D., Lockley, P., Luckett, D. and Wu, H. (2010). Canola competition for weed suppression. Proceedings of the 17th Australasian Weeds Conference, 26-30 September 2010, Christchurch, New Zealand, pp60-62.

Zand, E. and H. J. Beckie. 2002. Competitive ability of hybrid and open-pollinated canola (*Brassica napus*) with wild oat (*Avena fatua*). *Canadian Journal of Plant Science* 82:473–480.

# BENEFITS FROM BIOLOGICAL CONTROL OF WEEDS IN AUSTRALIA

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## ABSTRACT

Australia has a long and successful history in weed biological control, second only to the USA. A new book reviews all 73 weeds targeted in Australia, listing the >200 insect and pathogens released as biocontrol agents. Biocontrol programs have targeted agricultural, pastoral, rangeland, aquatic, and environmental weeds in tropical and temperate Australia. Despite so many releases over more than 100 years, there has been very little damage to non-weedy plants.

In an economic impact assessment done in 2006 on the 36 programs for which there was economic data, only nine gave few or no economic benefits. Thirteen programs resulted in very large economic benefits, including those against blackberry and lantana which had been considered failures. Biocontrol against parthenium started in 1977 and cost over $11 million, but benefits from reduced control costs and increased pasture production exceed $39 million. Parthenium is still abundant in central and north Queensland but is now much easier for landholders to manage, and the economic benefits are very great. Overall, biocontrol has returned annual benefits of $95.3 million from an annual investment of $4.3 million.

The Australian experience demonstrates that weed biocontrol is very cost-effective, most programs are successful and the risks are very small. Costs and risks are even smaller when using biocontrol agents already successful in another country. Asian countries could benefit from proven agents against weeds of importance in this region, such as parthenium, chromolaena, mikania, and mimosa, to gain the economic benefits already captured by Australia.

**Key words:** economic benefits, parthenium, chromolaena, mimosa

## INTRODUCTION

Biological control of weeds using introduced insects and, later, pathogens has a long and successful history in Australia (Julien *et al.* 2012). The first deliberate introduction was the cochineal insect *Dactylopius ceylonicus* in 1903, which failed but was followed by the release in 1914 of another strain which resulted in the successful control of drooping tree pear *Opuntia vulgaris*. This was followed in the 1920s by the introduction of up to 30 separate insect species, and the successful control of common pest pear *Opuntia stricta* by the moth *Cactoblastis cactorum*, and of other cacti by this moth and different *Dactylopius* species. Careful testing was undertaken with each insect to ensure that they would not feed on other

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plants. Australia was the first country in the world to use these tests, and has continued their use ever since.

After the great success of the prickly pear control, governments and scientists in Australia continued to support biological control, and by the 1980s Australia was a world leader in weed species targeted and new agents introduced (McFadyen 1998). Australia also led the world with the first deliberate introduction of a plant pathogen as a biocontrol agent, the rust *Puccinia chondrillinae* released in 1971 to control skeleton weed *Chondrilla juncea*. Despite the success of this introduction, and the complete absence of attack on non-target plants, doubts about the safety of pathogens stifled further introductions for 20 years. As a result of the complete blocking of the legal pathways, there were two illegal introductions, the rust *Puccinia xanthii* against Noogoora burr *Xanthium strumarium* in 1974, and in 1984 the rust *Phragmidium violaceum* against blackberry *Rubus fruticosus* agg.. The result was excellent control of Noogoora burr in the high rainfall coastal districts, but only poor control of blackberry The second legal introduction was the release of the rust *Puccinia abrupta* against parthenium *Parthenium hysterophorus* in 1991. Since then, at least 10 plant pathogens have been released, with varying success, but the majority of agents used were insects.

The 1970s and 1980s saw the successful control of the waterweeds water hyacinth *Eichhornia crassipes*, salvinia *Salvinia molesta* and pistia *Pistia stratiotes*, all with different species of weevils; of harrisia cactus *Harrisia martini* with a mealybug; of giant sensitive plant *Mimosa (invisa) diplotricha* with a psyllid; and partial or developing control of St John's wort *Hypericum perforatum*, various thistles, *Sida acuta*, and annual ragweed *Ambrosia artemisiifolia*. In the 20 years since, successful control is being achieved against ragwort *Senecio jacobaeae*, groundsel bush *Baccharis halimifolia*, bridal creeper *Asparagus asparagoides*, rubber vine *Cryptostegia grandiflora*, giant mimosa *Mimosa pigra*, and parthenium (Briese 2000, Julien *et al.* 2012).

Within the Asian-Pacific region, Australia is the leading country working with biocontrol of weeds of tropical and sub-tropical climates, and Australian expertise has helped in biocontrol programs in many countries in the region, resulting in some notable successes. This review of the benefits gained by Australia from 100 years of weed biocontrol is therefore relevant and important for all Asian-Pacific countries.

## REVIEW METHODS

In 2005 the Weeds CRC commissioned a major economic analysis of all weed biocontrol programs undertaken in Australia (Page and Lacey 2006). Data on the costs of research and releases was obtained from scientists working in biocontrol and with access to unpublished material including internal reports, field results, budgets and financial statements. Where data were unavailable or incomplete, the duration of the research in years and the number of staff employed (obtained from internal reports) was used to calculate costs, using a factor of Aus$300,000 per scientist-year whether employed in Australia or overseas. Only economic costs and benefits were considered: other benefits were listed but not included in the analysis. Completed programs where no agents were released or established were included and costs included in the analysis. All economic data throughout the study were converted to 2005 Aus$ values.

In 2010 a major review of all weed biocontrol programs in Australia was undertaken and will be published shortly (Julien *et al.* 2012). Numerous reviews of selected programs had been published over the years, but this will be the first comprehensive review since that by F. Wilson in 1960 (Wilson 1960). The intention was to collect together all essential information on each program, referring to earlier published material where this exists, and summarizing critical data such as duration and resources for each program, overseas exploration localities and dates, personnel involved, agents tested and released, and outcomes achieved. Much of this information is hidden in internal reports and never published, and, since the 1980s, is increasingly inaccessible as a result of old electronic data storage media that have never been adequately indexed or archived. This review therefore aimed to publish critical summaries, with each chapter written by scientists who were actively involved in the biocontrol programs and had access to the unpublished files.

## RESULTS

### Economic Study

The major message from the economic study (Page and Lacey 2006) was the huge overall benefit/ cost ratio, 23:1 for the 28 programs where data was available, an astonishing result. Even if the iconic prickly pear success is excluded, the overall benefit/ cost ratio is 12:1. Out of the total 36 programs, only nine were failures, ie resulted in few or no economic benefits.

Also important was the demonstration of the large economic benefits that result from even partial control of widespread weeds with high economic impacts, eg lantana and blackberry. Weed scientists tend to assume that if the weed is still a major problem despite biocontrol, then biocontrol has been a failure. But the economic analysis demonstrated that reductions in the vigour of a weed, or destruction of stands in some years but not all, still caused a significant reduction in the costs of managing the weed, or in the losses due to the weed. For example, it was calculated that $13.6 million has been spent on biocontrol of lantana (*Lantana camara*) in eastern Australia from 1914 to 2005. Losses due to lantana in 2005, in pasture, forestry, cattle poisoning and control costs, were at least $23.2 million per year, and this does not include biodiversity impacts in natural vegetation where control is not undertaken. As a result, although biocontrol is estimated to have resulted in only 10% reduction in lantana and only over the northern half of its range in Australia, this nevertheless results in sufficient savings in control costs and lost production to give an overall benefit/ cost ratio of 6:1 – ie $6 gained for each $1 invested. As biocontrol agents gradually increase and spead, these benefits continue to rise. Results for blackberry are similar.

A third finding was the importance of documenting the economic impact of target weeds at the start of a biocontrol program. It is essential to quantify the economic costs of the target weed at the start, so that the benefits from any reduction in its abundance can in turn be quantified. This is best done as an initial benefit/ cost study by independent economists prior to starting any biocontrol program, which should be part of the decision whether or not to undertake biocontrol (Jarvis *et al.* 2006). Such analyses clarify where data are not available, as well as identifying the critical issues for successful control, eg is total control required, or would any reduction in impact have important economic benefits. For most weeds, even non-production impacts have an economic aspect; if control was cheap or easy, the community would not permit weeds to overrun environmental areas. Economic impact is the sum of many factors: loss of agricultural productivity; actual and potential extent of infestation; spread rate; cost of removal; and frequency of recurrence. Initial measurement of these is essential to make future assessments possible. Analyses can then be repeated when more information is available (e.g. whether suitable agents can be found; spread rate of weed). Initial studies, using realistic probabilities of success based on historic rates for the country and type of weed, and including the full range of potential costs and benefits, are powerful tools to convince funding agencies to undertake a biocontrol program. Later analyses based on these data will clarify the true probabilities of failure and therefore the true return-on-investment for weed biocontrol programs.

The analysis also showed that program costs varied greatly. Some programs continued over decades, with years of overseas research and the employment of several scientists; the most expensive being that against *Mimosa pigra*, with a total cost of Aus$21.6 million. The cheapest successful program was against annual ragweed, with a total cost of only Aus$0.6 million. This cost was low because the successful agents were imported for the control of the closely related weed parthenium, and the only additional costs were for extra releases in ragweed areas. However, overall the median cost for each of the 17 successful programs was Aus$7 million and the duration was 14 to 27 years. This demonstrates that it is unrealistic to expect good results from programs run for a short time or 'on the cheap', except where agents have already been tested and successful in another country, eg the biocontrol of salvinia in Sri Lanka (Doeleman 1990), or the biocontrol of *Mimosa diplotricha* in Papua New Guinea (Kuniata and Korowi 2004).

**Overall Review**

A total of 73 weeds have been targeted for biocontrol in Australia, and between 1903 and 2010 more than 200 insect and pathogens have been released as biocontrol agents against these weeds. Biocontrol programs have targeted agricultural, pastoral, rangeland, aquatic, and environmental weeds in both tropical and temperate Australia. Despite so many releases over more than 100 years, there has been very little damage to non-weedy plants, with only one example of minor economic losses and none of environmentally significant damage.

Of these 73 programs, some (eg against *Mimosa pigra*, parthenium, lantana, skeleton weed, St John's wort and Paterson's curse *Echium plantagineum*) were large well-funded programs over many years, with many potential agents investigated and several imported and released. These weeds generally have a wide distribution in their native range with a large suite of native insects and pathogens to choose from. In other programs, the native range is very limited and as result, very few agents were found and less than five were released. However, some of these were nevertheless extremely successful, with one or two agents successfully establishing and resulting in almost complete control of the weed. Examples are the programs against docks *Rumex* spp., rubbervine, harrisia cactus, and the floating waterweed salvinia.

Several programs were hindered from the start by the presence of closely-related native plants or plants of economic value, e.g. fireweed *Senecio madagascariensis*, where there is a large complex of native senecios, or parthenium and annual ragweed which are both closely

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related to sunflower *Helianthus annuus*. On the other hand, other programs benefited from agents initially investigated for related weeds or by other countries, eg the successful control of annual ragweed using insects imported against parthenium weed, or control of alligator weed *Alternanthera philoxeroides* using insects already tested by the USA. At least one program, against the widespread tropical weed chromolaena *Chromolaena odorata*, was undertaken for countries in the Asian-Pacific region, by Australian scientists funded by Australian aid money, prior to any biocontrol program commencing within Australian territories. Australia has benefitted from reductions in the spread southwards of the weed and in the knowledge gained for the biocontrol program about to commence within Australia.

A result of major importance has been the complete absence of significant harmful impacts from the release of more than 200 plant-feeding insects and plant pathogens over the last 100 years. Minor damage has been caused by two agents released against lantana; the leaf-feeding beetle *Octotoma scabripennis* occasionally causes limited damage to commercially-grown herbs in the family Verbenaceae; and the sap-sucking bug *Acanophora compressa* damages the ornamental tree *Citharexylum spinosum* (Palmer *et al.* 2010). In both cases, there is no environmental impact as the plants attacked are not native to Australia, and the economic damage is very minor. On the other hand, the successful control of the prickly pears and of tiger pear *O. aurantiaca*, achieved between 1903 and the 1930s, by the release of cochineal insects *Dactylopius* spp. and the cactoblastis moth, is still just as successful 80 to 90 years later, with no evidence of the development of resistance in the weedy cacti nor of increased host range in the insects. Other successful programs, such as the release in 1970 of the rust against skeleton weed or of the cactus mealybug in 1974 against harrisia cactus, also continue to give excellent control 40 years later with no evidence of the development of non-target impacts or plant resistance.

**Implications for the Asian-Pacific Region**

The main message for weed management in the Asian-Pacific region is that Australia has already achieved successful biocontrol of several weeds which are major problems in one or more countries of the region. Importation of the successful biocontrol agents would be easy and relatively cheap, as the agents can be easily obtained in Australia and as their biology and host-specificity is already known. For example, *Mimosa diplotricha* has been successfully controlled in northern Australia by the sucking bug *Heteropsylla spinulosa*, and similar results have been obtained in PNG (Kuniata and Korowi 2004). The insect has been established in Fiji and the Solomon Islands, and recently in East Timor (McFadyen 2012), but there are many countries where this weed is a problem for agriculture and forestry, and which would benefit from introduction of the biocontrol agent. Similarly, the release of several insects and two rust pathogens have resulted in a significant reduction in the problem caused by parthenium weed in Australia, and releases of these agents should be considered in other countries where parthenium is a problem (Dhileepan and McFadyen 2012).

Another lesson is the large economic benefits from reduction in the vigour of a major weed, even if control is not complete. For example, in Indonesia and elsewhere, the weed chromolaena often replaced other weeds, such as lantana, when it first invaded. Once chromolaena has been successfully controlled by introduced biocontrol agents, lantana and other weeds may re-invade. However, control costs (or production losses) for chromolaena greatly exceed those from lantana; hence, there is still a benefit from its successful biological control even if other weeds subsequently re-invade.

With widespread invasive weeds, failure to use all successful control methods means that invasive plants continue to spread and cause increasing damage. In 2004, weeds cost the livestock industry in Australia $1.8 billion a year in lost production (Sinden *et al*. 2004), which represents many thousands of square kilometres of land effectively abandoned to weed invasion. The use of conventional control methods (herbicides, mechanical) results in increasing economic, environmental and health costs, especially in environmentally sensitive areas such as riparian lands or national parks.

The key messages from these two reviews, therefore, were that biological control of weeds is very cost-effective method and has given excellent returns-on-investment for Australian governments; it is safe; and the majority of programs achieve good levels of control. Governments in Asian-Pacific countries should put more effort and resources into biological control, starting with those weeds where success has already been achieved in Australia or elsewhere.

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## REFERENCES

Briese, D.T. (2000). Classical biological control. In Sindel B.M. (ed). *Australian Weed Management Systems.* R.G. and F.J. Richardson Publishers, Meredith. pp. 161-192.

Dhileepan, K. and Strathie, L. (2009). 20. *Parthenium hysterophorus*. In: Muniappan, R., Reddy, D.V.P. and Raman, A. (eds). *Weed Biological Control with Arthropods in the Tropics: Towards Sustainability.* Cambridge University Press, Cambridge. pp. 272-316.

Dhileepan, K. and McFadyen, R.E.C. (2012). *Parthenium hysterophorus* - parthenium. In: Julien, M., McFadyen, R. and Cullen, J. (eds). *Biological Control of Weeds in Australia*. CSIRO, Australia (In Press).

Doeleman, J.A. (1990). Biological control of salvinia in Sri Lanka: an assessment of cost and benefits. *Economic Assessment Series*. Australian Centre for International Agricultural Research, Canberra, Australia.

Jarvis, P.J., Fowler, S.V., Paynter, Q. and Syrett, P. (2006). Predicting the economic benefits and costs of introducing new biological control agents for Scotch broom *Cytisus scoparius* into New Zealand. *Biological Control* 39: 135-146.

ISBN Number: 978-0-9871961-0-1

Julien, M., McFadyen R. and Cullen, J. (eds). (2012). *Biological control of weeds in Australia.* CSIRO, Australia (In Press).

Kuniata, L.S. and Korowi, K.T. (2004). Bugs offer sustainable control of *Mimosa invisa* and *Sida* spp. in the Markham Valley, Papua New Guinea. In: Cullen, J.M., Briese, D.T., Kriticos, D.Y., Lonsdale, W.M., Morin, L. and Scott, J.K. (eds). *Proceedings of the XI International Symposium on Biological Control of Weeds*. 27 April-2 May 2003, Canberra. CSIRO Entomology, Canberra. pp. 567-573.

McFadyen, R.E.C. (1998). Biological Control of Weeds. *Annual Review of Entomology* 43: 369-93.

McFadyen, R.E.C. (2008). Winning the lottery: return on investment from weed biocontrol programs. In: van Klinken, R.D., Osten, V.A., Panetta, F.D. and Scanlan, J.C. (eds). *Proceedings of the 16th Australian Weeds Conference*. Queensland Weeds Society, Brisbane. Pp. 231=233.

McFadyen, R.E.C. (2008). Return on investment: determining the economic impact of biological control programmes. In: Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L. and Rector, B.G. (eds). *Proceedings of the XII International Symposium on Biological Control of Weeds*. CABI, Wallingford. pp. 75-82.

McFadyen, R.E.C. (2012). *Mimosa diplotricha* – giant sensitive plant. In: Julien, M., McFadyen, R. and Cullen, J. (eds). *Biological Control of Weeds in Australia.* CSIRO, Australia. (In Press).
Page, A.R. and Lacey, K.L. (2006). *Economic impact assessment of Australian weed biological control*. Technical Series No.10, CRC for Australian Weed Management, Adelaide.

Palmer, W.A., Heard, T.A. and Sheppard, A.W. (2010). A review of Australian classical biological control of weeds programs and research activities over the past 12 years. *Biological Control* 52: 271–287.

Raitzer, D.A. and Lindner, R. (2005). *Review of the returns to ACIAR's bilateral R&D investments.* Impact Assessment Series Report No. 35, Australian Centre for International Agricultural Research, Canberra.

Sheppard, A.W., Hill, R., DeClerke-Floate, R.A., McClay, A., Olckers, T., Quimby, P.C. Jr. and Zimmermann, H.G. (2003). A global review of risk benefit–cost analysis for the introduction of classical biological control agents against weeds: a crisis in the making? *Biocontrol News and Information* 24: 91–108.

Sinden, J., Jones, R., Hester, S., Odom, D., Kalisch, C., James, R. and Cacho, O. (2004). *The economic impact of weeds in Australia.* Technical Series 8, CRC for Australian Weed Management, Adelaide.

Wilson F. (1960). A review of the biological control of insects and weeds in Australia and Australian New Guinea. *Commonwealth Institute of Biological Control Technical Communication* 1: 51-68.

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# IMPACT OF HERBICIDE TOLERANT CROPS ON WEED MANAGEMENT IN THE ASIA PACIFIC REGION

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## ABSTRACT

This paper briefly reviews herbicide tolerance and its impact on weed management regimes, focusing on genetic modification technologies to derive novel herbicide tolerance in various crops worldwide, and how such HT crops may influence weed management strategies in the Asia Pacific region.

**Keywords:** Genetic modification, herbicide tolerance, weed management, biotechnology, Asia Pacific

## INTRODUCTION

Herbicide tolerant crops, contrary to popular belief, are not new. Nor are they restricted to genetically modified (GM) crops, also known as transgenic, genetically engineered, products of recombinant DNA technology (rDNA) or biotechnology. And sometimes they're called "Frankenfoods".

As we know, all crops are tolerant to at least some herbicides. Such tolerance might be entirely natural, or they could be created by humans using various traditional plant breeding methods. However, as 'herbicide tolerance' is so often used synonymously, if incorrectly, with GM technology, let's start by exploring that technology.

### What is Genetic Modification (GM) technology?

Recombinant DNA technology is a powerful means to transfer genetic material from one organism of any species to any other. The genetic material is usually a sequence of DNA coding for a particular trait, and the biotech breeder desires to introduce that trait into a host organism that currently lacks the desired trait.

One prominent example of an rDNA product is human insulin, used to treat diabetes. Since the early 20th Century when it was first purified, diabetics injected insulin extracted from dogs, rats and various farm animals, as such animals produce a version of insulin functionally similar to that produced by humans. Half a century later, in 1982, human insulin started being produced by *E. coli* bacteria into which the human DNA coding for insulin had been transferred. This human form of insulin, called Humulin™ rapidly replaced domestic animals as the insulin source, being better for the human diabetics, as the insulin was not just functionally similar to human insulin, it was exactly the same as human pancreas biosynthesized insulin. Plus, it saved countless farm animals, and also showed the public at large that human and bacteria DNA are not all that different.

The first example of using rDNA technology in the food realm occurred a few years after insulin (and many other rDNA based pharmaceuticals, which quickly followed), when in 1991 GM bacteria produced chymosin, a coagulating agent used in making cheese. rDNA-derived chymosin is now almost ubiquitous in industrial cheese making, and is popular with vegetarians as, unlike traditional rennet, does not come from animals.

The first GM crops came along a few years later. In 1994 in the USA, Calgene introduced FlavrSavr tomato to the market with great fanfare but was met with great indifference by American shoppers. The FlavrSavr was meant to provide summertime taste to wintertime consumers, as it had an antisense polygalacturonase gene to inhibit over-ripening, which ordinarily occurs between harvest and market when cultivation and consumption venues are distant. The concept was that the GM tomatoes would be grown in winter in southern States like Florida, then shipped ripe to northern markets in winter's deepfreeze grip, like Chicago. Unfortunately, the company suffered a number of setbacks, not least of which was choosing a tasteless tomato cultivar to engineer, and FlavrSavr failed in the marketplace. Other marketing strategies are more successful. In the UK, GM tomato paste in tins was quite successful in groceries, until anti-GM activists decided to threaten the retailers, when the otherwise successful GM paste was removed from market shelves.

Herbicide tolerance as an engineered trait came along soon after, led by Monsanto, who had been developing crop cultivars with rDNA engineered tolerance to Monsanto's glyphosate (the active ingredient in RoundUp™) herbicide. The strategic concept was commercially sound: glyphosate, as a broad spectrum, non-selective herbicide could not be used to control weeds in crops, because the chemical kills (or at least controls) all plants. But a farm field growing a glyphosate tolerant, "Roundup Ready ™ cultivar could withstand the usual dosage without damage to the crop, while controlling all other plants, giving complete weed control in one pass with one chemical.

Plus, RoundUp™ was already in widespread use by farmers as a burndown or complete kill for certain non-selective purposes, so farmers were already familiar with the chemical, the price was competitive and dropping, the chemical had relatively low toxicity to animals and was quickly degraded in the soil.

Other big chemical pesticide companies were active as well, developing GM Herbicide Tolerant (HT) crop cultivars matched to their own chemistries. DuPont, Hoescht, AgrEvo, Syngenta, BASF, Rhone-Poulenc and others all had GM HT R&D programs. And the list of crop species under development grew quickly, and included maize, soybean, canola, cotton, rice, and other major crop species. While HT was not the only GM trait being developed using rDNA in crops, it received a high profile, largely due to HT and big companies being specifically and preferentially targeted by activist groups.

Curiously, the use of GM technology in plants and crops has become controversial worldwide, with charges of "untested technology" and "unknown risks", while the use of the same

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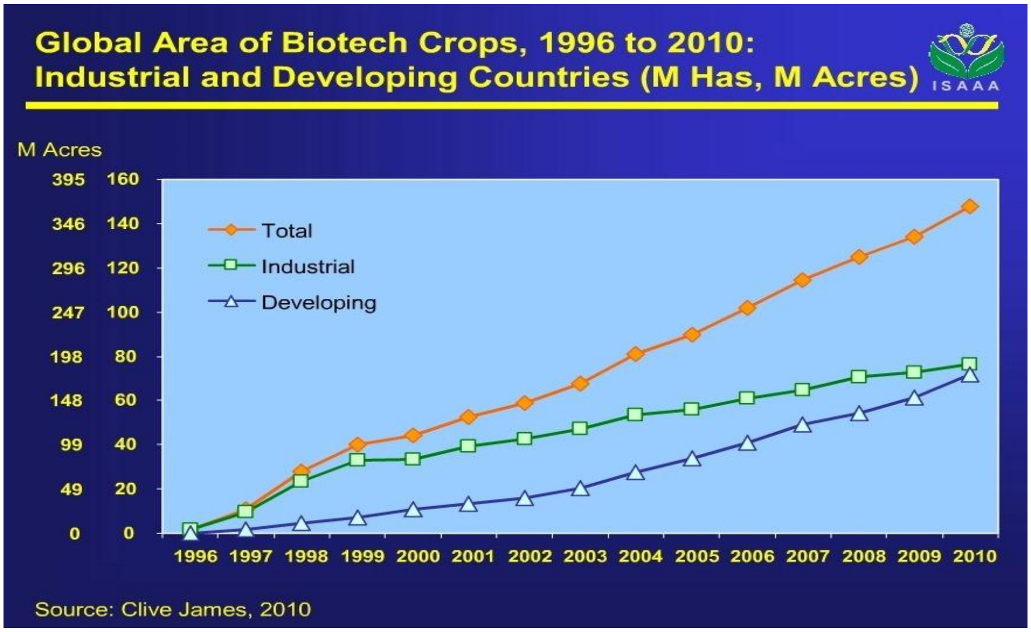
technology in pharmaceuticals and foods is widely accepted, even though the technology is exactly the same. Logically, because the basis of the anxieties is exactly the same, the same fears and anxieties should apply to pharmaceuticals as they do to foods. But logic is, unfortunately, overwhelmed by emotion and rhetoric in the public discourse of agricultural biotechnology.

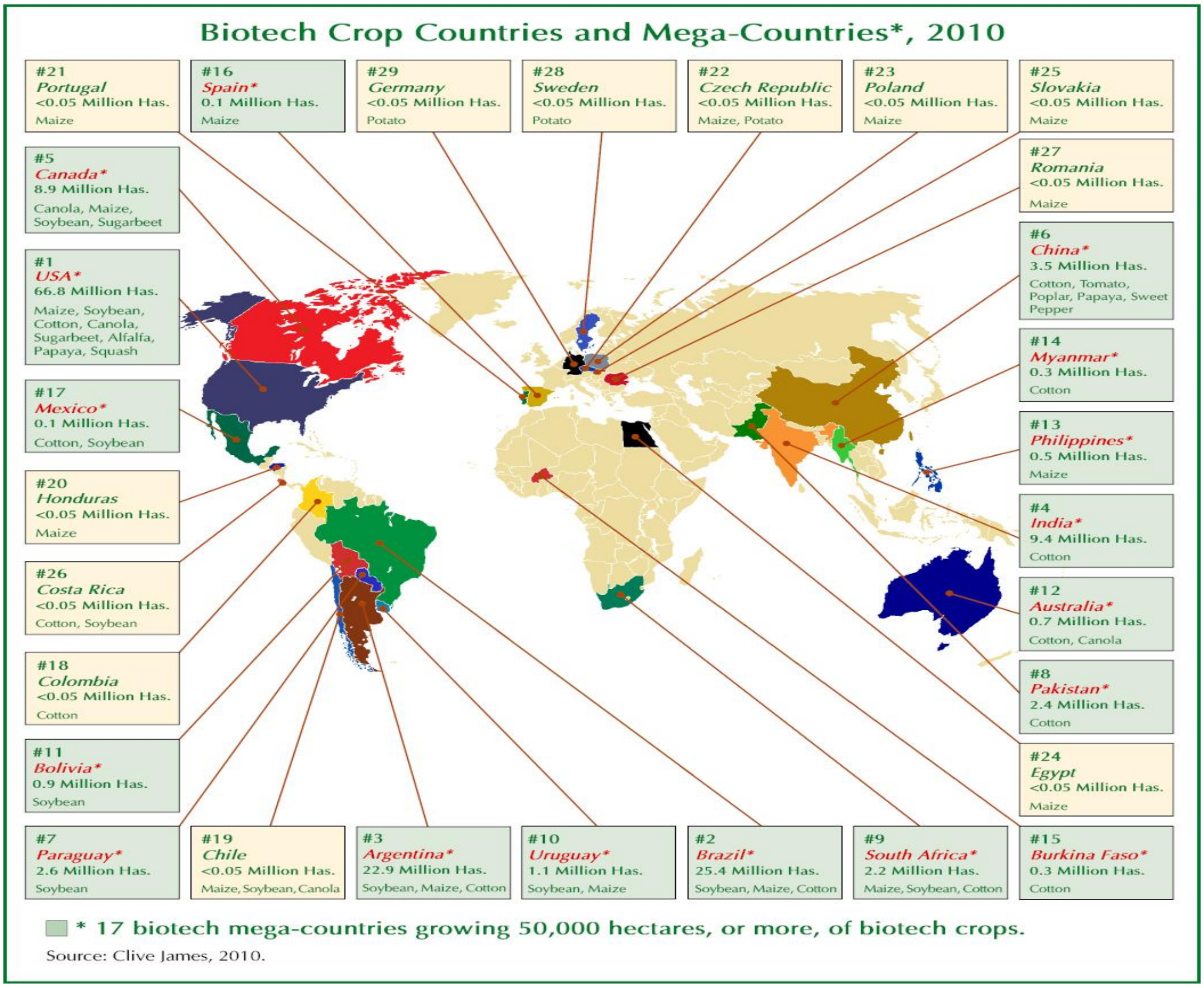
Since GM crops were introduced in 1994, the numbers of traits, species and regions growing them have all grown substantially (James, 2010).




**World Status of GM Crops**

Clive James (2010) and colleagues at ISAAA conduct annual surveys of GM crop adoption worldwide and analyse those data. Figure 1 shows the growth over the years of global area under GM crops, with segments differentiating industrial vs. developing countries (James, 2010).

**Figure 2** is a map of the world with GM cropping counties indicated, along with the specific GM crop type and total area under cultivation in each country. In 2010, for example, Australia cultivated 0.7Million Hectares of GM cotton and canola; New Zealand did not (legally) cultivate GM crops at all (James 2010).




## GM Crops in Asia Pacific

Several countries in the Asia-Pacific area cultivate GM crops. China is by far the most significant grower, with 3.5Million hectares of various GM traits in cotton, tomato, poplar, papaya, and sweet pepper (James, 2010). In addition, China has given regulatory safety certificates to GM cultivars of rice, maize and wheat. These are currently being subjected to mandatory testing in agronomic field trials prior to commercial release (BaoRong Lu, personal communication, 2011).

In the Philippines, GM maize engineered with Bt (for lepidopteran insect control) is the only genetically engineered crop under cultivation (James 2010). But results have been impressive, with the GM maize cultivars bringing in a 30% increase in yield, due mainly or exclusively to the insect control.

As mentioned, Australian farmers cultivate a substantial area of GM cotton and canola. Australia is also field trialing GM wheat and has a number of other GM species at various pre-commercial testing stages. Australia has a long history of growing triazine tolerant (T) canola, although not technically GM, so Australian scientists and farmers are familiar with

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management of novel herbicide tolerance traits in crops. This management experience should help in handling GM HT crops as they come more predominant.

New Zealand has been active in agricultural biotechnology research, but remains a “GMO-free” country as far as GM crop cultivation is concerned.

**Herbicide Tolerance**

Farmers need to control weeds somehow, or else risk losing a substantial portion of the crop to the competing plants, which are usually much more aggressive than the crop plants. Historically, farmers controlled weeds using physical means; tilling, hoeing or rogueing. But such physical methods are problematic in their own right - they are labor intensive, expensive, and environmentally damaging, especially when conducted on commercial sized fields. As a result, most commercial farmers - apart from organic farmers - now use synthetic chemical herbicides to control weeds.

Herbicide tolerance, as a trait of agronomic significance, is not new. Nor is HT exclusive to GM crops. In the 1940s, auxin based chemicals were noted to be more damaging to broadleaf (dicot) plants than grassy (monocot) leaf plants, giving rise to chemical weed control in cereal crops for the predominantly broadleaf weeds. The first and still ‘flagship’ auxin herbicide, 2,4-D, has been used as a selective herbicide since WWII, mainly to control broadleaf weeds in naturally-tolerant cereal crops.

In the years following, a series of different kinds of synthetic herbicides with different features were developed. Some herbicides are more effective against grassy plants, so are used to control grassy weeds in broadleaf crops.  And other chemical families or groups provide different mechanisms of action for more selective weed control properties. In every case, herbicide tolerance within the crop species allows selective herbicide weed control.

And herbicide tolerant weeds have been with us as long as there have been herbicides, either due to the weedy species being naturally tolerant to the chemical, or having acquired tolerance through, for example, spontaneous mutation.

As reported in Hanson *et al.* (2011), no herbicide is completely effective against all weed species and completely benign to the crop plants. There is usually at least some degree of tolerance in weeds, and some degree of susceptibility in the crop plants. Good management means using a herbicide or combination of different herbicides to effectively control the weeds while minimizing the damage to the crop. This management strategy will differ according to crop type, region and spectrum of weeds present in a particular farm. Herbicides kill or retard plant growth by any of several methods; herbicides can be classified based on specific ‘mechanism of action’. For example, some herbicides act by interfering with enzymes within the plant, such as the Group 2 herbicides, which bind to (and thus inactivate) acetolactate synthase, ALS. With ALS inactivated, the plant cannot synthesize branched-chain amino acids (valine, leucine, and isoleucine) and so cannot sustain growth; the susceptible plant starves to death. In contrast, Group 4 herbicides (which includes the auxin 2,4-D) work as growth regulators; 2,4-D stimulates rapid uncontrolled growth in susceptible plants and so the plant grows beyond its ability to sustain the growth (Hanson *et al.* 2011).

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### Traditional Breeding for Herbicide Tolerance (Not GM)

Eventually, evolution will generate resistance in any plant population exposed to a given herbicide at less than lethal doses. So another approach in conventional breeding for herbicide resistance is to expose the crop plants to sublethal doses of the relevant herbicide and select the best performing survivors over several generations and spray regimes (Hanson *et al.* 2011).

Also, the crop species germplasm of a given crop species could be surveyed, as some varieties, breeding lines or stock may display a higher degree of resistance than the crop cultivars. Identifying the more resistant lines and introgressing the resistance genes through traditional crossing may be used to move the improved resistance genes into commercial cultivars (Hanson *et al.* 2011).

In taking a more direct hands-on strategy to acquiring herbicide tolerance in a crop, one successful approach is to alter the herbicide's molecular target. In the case of Group 2 herbicides, for example, changing the structure of the target ALS enzyme such that it has less binding affinity for the herbicide, without unduly sacrificing enzymatic function, will render the previously susceptible plant tolerant to the Group 2 herbicides.

An alternative mechanism to acquire herbicide tolerance is by interfering with the delivery of the chemical herbicide to the target. The mechanism could be physical, such as having a thick cuticle to impede entry of the herbicide into the plant cells, or it could be biochemical, for example by producing an enzyme that binds or metabolizes the herbicide. Using the Group 2 ALS inhibitor example again, the ALS enzyme in linseed flax is susceptible to Group 2 herbicides, but when the herbicide is sprayed on the plant, an enzyme in the linseed plant breaks down the herbicide molecule before it can bind to the ALS target enzyme, rendering the plant tolerant of the herbicide.

In practice, spontaneous mutations generated herbicide resistant commercial cultivars. Australia is very familiar with triazine tolerant (TT) canola, which was first developed from a spontaneous mutant *Brassica rapa* weed growing in a farmer's field in Quebec. The farmer sprayed the field with triazine to kill the various weeds and noticed a weed plant survivor. Plant breeders used that sole surviving plant as a parent in a canola breeding program and derived the entire series of triazine tolerant canolas. Although coming with a severe yield deficit, certain farmers continue to prefer the TT cultivars as they are a good fit with the farmer's management and agronomic practices.

Instead of waiting for fortuitous spontaneous mutations, breeders can also help evolution along by inducing mutations, with treatments of ionizing radiation, EMS or other mutagenic chemicals, etc. The method is simple: start with as large a population of the desired species as you can handle, expose them to the mutagenic agent, grow out a progeny population and spray with the relevant herbicide to which you seek novel tolerance. Survivors may or may not be true genetic mutants, and even mutants may not be suitable for commercial release, as the mutagen likely perturbed other functions of the plant as well, leading to a diminution of some important feature, like reduced yield (as in the triazine tolerant canolas).

Several herbicide resistant commercial crop cultivars were developed using induced mutagenesis. One of the most prominent is the wheat cultivar “Above” which mutated a novel genetic resistance to imidazolinone (Group 2) herbicides.

Pre-GM biotechnology has also been used to creating *de novo* herbicide resistance. In the 1980s, prior to rDNA technologies adapted to transform higher plants, several groups were growing crop plant cells *in vitro*, then adding the active ingredient chemical from various herbicides to the culture medium, and then regenerating whole plants from surviving cell pockets (see e.g. Jordan and McHughen, 1987). In some cases, the progeny of the regenerants also showed enhanced resistance to the herbicide. However, whether any of these lines were commercialized is unclear.

Somaclonal variation is a phenomenon in which plants cells growing *in vitro* are observed to spontaneously mutate (first reported by Australians working with wheat cultures (Larkin and Scowcroft, 1981)). Somaclonal variants with herbicide tolerance have been selected from cell lines growing in vitro which were then regenerated into whole plants. In some rare cases, the variant plant lines founded new herbicide resistant cultivars, mainly crops with resistance to Group 2 herbicides such as BASFs “Clearfield” series of crop cultivars, including canola, sunflower, rice, corn wheat and even lentils (Hanson *et al.* 2011).

**Herbicide Tolerance Using GM**

Over the past 20 years, the application of rDNA technologies to agriculture has provided a series of new herbicide tolerant crops, aka HT GMOs. Genes conferring tolerance to several different kinds of herbicides have been isolated, cloned and transferred to various crop plant species, including glyphosate (the active ingredient in RoundUp™), glufosinate (Liberty™), 2,4-D, bromoxynil, sulfonulureas and imidizolinones. The number of genetically engineered crop species is relatively large, but only a small fraction of those are in actual commercial production.

In fact, the majority of those HT GMO crops in commercial production are soybeans, maize, canola and cotton tolerant of either glyphosate or glufosinate. And those four crops and two herbicides account for almost 70% of the world’s GM crops (James, 2010). Other crop species genetically engineered for herbicide tolerance include tobacco, alfalfa, sugarbeet, rice, wheat, and linseed flax, and of these, only alfalfa and sugarbeet are currently in legal commercial production.

GM technology is slated to expand rapidly worldwide, with increasing numbers of crop species, GM traits, countries of cultivation and countries of development and release, with an estimated 120 GM crops under cultivation somewhere on the planet by 2015 (Stein and Rodriguez-Cerezo, 2009). Recent analyses of the economic impacts of growing GM crops explains why the expectation for continued growth is so confident: farmers growing GM crops have seen tangible economic gains as well as non-pecuniary benefits. For example, farmers growing HG HT soybeans in 2009 saw an economic benefit of 2.7%, 0.6 % for GM HT maize, 0.13% for GM HT cotton and 7.1% for GM HT canola (Brookes and Barfoot, 2011). However, HT crops, while perhaps expanding their global acreage, are unlikely to expand very much in terms of specific chemistries. That is, as more countries allow GM cropping, the current pool of HT GM crops and chemistries will be primarily utilized, thus expanding the area of HT GM crops cultivated, but the development of new genes conferring tolerance to new herbicides has stalled. We can, however, expect release of HT cultivars of some additional crop species for which GM HT is not currently commercialized, such as GM HT rice and wheat, and these may occupy substantial areas of cultivation.

**Impacts of HT GM crops**

The number of independent investigations into the various impacts of GM HT crops is accumulating. The reports range from standard small scale academic reports with publication in peer reviewed journals to major studies sponsored by national scientific societies.

Probably the largest of the peer reviewed studies is that from the US National Academy of Sciences. In 2010, they released their opus, two years in the making, on the impact of GM crops on farm sustainability (NAS, 2010). Because HT is the dominant trait in current GM crops in the USA, a primary focus of this study was on the impacts of cultivating those HT GM crops, on farmers, on the environment and on society, with a view to understanding the ultimate effect on farm sustainability.

One of the primary conclusions reached was that GM crops generally have fewer adverse environmental effects than do non-GM crops. A second is that GM HT crops facilitate soil conservation efforts, particularly reduced tillage, which then leads to improved soil and groundwater quality. On this latter point, Carpenter (2010) recorded a 25% drop in pesticide leaching in GM HT cotton fields in North Carolina compared to non GM cotton fields.

Economically, GM crop farmers enjoy higher yields and lower production costs. Among the non-monetary benefits noted are the increased farmworker safety (due to a shift away from more toxic chemicals, a benefit first documented by Fernandez-Cornejo and Caswell 2006)) and greater flexibility in weed control management (NAS 2010).

There were also concerns recorded for the potential for negative impacts. The main one was the inevitability of weeds evolving resistance to the primary herbicides used in GM HT crop systems, notable glyphosate (NAS 2010). Glyphosate dominates the herbicide market, capturing 30% of all herbicides sold globally (Bonny 2011). Populations of weeds with novel glyphosate tolerance have already been recorded, and they can present management problems for farmers trying to control those weeds (Nandula *et al.* 2005). Indeed, glyphosate tolerant weed populations have been reported around the world, including ryegrass (*Lolium perenne*) (Pratley *et al.* 1999) and liverseed grass (*Urochloa panicoides*) (Preston and Boutsalis 2008) in Australia, goosegrass (*Eleusine indica*) (Lee and Ngrim 2000) in Malaysia, and horseweed (*Conyza canadensis*) (van Gressel, 2001; Shrestha *et al.* 2007) and pigweed (*Amaranthus palmeri*) (Culpepper *et al.* 2006) in several parts of the USA. A helpful tool is the International Survey of Resistant Weeds, a searchable database online at www.weedscience.org.

It is important to note here that GM HT crops did not give rise to these glyphosate tolerant weeds. But the availability and success of glyphosate tolerant crops will inevitably increase the opportunity for glyphosate overuse, leading to additional populations of weeds with glyphosate tolerance. Bonny (2011) provides several explanations:

- The success of glyphosate tolerant crops inexorably leads to the expansion of the use of glyphosate (there is no advantage to growing glyphosate tolerant cultivars without spraying them with glyphosate);
- with glyphosate being a simple chemical and now being off patent, inexpensive generic versions are readily available in some areas;
- the rise in popularity of conservation tillage encourages glyphosate use;
- the rise in non-agricultural uses of glyphosate, as a non-selective vegetation control for roadsides, power lines, recreational grounds, etc.

The same opportunity to derive weeds with herbicide tolerance is true for all other herbicide tolerant crops, whether GM or traditional.

Also, not all farmers end up reducing the amount of herbicide used as, in certain cases, depending on the type of weeds present, the farmer may use more herbicide than he or she used previously. In such cases, the farmer should consider if he or she is getting value from the new cultivar and consider reverting to prior practice. Of course, simple comparisons of herbicide quantities are not the whole story, as other factors come to bear. It may be that a somewhat larger absolute quantity of glyphosate provides better weed control, is less expensive and is more environmentally benign than the smaller amount of the previously used herbicide.

Another potential problem, not directly related to the agronomic cultivation of GM crops, is the potential negative impact of growing GM crops for export to non-GM markets. This is a difficult topic to discuss scientifically, as it is inherently a non-scientific issue. International political intrigue plays a major role in this, and the rules of international trade seem to shift constantly. Nevertheless, farmers should be aware of their markets and market preferences.

The primary concern raised in the US report was on increasing weed problems arising due to evolution of weeds with resistance to glyphosate, or weed populations with less susceptibility to glyphosate become positively selected in areas where glyphosate is the exclusive herbicide, and then the weeds become established. Of course, as the study notes, these issues are neither new nor exclusive to GM HT crops, but do emphasize the need to strategically manage herbicide usage to assure longterm efficacy of glyphosate as a weed control measure (NAS 2010).

Other studies of economic impacts of GM cropping may differ in scope, but reach similar conclusions. Brookes and Barfoot (2011) looked at global impacts from 1996 to 2009. They note dramatic benefits for most farmers of GM crops, including both increases in income and reductions in expenditures. Others, particularly in non-peer reviewed publications (e.g. Benbrook, 2009; Gurian-Sherman, 2009) argue that not all GM HT farmers realize such benefits and in fact actually increase the use of herbicides. Unfortunately, such authors fail to explain why farmers would continue to use an herbicide regime inferior to what they practiced before.

In some developing countries, farmers of GM HT soybean will save a portion of their harvested seeds for replanting, thus circumventing the usual expectation that farmers will buy fresh seed each year (Qaim, 2009).

Collectively, these cited studies consistently reached a number of important common findings and conclusions, many or most of which are relevant to weed control with HT crops in the Asia-Pacific realm.

**Weed Management and GM Technology: Herbicide Tolerance**

As mentioned previously, all crops are tolerant to at least some herbicides, hence farmers, agronomists and weed scientists have a long history of weed control in herbicide tolerant crops using selective herbicides. The problems and challenges are not new with GM HT crops, so the same old management strategies apply.

As noted in various studies, the biggest challenges include evolution or derivation of herbicide tolerant weed populations, thus compromising the utility of the respective herbicide in controlling that weed. Farmers encountering such weed populations have to change practices, to revert to older, more toxic or environmentally damaging chemicals, to re-acquire effective weed control (Lemaux, 2009).

Major issues include evolution of herbicide resistance by weeds, and escape of HT genes from the GM crop, either by seed (and becoming problems as volunteers or feral populations) or by outcrossing to weedy relatives, with the consequence of the hybrid establishing a population of HT weeds. Evolution of resistance in weedy species is well documented and can be expected to occur with the increasing cultivation of GM HT crops. Indeed, such populations are already being documented. And such eventualities are also well known from conventional selective herbicide histories, especially weed populations overcoming Group 2 herbicides in conventional agronomic practice. And there's no reason to suspect GM HT crops will be any different in this respect: if the use of a specific herbicide is not managed properly, by not overspraying or rotating, then weeds will eventually evolve and overcome that chemical.

Outcrossing of HT genes from GM crops to weedy species presents a slightly more interesting wrinkle with respect to the Asia Pacific region. Of course, this source of herbicide tolerance in weed populations is also well known, and GM HT crops can be expected to behave in the same way as non-GM versions of the same species. That is, outcrossing to weedy relatives is a function of pollen flow, presence of weedy relatives and opportunity for plant sexual trysts. What makes it interesting is that the of the main GM HT crops in cultivation, soy, maize, cotton and canola, only canola has suitably weedy relatives in most regions of cultivation to present the appropriate opportunities for HT gene escape and introgression. There are few or no weedy relatives of soy or maize in areas where they are cultivated, and the threat for cotton is minimal. But canola is grown amid many other interfertile *Brassica* species, some domesticated and some less so. And the experience so far shows GM HT canolas are just as promiscuous as their non-GM relatives (Warwick *et al.* 2003; Beckie *et al.* 2003).

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In the main canola cultivation region of Canada, canola plants with three novel herbicide resistance genes have been identified, a result of multiple outcrossings (Hall *et al.* 2000). Interestingly, such hybrids house glyphosate and glufosinate resistant transgenes, and also a non-transgene HT trait for ALS inhibitors. Not only does this illustrate the need to carefully manage herbicide usage and resistance in highly outcrossing species with weedy relatives in proximity, it shows that outcrossing and trait escape is not limited to GM crops or traits.

Canadian farmers dealing with the multi-HT weeds in their canola crops cannot use either glyphosate, or glufosinate, or imidazoninone to control these ‘superweeds’ with multiple HT gene stacks. But conventional canola farmers cannot use these herbicides either, as a conventional canola crop would succumb as readily as the weeds if they were sprayed with glyphosate, glufosinate or imidazoninones. Instead, these farmers revert to older weed control practices, for example using 2,4-D or bromoxynil, and spray to remove the superweeds when they appear in the rotational crop (typically a cereal).

There are management strategies to delay the onset of weeds with novel tolerance, and there are management strategies to deal with the rise of weedy populations with tolerance to herbicides that previously controlled them (NAS, 2010, Ronald, 2011, Hanson, 2011). Crop rotations and herbicide group rotations are clearly crucial. Using appropriate doses and tank mixes are important. Walking the fields with an eye to unusual or unexpected weeds will help identify potential problems and resolve them more easily. When a population of weeds survives a treatment that would ordinarily control them (indicating a possible genetic HT in that weed population), farmers should eradicate the weeds with a different herbicide, or at least combine a different herbicide with the glyphosate or glufosinate, or they should seed that field to a different crop next season and spray with a herbicide appropriate to control that the weed and appropriate for that crop species. None of these are new or limited to GM HT crops or weeds, so the same good management practices that we’ve advocated for years remain the first line of defence against new or more aggressive weed populations.

## CONCLUSIONS

The good news is that GM HT crops, in general, are good news in that they do deliver documented benefits of increased productivity and profitability in an environmentally sustainable manner. But, like any regime involving chemical weed control, they do need proper and attentive management.

The other good news is that the management strategies are the same as they have been for weed control before GM technology came along.

If weed scientists, agronomists and farmers merely continue to apply good science, good stewardship principles and good common sense, GM HT crops can be safely and effectively cultivated, with weed control greatly simplified.

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## REFERENCES

Beckie, Hugh J., Ginette Séguin-Swartz, Harikumar Nair, Suzanne I. Warwick . (2003). Gene flow in commercial fields of herbicide resistant canola (*Brassica napus*). *Ecological Applications* 13(5):1276–1294.

Beckie, Hugh J., Ginette Séguin-Swartz, Harikumar Nair, Suzanne I. Warwick and Eric Johnson (2004) Multiple herbicide–resistant canola can be controlled by alternative herbicides. *Weed Science* 52: 152-157.

Benbrook, C.M. (2009). *Impacts of Genetically Engineered Crops on Pesticide Use in the United States: The First Thirteen Years;* The Organic Center, Boulder, CO, USA.

Bonny, S. (2011). Herbicide-tolerant transgenic crops: impacts on pesticide use, issues, and prospects given weed resistance. *Sustainability* 3: (in press)

Brookes and Barfoot (2011). *GM crops: global socio-economic and environmental impacts.* PG Economics Ltd., Dorchester UK. http://www.pgeconomics.co.uk/

Carpenter, J.E. (2010). Peer reviewed surveys indicate positive impact of commercialized GM crops. *Nature Biotechnology* 28:319-321.

Culpepper, A.S., Grey, T.L., Vencill, W.K., Kichler, J.M., Webster, T.M., *et al*. (2006). Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Sci.* 54:620-26.

Fernandez-Cornejo and Caswell (2006). The first decade of genetically engineered crops in the United States, pp. 1–30. In: *Economic Information Bulletin,* ed. United States Department of Agriculture Economic Research Service. USDA, Washington, DC.

Gurian-Sherman, D. (2009). *Failure to yield*. Union of Concerned Scientists. UCS Publications, Cambridge, MA, USA.

Hall, L., Topinka, K., Huffman, J., Davis, L. and Good, A. (2000). Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science* 48: 688-694.

Hanson, B.D., Fischer, A., McHughen, A., Jasieniuk, M., Shrestha, A. and Jhala, A. (2011). Herbicide resistant weeds and crops. In: *Principles of Weed Science, 4th ed*. (in press).

James, C. (2010). *Global Status of Commercialized Biotech/GM Crops: 2010*. ISAAA Briefs 42-2010. 488 ISAAA (International Service for the Acquisition of Agri-biotech Applications): Ithaca, NY.

Jordan, M.C., McHughen, A. (1987). Selection for chlorsulfuron resistance in flax (Linum usitatissimum) cell cultures. *Journal of Plant Physiology* 131:333-338.

Larkin, P.J. and W.R. Scowcroft. (1981). Somaclonal variation: a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics* 60:197-214.

Lee, L.J., Ngim, J. (2000). A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. *Pest Manag. Sci.* 56:336–39.

Lemaux, P. G. (2009). Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part II). *Annual Review of Plant Biology* 60: 511-559.

Nandula, V.K., Reddy, K.N., Duke, S.O. and Poston, D.H. (2005). Glyphosate-resistant weeds: current status and future outlook. *Outlooks Pest Manag.* :183-187

National Academy of Sciences US. (2010). *Impact of Genetically Engineered Crops on Farm Sustainability in the United States*. National Academies Press, Washington, DC

Pratley, J., Urwin, N., Stanton, R., Baines, P., Broster, J., *et al*. (1999). Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. *Weed Sci.* 47:405–11

Preston, C., and Boutsalis, P. (2008). *Another pesticide resistant weed found.* Weeds CRC. **http://www.sciencealert.com.au**

Qaim, M. (2009). The Economics of Genetically Modified Crops. *Annual Review Resource Economics* 1:665–93.

Ronald, P. (2011). Plant Genetics, Sustainable Agriculture and Global Food Security. *Genetics* 188: 11–20.

Shrestha, A., Hembree, K.J. and Va, N. (2007). Growth stage influences level of resistance in glyphosate resistant horseweed. *Calif. Agric.* 61:67–70

Stein, A. J., and Rodriguez-Cerezo, E. (2009). The global pipeline of new GM crops: implications of asynchronous approval for international trade, pp. 1–114 in: *JRC Scientific and Technical Reports*, edited by Joint Research Centre European Commission, Institute for Prospective Technological Studies. Joint Research Centre, Institute for Prospective Technological Studies, Seville, Spain.

VanGessel, M.J. (2001). Rapid Publication: Glyphosate-resistant horseweed from Delaware. *Weed Sci.* 49:703–5

Warwick, S.I., Simard, M-J., Légère, A., Beckie, H.J., Braun, L., *et al.* (2003). Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. *Theoretical and Applied Genetics* 107:528–39.

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# IMPACT OF *MIKANIA MICRANTHA* ON CROP PRODUCTION SYSTEMS IN VITI LEVU, FIJI

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## ABSTRACT

A study assessing the economic impacts of *Mikania micrantha* Kunth. ex. H.B.K. (hereafter, mikania) was conducted using a targeted questionnaire survey in the high rainfall eastern and moderate rainfall western regions of Viti Levu. The survey questionnaire was distributed to 320 (with 59% response rate received) and 275 (with 40% response rate received) farmholders in high rainfall eastern and low rainfall western regions of Viti Levu, respectively. Outcomes of the survey revealed that respondents recognised the negative impact of mikania with a large majority (94% in root crop areas and 100% in sugarcane areas) indicating that they control the weed to prevent crop loss while (76% in root crop areas and 97% in sugarcane areas) do so to prevent the spread of mikania to other areas. A high proportion of respondents in root crop (57%) and sugarcane (66%), indicated that herbicides were the most frequently used management tool for mikania. Controlling weeds was also considered by farmholders as a costly activity, with 35% respondents in root crop areas and 29% respondents in sugarcane areas reporting that they spend AUD \$31.00 and \$21.00 ha$^{-1}$ on controlling mikania infestations in root crop and sugarcane areas, respectively. Mikania is mostly controlled in production areas leaving non-production areas as reservoirs for reinfestation. Efforts may be best concentrated in managing the weed in both production and non-production areas. In addition, research using an effective biological control agent(s) for mikania in non-production areas would assist in reducing the density and area of infestation of the weed.

**Key words:** *Mikania micrantha*, questionnaires, crop loss, root crop, sugarcane

## INTRODUCTION

*Mikania micrantha* Kunth. (Asteraceae; hereafter, mikania), is native to Central and South America where the majority of the genus is found (Holmes, 1995; Ruas *et al*., 2000). Mikania can reproduce both by its windblown seeds and creeping stems which root at the nodes (Zhang *et al*., 2004). It was introduced into the Asia and Pacific region probably by human intervention (Waterhouse, 1994) and has become one of the major weeds of plantation crops and home food gardens (Abraham and Abraham, 2005; Macanawai *et al*., 2010).

Suppression of crop growth and yield loss caused by mikania has been reported in rubber (*Hevea brasiliensis* (A. Juss.) Muell. Arg; Watson *et al*., 1964) and oil palm (*Elais guineensis* Jacq.; Caunter and Lee, 1996) in Malaysia and in tea (*Camellia sinensis* (L.) Kuntze (Barbora, 2001; Singh, 2008), pineapple (*Ananas comosus* (L.) Merr. and banana (*Musa* spp.; Abraham *et al*., 2002) in India.

In Fiji, mikania was first reported in 1907 and was found infesting sugarcane fields (Knowles, 1907). Since then, mikania has become a menace to both mid and long-term crops such as sugarcane (*Saccharum officinarum* L.), taro (*Colocasia esculenta* (L.) Schott, cavendish banana *Musa* spp. (AAA group) and cassava *Manihot esculenta* (Robinson and Singh, 1973; Garnock-Jones, 1978; Macanawai *et al*., 2010). The sugarcane farming areas in Viti Levu are centered around Rakiraki, Tavua, Ba, Lautoka, Nadi and Sigatoka towns (Figure 1). These areas are considered to be within the moderate rainfall region of Viti Levu, which receives a monthly average of 206 hours of sunshine, 21 minimum and 30 °C maximum temperature and 170 mm rainfall (Fiji Meteorological Department, 2008). The wetter region of Viti Levu is located in the eastern side of the island covering Naitasiri, Tailevu, Serua, Namosi and Rewa provinces (Figure 1). This area receives a monthly average of 154 hours of sunshine, 22 minimum and 29 °C maximum temperature and 244mm rainfall (Fiji Meteorological Department, 2008).

Although, the presence of mikania is obvious in many cropping systems due to its creeping and smothering habits, its on-farm impact as perceived by farmers in the Pacific region has not been formally documented. This study is a timely investigation into farmholders' and stakeholders views about the weed. Such information would be helpful in informing decision makers regarding the management of mikaniaweed should be considered before making decisions to support any project to manage the weed at local or national level. The objective of this study is to evaluate root crop and sugarcane farmers' perceptions regarding the impacts of mikania and its management on crop production systems in Viti Levu by using questionnaire survey.

## MATERIALS AND METHODS

### Study Areas

The four provinces surveyed in the eastern region (high rainfall areas) of Viti Levu surveyed were Naitasiri, Tailevu, Namosi and Serua. These provinces were selected as they represent the main root crop production areas in the region. In the sugarcane farming regions (modedrate rainfall areas), the areas surveyed were parts of Ra and Ba provinces covering Rakiraki, Tavua, Ba, Lautoka and Nadi towns.

### Farmholders Survey

A targeted survey was undertaken between June and July 2009 to identify and quantify the on-farm impacts of mikania to crop farmers in the eastern region of Viti Levu Fiji. The survey questionnaire used in this study adapted a structure used in assessing the economic impact of lantana (*Lantana camara* L.) in Australia (AECGroup Limited, 2007). Our questionnaire in the current study consisted of several question categories including (1) farmholders profile,

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(2) information on land use, (3) density and extent of mikania infestation (4) control methods used and cost of controlling mikania and (5) other economic impacts of mikania. This approach is often the only cost-effective and feasible way to reach a number of respondents large enough to allow statistically analyses to be conducted (Batjes and Cummings, 2003).

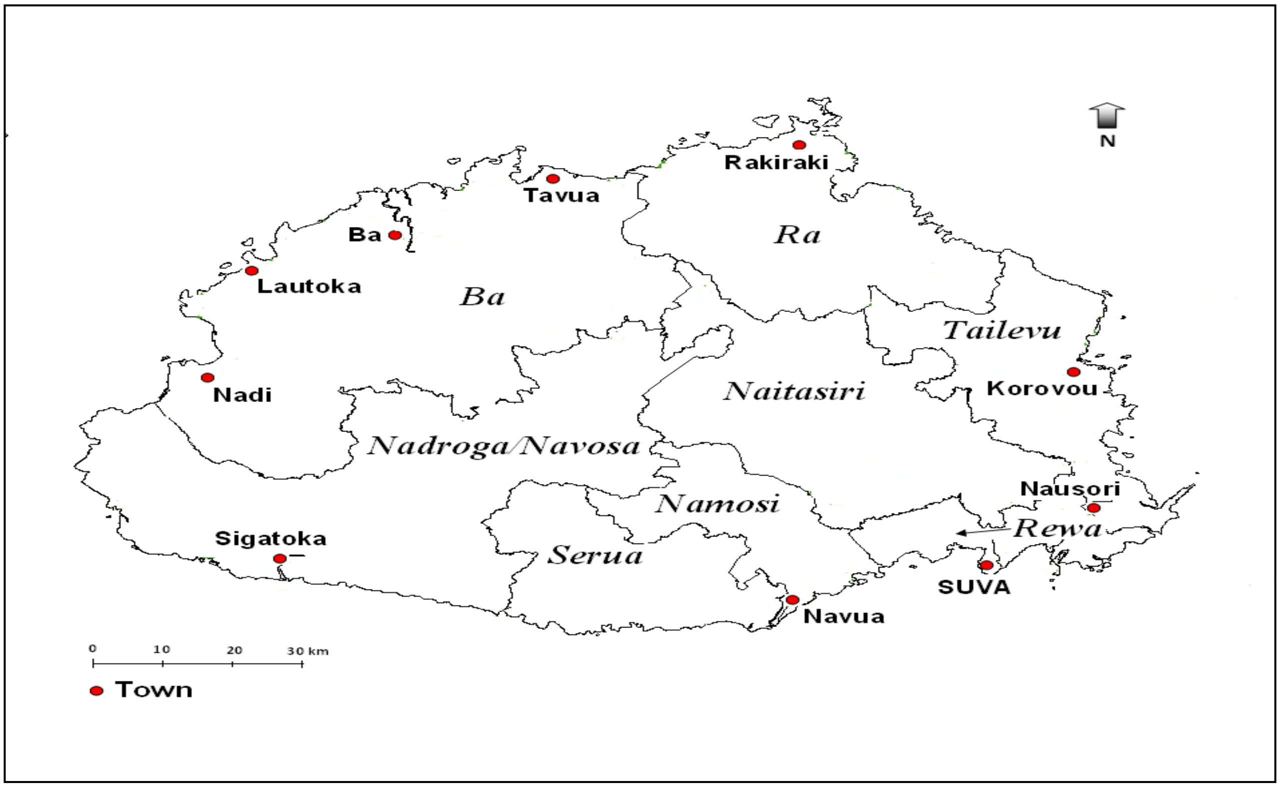


**Figure 1.** Map of Viti Levu, showing the regions surveyed. Sugarcane farming areas surveyed covering parts of Ra and Ba provinces which includes areas around Rakiraki, Tavua, Ba, Lautoka and Nadi towns and root crop production areas covering parts of Serua, Namosi, Naitasiri and Tailevu provinces.

The questionnaire was first prepared in English and then its Fijian translation was also constructed to help Fijian farmers better understand the questions. Considering the ethical aspects of this kind of research, the survey questionnaire was cleared in accordance with the ethical review guidelines and processes of The University of Queensland, Australia. Approval was granted by the Head of the Agriculture Extension Department in Fiji's central division to conduct the survey and engage the agriculture extension officers in the field survey. Assistance was also sought from the office of the Sugarcane Growers Council in Rakiraki, Tavua, Ba, Nadi and Lautoka to facilitate in distributing questionnaires to sugarcane growers. The Agriculture Extension and Sugarcane Growers Council staff work closely with farming communities and have good knowledge of the number and status of farmers in their localities including acreage they have and crops they produce. Staff engaged in the survey were briefed on the questionnaire protocol to ensure they understand the requirements of the survey and have confidence in assisting farmers on the technical aspects of the questionnaires when needed. The questionnaires were hand-delivered to the farmers and collected from farmers by the staff. Due to unreliable postal and communication services in the surveyed areas, hand distribution of the questionnaires approach was chosen over a postal survey. A total of 320 questionnaires were randomly distributed to farmholders in Naitasiri, Tailevu and Serua/Namosi provinces and 275 to sugarcane growers around Rakiraki, Tavua, Ba, Lautoka and Nadi. As part of the questionnaire documentation, the participants' declaration and consent form was included where farmers signed their names, dated and countersigned by respective surveyors. Survey results were processed using Microsoft Excel and Microsoft Access.

## RESULTS

Of the 320 questionnaires sent to farmers in root crop areas, 164 (59%) responses were received while 92 (40%) were received from the 275 questionnaires sent out to farmers in the sugarcane areas.

### Mikania Occurrence and Land Information

A large proportion of respondents (25.5%; n = 42) in root crop areas indicated that mikania plants have been growing on their land for 20-50 years, with 64% (66) of respondents indicating that mikania affects the profit of their farm. In sugarcane areas, 25% (n = 23) of respondents state that mikania has been existing on their farms for 20-50 years, with 84.8% (n = 78) of respondents reporting that mikania affected the profit of their farm

### Mikania Density and Extent Of Infestation

Forth-six percent (n = 76) of 164 respondents) of respondents in root crop farming areas reporting that over the past five years, the area infested with mikania has decreased. Similarly, in sugarcane farming areas 45% (n = 41) indicating that over the past five years the area infested with mikania has increased.

### Farmers' Perception on Control

In the root crop areas, 100% (n = 160) of respondents indicating that they control mikania in production areas. About 58% (n = 81) did not control mikania in non-production areas. About 74% (n = 91) did not control mikania in natural reserve and *c*. 2 % (n = 2) were not sure. About 63 % (n = 78) did not control mikania in other public areas and *c*. 1% (n = 1) was not sure. In sugarcane areas, 92% of respondents (n = 85) indicated that they control mikania in production areas. About 56% (n = 47) indicated that they control mikania in non-production. About 66% (n = 48) did not control mikania in natural reserve and *c*. 52 % (n = 38) did not control mikania in other public areas.

### Perception on Reasons For Control

In root crop production areas, 98% (n = 155) of respondents indicating that they control mikania in production areas to prevent crop loss and 99% (n = 127) of respondents control mikania in production areas to prevent its spread to other areas. In the non-production areas, 96% (n = 52) of respondents indicated that they control mikania in non-production areas to prevent its spread to production areas and 76% (n = 32) control mikania in non-production areas to maintain the environment. In sugarcane areas all respondents (84) indicated that they control mikania in production areas to prevent crop loss and 97% (n = 73) of respondents indicated that they control mikania in production areas to prevent its spread to other areas. In the non-production areas, 98% (n = 44) of respondents indicating that they control mikania in non-production areas to prevent its spread to production areas and 95% (n = 35) control mikania in non-production areas to maintain the environment

**Perception on Methods of Control**

Respondents used a number of methods to control mikania and herbicides was the most common method used in both production and non-production areas in root crop and sugarcane cropping regions (Table1).

**Table 1.** The percentage of respondents who use different control methods in production and non-production areas in both root crop and sugarcane regions.

| Weed control method | Responses (%) | | | |
|---|---|---|---|---|
| | Root crop | | Sugarcane | |
| | Production areas (n = 158) | Non-production areas (n = 45) | Production areas (n = 85) | Non-production areas (n = 44) |
| Herbicides | 79 | 89 | 85 | 86 |
| Physical | 72 | 69 | 85 | 80 |
| Fire | 18 | 44 | 29 | 41 |
| Grazing | 0 | 2 | 0 | 2 |

**Farmers' Perception on Costs Incurred**

Respondents estimated that costs to control mikania increased with the level of infestation irrespective of the region. Costs increased from c. \$30 ha$^{-1}$ for light infestation in root crop areas to over \$45 ha$^{-1}$ for heavy infestations (Figure 2).

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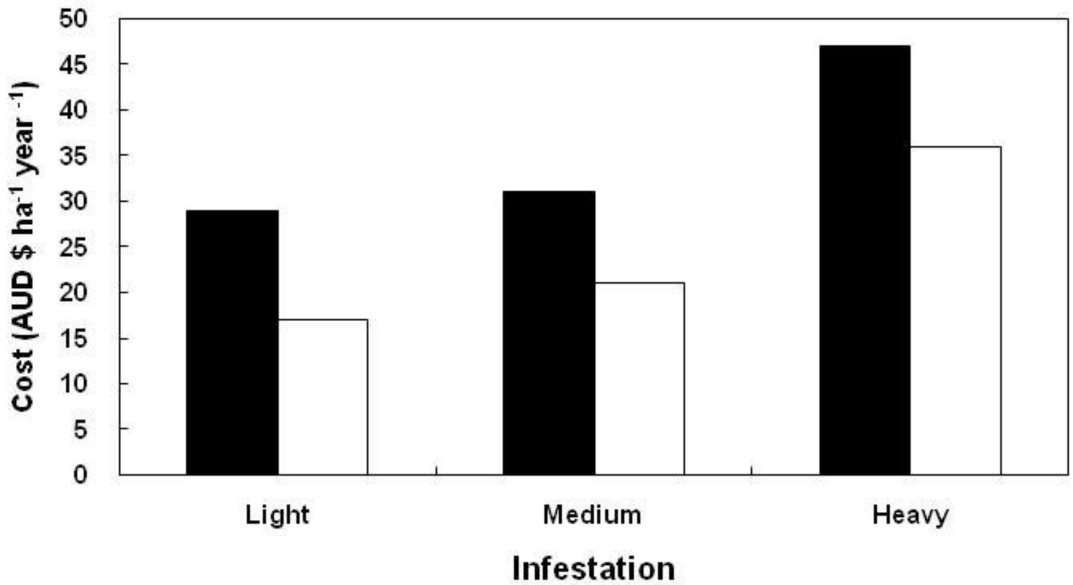


**Figure 2.** The average cost of controlling light (10,000 plants ha$^{-1}$), medium (20,000 plants ha$^{-1}$) and heavy (> 30,000 plants ha$^{-1}$) infestation of mikania in root crop (■) and sugarcane (□) farms in Viti Levu Fiji.

### Farmers' Perception on Other Benefits

Mikania is perceived to have other benefits such as its uses for traditional medicine, soil improvement and livestock feed. Ninety four percent (n = 135) of respondents in root crop areas and 42% (n = 31) in sugarcane areas indicating that mikania is useful as a traditional medicine. Sixty eight percent (n = 44) of respondents in root crop areas and 21% (n = 15) in sugarcane areas indicating that mikania is useful for soil improvement. Fewer respondents in root crop (21%; n = 16) and sugarcane (34%; n = 24) specified that mikania is useful as a livestock feed.

## DISCUSSION

### Farmholders' Land Information and Mikania Occurrence

Mikania has been occurring on root crop, banana and sugarcane farms for around 50 years. This suggests that mikania is well known to the respondents and is compatible with soil and environmental conditions in these regions. Not all fields were infested with mikania as the survey showed that *c*. 38 and 52% of existing land area cultivated with root crops and sugarcane respectively were not infested. This implies that the extent of spread of mikania in the high rainfall region is more prevalent than in the moderate rainfall region of Viti Levu.

### Farmers' Perception on Control

There were more respondents that did not control mikania in public areas, natural reserves and public areas in the root crop region than in the sugarcane region. This may be attributed to the higher proportion of respondents in the root crop areas (high rainfall) indicating the significance of the weed for traditional medicine and soil fertility than respondents in sugarcane region (moderate rainfall). Herbicide was the most commonly used control method in both production and non-production areas in both root crop and sugarcane regions. This is probably due to the better control achieved from the use of herbicide, as opposed to other methods such as slashing where the remnants of the plant can regrow. In the root crop region, some respondents have specified that they used glyphosate and paraquat, two non-selective herbicides, for controlling mikania on their farms where as sugarcane farmers used numerous selective herbicides including 2,4-D, diuron, MCPA and 2,4-D + dicamba which can kill mikania but are ineffective on sugarcane (Szmedra, 1999).

This study has demonstrated that there is a substantial cost associated with mikania infestations in crop production in the surveyed areas and cost farmers on average *c*. AUD $31 ha$^{-1}$ in root crop and AUD $22 ha$^{-1}$ in sugarcane production. The majority of respondents in both root crop and sugarcane regions do not control mikania in non-production areas, natural reserve and other public areas because they perceived control activities to be a waste of time and financial resources. However, there are some perceived benefits of the species which caused them not to actively control the weed in non-production areas. These perceived benefits include improvement of soil fertility and use of mikania for traditional medicine, livestock feed and ground cover. These areas could then be a reservoir for mikania and if they are not managed, will continue to be a source for new infestations in production areas via fragmented stem sections and wind-blown seeds. It is essential that a cost-effective and sustainable management strategy is considered to reduce and maintain mikania populations. The outcomes of this study could form part of a suit of information that may be required for an integrated weed management strategy involving all control options, including biological control and legislation. Further investigation into the extent, distribution and spread potential of mikania in the whole country also needs to be explored.

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## REFERENCES

Abraham, M. and Abraham, C. T. (2005). Biology of Mile-a-minute weed (*Mikania micrantha* HBK), an alien invasive weed in Kerala. *Indian Journal of Weed Science* 37: 153-154.

Abraham, M., Abraham, C.T. and George, M. (2002). Competition of Mikania with common crops of Kerala. *Indian Journal of Weed Science* 34: 96-99.

AECGroup Limited (2007). Economic Impact of Lantana on the Australian Grazing Industry.Report for the Department of Natural Resources and Water. Department of Natural Resources and Water (Queensland Government). Brisbane, Australia.

Barbora, A. C. (2001). Weed control in tea plantations: current scenario in Northeastern India. In Sankaran, K. V., Murphy, S. T. and Evans, H. C., (eds). *Proceedings of Workshop on Alien Weeds in Moist Tropical Zones: Banes and Benefits*, 2-4 November 1999. Kerala India. Kerala Forest Institute India and CABI Bioscience UK., 107-111.

Batjes, K. and Cummings, S. (2003). Smart Tools for the Evaluation of Information Products and Services: A Collaborative Initiative. *Information Development* 19: 127-136.

Caunter, G. I. and Lee, K. C. (1996). Initiating the use of fungi for biocontrol of weeds in Malaysia. In Moran, V. C. and Hoffmann, J. H., (eds). *Ninth International Symposium on Biological Control of Weeds*. University of Capetown, Republic of South Africa. 249-252.

Fiji Meteorological Services (2008). Climate Data and Products. http://www.met.gov.fj. Viewed 15 November 2008.

Garnock-Jones, P. J. (1978). Plant communities on Lakeba and southern Vanua Balavu, Lau Group, Fiji. *Royal Society of New Zealand Bulletin* 17: 95-117.

Holmes, W. C. (1995). Review preparatory to an infrageneric classification of Mikania (Tribe; Eupatorieae). In Hind, D., C. Jeffrey and G.V. Pope (eds.) *Advances in Systematics,pp. 239-254.* Royal Botanical Gardens, Kew.

Knowles, C. H. (1907). *Mikania scandens* in Fiji. *Bulletin of Miscellaneous Information (Royal Gardens, Kew),* 1907: 306.

Macanawai, A. R., Day, M. D., Tumaneg-Diete, T. and Adkins, S. W. (2010). Frequency and density of *Mikania micrantha* and other weeds in taro and banana systems in eastern Viti Levu, Fiji. *Seventeenth Australasian Weed Conference.* Christchurch, New Zealand. 26-30 September 2010.

Rajkhowa, D. J., Barua, I. C., Bhyyan, R. P. and Yaduraju, N. T. (2005). Weed Management in Tea, http://www.nrcws.org/WM-Tea.pdf, Viewed 15 Feb 2010.

Robinson, J. B. D. and Singh, J. M. (1973). Effects and economics of weed control in bananas. *Fiji Agricultural Journal* 35: 85-88.

Ruas, P. M., Ruas, C. F., Maffei, M. D., Marin-Morales, M. A. and Aguiar-Perecin, M. L. R. (2000). Chromosome studies in the genus Mikania (Asteraceae). *Genetics and Molecular Biology* 23: 979-984.

Singh, M. K. (2008). Biological pollution: impact and control of invasive alien weeds in North east region - A review. *Environment and Ecology* 26: 983-988.

Szmedra, P. (1999). Bittersweet harvest: the social costs of pesticide use in Fiji sugarcane production. *Journal of South Pacific Agriculture* 6: 34-48.

ISBN Number: 978-0-9871961-0-1

Waterhouse, D. F. (1994). *Biological Control of Weeds: Southeast Asian Prospects,* ACIAR, Canberra, Australia.

Watson, G. A., Wong, P. W. and Narayanan, R. (1964). Effects of cover plants on soil nutrient status and growth of Hevea. A comparison of leguminous creepers with grasses and *Mikania cordata*. *Journal of Rubber Research Institute of Malaysia* 18: 80-85.

Zhang, L. Y., Ye, W. H., Cao, H. L. and Feng, H. L. (2004). *Mikania micrantha* H. B. K. in China - An overview. *Weed Research* 44: 42-49.

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# THE IMPACT OF RAINFALL UPON POLLINATION AND REPRODUCTION OF *MIKANIA MICRANTHA* IN VITI LEVU, FIJI

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## ABSTRACT

A pollination and seed production study of *Mikania micrantha* Kunth was undertaken in Viti Levu, Fiji to determine the impact of rainfall upon the reproductive potential of this weed. Twenty-one insect taxa belonging to 17 families visited the field sites during the three days of study. Out of the total individual visitors (n = 1255), the order Diptera was the most abundant (38%), followed by Hymenoptera (34%), Lepidoptera (27%) and Coleoptera (0.5%). In the Hymenoptera, the honeybee (*Apis mellifera* L.) was the most abundant visitor (58% of all Hymenoptera visits) and was observed to have the longest foraging time than any other floral visitors. Wet weather reduced the number of floral visits by all taxonomic groups and thus influenced the production of filled seed. There was a greater proportion of filled seed produced by mikania plants per $m^2$ in the moderate rainfall region (61%) than in high rainfall region (51%). There was an average of about 94,500 viable seed $m^{-2}$ produced in both regions. Rainfall has an important role to play in the filling of seed of mikania but seems to have no effect upon overall seed production.

**Key words:** *Mikania micrantha*, pollination, *Apis mellifera*, reproductive capacity, germination, Viti Levu.

## INTRODUCTION

*Mikania micrantha* Kunth (Asteraceae; here after, mikania) in its native range produces a large amount of viable seed which is thought to be a result of an efficient cross pollination mechanism (Hong *et al*., 2007) achieved in part by the high abundance of appropriate native pollinators (Leppik, 1977; Ueckermann and van Rooyen, 2000). Similarly, in its introduced range mikania has the ability to produce many seeds and a single mikania plant had been reported to produce up to 54,000 seeds annually (Abraham and Abraham, 2005). The flowerhead or capitulum of mikania can produce vast amounts of pollen (Hong *et al*., 2007). However, without the appropriate transfer of this pollen from flower to flower, the fertilization process will not take place as it is a predominantly cross pollinated species (Hong *et al*., 2007). It is thought that insects play a dominant part in this cross pollination process and therefore, contributes if only indirectly, to the spread of this weed (Cerana, 2004; Hong *et al*., 2007). So far, very few studies have been undertaken on the pollination process in any species of the genus *Mikania*, and no studies have been undertaken on *M. micrantha* in its introduced range.

Therefore, the main aims of this study are to investigate the pollination and reproductive biology of mikania in Fiji by determining the insects responsible for pollinating mikania in a moderate rainfall region and then the reproductive output in both a high and moderate rainfall regions. By knowing the full reproductive potential of mikania will not only enhance our knowledge on its reproductive biology and population dynamics but also will assist in the development of mikania management approaches for Fiji, and such information could be extended and used in other Pacific Island countries.

## MATERIALS AND METHODS

### Pollination

<u>Study site</u>

During the middle of the mikania flowering season in June 2008, a randomly selected sugarcane farm (17°51'01.2"S 177°20'23.4"E) at Yako, about 12 km south of Nadi, and heavily infested with mikania was selected for study. This farm is in the western region of Viti Levu and receives ‘moderate’ annual rainfall of *c*. 2,000 mm and annual sunshine of *c*. 2,300 hours (Fiji Meteorological Services, 2008). Two sites, 10 m apart along the roadside of this farm, having mikania inflorescences covering at least a 1 $m^2$ area, were identified for the study. A 1 $m^2$ quadrat was placed on top of the mass of mikania inflorescences at each site to mark the area in which insect observation were to be made.

<u>Pollinator observation</u>

Within each quadrat, observations were made on insect visits to the marked inflorescences every hour for 15 minutes from 8 am to 5 pm, everyday for three consecutive days (i.e. a total of 30 pollinator counting events were undertaken over a three day period. The identity of insects and the number of visits of each insect visiting the mikania inflorescences within the quadrat was recorded over this time. Trapping was undertaken using a hand net and the insects collected in glass tubes containing methyl alcohol. These specimens were mounted and hand carried to Brisbane, Australia. Upon arrival at Brisbane Airport, the specimen were inspected by the Australian Quarantine Inspection Services and later released to the University of Queensland and identified to the genus and to the species level, whenever possible. Other identification references consulted were, Butterflies of the Fiji Islands (Prasad and Waqa-Sakiti, 2007) and Moths in Fiji (Clayton, n.d.).

### Reproductive capacity

<u>Study sites</u>

During the middle of the mikania flowering season in June 2010, five sites in each of the ‘high rainfall’ (annual rainfall of 3,000 mm; Fiji Meteorological Services, 2008) and the ‘moderate rainfall’ (annual rainfall of 2,000 mm; Fiji Meteorological Services, 2008) regions of Viti Levu were randomly selected for this part of the study. Each site had mikania infestations growing at the edges of a field with inflorescences covering at least 1 $m^2$ and having inflorescences that were senescing (i.e. becoming dry and turning brown) which indicated that they had been fertilised (Hong *et al*., 2008). From each site, 24 inflorescences (covering *c*. 4 x 3 cm surface area) were randomly selected and cut from the plant using a pair of scissors. The inflorescences were then placed into paper bags and transported to the laboratory at Koronivia Research Station where they were viewed under a dissecting microscope to count the number of filled, and potentially viable seeds present (as described below).

<u>Seed production assessment</u>
Capitula (flower heads) were removed from the inflorescences and gently opened using a scapel blade and forceps to expose the individual florets. A dissecting microscope was used to aid the counting of the individual seeds contained within each capitulum. Filled seeds (black) and empty seeds (yellowish; Hong *et al*., 2008) were counted and recorded separately. A longitudinal dissection of a small subset of the seed using a dissecting microscope confirmed that only the black seeds were filled (i.e. containing cotyledons and presumably an embryonic axes), while the yellow and green ones were unfilled. The total number of filled (black) and unfilled (yellow and green) seed per capitulum was then determined. The average percentage of filled seed at each site was calculated by adding the number of filled seed in each capitulum and divided by the total number of seed in each capitulum collected from each site and multiply by 100. The average (pooled) percentage of filled seed for each region (high and moderate rainfall region) was calculated by adding all the filled seed and divide by the total number of seed collected from the five sites in each region and multiply by 100 and this value was then used for the comparisons in the proportion of filled seed between high and moderate rainfall regions. In addition the number of capitulum and filled seed in a $m^2$ was also calculated using the formulas below.

Number of capitulum $m^{-2}$ = ———————— ÷ 0.0012

Where 24 is the number of inflorescences sampled from each site and the value 0.0012 is derived from the area studied (i.e. 12 $cm^2$/10,000 $cm^2$) in extrapolating the number of capitula in a 12 $cm^2$ (top view) surface area of inflorescences into a $m^2$ of inflorescence mass. Considering that all the seed in a 12 $cm^2$ inflorescences would drop straight down to the ground when dehisced.

FS $m^{-2}$ = Number of capitulum $m^{-2}$ x Average number of FS $capitulum^{-1}$

Where FS refers to number of filled seed.

**Experimental Design and Statistical Analysis**

For each pollinator, the mean number of hourly visits was calculated over the 30 observation times for each of the two sites. All data sets for the pollination study and for percentage of filled seed were then square root and arcsine transformed, respectively to normalise the data set. A factorial ANOVA for site and time of floral visitation effect and seed filled was analysed using Minitab® version 16, 2010 Minitab Inc. The mean values for seed filled were separated by using the Fisher's Least Significant Difference test at $p < 0.05$.

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## RESULTS

### Pollination

Flowering of mikania at the two sites occurred from late April to late September which coincided with the cooler (28.3 ± 0.1 to 20.7 ± 0.1 °C) and drier (653 ± 53 mm rainfall) season for that region. The total number of visits was 1,255 of which, Diptera was the most abundant visiting order (38.0%), followed by the Hymenoptera (34.3%), Lepidoptera (27.2%) and Coleoptera (0.5%). Many of the Diptera visitations were observed during the cloudy and rainy periods (Table 1).

In the order Hymenoptera, *Apis mellifera* L. (honeybee) was the most abundant visitor recording 58.0% of all Hymenoptera visits. On average, the highest number of *A. mellifera* visits was observed at *c*. 9.00 am. While about 28 to 40 *A. mellifera* individuals visited the inflorescences every hour from 9.00 to 4.00 pm. It was observed that *A. mellifera* seem to have a much longer foraging time when compared to other floral visitors (visual observation) but the frequency of *A. mellifera* and other species visitations was reduced when it rained (data not shown). There was a significant difference between the times visited by *Ichneumon* spp. (wasps). On average, there was an increase in frequency of visits from 8.00 am onwards, and it peaked at *c*. 2.00 pm, before rapidly decreasing from 3.00 pm to the end of the day. The presence of large numbers of the moth *Piletocera pseudadelpha* Meyrick moth in early morning and late afternoon was also obvious.

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**Table 1.** Floral visitors to mikania inflorescences (2 x 1 $m^2$) and measured over 3 consecutive days from 8.00 am to 5.00 pm at a sugarcane farm in Yako, Nadi in the moderate rainfall region of Fiji.

| Order | Genus/ Species | Common name | Floral visits |
|---|---|---|---|
| Diptera | *Telostylinus* sp. | | 478 |
| | Tachinids | Tachina flies | |
| | *Odontomyia* sp. | Soldier Fly | |
| | *Chrysomya* sp. | Hairy maggot blowfly | |
| | *Lucilia* sp. | Blowfly | |
| | *Sarcophaga aurifrons* Macquart | Flesh fly | |
| | *Chlorops* sp. | Grass fly | |
| | | Mosquito | |
| | | Hoverflies | |
| | *Musca domestica* L. | House fly | |
| Hymenoptera | *Apis mellifera* L. | European honey bee | 226 |
| | | Ichneumon wasp | 162 |
| | | Ants | 42 |
| Lepidoptera | *Euploea* sp. | | 341 |
| | *Hypolimnas bolina* L. | Common eggfly | |
| | *Melanitus leda* L. | Common evening brown | |
| | *Xois sesara* Hew. | | |
| | *Zizula* sp. | | |
| | *Catopsilia pomona* Fabricius | | |
| | *Piletocera pseudadelpha* Meyrick | | |

| | | | |
|---|---|---|---|
| Coleoptera | *Coccinella transversalis* Fabricius | Lady bird beetle | 6 |

### Reproductive capacity

Comparison between high and moderate rainfall regions

In the high rainfall region, an average of 1,246 capitula from the 24 inflorescences (*c*. 52 capitula per inflorescence) were examined in each of the five sites while in the moderate rainfall region, an average of 1,239 capitula from the 24 inflorescences (*c*. 51 capitula inflorescences $^{-1}$) were examined in each of the five sites. There was a significantly greater percentage of filled seeds (61 %) found in the inflorescences of mikania plants in the moderate rainfall region as compared to the high rainfall region (51 %; $F_{1, 238} = 20.26$; $P < 0.001$). However, the numbers of seed produced were 98,134 m$^{-2}$ in the moderate rainfall region and 90,825 m$^{-2}$ in the high rainfall region. The number of seed in a capitulum ranged from 1 to 7 with the highest proportion of capitula (94 %; n = 11,644) carrying four seeds in both high and moderate rainfall regions (Figure 1).

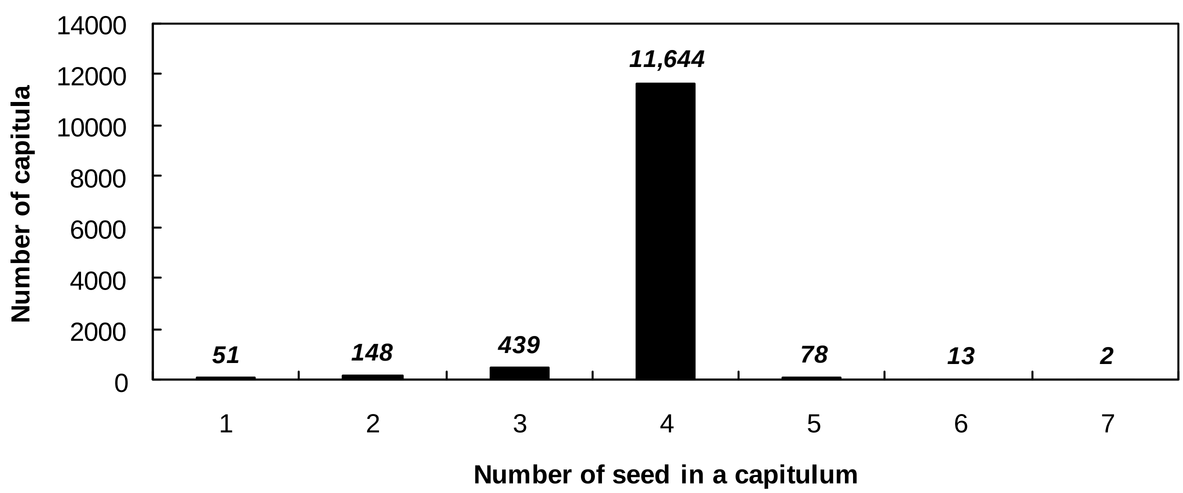


**Figure 1.** The number of seed found in a flower head (capitulum) estimated from 12,375 capitula investigated, with the total number of capitula carrying each respective number of seed and estimated from mikania inflorescences in both the high and moderate rainfall study regions of Viti Levu.

## DISCUSSION

### Pollination

While many local insects visited mikania inflorescences, the number of visits reduced when heavy rain was falling (personal observation). Even in the moderate rainfall region, there was only 61 % of filled seeds and rainfall could have been one of the reasons for flowers not being pollinated. Floral visits observed took place throughout the day, with *A. mellifera* visiting for

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most of the day, except for early morning and late afternoon, while flies visited all day long, including early morning and late in the afternoon. However, wasps preferred to visit mikania inflorescences during the middle of the day while the Lepidoptera paid some visits during the day but mostly in the evening and early mornings. In another study, *A. mellifera* visited *Mikania glomerata* inflorescences more often than 10 other plant species belonging to the Asteraceae (Cortopassilaurino and Ramalho, 1988). This may suggest that inflorescences of *Mikania* species attract *A. mellifera* more than other co-occurring Asteraceae species. Similarly in the present study, *A. mellifera* was the most frequent single insect visitor to mikania inflorescences and its long foraging time during a single visit (personal observation) may demonstrate its potential as a major pollinator of mikania inflorescences. Most insects visit mikania flowers during the day and in the event of heavy rainfall during the day will reduce the number of pollination visits and may lead to a reduction in seed set.

**Reproductive Capacity**

The flowering season for mikania in Viti Levu occurs between April to September and this coincides with a cooler, drier period in Fiji. This season is probably the most suitable for flowering in Fiji with all reproductive development and fruit dehiscence completed before the wet season begins in October. This drier period also makes this time a more suitable one for pollination to occur as insect visits tend to decrease with rain. The overall proportion of filled seed in a capitulum of a mikania inflorescence in Viti Levu Fiji was *c*. 58 % (n = 2.3), however, it is unknown if this is similar to its native range as such studies have not been undertaken. This indicates that there was still a significant proportion of empty seed in each capitulum which suggests that the stigma of these florets did not receive enough viable pollen or alternatively the seed did not develop after fertilization took place and rainfall could have been one reason for this.

The proportion of filled seed produced by mikania inflorescences in the moderate rainfall region was 10% higher than that produced in the high rainfall region of Viti Levu. This variation may be due, in part, to the availability of pollinators and the quality of the pollination process (Hernandez, 2008). Mikania is a self-incompatible plant species which means it needs to be pollinated by insects (Hong *et al*., 2007). A lack of insect pollinators would probably mean reduced pollination, which in turn leads to reduced seed set. Other contributing factors to a low pollination rate may include certain aspects of the local environment viz. low soil nutrient status, poor plant health (high abundance of inflorescences usually indicates good plant health; Hall, 2007). The lower number of filled seed produced in the high rainfall region may have resulted from the duration and intensity of rainfall. There were *c*. 2.3 times more rainfall falling in this region than the moderate rainfall region during the mikania flowering period (Fiji Meteorological Services 2008). Rainfall reduces flight frequency and therefore floral visitations for many pollinators (González *et al*., 2009; Hegland *et al*., 2009). So the absence of pollinators when the stigmatic surfaces are ready to receive pollens would negatively affect the production of seed. In addition, the prolonged wet and damp conditions in the high rainfall region may have caused secondary infections on mikania inflorescences causing fruit-set failures resulting in yield loss as occurred in several other plant species (Rossi *et al*., 2007; Xu *et al*., 2009). The range of seeds found in each capitulum of mikania inflorescences in high rainfall region varied from one to seven, but with the vast majority of capitula carrying a maximum of four seed (Figure 1). This is the first record of the amount of seed produced in each capitulum of mikania in its introduced range.

This study has demonstrated that a 1 $m^2$ of mikania inflorescences could produce an average of *c*. 94,500 viable seeds in both the high and the moderate rainfall regions of Viti Levu. However, this reproductive capacity was *c*. 75,500 less than the 170,000 seed $m^{-2}$ reported on mikania from China (Zhang *et al*., 2004). This large variation is probably due to the inclusion of empty seeds in the number of seed $m^{-2}$ reported in China. In the current study, the average number of empty seed $m^{-2}$ was *c*. 74,700, therefore the total number of seeds $m^{-2}$ (filled and empty) was 169,200.

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## REFERENCES

Abraham, M. & Abraham, C. T. (2005). Biology of Mile -a-minute weed (mikania micrantha HBK), an alien invasive weed in Kerala. *Indian Journal of Weed Science,* 37, 153-154.

Cerana, M. M. (2004). Flower morphology and pollination in Mikania (Asteraceae). *Flora* 199: 168-177.

Clayton, J. A. (n.d.). Moths in Fiji. http://www.usp.ac.fj/fijimoths/index.php. Viewed 20 September 2009.

Cortopassilaurino, M. & Ramalho, M. (1988). Pollen harvest by Africanized *Apis mellifera* and *Trigona-spinipes* in Sao-Paulo Botanical and ecological views. *Apidologie* 19: 1-24.

Fiji Meteorological Services (2008). Climate Data and Products. http://www.met.gov.fj. Viewed 15 November 2008.

González, A. M. M., Dalsgaard, B., Ollerton, J., Timmermann, A., Olesen, J. M., Andersen, L. & Tossas, A. G. (2009). Effects of climate on pollination networks in the West Indies. *Journal of Tropical Ecology* 25: 493-506.

Hall, F. C. (2007). Variation in shrub and herb cover and production on ungrazed pine and sagebrush sites in Eastern Oregon: A 27-year photomonitoring study. *USDA Forest Service - General Technical Report PNW-GTR*, 1-48.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A. L. & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12: 184-195.

Hernandez, L. F. (2008). Visit path pattern of the honeybee (*Apis mellifera* L. on the sunflower capitulum-correspondence with the location of seedless and incompletely developed fruits. *Helia* 31: 1-16.

Hong, L., Shen, H., Ye, W. H., Cao, H. L. & Wang, Z. M. (2007). Self-incompatibility in *Mikania micrantha* in South China. *Weed Research* 47: 280-283.

Hong, L., Shen, H., Ye, W. H., Cao, H. L. & Wang, Z. M. (2008). Secondary pollen presentation and style morphology in the invasive weed *Mikania micrantha* in South China. *Botanical Studies* 49: 253-260.

Leppil, E. E. (1977). The evolution of capitulum types of the Compositae in the light of insect-flower interaction. In:Heywood, V. H. ; Harborne, J. B. & Turner, B. L.(eds.) : *The biology and chemistry of the Compositae 1.Acad. Pr.,* London. 62–89.

Prasad, S. R. & Waqa-Sakiti, H. 2007. *Butterflies of the Fiji Islands.* The University of the South Pacific, Suva, Fiji. 57p.

Rossi, V., Bolognesi, M. & Giosue, S. (2007). Influence of weather conditions on infection of peach fruit by *Taphrina deformans*. *Phytopathology* 97: 1625-1633.

Ueckermann, C. & Van Rooyen, M. W. (2000). Insect pollination and seed set in four ephemeral plant species from Namaqualand. *South African Journal of Botany* 66: 28-30.

Xu, X. M., Robinson, J. D. & Berrie, A. M. (2009). Infection of blackcurrant flowers and fruits by *Botrytis cinerea* in relation to weather conditions and fruit age. *Crop Protection* 28: 407-413.

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# VEGETATIVE GROWTH AND DEVELOPMENT OF *MIKANIA MICRANTHA* IN TARO AND CASSAVA PRODUCTION IN VITI LEVU, FIJI

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**Abstract**

A growth analysis study was conducted on *Mikania micrantha* Kunth stem sections grown between crop rows and at the edges of taro (*Colocasia esculenta* (L.) Schott) and cassava (*Manihot esculenta* Crantz) fields at Koronivia Research Station, Viti Levu Fiji. The relative stem growth rate of the longest stem of mikania in the taro field was 3.4 and 2.7 cm cm$^{-1}$ day$^{-1}$ at the edges and in between crop rows, respectively. While in the cassava field, the relative stem growth rate was 2.7 and 3.8 cm cm$^{-1}$ day$^{-1}$ for between crop rows and at the edges, respectively. Mikania plants growing at the edges of the field had greater relative leaf, stem and root dry matter growth rates than plants growing in between crop rows of both crops. These differences in plant performance may be attributed to the photosynthetically active radiation (PAR) that was available for interception at the edges of the crop but less so within the crop as well as a greater nutrient availability at the edges compared to between the crop rows.

**Key words**: *Mikania micrantha*, *Colocasia esculenta*, *Manihot esculenta*, relative growth rate

## Introduction

*Mikania micrantha* Kunth (Asteraceae) was first recorded in Fiji in 1907 as *Mikania scandens* Willd (Knowles, 1907) and was later reported under its current name in 1942 by A.C Smith (Parham, 1959). According to Knowles (1907), mikania was mainly found along roadsides, climbing on trees, posts and forming dense mats on the ground and into *Saccharum officinarum* L. (sugarcane) crops in the high rainfall region of Viti Levu, suggesting that the weed was probably introduced well before this date. Its invasiveness is attributed to its ability to produce vast amounts of seed as well as reproduce vegetatively (Holm *et al*., 1991; Macanawai *et al*., 2010). Recently, mikania was reported to be infesting taro (*Colocasia esculenta* (L.) Schott) and cassava (*Manihot esculenta* Crantz; Macanawai *et al*., 2010), two of the most economically important food crops in Fiji (Ministry of Primary Industries, 2010). When mikania is growing in a cropping situation, various physical and environmental factors (such as the degree of shading, the levels of soil moisture and nutrients) may affect its vegetative growth (Kami *et al*., 2010; Sugiyama and Gotoh, 2010).

Several previous studies have documented the vegetative growth rate of mikania in non-cropping situations but measurement within crops is relatively unknown. In monoculture, the vegetative growth rate of mikania has been reported to be about 7.0 cm $day^{-1}$ during the rainy season in China (Zhang *et al*., 2004), 8.0 to 9.0 cm $day^{-1}$ in India (Choudhury, 1972) and 1.1 to 3.7 cm $day^{-1}$ in Fiji (Day *et al*., in press). As there hasn't been any comparative studies on the vegetative growth rate of mikania within crops and at the edges of crops, the aim of this study was to gain an insight into how vegetative growth rates of mikania will differ in two cropping (taro and cassava) systems, and within a crop and at the edges of a crop. This will be undertaken with a view to better understanding its invasiveness and then to identify better management options for this weed in these cropping systems.

## Materials and Methods

### Growth and development in crops

#### <u>Stem section preparation</u>

Eight hundred 15 cm long stem sections of mikania, each with two nodes and of uniform diameter and of the same physiological state of development, were cut from a 12 $m^2$ mikania infested area at Koronivia Research Station (KRS), Viti Levu, Fiji. Each stem section was planted into a 200 mL pot (7 cm diameter), filled with a moistened Yates GroPlus multi-purpose potting mix (containing an organic-based compost and a full range of fertilizers) and placed onto a bench in a glasshouse. The pots were watered regularly to field capacity for three weeks, by which time new shoots had already formed at the upper node. Thirty of these plantlets, of uniform size were randomly selected and used for an initial dry weight determination. The leaves, stems and roots of each of these 30 plantlets were separated and dried in an oven at 70˚ C for 72 hours. From the remaining 770 plantlets, 600 were selected for uniform size and health, and transferred to a field site that was *c*. 1 km away. Upon transplanting into the field (day 0), the initial morphological state of each plantlet was

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determined by measuring the length of the longest side shoot and counting the number of leaves, nodes, shoots and rooted nodes present.

## Crop field description

The field site was prepared in April 2010 at the KRS farm, and consisted of two separate blocks of clay loam soil, with established taro or cassava crops. The cassava (800 $m^2$: 18° 02' 50.2" S, 178° 32' 10.0" E) and taro (600 $m^2$: 18° 02' 50.7" S, 178° 31' 53.3" E) fields had been prepared mechanically and the crops planted manually into rows (each *c*. 1.5 m apart). At the time of transplantation, the cassava and taro crops were both five months old and *c*. 1.5 m and 0.7 m in height, respectively. Both field sites were free from all other weeds at the commencement of the study. In each crop, two planting locations were selected, the first was between rows of the crop, in which 25 mikania plantlets were planted 50 cm apart in six rows. The second planting location was on the edges of the field, where six replications of 25 mikania stem sections were also planted 50 cm apart and *c*. 1.0 m away, parallel to the outside row of the field.

Eight soil cores (each 15 cm deep x 7 cm diameter) were randomly taken from each treatment (i.e. within and at the edges of each crop) at the time of transplantation. The soil samples collected were bulked together into polythene bags, air dried at room temperature and sieved with a 2 mm sieve. They were then analysed for their nutrient content at the Fiji Agricultural Chemistry Laboratory at KRS, using a standard soil testing approach. The soil pH was assessed from a 1:1 soil to water suspension. Carbon content was determined by the method of Walkley-Black which involves extraction with dichromate (Nelson and Sommers, 1982). The total N was analysed using the semi micro Kjedhal method. Exchangeable bases were determined from neutral 1M ammonium acetate (Blakemore *et al*., 1987). Soil phosphorus was extracted by using a sodium bicarbonate extraction. The level of the photosynthetically active radiation (PAR) perceived in the centre of the fields and at the edges of the field was measured on a number of clear days from (*c*. 12.00 to 1.00 pm) using a PAR light meter (PAR GASP-03 G11162). Sensors were placed 30 cm above the soil surface at about the same height as the mikania plants creeping on the soil surface and was conducted at four randomly selected places in each crop, within each treatment unit, and averaged for that crop.

### Experimental Design and Statistical Analysis

The relative growth rate (RGR) data were log-transformed while the data sets on leaf number, nodes, shoot and rooted nodes were all square root-transformed prior to ANOVA to satisfy analysis requirements. The relative growth rate (fresh or dry matter) was calculated by using this formula (Radford, 1967):

$$\textbf{RGR} = \frac{-}{-}$$

Where $F_2$ is the final length of the stem or the final dry matter weight of the leaf, stem or root samples, $F_1$ is the initial length of the stem or initial dry matter weight of the leaf, stem or root at day 0 and $t_2$ is the final day of measurement when $F_2$ was taken and $t_1$ is the initial day of measurement at day 0. Data were analysed using Minitab® version 16, 2010 Minitab Inc.

## Results

### Growth and development in crops

There was a significant difference in the relative stem growth rate (cm $cm^{-1}$ $day^{-1}$) of mikania in between rows and at the edges of both taro ($F_{1,46}$ = 5.03, P < 0.05) and cassava ($F_{1,46}$ = 8.78, P < 0.01) fields. The relative stem growth rate of mikania was 3.4 and 2.7 cm $cm^{-1}$ $day^{-1}$ at the edges and in between rows of taro field, respectively while in cassava, the relative stem growth rate was 2.7 and 3.8 cm $cm^{-1}$ $day^{-1}$ at the edges and between the rows, respectively. There were significant differences in the relative whole plant dry matter growth rate of mikania plants in the between rows and at the edges of the taro crop ($F_{1,11}$ = 11.372, P < 0.01) and at the cassava crop ($F_{1,11}$ = 5.128, P < 0.05) fields. The dry matter growth rate of mikania at the edges of taro and cassava field were *c*. 150 and 100 mg $g^{-1}$ $day^{-1}$, respectively where as in between the rows of the fields, it was 80 mg $g^{-1}$ $day^{-1}$ for taro and 50 mg $g^{-1}$ for cassava.

### Soil chemical properties and light intensity

The soil pH between the edges and in between rows of taro and cassava fields were similar. However, there were more macro nutrients at the edges than in the in between rows of the fields of both crops (Table 1). There was a significant difference between the three stages of crop growth in the PAR intercepted by mikania at the edges ($F_{2,9}$ = 272.36, P < 0.001) and also in between rows of the cassava field ($F_{2,9}$ = 15.12, P < 0.01; Table 2). There was also a significant difference between PAR intercepted by mikania at the edges and in between rows of the field ($F_{1,22}$ = 41.99, P < 0.01; Table 2). In taro, there was also significant difference between the three stages of crop growth in the PAR intercepted by mikania at the edges ($F_{2,9}$ = 234.91, P < 0.001) and in between rows of the field $F_{(2,9)}$ = 22.08, P < 0.001; Table 2). There was also a significant difference between PAR intercepted by mikania at the edges and in between rows of the taro field ($F_{1,22}$ = 5.6, P < 0.05; Table 2). For both crops and treatment areas there were greater PAR intercepted at days 0 to 18 than from days 19 to 56. Furthermore, greater PAR was intercepted at the edges than in between rows of both crops (Table 2).

**Table 1.** The soil chemical properties found within crops rows and at the edges of taro and cassava fields. The measurements were taken before planting mikania plantlets.

| **Soil parameters** | **Taro** | | **Cassava** | |
|---|---|---|---|---|
| (mg $kg^{-1}$ unless otherwise stated) | **In between rows** | **Edges** | **In between rows** | **Edges** |
| pH | 5.3 | 5.3 | 5.5 | 5.6 |
| Total Carbon (%) | 3 | 3 | 1 | 1 |
| Total Nitrogen (%) | 0.2 | 0.3 | 0.1 | 0.1 |
| Available P | 1 | 1 | 15 | 19 |
| $Ca^{2+}$ | 2,570 | 2,658 | 2,334 | 2,365 |
| $Mg^{2+}$ | 820 | 937 | 699 | 821 |
| $K^{+}$ | 59 | 133 | 196 | 209 |
| $Na^{+}$ | 46 | 48 | 51 | 43 |
| $Fe^{3+}$ | 148 | 141 | 124 | 117 |
| $Mn^{2+}$ | 19 | 13 | 50 | 55 |
| $Cu^{2+}$ | 9 | 9 | 8 | 8 |
| $Zn^{2+}$ | 3 | 3 | 3 | 4 |

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**Table 2.** The photosynthetically active radiation (PAR) measured 30 cm above the soil surface at about the same height as the mikania plants in the in between rows and edges of taro and cassava fields. The PAR values were average values from four x 14 readings and ± two standard errors from the mean. Values within each crop and each treatment with same letters are not significantly different at $P < 0.05$.

| Crop | Stages of crop growth (days) | PAR (µmol $m^{-2}s^{-1}$) | |
|---|---|---|---|
| | | In between rows | Edges |
| Taro | 0-18 | 899 ± 82 *a* | 1,512 ± 58 *a* |
| | 19-36 | 573 ± 20 *b* | 837 ± 44 *b* |
| | 37-56 | 374 ± 15 *c* | 525 ± 20 *c* |
| | Overall (0-56) | 618 ± 42 | 967 ± 63 |
| Cassava | 0-18 | 137 ± 10 *a* | 1,239 ± 42 *a* |
| | 19-36 | 98 ± 7 *b* | 675 ± 41 *b* |
| | 37-56 | 63 ± 4 *c* | 419 ± 17 *c* |
| | Overall (0-56) | 99 ± 6 | 785 ± 52 |

**Discussion**

The relative stem growth rate of mikania at the edges was similar for both taro and cassava and also the relative stem growth rate of mikania between crop rows for both taro and cassava was the same. This indicates that mikania has the potential to compete successfully against. Although the relative growth rate of taro and cassava were not assessed, the growth rate of mikania from this study indicates the considerable growth potential of this weed to compete certain crop species both at the edges and in between crops including those of the present study in the Fiji cropping environment.

The higher levels of PAR recorded on the edges of both fields is reflected in the growth of mikania, with a larger number of leaves produced by mikania plants growing in between rows of both fields than at the edges. This suggests that mikania allocates more resources to produce more new leaves when it is under lower light conditions than when there is ample light. A similar response to low light is used by many plant species to enable them trap more light to sustain growth (Bazzaz *et al*., 1987). However, the significant differences in dry matter growth rate of mikania grown at the edges and those in between rows of both taro and cassava fields is likely to be due to the variation in the intensity of light intercepted by the plants in the two treatments, as light is a major determinant of plant growth (Kami *et al*., 2010; Nishimura *et al*., 2010). This study has also found that, as the number of rooted nodes increased, the number of shoots produced also increase, which indicates that an association exists between the rooted nodes and production of shoots in mikania. This response has also been observed in five stoloniferous species belonging to five different families including Asteraceae (Thomas and Hay, 2004).

The greater dry matter growth rate of mikania plants grown at the edges than those plants grown in between rows of both taro and cassava field, may also be attributed to the larger quantity of soil chemical nutrients present at the edges of the field as fertile soil produces more vigorous high yielding plants than poor soil (Sugiyama and Gotoh, 2010; Useche and Shipley, 2010).

The shading effect from crops and other weeds would explain the reduced light intensity within the crop and may have contributed to the reduction in biomass production of mikania. This study has also given some insights into the reason for the large occurrence of mikania at the edges, rather than within the crops (field observations). The assessment was conducted for only 56 days and some mikania vines were found scrambling on the canopy of both crops. This demonstrated that mikania can compete aggressively against the two crops for limited resources in soils similar to the current study and delay in its control may pose a real threat to the cost of crop production. The vigorous growth habit of mikania at the edges of the field shows its invasive potential and smothering habit if not managed and an ability to act as a reservoir for dispersal units including both stem sections and seeds.

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## References

Bazzaz, F. A., Chiariello, N. R., Coley, P. D. and Pitelka, L. F. (1987). Allocating Resources to Reproduction and Defense. *BioScience* 37: 58-67.

Blakemore, L.C., Seale, P.L. and Daly, B.K. (1987). Methods for chemical

analysis of soils. *New Zealand Soil Bureau Scientific Report 80.* 103 p.

Holm, L. G., Plucknet, D. L., Pancho, J. V. and Herberger, J. P. (1991). *Mikania cordata (Burm.f.) B.L. Robinson, Mikania scandens (L.) Willd., and Mikania micrantha HBK Asteraceae (also compositae), Aster family. The World's Worst Weeds, Distribution and Biology.* The University of Hawaii Press.

Kami, C., Lorrain, S., Hornitschek, P. and Fankhauser, C. (2010). Light-regulated plant growth and development. *Plant Development.* San Diego: Elsevier Academic Press Inc.

Knowles, C. H. (1907). *Mikania scandens* in Fiji. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)* 1907: 306.

Macanawai, A. R., Day, M. D., Tumaneng-Diete, T. and Adkins, S. W. (2010). Some factors that may influence the invasiveness of *Mikania micrantha* Kunth. ex. H.B.K. in Fiji. *Seventeenth Australasian Weed Conference* Christchurch, New Zealand.

Ministry of Primary Industries, (2010). Agriculture Crop Production 2009. http://www.agriculture.org.fj. Viewed 17 February 2011.

Nelson, D. W. and Sommers, L. E. (1982). Total carbon, organic carbon and organic matter. *In:* AL., P. A. L. E. (ed.) *Method of Soil Analysis (Part 2). 2nd Edition.*: Agro. Publi. # 9. A. S. A. Madition, Wisconsin. pp. 539 – 580.

Nishimura, E., Suzaki, E., Irie, M., Nagashima, H. and Hirose, T. (2010). Architecture and growth of an annual plant *Chenopodium album* in different light climates. *Ecological Research* 25: 383-393.

Parham, J. W. (1959). *Weeds of Fiji.* , Department of Agriculture, Fiji. Bulletin no. 35.

Radford, P. J. (1967). Growth analysis formulae - their use and abuse. *Crop Science* 7: 171.

Sugiyama, S. and Gotoh, M. (2010). How meristem plasticity in response to soil nutrients and light affects plant growth in four Festuca grass species. *New Phytologist* 185: 747-758.

Thomas, R. G. and Hay, M. J. M. (2004). Evidence suggests plagiotropic clonal species have evolved a branching physiology emphasizing regulation by nodal roots. *Evolutionary Ecology* 18: 409-428.

Useche, A. and Shipley, B. (2010). Plasticity in relative growth rate after a reduction in nitrogen availability is related to root morphological and physiological responses. *Annals of Botany* 106: 617-625.

# THE DEVELOPMENT AND IMPLICATIONS OF HERBICIDE RESISTANCE IN AQUATIC PLANT MANAGEMENT

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## INTRODUCTION

Aquatic plants are among the most unique and potentially understudied plants in the world. Aquatic communities represent incredibly diverse ecosystems that perform many vital natural and anthropogenic functions – fisheries, navigation, water supply, recreation, and hydropower generation (Gettys et al., 2009). Most often these anthropogenic requirements lead to manipulations of the aquatic environment, such as reservoir creation, changes in water fluctuation – intensity and frequency, removal of native freshwater vegetation and most importantly - alterations in water chemistry (Madsen, 2009).

These changes often lead to excessive aquatic plant growth, particularly from introduced exotic species. Problematic infestations generally require varying degrees of management. Managing plants in the aquatic environment is challenging and requires a multi-pronged approach, integrating biological, cultural, mechanical and chemical control methods. This paper will provide a brief overview of aquatic plants and management techniques, but chemical control through the use of aquatic herbicides, including the development and implications of herbicide resistance, will be the primary focus of this article.

### Overview of Aquatic Plants and Management Techniques

Aquatic plants can be classified as macrophytic (visible to the naked eye) and microphytic (phytoplankton – microscopic). Phytoplanktons reside as free-floating suspensions throughout the water column while macrophytes require the water's surface or a soil/hydrosoil interface for growth. Emergent plants are rooted to the moist bank of a water body or in the shallow sediment and have leaves and stems that penetrate above or lie on the surface of the water. These plants are physiologically well adapted to changes in water levels and include such species as cattails (*Typha* spp.), pickerelweed (*Pontederia cordata*), the water lilies (*Lotus; Nuphar* and *Nelumbo spp*.) and many aquatic grasses including torpedograss (*Panicum repens*) and paragrass (Urochloa spp.).

Floating plants reside completely on the waters surface, with roots extending into the water column that absorb water and nutrients. The most common and problematic species of this classification is water hyacinth (*Eichhornia crassipes*), but also include the duckweeds (*Lemna spp.*), alligatorweed (*Alternanthera philoxeroides*), water lettuce (*Pistia stratiodes*) and salvinia (*Salvinia spp.*). Submersed aquatic macrophytes live completely within the water column. They are rooted to the hydrosoil and grow through the water column to the surface. Light is the major limiting factor for the growth and establishment of these species which

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include hydrilla (*Hydrilla verticillata*), elodea (*Egeria/Lagarosiphon spp.*), milfoils (*Myriophyllum spp.*), pondweeds (*Potamogeton spp.*), and several others.

Several methods can be utilized to provide control of nuisance aquatic vegetation. The first and most effective technique is prevention. While natural spread is common within adjoining waterbodies, long-distance dispersal, particularly of problematic weeds, is through humans. Dredging, mechanical harvesting, boat trailers and the incredible increase in internet trade of exotic water plants all contribute to weed spread into vulnerable areas (Richardson, 2008). Educational awareness, coupled with monitoring and rapid response has been effective for certain species in the U.S. (giant salvinia - *Salvinia molesta*) thus far, but the decrease in governmental funding in this area is likely to result in increased infestations.

Mechanical control methods include various types of cutters, shredders, dredgers, suction hoses, and harvesters. The underlying premise of these devices is to physically remove or cut up the plant to provide a measure of control. While effective for certain areas and situations, mechanical control is often expensive and generally results in only short-term control. Moreover, mechanical control may actually increase infestations through fragmentation. It may, however, allow the reduced use of herbicides or biological control agents, such as the triploid grass carp (*Ctenopharyngogon idella*).

The sterile grass carp is the most widely used biological control and is very effective on soft tissue plants, particularly hydrilla. However, stocking rates as a function of infestation levels or plant community type is still questionable in areas where some submersed aquatic vegetation is desired. Other insect bio-control agents have been very effective, but wide-spread control across large geographic ranges is limited for many species.

Benthic barriers, drawdowns, shading, limiting nutrient influx or nutrient inactivation are considered physical methods of control and can be very effective, but not applicable or possible for all aquatic situations. Finally, chemical control through the use of approved aquatic herbicides is utilized by many aquatic plant managers, but this approach also has its challenges and pitfalls.

**Overview of Herbicides Used for Aquatic Plant Management**

Regardless of control tactic, an integrated management plan should be the first step in any aquatic plant control strategy. Site assessment, intended use or uses of the waterbody and long-term consequences of control are critical factors to be determined. Herbicide selection, including timing, rate and use pattern is also paramount. Currently there are 14 products registered for aquatic use in the United States. Table 1 reflects the herbicide active ingredient, mode-of-action, and general use pattern/plants controlled.

Aquatic herbicides represent a very small number of chemicals compared to terrestrial agricultural situations. These materials fall into two primary categories, contact and systemic, which help to define herbicide movement and behavior within aquatic plants. These terms are also used somewhat erroneously to describe herbicide persistence in the aquatic environment.

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Contact time is the time needed for the herbicide to be in contact with the plant to cause the desired lethal effects. As a general rule, contact herbicides require a few hours to days, while systemic materials require several days to weeks for control.

Contact Herbicides

Contact herbicides are generally applied at use rates in parts per million (ppm) and provide fast-acting control of floating and submersed species. Contact herbicides, as their name suggests, provide control of those plant tissues that are contacted by the herbicide.

Once the herbicide is absorbed into the plant tissues, it is not moved or translocated to other parts of the plant. For this reason, contact herbicides must be applied in manner that ensures good coverage. They have limited activity on emergent and littoral plants.

**Table 1.** Registered herbicides for use in aquatic plant management in the United States

| Active Ingredient | Mode-of-Action | Usage Pattern & Plants Controlled |
|---|---|---|
| 2,4-D | Synthetic auxin - systemic activity | Foliar for floating plants – hyacinth, and also submersed milfoil |
| Acrolein | Membrane disruption – contact activity | General submersed vegetation control in canal systems |
| Bispyribac-sodium | Inhibition of ALS enzyme – systemic activity | Submersed aquatic weeds |
| Carfentrazone-ethyl | Protox enzyme inhibition – contact activity | Foliar for floating plants and algae |
| Coppers (chelate, sulfate) | Membrane disruption – contact activity | Primarily algae control, some submersed plant control |
| Diquat | Photosystem electron diversion – contact activity | Foliar for floating plants – salvinia, duckweeds, also submersed control |
| Endothall | Membrane disruption – contact activity | Submersed plant control – hydrilla, milfoils |
| Flumioxazin | Protox enzyme inhibition – contact activity | Submersed plant control and algae – particularly effective on cabomba |
| Fluridone | Pigment synthesis inhibition – systemic | Submersed plant control – hydrilla and milfoils, entire water body treatments |
| Glyphosate | Inhibition of ESPS enzyme – systemic activity | Foliar plant control and emergent ditchbank weeds, no activity in water |

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| | | |
|---|---|---|
| Imazamox | Inhibition of ALS enzyme – systemic activity | Foliar plant control, particularly cattails and some submersed control |
| Imazapyr | Inhibition of ALS enzyme – systemic activity | Foliar and emergent ditchbank weeds, limited submersed activity |
| Penoxsulam | Inhibition of ALS enzyme – systemic activity | Primarily submersed plant control – hydrilla, others |
| Triclopyr | Synthetic auxin - systemic activity | Foliar for floating plants – hyacinth, and also submersed milfoil |

These plants possess extensive below surface tissues such as rhizomes (water lilies, cattails) and will regrow rapidly because the herbicide is not translocated. Furthermore, contact herbicides dissipate quickly in the aquatic environment – therefore the regrowth of the target weed escapes the lethality of the once present herbicide.

Dense plant growth on the surface often requires more than one treatment because the herbicide is absorbed into the upper plants, killing those plants, but because of rapid dissipation, those plants below the initial surface are able to continue growing. This is most often observed with the duckweeds and salvinia, where several layers of plants can be matted together on the surface.

For submersed applications with contact-type herbicides, the herbicide must be in contact with the target plant in the range of hours to a few days to provide control. Plant uptake of these herbicides is very rapid, so applications must be close to the target plants to achieve good control. Contact herbicides for submersed plant control are generally used for site specific plant removal such boat ramps, shallow zones along shorelines, channels or other specific areas. Some whole lake treatments can be made with these types of herbicides, but the entire area must be treated. For this reason whole lake treatments are limited due to logistics and cost.

One of the oldest herbicides used for weed control is copper and copper salts. In the late 1800's, researchers found copper salts effective for selective broadleaf weed control in cereals. Selectivity was achieved primarily through differential uptake, whereby the copper salts rolled down and off the vertical leaves of the cereals and was retained on the horizontal surface of the broadleaf weeds.

In the aquatic environment, differential uptake is also the key to selective control. This herbicide is primarily used for algae control; because algae have the greatest surface to volume ratio, more uptake occurs in algae compared to more complex macrophytes. Copper has also been used to increase the effectiveness of other herbicides such as diquat. Copper is a general toxin and is very rate dependent for activity. As the levels of copper increase, normal cell function such as co-factor mediated enzyme reactions and cell membrane integrity are compromised.

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Copper is used as a chelated compound or as copper sulfate. The sulfate salt is considered to be more toxic due to increased uptake and is limited in areas devoid of desirable fish populations. This herbicide is rapidly absorbed into tissues and control is nearly immediate. As such, contact time for this herbicide ranges from a few hours to a day or so.

Acrolein herbicide is a general plant toxicant that is used for total vegetation control in irrigation canals. This herbicide is rapidly absorbed by submersed plant tissues and reacts with sulfhydryl groups in a variety of plant biochemical functions. Acrolein is not very effective on terrestrial plants due to limited uptake, but very little tolerance/selectivity is observed with submersed aquatic plants.

Diquat has been registered for aquatic weed control since the 1960's for foliar and submersed weed control. This herbicide is used for floating weeds such as water lettuce, salvinia, and duckweeds. Complete coverage is required for good control to ensure that all portions of the plant come in contact with the herbicide. In situations where the plants are crowded or layered, more than one application is generally needed. Submersed applications rely on the herbicide dispersing quickly within the water column as diquat is rapidly absorbed by the plant tissues. In situations where there is dense plant growth, trailing hoses are used to place the herbicide several feet below the water surface.

Diquat is very effective on the milfoils, elodea, certain pondweeds and naiads (*Najas spp.*). Diquat is also effective on hydrilla but requires the addition of copper; the actual mechanism of this beneficial interaction on this species is unknown. Diquat interferes with the light reactions of photosynthesis, specifically by accepting electrons from photosystem (PS I) and passing this energy to oxygen – forming radical oxygen. This reaction is continuous, and radical oxygen reacts with lipids in the membranes, causing leakage and cell lysis and death.

Endothall is used primarily for submersed weed control and is formulated as a potassium salt or as an alkyamine salt. The latter is much more efficacious, providing filamentous algae control. However, concerns over fish toxicity result in the use of the potassium salt for most situations. Endothall is applied in a similar manner to diquat and is very effective on hydrilla, curly-leaf pondweed, milfoil and several other species. The mechanism of action of endothall is unknown, but appears to be membrane active, causing rapid cellular leakage.

Flumioxazin and carfentrazone-ethyl are two relatively new herbicides that have been introduced to the aquatic market in the United States. These herbicides cause a buildup of cytoplasmic protophorinogen which is rapidly converted into light accepting protoporphin IX. Because of the buildup in the cytoplasm, the energy cannot be utilized by the light reaction centers in the chloroplast. As such the energy is passed to oxygen creating singlet oxygen. This radical oxygen species then causes similar effects observed with diquat, including membrane perturbation and rapid cell death. Carfentrazone is labeled for floating plant control including water lettuce and salvinia. Flumioxazin is very effective for certain submersed species – especially cabomba, and filamentous algae.

<u>Systemic Herbicides</u>

Systemic herbicides, as the name suggests, translocate throughout treated plants. In aquatic habitats, these materials move within the phloem tissues and generally accumulate in areas

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of new growth. Systemic herbicides are used for floating, littoral/ditchbank and submersed plants. Herbicide translocation in aquatic plants is not well understood and will be discussed in more detail with respect to each individual herbicide.

Glyphosate is a non-selective herbicide used routinely for ditchbank weed management and provides good control of many grasses and other perennial species. It also provides good control of most floating plants and emergent species such as lilies. Glyphosate has no soil activity and no activity in water. This material will translocate to growing regions of the plant, accumulating in the apical meristem. Recent research suggests glyphosate translocation is limited in stems that extend from the shoreline that dip below the water surface and then resurface some distance away (MacDonald et al. 2005).

These authors also demonstrated low levels of herbicide leakage with glyphosate into the surrounding water. It has been speculated that glyphosate applied to littoral vegetation ‘leaks’ out of the plant tissues once applied, and this is the reason for poor control in some cases. However, research has shown that glyphosate and imazapyr do not leak from plant tissues, suggesting poor translocation or initial uptake is the reason for poor control. Mechanistically, glyphosate blocks the formation of essential aromatic amino acids. This leads to a cessation of growth and eventual plant death.

Imazapyr, imazamox, bispyribac and penoxsulam are four recent herbicides labeled for use in the aquatic sector in the United States. These herbicides act in a similar manner on the same target enzyme (acetolactate synthase); as such these materials are commonly referred to as ALS herbicides.

Imazapyr and imazamox are used primarily for littoral weed control, and both compounds are particularly effective on Typha. Imazapyr is generally used for emergent plant control, especially littoral zone and wetland grasses such as torpedograss, phragmites and giant reed (*Arundo donax*). It is considered to be non-selective and has considerable soil activity and persistence. Imazapyr has limited activity in the water for submersed plant control. Imazomox is also used for emergent plant control, and is more selective compared to imazapyr. However, it can be used for submersed control of hydrilla where it provides suppression for several months.

Penoxsulam is primarily used for submersed plant control and is very effective on several species including hydrilla. It also appears to have some floating plant control from in-water applications, suggesting this herbicide is taken up by the roots of floating plants.

Bispyribac is the most recent registered herbicide for submersed plant control. Due to the recent registration of these herbicides, much is unknown regarding selectivity and effective use patterns for all aquatic situations.

2,4-D and triclopyr are growth regulator herbicides that mimic the plant growth hormone auxin. Effected plants undergo uncontrolled growth and eventually die. These materials are used for broadleaf and woody littoral zone and wetland species. Both products are also very effective for control of the floating species water hyacinth and the submersed Eurasian water milfoil. Both of these materials are rapidly absorbed by leaf tissues from foliar applications

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and moved throughout the plant. In submersed applications, the herbicide is absorbed directly in shoot and leaf tissue, but the extent of translocation is unknown.

Fluridone is one of the most widely used herbicides for submersed control of hydrilla and milfoils. This herbicide blocks the formation of carotenoids, leading to the degradation of chlorophyll and white/bleached tissues. Unlike the other systemic herbicide labeled in aquatics, fluridone does not translocate in phloem tissues. Many submersed species lack functional xylem, so translocation of fluridone is not possible – thus leading to the conundrum of this herbicide being designated as systemic. It is xylem mobile from root uptake in terrestrial situations, and is often absorbed by littoral species, causing temporary bleaching. Fluridone does provide control of certain floating species, but it is unclear as to how the herbicide is being moved/absorbed into these plants.

Submersed aquatic species are the most difficult to control with either contact or systemic herbicides. In either classification, the concept of concentration exposure time (CET) is important to understand. Contact herbicides directly affect all plant tissues, so the key issue is having the herbicide in a lethal concentration for a long enough time to be absorbed. A CET of hours to a few days is long enough to affect control. However, with systemic herbicides, CET is much more critical. These herbicides do not equally affect all tissues of the plant, but directly affect new growth.

More importantly these herbicides do not impact existing plant tissues. The herbicide must be present in a sufficient dose and for a long enough time to prevent new growth. If CET is compromised through a loss of a lethal rate, the plant will be able to recover. Generally exposure time cannot be overcome since with higher rates; once the critical rate is met, additional herbicide is not needed. Higher rates do extend exposure time, but most plant managers prefer to add herbicide (bump treatments) when needed. Another interesting quandary concerning systemic materials, is whether the herbicide is concentrated within plant tissues, or simply equilibrates with the surrounding water.

**Resistance and Tolerance in Aquatic Plants**

There has been a great deal of confusion with respect to herbicide tolerance and resistance. Resistance is defined by the Weed Science Society of America as a plant population that has changed to resist a once lethal dose of an herbicide. Tolerance is defined as a plant population that has always resisted or 'tolerated' an herbicide when used at labeled rates. The development of resistance in terrestrial cropping systems is characterized by a shift in the rate required to provide a comparable level of control.

In years past a 10-fold shift in the rate needed to provide control was considered to be true resistance. However more recent definitions state a shift in rate that no longer allows the herbicide to be effectively and safely used in the labeled situation. In some cases the herbicide is no longer effective at reasonable rates; in others the rate that will provide control will not allow for crop tolerance.

Plants tolerate herbicides through a variety of mechanisms. These are most closely studied in crops, where tolerance is essential. Placement is used to limit absorption or uptake in desirable species, and is not a viable option for many aquatic applications. Limited uptake and or translocation are other mechanisms of tolerance where the herbicide is not present at the site of activity at a lethal concentration.

Metabolism of the herbicide includes breakdown or conjunction –sequestration which also provides a measure of tolerance. Finally, the active site – usually an enzyme, does not allow the herbicide to bind and cause inactivation. These mechanisms explain how certain plants – both crops and weeds are not affected by an herbicide. When resistance to an herbicide occurs, one or more of these mechanisms is responsible.

Selectivity of herbicides in the aquatic environment is not well understood, particularly as it relates to terrestrial systems. This is partially due to the difficulties with studying herbicide activity in aquatic plants but also the unique physiology of these plants. Phloem and xylem movement is limited and many traditional anatomical features such as cuticle and vascular tissues are vestigial or lacking.

There are specific examples where very similar plants react completely different to the same herbicide. The most notable example is hydrilla and elodea. Hydrilla is very susceptible to endothall herbicide and moderately effected by diquat, while the opposite occurs for elodea. Both plants are in the same plant family, grow in similar habitats and are almost indistinguishable. Endothall and diquat are non-selective herbicides in their mode of action, yet major differences result when applications are made to these two species. Since metabolism has not been reported with either herbicide, differential uptake appears to be the most plausible explanation.

Resistance to three herbicides has recently developed thus far in aquatics in the United States. The most notable was the development of fluridone resistance by the dioecious biotype of hydrilla in the late 1990's. This occurred in central Florida and has spread throughout much of the state. Research confirmed resistance is due to, in part, to an amino acid substitution in the gene coding for phytoene desaturase (Puri et al. 2007). However, resistance ranges from 3-fold to 7-fold with the same genetic change, indicating some other factor may be involved in resistance.

A similar situation has arisen with hybrid watermilfoil in the state of Michigan. Hybrid milfoils result from a cross between Eurasian and northern watermilfoils and have only recently been documented in the northern mid-western U.S. However, it is unclear whether resistance has developed to an existing population, or whether inherent tolerance is a result of the development of the hybrid. Studies to elucidate the level of tolerance and associated mechanisms are currently being conducted.

Resistance to diquat has developed in spotted duckweed (*Landoltia punctata*), also in central Florida (Koschnick et al. 2006). Unlike fluridone, the level of resistance to diquat is greater than 50-fold. Interestingly, resistance can be overcome with the addition of copper. This lead to the speculation that limited uptake was responsible for the resistance, although studies could not elucidate the exact mechanism. Hydrilla has also developed resistance to endothall herbicide, once again in central Florida (Berger et al 2011). The mechanism of resistance is

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still unclear and studies are currently being conducted on this phenomenon, and the level of resistance appears to be 3 to 5-fold.

In all cases of resistance in aquatics thus far, the underlying issue has been continuous use and /or long-term exposure to the herbicide. This has been the case for most resistance developments in terrestrial cropping systems and it is not surprising that this is same in the aquatic environment. The major difference is the development of resistance without genetic recombination, especially with dioecious hydrilla. This biotype only reproduces asexually in the U.S. so the development of resistance was thought to be minimal, given the clonal and theoretically identical plants. However, it was discovered that genetic variability exists in asexually propagated hydrilla and more than likely other predominantly vegetative species.

Resistance to herbicides in the aquatic environment poses severe implications to plant managers. There are very few herbicides registered for use in these areas and the loss of one herbicide can be extremely detrimental and result in the inability to control a particular species. An integrated approach utilizing non-chemical methods of control is always desirable and will prevent or substantially reduce the potential for resistance development.

The use of herbicides with different modes of action is also a proven method for deterring resistance. However, aquatic herbicide registration and labeling is very difficult so the option of readily available alternative herbicides is not always a feasible option.

An additional area of research is a more fundamental understanding of the activity and physiological mechanisms of herbicides within aquatic plants. Herbicide activity is closely linked with physiology and biochemistry, but aquatic plants are vastly different from typical terrestrial plants and cropping systems. The key to managing herbicide resistance in the aquatic plant environment is the same as terrestrial systems – rotating herbicide chemistries and alternative weed control strategies, and understanding the underlying mechanisms of herbicide activity.

## REFERENCES

Berger, S., G. E. MacDonald and M. D. Netherland (2011). Suspected endothall tolerant hydrilla in Florida. Proceedings Southern Weed Science Society. Vol. 64. *In press.*

Gettys, L. A., W. T. Haller, and M. Bellaud (2009). Biology and control of aquatic plants: a best management practices handbook. Aquatic Ecosystem Restoration Foundation, Marietta, GA. 210 pages.

Koschnick, T. J., W. T. Haller, and L. Glasgow (2006). Documentation of landoltia (*Landoltia punctata*) resistance to diquat. Weed Science, 54:615-619.

MacDonald, G. E., K. A. Langeland, and D. L. Sutton (2005). Torpedograss (*Panicum repens*) management in Florida. Proceedings Southern Weed Science Society, 58:256.

Madsen, J. (2009). Chapter 1: Impact of invasive aquatic plants on aquatic biology. In: Biology and control of aquatic plants: a best management practices handbook (Gettys, L.A., W.T. Haller, and M. Bellaud, Eds.). Aquatic Ecosystem Restoration Foundation, Marietta, GA. 210 pages.

Puri, A., G. E. MacDonald, F. Altpeter, and W. T. Haller. (2007). Mutations in phytoene desaturase gene in fluridone-resistant hydrilla (*Hydrilla verticillata*) biotypes in Florida. Weed Science, 55:412-420.

Richardson, R.J. 2009. Aquatic plant management and the impact of emerging herbicide resistance issues. Weed Technology,. 22:8-15.

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# ALLOMETRY AND GROWTH PATTERNS OF *SCIRPUS GROSSUS* L. ON PEAT

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## ABSTRACT

*Scirpus grossus* L. is a principal rhizomatous weed in the rice fields, drainage and irrigation canals, river banks, abandoned rice fields and wasteland in Malaysia. This study describes the modular dynamics, spatio-temporal growth patterns of aerial plant and sub-terranean rhizome populations of this scourge on fertilized and unfertilized peat soils. The NPK fertilizer application at 100:30:30 ha$^{-1}$ resulted in more robust aerial plant growth of *S. grossus* with 126.75 ramets m$^{-2}$ (mean dry aerial bioamass of 2.32 g plant$^{-1}$) compared with 117.83 ramets m$^{-2}$ (1.63 g plant$^{-1}$) in unfertilized plots 24 weeks after planting of the mother plant. Mean ramets mortality was significantly higher in unfertilized plots at 30.3 ramets m$^{-2}$, while in the fertilized plots this was only 8.7 ramets m$^{-2}$, resulting respective net populations of 116.08 ramets m$^{-2}$ and 87.5 ramets m$^{-2}$ in fertilized and unfertilized plots. Flowering set in earlier among ramets in fertilized plots with 51.58 ramets m$^{-2}$ *vis-a-vis* 38.75 ramets m$^{-2}$, 24 weeks after transplanting of the mother plant in unfertilized plots. Fertilizer applications did not register any significant difference in mean plant height, chlorophyll contents, and chlorophyll fluorescence measurements *vis-a-vis* those devoid of fertilizer application. The time- and space-mediated clonal growth of *S. grossus* did not register any significant preferential directionality and dispersion of aerial plants and their sub-terranean rhizomes irrespective of fertilizer regimes, but rather displaying opportunistic resource capture by aerial and sub-terranean modules.

**Keywords:** *Scipus grossus* L., modules, modular growth, fertilizer application, sub-terranean rhizomes.

## INTRODUCTION

Rhizomatous plants grow and reproduce vegetatively by rhizomes. Vegetative branches are formed from the reiteration of the basic units, while flowers and inflorescences come from the reiteration of units bearing modified leaves. The population dynamics of many rhizomatous plants is dominated more by the flux of clonal modules. The ability of a single genotype to form fragmented phenotypes is just one of the variants in the life patterns of modular organism (Harper & Bell, 1979). The process of new growth is often subjected to different pressures, including the change in soil nutrients, and resource capture ability among individual plants and their modules.

It has been well documented in the literature that nitrogen, potassium, and phosphorous are important macroelements for healthy plant growth, in addition to other macro- and microelements (Baki1988; Huang, *et al.* 2004 ). Nitrogen is present in all the macromolecules in the cell, such as amino acids, proteins, lipids and carbohydrates. Probably more importantly, nitrogen concentration in green vegetation is often related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes, namely photosynthesis (Daughtry *et al.* 2000). Recently, Huang, *et al.* (2004) has shown in a study on rice seedlings that nitrogen deficiency brought about adverse effects on the chlorophyll content of the leaves and chlorophyll fluorescence, both of which are good indicators of photosynthetic capacity. Thus nitrogen deficiency in soils will result in plants exhibiting limited growth and deficiency symptoms such as chlorosis. Many studies have shown that a significant increase in growth rate of plants will occur with the application of nitrogen (Ozer 2003). Baki (1988) reported that additions of phosphate appeared to enhance the rate of flowering in *S. grossus.*

*Scirpus grossus* is a pan-tropical weed in the rice fields, drainage and irrigation canals, river banks, abandoned rice fields and wastelands in Malaysia and elsewhere. There is a paucity of information on the population biology of this scourge in the literature. This study reports on the allometry, modular dynamics, and spatio-temporal growth patterns of aerial plant modules and sub-terranean rhizome populations of this weed on fertilized and unfertilized peat soils.

## MATERIALS AND METHODS

### Plant Establishment and Care

Synthetic populations of *S. grossus* were established on peat soils in the Malaysian Agriculture Research Development Institute (MARDI) Research Station, Jalan Kebun, Klang (3.00$^o$ N /101.30$^o$ E), Malaysia for 24 weeks commencing on 24 February 2010. Cohorts of young ramets at 2-3-leaf of *S. grossus* were obtained from rice fields of Tanjung Karang, Selangor. Each ramet was planted in the centre of a plot measuring 2m x 2m, previously demarcated and lined with 5cm x 5cm grids and sub-plots **(Fig. 1).** Fertilizer applications with Nitrophoska Blue Special NPK fertilizer at the rate of 100:30:30 were made one week prior to planting. A set of 3 replicated plots with fertilizer application was allocated with while another 3 sets served as a control. Watering of the plots was made twice daily, one in the morning and the other in the late afternoon using a fine rose fitted to a water hose. No weeds were allowed to grow in the plots during experimentation.

### Data Acquisition and Management

The clonal growth of *S. grossus* based on the number of emerged plants in each plot, its location, and heights was recorded on a weekly basis for 24 weeks. Likewise for the number of dead plants and their positions in each plot were also recorded. The phenology of *S. grossus* was also recorded, taking into account the time of flowering after planting, and the number flowers were recorded on a weekly basis. The chlorophyll content in the leaves was determined at the end of planting using a Minolta SPAD meter. From each plot 15 leaves were randomly selected for measurement. In the case of the chlorophyll fluorescence which has been well documented to be closely related to photosynthetic capacity and quantum yield was recorded using a Hansatech (UK) Photosynthetic Efficiency Analyzer. Variable (Fv) and maximum fluorescence (Fm) readings were taken and the Fv/Fm values, which correspond to quantum efficiency, were calculated. A minimum of five readings per plot for each treatment were taken and the average determined.

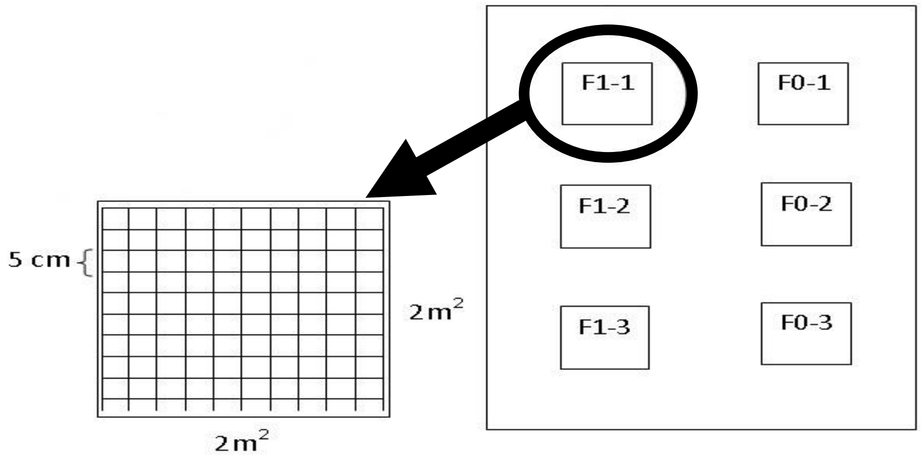


**Figure 1.** Experimental design and quadrats arrangement of *Scirpus grossus* in MARDI Research Station, Jalan Kebun, Selangor, Malaysia. $F_0$ – No fertilizer application; $F_1$, NPK applied.

After 24 weeks of experimentation, the plants were harvested by dismembering them into leaf, stem, and inflorescence components, and their dry weights were determined. Ten flowering plants taken at random were harvested from each plot. These components were subjected to chemical analysis. The sub-terranean rhizomes remained intact by ensuring that no rhizome damage was inflicted during harvest of the aerial plant parts.

As for the spatio-temporal growth pattern, ramification and mapping of the architecture of sub-terranean rhizomes, a water hose with strong pressure was used to wash way the peat soils to expose the rhizomes. Extra care was instituted so as not to damage the exposed rhizomes during this operation. The exposed rhizomes were mapped by measuring inter-nodal lengths of each rhizome, and noting the precise positions of the harvest plants. These data were transferred into the data logger, and together with the weekly data on the precise spatio-temporal positions of emerged plants of *S. grossus*, computer generated subterranean rhizome architectures were produced. The computer program used was AutoCAD 10. These were generated based on time- and space-mediated architecture of the weed at 1, 2, 3, 4, 5 and 6-months old.

The directionality and dispersion patterns of sub-terranean modules of the weed with time and space were also analyzed using Circular Statistics (Zar 2006). For this purpose, the computer-generated maps of emerged plants and rhizomes in the fertilized and unfertilized plots for 1, 2, 3...6 months old *S. grossus* were each arbitrarily divided into 8 sub-sectors with respect to the geographical north. The number of emerged individuals and the length of each rhizome in each sub-sector were recorded. The analysis were based on Rayleigh's *r* and Rayleigh's *z*values, and mean angle of dispersion (Zar 2005) in the assessment whether there is a particular preferential direction of growth of the emerged plants and the subterranean rhizomes, or otherwise with respect to the geographical north.

The rates of spread (based on the number of emerged plants or ramets, and length of rhizomes way from the mother plants) were also calculated. This was done based on the computer-generated maps described earlier. The plants were group according to their concentrations in six concentric circles, each circle representing the mean monthly individuals established from the single mother plants. This analysis is to assess whether the rates of emergence of individual ramets and their associated rhizomes were different or otherwise as they move away from the mother plant as a strategy to avoid self-crowding and minimize mean density of emerged individuals from each other with time.

The appropriate data were subjected to ANOVA and their treatment means were tested for significant difference, if any, using HSD and *t*-tests (Zar 2006).

## RESULTS

### General Clonal Growth Patterns

*Scirpus grossus* plant reiterates by rhizomatous growth and branches from a single mother plant. As shown in (Figure 2), the best period of clonal growth in general is between 10-18 weeks. The best period of clonal growth in fertilized soils was at week $12^{th}$ while in unfertilized soils it was at week $13^{th}$. At the end of the 24 weeks of study period, the total average gross number of emerged ramets in fertilized soils were 126.75 ramets/$m^{-2}$ and 117.83 ramets/$m^{-2}$ in unfertilized soils.

The mortality rate recorded was 30.33 ramets in unfertilized soils and 8.67 ramets in fertilized soils (Figure 3),while the real rate showed 87.5 ramets/$m^{-2}$ in unfertilized soils and 116.08 ramets/$m^{-2}$ in fertilized soils (Figure 4a & 4b).

The results for subsequent recruitment of shoot modules appeared convergent (Table 1) where the highest average plant height in unfertilized soils was 161.67 cm while in fertilized soils it was 160.67 cm.

Plants growing in unfertilized soils started to flower 16 weeks after transplanting, while in fertilized soils, *S. grossus* started to flower at week 13. At the end of the 24 weeks period study, the average number of flowering ramets in unfertilized soils stood at 38.75 ramets/$m^{-2}$ *vis-a-vis* 51.58 ramets/$m^{-2}$ for those devoid of fertilizer application (data not shown).

Table 1 show the dry biomass of selected plant parts of *S. grossus* taken after harvest at 24 weeks after transplanting displaying measurable differences according to fertilizer regimes. In unfertilized soils: the leaves were 6.90 g, and the stems, 7.99 g whilst the flowers were 1.92 g in weight. In fertilized soils these were, 9.73 g (leaves), 10.51 g (stems) and 2.77 g for flowers.

Chlorophyll content in leaves has always been regarded as a measure of the health status of a plant. For example plants deficient in nitrogen will exhibit chlorosis and the leaves will be less green than a healthy plant. (Table 1) shows that the chlorophyll content in leaves of plant growing in fertilized soil was slightly higher (46.51) than that recorded in leaves of the control plant (49.48). However this difference was not significant. Similarly, in the case of chlorophyll

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fluorescence, to determine to photosynthetic capacity of the leaves, there was very little difference between the two sets of plants.

**Table 1.** General growth patterns of *Scirpus grossus* in unfertilized and fertilizer soils.

| | Unfertilized peat | Fertilized peat |
|---|---|---|
| **Gross plant number** | 117.83 ± 60 $/m^{-2}$ | 126.75 ± 48$/m^{-2}$ |
| **Mortality number** | 30.33 ± 22.25 $/m^{-2}$ | 8.67 ± 11.75$/m^{-2}$ |
| **Net plant number** | 87.5 ± 37.75 $/m^{-2}$ | 118.08 ± 36.25 $/m^{-2}$ |
| **Flowers number.** | 38.75 ± 67.75 $/m^{-2}$ | 51.58 ± 66.75$/m^{-2}$ |
| **Plant height** | 161.67 ± 51 cm | 160.67 ± 44 cm |
| **Chlorophyll content** | 46.51 ± 3.16) SPAD | 49.48 ± 7.1 SPAD |
| **Chlorophyll fluorescence** | 0.76 ± 0.016 | 0.77 ± 0.038 |
| **Leaf weight (g)** | 6.9 ± 5.7 | 9.7±4.9 |
| **Stem weight (g)** | 7.9 ± 4.4 | 10.5 ±6.7 |
| **Florescence (g)** | 1.9 ±1.0 | 2.8 ±2.2 |
| **No. inflorescence*** | 152.4 ± 36.6 | 211.0 ± 28.8 |

* after 24 weeks

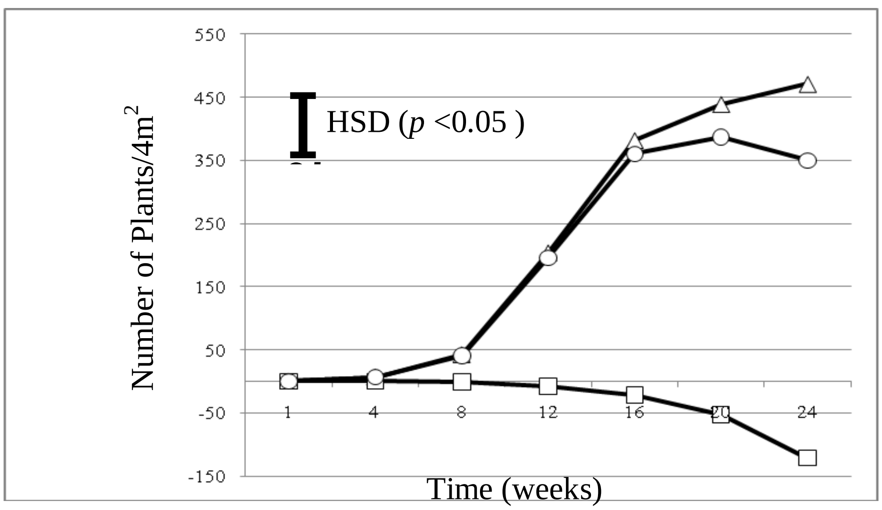


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**Figure 2a.** Population fluxes of *Scirpus grossus* on unfertilized peat.
Natality (▲), Mortality (■), Net population (●).

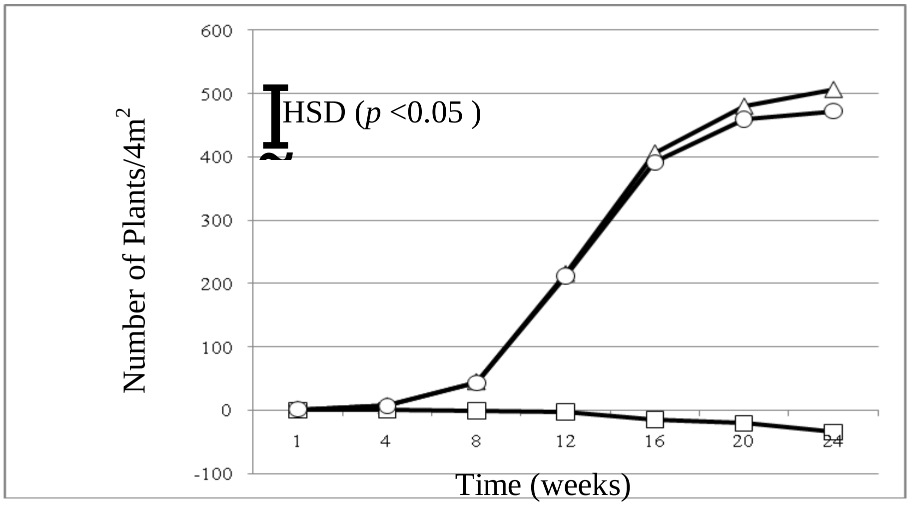


**Figure 2b.** Population fluxes of *Scirpus grossus* fertilized peat. Natality (▲), Mortality (■), Net population (●).

## Population Spread and Sub-Terranean Rhizome Architecture

Figure 3a shows the time-mediated growth of subterranean rhizomes of *S. grossus.* in unfertilized soils, showing the best period 3 month after sowing of the mother plant. The parallel figure for fertilized soils was 4 months after sowing of mother plant (Figure 3b). The time-mediated emergence of ramets in both fertilized and unfertilized peat soils are shown in Figs. 4a and 4b. Invariably, more ramets were recorded in fertilized soils than those in the unfertilized counterparts, indicating the stimulatory effects of fertilizer application of the growth and proliferation of *S. grossus*.

## Directionality and Dispersion Analyses

Dispersion analysis of subterranean rhizomes by circular statistics on *S. grossus* generated no special preferences in the direction of modules or emerged ramets as explained by the Rayleigh's *r*, Rayleigh's z, and mean angle of dispersion (Table 2). However, there were heavier concentrations of ramets in the eastern sector of the plot, presumably due to phototropic effect of sunlight (Figure 5)

## DISCUSSION

This study was an attempt to determine the effect of NPK fertilizer on the structural life *S. grossus* L. on peat. It showed details of different growth patterns. The best period of clonal growth in general was between 10-18 weeks, an outcome similar to the results mentioned by (Baki 1988). This phenomenon is probably due to the finite amount of resource in the soil, diminishing with time. In addition to this although there may be resources still available, the

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plants are ageing and the leaves start to show reduced effective photosynthesis. Furthermore the assimilatory activity of the plant may have been approaching the compensation point with the respiratory burden of accumulated support tissue.

The results showed that the use of fertilizers had a significant impact on content in growth parameter but others did not show a significant impact. The addition of NPK fertilizer had a significant effect on clonal growth where it dramatically increased the population flux of the weed. Similarly, the NPK fertilizer caused a decrease in the number of deaths, and this was similar to the findings of Baki (1988) who studied the structural demography and growth patterns of *S. grossus.*

The NPK fertilizer, which contains 30% of phosphate, also increased the flowering rate of the weed. A similar observation was reported by Baki, (1988). In addition, the NPK fertilizer helped to strengthen the plant and this was observed in the significant increase in the weights of the various plant parts in fertilized soils.

However with regard to other measurements made, the fertilizer treatment did not have a significant impact and this included plant height. Baki (1988) explained the height of *S. grossus* was affected by the depths of inundation.

In addition to this NPK fertilizer treatment had minimal effect on the leaf chlorophyll content and chlorophyll fluorescence. This probably indicates that the soil on which the rice plants were grown had sufficient macro and micro nutrients to support healthy growth

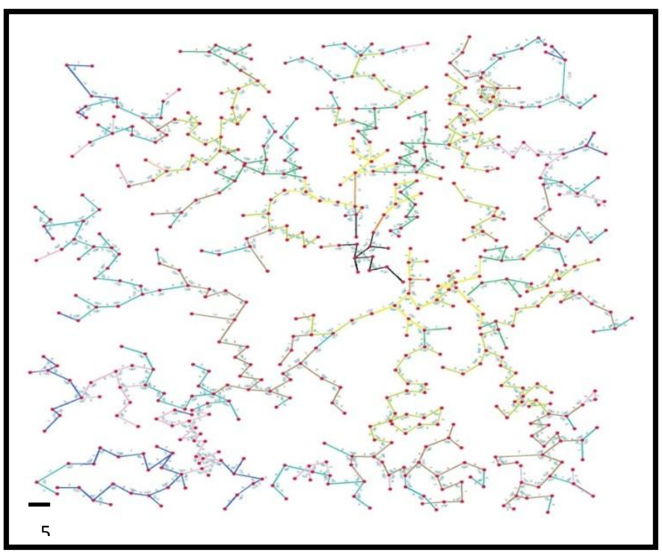


**Figure 3a.** Time-mediated growth of subterranean rhizomes of *Scirpus grossus* in unfertilized peat soil ($F_0$) 6 months after planting of mother plant. Black, 1st, 2nd week; Orange, 3rd, 4th week; Yellow, 5th and 6th week; Dark green, 7th and 8th week.; Olive green 9th, 10th week; Blue, 11th, 12th week; Brown, 13th, 14th week; Pink, 15th, 16th week; Dark blue, 17th, 18th week; Purple, 19th, 20th week; Grey, 21st, 22nd week.

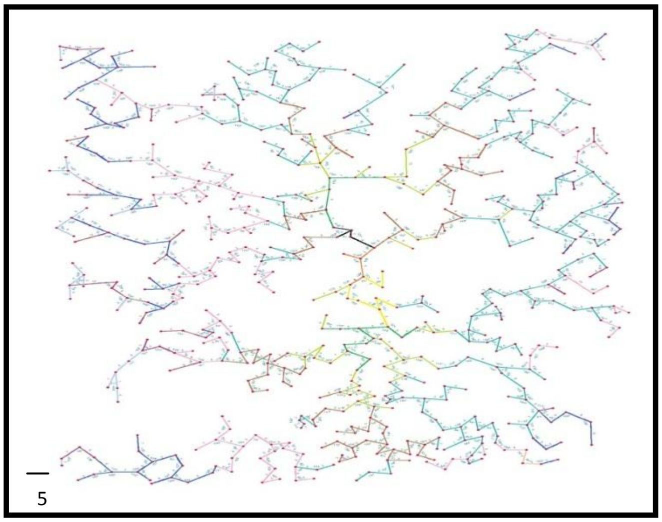


**Figure 3b.** Time-mediated growth of subterranean rhizomes of *Scirpus grossus* in fertilizer peat soil ($F_1$) 6 months after planting of mother plant. Black, 1st, 2nd week; Orange, 3rd, 4th week; Yellow, 5th and 6th week; Dark green, 7th and 8th week.; Olive green 9th, 10th week; Blue, 11th, 12th week; Brown, 13th, 14th week; Pink, 15th, 16th week; Dark blue, 17th, 18th week; Purple, 19th, 20th week; Grey, 21th, 22th week.

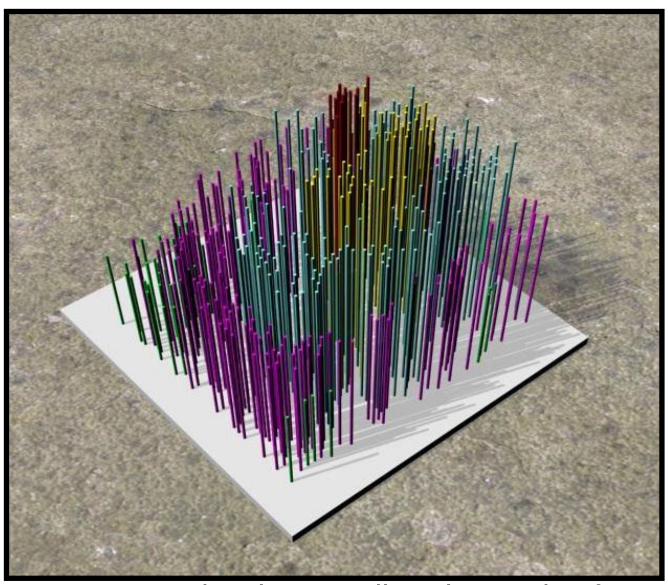

**Figure 4a.** The time-mediated growth of ramets in *Scirpus grossus* in unfertilized peat soil ($F_0$) 6 months after planting of the mother plant. Red, emerged ramets in the 1st month; Yellow, emerged ramets in the 2nd month; Blue, emerged ramets in the 3rd month; Purple, emerged ramets in the 4th month; Green, emerged ramets in the 5th month and Black, emerged ramets in the 6th month.

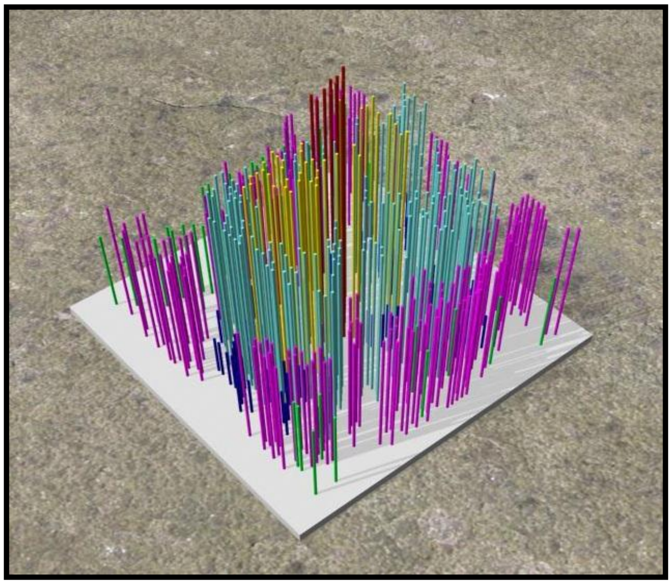

**Figure. 4b.** The time-mediated growth of ramets in *Scirpus grossus* in fertilizer peat soil ($F_1$) 6 months after planting of the mother plant. Red, emerged ramets in the 1st month; Yellow, emerged ramets in the 2nd month; Blue, emerged ramets in the 3rd month; Purple, emerged ramets in the 4th month; Green, emerged ramets in the 5th month; and Black, emerged ramets in the 6th month.

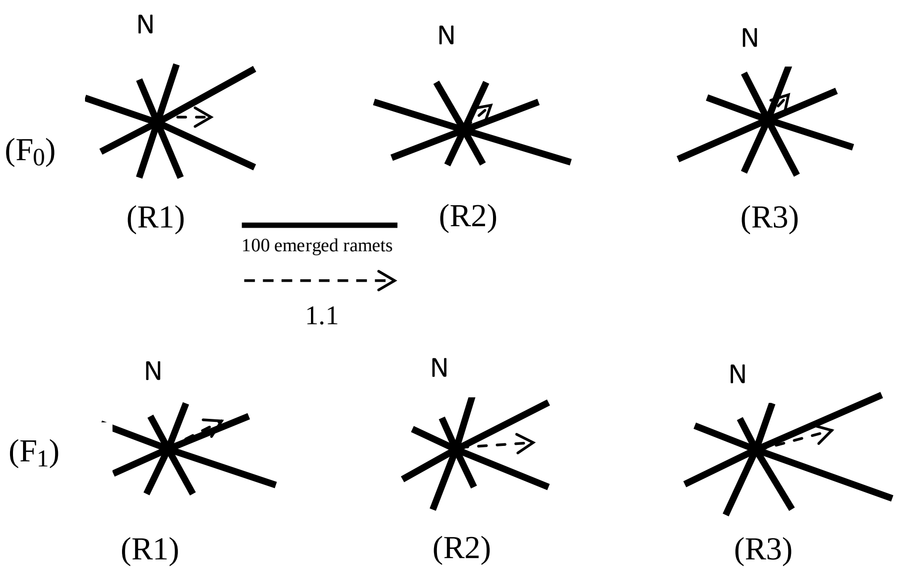


**Figure 5.** Dispersion analysis of emerged ramets of *Scirpus grossus* by circular statistics in unfertilized soil ($F_0$) and fertilizer soil ($F_1$). N, geographical north; mean angle of dispersion.

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| | | Attributes | | |
|---|---|---|---|---|
| **Parameter** | Replicate | r | Z | θ° |
| **Fertilized Soils** | R1 | 51.739 | 6.529 | 88.08 |
| | R2 | 58.195 | 6.653 | 39.34 |
| | R3 | 111.747 | 20.743 | 23.20 |
| **Unfertilized soils** | R1 | 78.361 | 10.887 | 65.12 |
| | R2 | 15.862 | 0777 | 82.39 |
| | R3 | 21.235 | 0.857 | 75.57 |

**Table 2.** Degree of directional and dispersion in the circular distributions of emerged ramets around the mother plant of *Scirpus grossus* in fertilized ($F_1$) and unfertilized peat soils ($F_0$) as measured by selected attributes.

**REFERENCES**

Baki, B. B. (1988). The structural Demography and Growth Patterns of *Scirpus grossus* L. *Proc. National Seminar and Workshop on Rice Field Weed Management, Penang,* pp. 299-314.

Daughtry, C. S. T., Walthall, C. L., Kim, M. S., Brown de Colstoun, E. & McMurtrey III, J. E. (2000). Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment*, 74:229– 239.

Harper, J.L. & Bell, A.D. (1979). The Population Dynamics Of Growth Form In Organisms With Modular Construction. *Proc*. *Symposium of the British Ecological Society* 20:29-52.

Ozer, H. 2003. Sowing date and nitrogen rate effects on growth, yield and yield components of two summer rapeseed cultivars. *European Journal Agronomy* 19: 453-463.

Zar, J.H. 2006. *Biostatistical Analysis.* 5th Edition, Prentice Hall Inc., Euglewood Cliffs, N.J.

# CHLOROPHYLL FLUORESCENCE MICROSCREENING AS A RAPID DETECTION METHOD FOR HERBICIDE RESISTANCE IN GRASS WEEDS IN NORTH CHINA PLAIN WINTER WHEAT PRODUCTION SYSTEMS AND BEYOND

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## ABSTRACT

The North China Plain (NCP) is one of the most important winter wheat production areas in the world. A double cropping system of winter wheat followed by summer maize in one year is the most common cropping practice in the NCP. However new crops and agricultural practices including chemical weed control measures were recently introduced in this area. *Alopecurus spp., Aegilops squarrosa* L. and *Bromus japonicus* Thunb. were found to be the most abundant grass weeds in the NCP winter wheat production system. In 2008 and 2009, *A. japonicus* seeds were collected from different locations in the NCP to conduct herbicide efficacy studies. Besides conventional glasshouse bioassays a rapid herbicide resistance test has been developed and tested. This new resistance test is based on chlorophyll fluorescence microscreenings for evaluation of the efficacy of herbicides on grass weeds grown in tissue culture plates filled with an agar-herbicide solution. In glasshouse bioassays for chlorotoluron a resistance factor of 3.5 was found for one of the NCP biotypes compared to the sensitive control biotype. The chlorophyll fluorescence microscreening clearly verified this result. Further studies showed that this method is also suitable for other herbicide modes of action like ALS and ACC inhibitors. Furthermore this method is easily transferable to other important grass weeds. Using the chlorophyll fluorescence assay, it becomes possible to evaluate efficacy for a large number of biotypes with a minimum requirement of time and space. Therefore it is well suited for high throughput resistance screenings, especially at locations where glasshouse space is a limiting factor. An accelerated identification of resistant grass weed biotypes and thus a prompt resistance management plan for the field will be of great importance for the North China Plain and other intensive agricultural areas in the world.

**Key words:** herbicide resistance, *Alopecurus japonicus* Steud., chlorophyll fluorescence, herbicide bioassay

## INTRODUCTION

The North China Plain (NCP) is the most important agricultural area in China. It comprises parts of the six provinces Beijing, Tianjin, Hebei, Shandong, Henan, Anhui, and Jiangsu and covers an area of 31 million hectares. More than half of this area is used for agriculture which accounts for only 20% of the total agricultural area in China (Liu et al., 2001), but 50% of the

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total Chinese winter wheat production (Wu et al., 2006). Winter wheat (*Triticum aestivum* L.) production in the NCP is implemented as a double cropping system of winter wheat followed by summer maize (*Zea mays* L.) over one year.

In 2009 and 2010 weed surveys were conducted in the NCP to investigate the major weed species in winter wheat (*Triticum aestivum*). As one of the most abundant grass weeds Japanese foxtail (*Alopecurus japonicus*) was widely distributed. In 2009 seeds of *A. japonicus* were collected from three different locations in the NCP for herbicide efficacy studies. For the efficacy studies an herbicide resistance microscreening method was developed, based on chlorophyll fluorescence measurements. Rapid herbicide resistance screening methods are receiving increased attention, because worldwide the number of herbicide resistant weeds is rapidly increasing and therefore more accelerated screening methods become necessary. Beside molecular genetic analysis, there are several test methods described in literature; for example, the “Rothamsted Rapid Resistance Test” (Moss 2000) or the Syngenta RISQ test (Kaundun et al. 2011). Except for molecular genetic analysis, all methods have several commonalities including lack of useful methodology to quantify herbicide resistance or difficult, time-consuming method for measuring plant response to herbicides. Chlorophyll fluorescence measurement has been widely used in weed science, especially in mode of action studies and crop sensitivity studies (Korres et al. 2003, Abbaspoor et al. 2007).

The aim of this study is to introduce and evaluate the chlorophyll fluorescence microscreening test to accelerate the detection of herbicide resistance with a minimum requirement of space and labor. Furthermore we wish to jointly utilize existing chlorophyll fluorescence based herbicide screening and photosynthesis evaluation methods and to develop them further. The method used in this study describes the effect of chlorotoluron upon *A. japonicus*.

## MATERIALS AND METHODS

### Seed Origin and Seed Germination

*A. Japonicus* seeds were collected from different locations in the North China Plain. Three biotypes originating from the Hebei province (subsequently named Hebei1, Hebei2 and Hebei3) and one biotype originating from the Jiangsu province (subsequently named Jiangsu1) were obtained. The collected biotypes were compared to a chlorotoluron sensitive biotype (subsequently referred to as sensitive).

The collected seeds were pre-germinated on three folds of filter paper in glass petri dishes. For germination, 6.0 mL of nutrient solution, formulated according to Pedas et al. (2005), was added to each petri dish. The petri dishes were placed in a growth cabinet at a day/night cycle of 18/10°C,12/12h.

### Herbicide Treatment

After cotyledons were fully developed, plants were transplanted into six-well tissue culture plates (TPP, Switzerland). Each well was filled with 3 ml of a 0.4% Agar solution (Micro Agar, Duchefa, Germany). The same nutrient solution as used for seed germination was used to prepare the plant Agar in order to guarantee optimal nutrient availability. Chlorotoluron

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(Lentipur™, 700 g a.i. $L^{-1}$) was added to the Agar solution at nine concentrations, ranging from 0.18 µM a.i. to 0.00070313 µM a.i.. Each treatment was repeated six times.

### Chlorophyll Fluorescence Instrumentation and Measurement Routine

Chlorophyll fluorescence parameters were analyzed with an IMAGING-PAM *M-Series* Chlorophyll Fluorometer (Heinz Walz GmbH, Germany). For evaluation of herbicide efficacy, maximum quantum efficiency of PS II photochemistry, is defined as *Fv/Fm*. It is calculated by the equation $Fv/Fm = (Fm - Fo)/Fm$, were *Fm* is the maximal fluorescence yield and *Fo* the dark fluorescence yield. For determination of *Fo*, plants were dark adapted for the duration of 30 minutes prior to the measurement. The measurement was carried out in a dark room under green illumination to avoid other phytosynthetic active radiation except that emitted by the IMAGING PAM light source. After dark adaption, leaves were illuminated with a light saturation pulse of 580 µM $m^{-2}$ $s^{-1}$ and a wavelength of 450 nm for *Fv/Fm* determination.

In unstressed leaves *Fv/Fm* is approximately 0.83. This value is independent of the plant species. Lower values indicate that a proportion of the PS II reaction centers are inhibited due to stress conditions, for example due to the presence of a herbicide. This phenomenon is called photoinhibition (Strasser and Stirbet 2001). Beside other fluorescence parameters *Fv/Fm* is suitable especially for the study of PS II inhibitors (Korres et al. 2003).*Fv/Fm* determination began 3 HAT (HAT, hours after transplanting) and was repeated at 24 HAT, 48 HAT, 72 HAT, 120 HAT and 192 HAT, for analysis of dose response relationships.

### Whole- Plant Herbicide Efficacy Studies

For validation of the chlorophyll fluorescence microscreening experiment results, standard glasshouse dose-response studies were carried out. Therefore seeds were pre-germinated in vermiculite and transplanted to plastic pots of 9x9 cm size after the cotyledon was fully developed. In each pot 2 plants were transplanted. Chlorotoluron (Lentipur™, 700 g a.i. $L^{-1}$) was sprayed in nine descending dosages ranging from 2100 g a.i. $ha^{-1}$ to 8.2 g a.i. $ha^{-1}$ 10 days after transplanting in a precision application chamber (Aro, Langenthal, Switzerland). The nozzle (8004 EVS, Teejet Spraying Systems Co., Wheaton, IL, USA) was calibrated to spray a volume of 400 L $ha^{-1}$ resulting in a speed of the nozzle of 800 mm $s^{-1}$, a distance from the sprayed surface of 500 mm and a spraying pressure of 300 kPa. Each treatment was replicated 4 times. Before and after the herbicide treatment, pots were placed in a glasshouse at a day/night cycle of 18/10°C,12/12h. The plants were cut at ground level 12 days after herbicide treatment and dried at 80°C for 48 h for dry weight determination.

### Statistical Analysis

Statistical analysis was carried out with the statistical software *R* (*R* Development Core Team 2011) and the R extension package *drc* (Ritz and Streibig 2005). For the analysis of dose response relationships the non-linear four parameter model after Streibig (1988) was used. The model follows the equation: $y = C + ((D - C) / (1 + exp(b\ ln(x / ED50))))$, where y represents the plant response (Fv/Fm or dry weight), D the upper limit of the curve, C the lower limit, and b is proportional to the slope around ED50, the dose at which the plant response is reduced by 50%. Dose–response curves were compared by horizontal

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assessment (*F* test, α=0.05) after data normalization according to Streibig (1995). All experiments were repeated twice.

## RESULTS

### Chlorophyll Fluorescence Microscreening Herbicide Efficacy Tests

The time response analysis of the sensitive biotype showed a reduction of *ED50* and *ED90* values with increased time of exposure (Figure 1). In order to determine the optimal timing for dose response comparison among biotypes, data was analysed with regard to the timing, where the maximum herbicide dosage resulted in a 90% *Fv/Fm*- reduction *(ED90*). Therefore *ED90* was reached 48 h after transplanting (Table 1). The standard error decreased over time and therefore horizontal comparison between biotypes was not be calculated earlier than 48 HAT in order to get meaningful results.

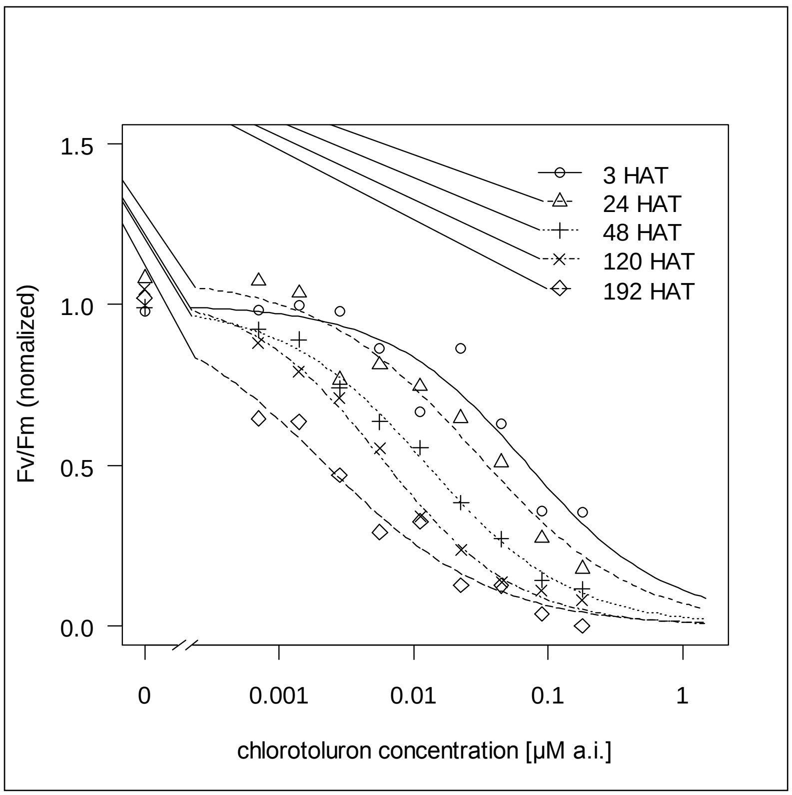


**Figure 1.** Chlorotoluron dose-response relationship for the sensitive biotype in dependency of the timing of *Fv/Fm* measurement (HAT, hours after transplanting).

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**Table 1.** Effective dosages of chlorotoluron causing a 90% (*ED90*) reduction of *Fv/Fm* over time in sensitive biotypes.

| | Estimate $ED90_{Fv/Fm}$ [µM a.i.] | Std. Error |
|---|---|---|
| 3 HAT | 0.92961 | 2.0806 |
| 24 HAT | 0.551237 | 0.6135 |
| 48 HAT | 0.185193 | 0.2056 |
| 120 HAT | 0.073922 | 0.0663 |
| 192 HAT | 0.049844 | 0.0462 |

For the tested *A. japonicus* biotypes Hebei2, Hebei3 and Jiangsu1 the horizontal curve assessment resulted in no significant differences (Table 2). The Hebei1 biotype showed an increased tolerance to chlorotoluron compared to the Sensitive biotype (Figure 2) resulting in a resistance factor ($ED50_{Hebei1}/ED50_{Sensitive}$) of *RF*=3.39 (Table 2).

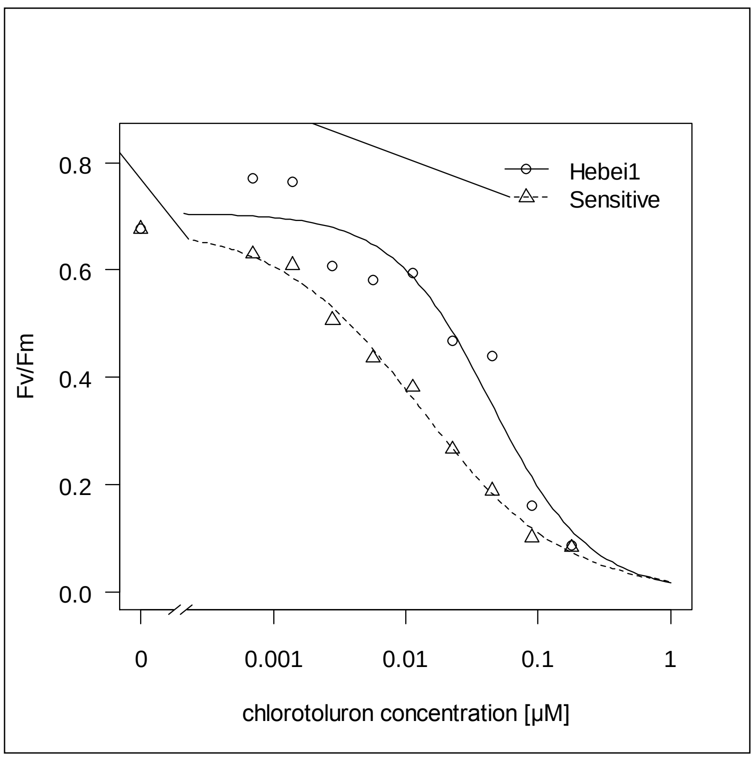


**Figure 2.** Chlorotoluron dose-response relationship for the sensitive biotype compared to the Hebei1 biotype. $ED50_{Sensitive}$=0.0129 µM a.i., $ED50_{Hebei1}$=0.0439 µM a.i.

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**Table 2.** Resistance factor calculations based on *Fv/Fm*- determination for the evaluated *A. japonicus* biotypes, p<0.05 indicating significant differences in *ED50* values.

| ED50/ED50 | Estimate | Std. Error | p-value |
|---|---|---|---|
| Hebei1/Sensitive: ED50/ED50 | 3.39 | 0.9211 | 0.0107 |
| Hebei2/Sensitive: ED50/ED50 | 0.79 | 0.1418 | 0.1324 |
| Hebei3/Sensitive: ED50/ED50 | 0.77 | 0.1411 | 0.1070 |
| Jiangsu1/Sensitive: ED50/ED50 | 0.98 | 0.1681 | 0.1390 |

## Whole Plant Herbicide Efficacy Studies

All tested *A. japonicus* biotypes were controlled sufficiently by application of chlorotoluron. For the Hebei2, Hebei3 and Jiangsu1 biotypes the horizontal curve assessment resulted in no significant difference compared to the sensitive biotype (Figure 3, Table 3).

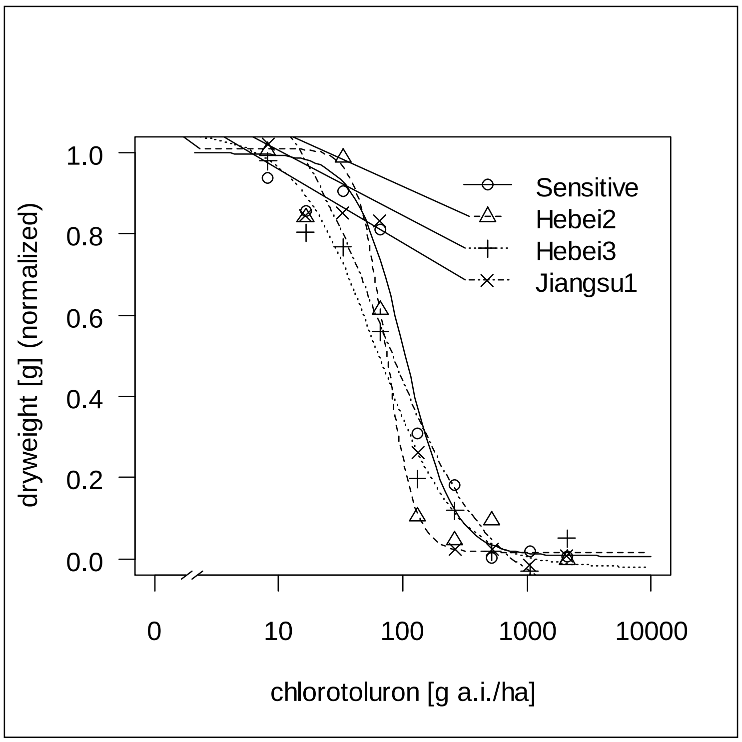


**Figure 3:** Whole-plant chlorotoluron dose- response results for the *A. japonicus* biotypes Hebei2 and Hebei3 and Jiangsu1 compared to the sensitive biotype.

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**Table 3.** Resistance factor calculation based on whole-plant dose-response studies for the evaluated *A. japonicus* biotypes; $p<0.05$ indicating significant differences in ED50 values.

| ED50/ED50 | Estimate | Std. Error | p-value |
|---|---|---|---|
| Hebei1/Sensitive: ED50/ED50 | 3.52 | 0.2235 | 0.0023 |
| Hebei2/Sensitive: ED50/ED50 | 0.70 | 0.1756 | 0.0952 |
| Hebei3/Sensitive: ED50/ED50 | 0.59 | 0.1722 | 0.0515 |
| Jiangsu1/Sensitive: ED50/ED50 | 0.63 | 0.2361 | 0.1259 |

The Hebei1 biotype showed an increased tolerance to chlorotoluron compared to the Sensitive biotype (Figure 4) resulting in a resistance factor ($ED50_{Hebei1}/ED50_{Sensitive}$) of RF=3.5 (Table 3).

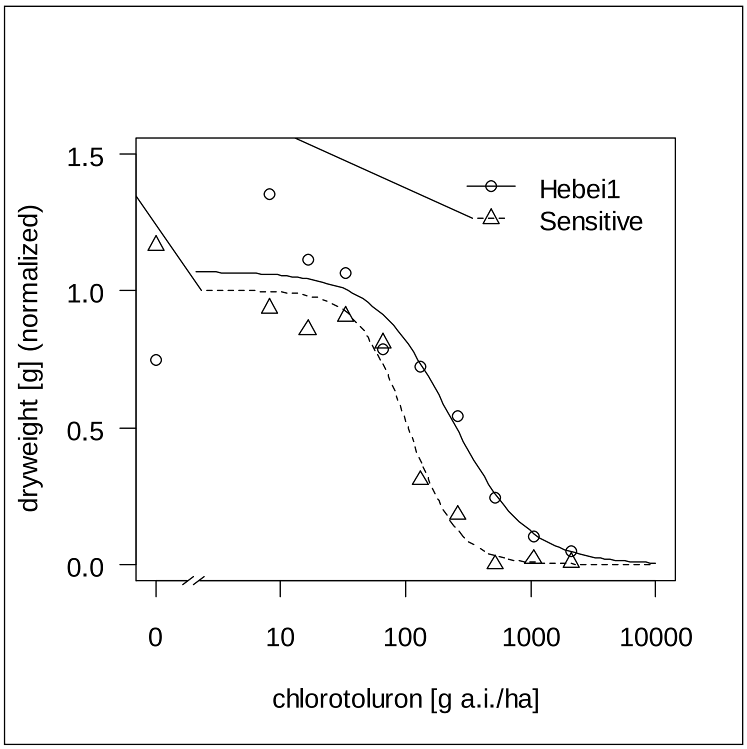


**Figure 4.** Whole-plant chlorotoluron dose response results for the *A. japonicus* biotypes Hebei1 compared to the sensitive biotype. $ED50_{Sensitive}$=65.68 g a.i. ha$^{-1}$, $ED50_{Hebei1}$=230.97 g a.i. ha$^{-1}$.

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## DISCUSSION

All biotypes under evaluation were controlled by chlorotoluron sufficiently even at lower dosages than the recommended maximum dosage of 2100 g a.i. $ha^{-1}$. The Hebei1 biotype required the highest dosage to gain 90% control of 1134.51 g a.i.; under field conditions it is assumed the full dosage is needed to control this biotype completely. All other biotypes were controlled under field conditions even with lower dosages of chlorotoluron than recommended.

The timing of 48 HAT for *Fv/Fm* measurement seems to be a good compromise between reduction of standard error and the evaluation of the maximum differences between the biotypes.The results of the whole-plant herbicide efficacy study verified clearly the results of the chlorophyll fluorescence microscreening. This new method can serve as a prospective rapid resistance test not only for chlorotoluron, but for other inhibitors. Further experiments already demonstrated that this method is also applicable for ALS- and ACCase herbicides and transferable to other grass weeds like *Bromus japonicus, Lilium rigidum* and *Apera spica-venti.* For this novel rapid resistance test, neither a glasshouse nor a precision track sprayer was necessary. Results available within 72 h after transplanting are crucial for testing during the field production season. This makes it possible for farmers to adjust weed control decisions within the season if weed seedlings from the field are directly transferred into the chlorophyll fluorescence microscreening test system.

## ACKNOWLEDGEMENTS

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## REFERENCES

Abbaspoor, M., Streibig, J.C. (2007). Monitoring the efficacy and metabolism of phenylcarbamates in sugar beet and black nightshade by chlorophyll fluorescence. Pest Management Science 585: 576-585.

Appenroth K.J., Stockel J., Srivastava A. and Strasser R.J. Multiple effects of chromate on the photosynthesis apparatus of Spirodela polyrhiza as probed by OJIP chlorophyll A fluorescence measurements. Environ. Pollut. 115:49–64.

Kaundun, S.S., Hutchings, S-J., Dale, R.P., Bailly, G.C., Glanfield, P. (2011). Syngenta RISQ test: a novel method for detecting resistance to post-emergence ACCase and ALS inhibitor herbicides in grass weeds. Weed Research 51:284-293.

Korres, N.E., Froud-Williams, R.J., Moss, S.R. (2003). Chlorophyll fluorescence technique as a rapid diagnostic test of the effects of the photosynthetic inhibitor chlorotoluron on two winter wheat cultivars. Annals of Applied Biology 143:53-56.

Moss, S.R. (2000). The “Rothamsted Rapid Resistance Test” for detecting herbicide-resistance in annual grass-weeds. Weed Science Society of America Annual Meeting, Toronto, 40, Abstract 102

Pedas, P., Hebbern, C.A., Schjoerring, J.K., Holm, P.E., Husted, S. (2005). Differential capacity for high-affinity manganese uptake contributes to differences between barley genotypes in tolerance to low manganese availability. Plant Physiology 139:1411–1420.

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ritz, C. and Streibig, J. C. (2005). Bioassay Analysis using R. J. Statist. Software, Vol 12:5.

Strasser R.J. and Stirbet A.D. (2001). Estimation of the energetic connectivity of PS II centres in plants using the fluorescence rise O–J–I–P; fitting of experimental data to three different PS II models. Math Comput Simul 56:451–461.

Streibig, J.C., Walker, A., Blair, A.M., Anderson-Taylor, G., Eagle, D.J., Friedländer, H., Hacker, E., Iwanzik, W., Kudsk, P., Labhart, C., Luscombe, B.M., Madafiglio, G., Nel, P.C., Pestemer, W., Rahman, A., Retzlaff, G., Rola, J., Stefanovic, L., Straathof, H.J.M., Thies, E.P., (1995). Variability of bioassays with metsulfuron-methyl in soil. Weed Research 35:215–224.

Streibig, J. (1988). Herbicide bioassay. Weed Research 28:479-484

# EFFECT OF REDUCED RATE OF APPLICATION OF A HERBICIDE, PROMETRYN+THIOBENCARB DURING DRAINAGE PERIOD ON IMPROVEMENT OF WEED MANAGEMENT IN DIRECT SEEDED RICE IN AKITA, NORTHERN JAPAN

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## ABSTRACT

A combination product of a granule herbicide of prometryn 0.80% and thiobencarb 8.0% (hereafter PT) which is not a one-shot herbicide, has been recommended in direct seeded rice fields in order to suppress *Echinochloa* spp. from seeding to emergence stage during the drained period just after seeding rice . The herbicides have a recommended application rate range of 0.32 kg + 3.2 kg to 0.48 kg + 4.8 kg a.i. $ha^{-1}$. Changes in efficacy and crop injury on direct seeded rice in a wet condition with reduced application rate of PT were evaluated under field and greenhouse conditions in Akita Prefecture, northern Japan. The aim of the study was to identify the herbicide rates required to avoid herbicidal injury to rice seedlings caused by unexpected rain, and to assess the sequential herbicidal efficacy of one-shot herbicides, which are major tools for weed management in direct seeded rice, applied under re-flooded condition after establishment of rice seedlings. With application of PT reduced to 0.16 kg + 1.6 kg a.i.$ha^{-1}$, sufficient herbicidal efficacy was obtained without injury to rice seedlings during the drained period after seeding. Effective control of weeds during the drained period using reduced rate of PT will ensure a sequential treatment of one-shot herbicide which can be applied after reflooding to provide good control of a range of weed species.

**Keywords:** drainage period, direct seeded rice, granule of prometryn 0.80% and thiobencarb 8.0%, one-shot herbicide

## INTRODUCTION

In Akita prefecture located in northern Japan, rice farmers who need labor saving and who wish to increase scale of farming, are trying to adopt direct seeding cultivation in wet condition. However, with this method of planting, rice yields are five to ten percent lower than in mechanical transplanting, the main method used in rice production. Therefore, these farmers need improvements for stable and high yields in direct seeded rice.

Difficulties in direct seeding in northern Japan are reliable establishment of rice seedlings and control of weeds. To prevent bird damage, seeds are buried by mechanical seeder and in order to accelerate emergence of rice seedlings under oxidized soil condition, drainage is strongly recommended. The drainage duration is determined according to the degree of paddy soil reduction. When weed management during the drainage period was unsuitable, emergences of weeds would be accelerated and that of rice would be suppressed, resulting in inadequate herbicidal efficacy in one-shot herbicide applied after re-flooding, because of missing appropriate application time.

A granule herbicide combination product of prometryn 0.80% and thiobencarb 8.0% (PT) has been registered for direct seeded rice fields as pre- and post-emergence treatment during the drainage period just after seeding, with the recommended application rate ranging from 0.32 kg + 3.2 kg to 0.48 kg + 4.8 kg a.i.ha$^{-1}$. However, PT can cause accidental herbicidal injury to rice seedlings when surface water remains (Yamamoto and Kikuchi 2006) through unexpected rain and inadequate drainage after seeding. It is necessary to prevent herbicidal injury without reducing herbicidal efficacy in PT for securing available time of one-shot herbicide application at the re-flooded time after drainage. Therefore, changes in herbicidal efficacy and injury with reduced application rate of PT were evaluated under field and greenhouse conditions in Akita prefecture, northern Japan.

## MATERIALS AND METHODS

### Influences of drainage of surface water on establishment of direct seeded rice applied with reduced rate of PT herbicide; Greenhouse examination

Thirty-four seeds of rice, *Oryza sativa* cv. Akitakomachi, coated with calcium peroxide were seeded to a depth of 5 mm in a plastic container (width: 30 cm, length: 40 cm, depth: 5 cm), and filled with puddled soil, on 13th May 2006. After seeding in a wet condition, PT was applied at a reduced rate of 0.16 kg + 1.6 kg a.i.ha$^{-1}$ and a recommended rate with 0.32 kg + 3.2 kg a.i.ha$^{-1}$, respectively. An untreated control was included. Treatments were applied under drained or saturated with 2-3 mm of water conditions, with three replications. Number of emergence and leaf number of rice plants were measured at 5 (18th May) and 21 (3rd June) days after seeding (DAS), respectively.

### Influences of reduced application rate of PT herbicide on herbicidal efficacy to major weed species under sequential application with one-shot herbicide; Field examination

Seeds of rice cv. Akitakomachi, coated with calcium peroxide were seeded in paddy fields of Gray Lowland soils on 15th May 2006, at a rate of 40 kg ha$^{-1}$ in Nikaho city, Akita prefecture. Surface water was drained after seeding for 15 days in order to accelerate emergence of rice and was irrigated again on 30th May. Two reduced rates of PT were applied, 0.16 kg + 1.6 kg and 0.24 kg + 2.4 kg, and the recommended rate of 0.32 kg + 3.2 kg a.i.ha$^{-1}$, including an untreated control on 17th May, at the beginning of the drainage period. One-shot herbicide, a granule of cyhalofop-butyl, pyrazosulfuron-ethyl, buromobutide and mefenacet, was applied sequentially on 10th June. Leaf number of weeds, injury to rice seedlings by visual examination and herbicidal efficacy for major species including barnyard grass (*Echinochola* spp.), needle spikerush (*Eleocharis acicularis*), false pimpernel (*Lindernia* spp.), bulrush *(Scirpus juncoides* var. *ohwianus*), *Monochoria vaginalis* var. *plantaginea* (hereafter *Monochoria*) and *Cyperus* spp. were evaluated 15 days after application of PT (2nd June) and 30 days after treatment of one-shot herbicide (10th July). There was no replication in this examination.

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# RESULTS

## Greenhouse examination

<u>Influence of reduced PT on rice establishment</u>

Reduction in number of established rice plants by existence of surface water was not recognized for plots with reduced rate of PT at 5 DAS and 21 DAS as shown in Figures 1 and 2, respectively, though it was observed in the plot of recommended rate under saturated condition at 21 DAS (Figure 2).

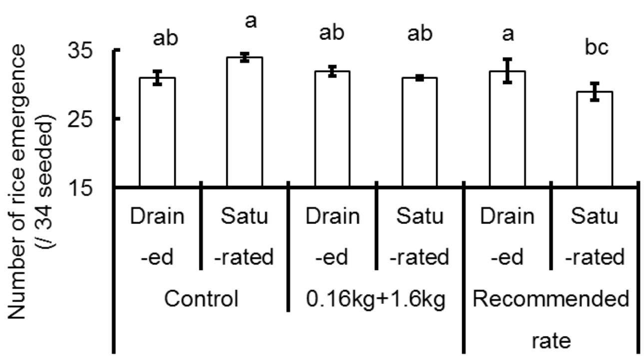


**Figure 1.** Influence of surface water and reduced application rate of PT to rice establishment at 5DAS. Bar indicates SE. Different letter indicate $p<0.05$, by Tukey's HSD test.

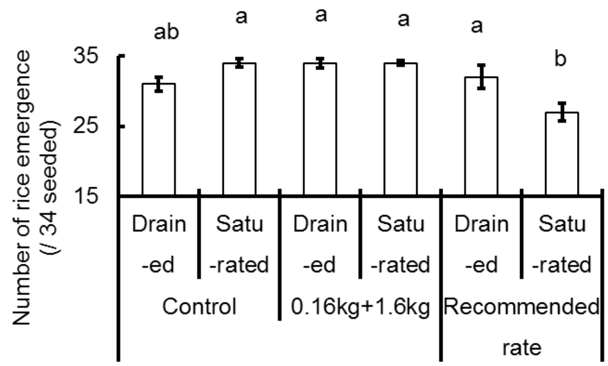


**Figure 2.** Influence of surface water and reduced application rate of PT to rice establishment at 21DAS. Bar indicates SE. Different letter indicate $p<0.05$, by Tukey's HSD test.

<u>Influence of reduced PT on leaf number of rice seedlings</u>

Leaf number of rice under reduced rate of PT was not influenced by existence of surface water at 5 DAS and 21 DAS as shown in Figures 3 and 4, whereas in the plots at the recommended rate at 5 DAS leaf number under saturated condition was suppressed compared to that under drainage condition (Figure 3).

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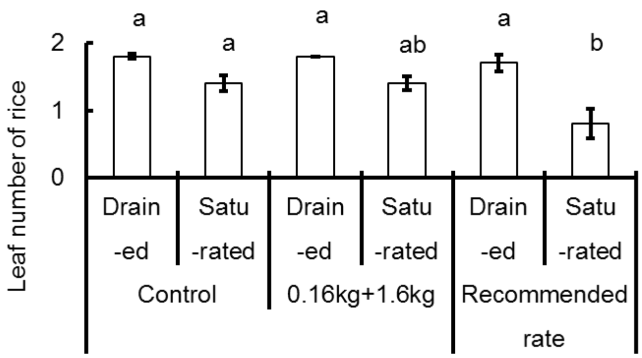


**Figure 3.** Influence of surface water and reduced application rate of PT on leaf number at 5DAS. Bar indicates SE. Different letter indicate p<0.05, by Tukey's HSD test.

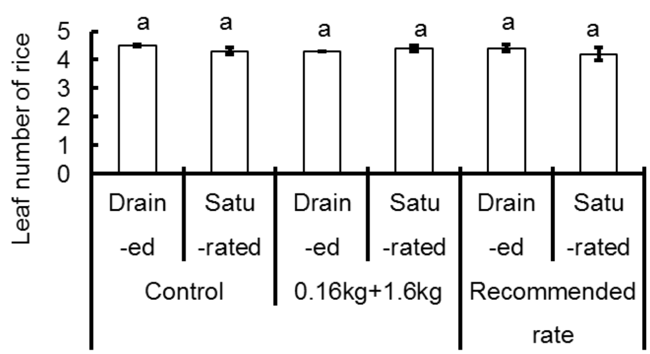


**Figure 4.** Influence of surface water and reduced application rate of PT on leaf number at 21DAS. Bar indicates SE. Different letter indicate p<0.05, by Tukey's HSD test.

## Field examination

### Growth stages of weeds at herbicide application

Weeds had not emerged at the time of PT application. At the application time of one-shot herbicide, leaf number of barnyard grass and bulrush were 2 and 1.5 for the plots of reduced rate of 0.16 kg + 1.6 kg a.i.ha$^{-1}$ and control plots, respectively, and these weeds were not observed in the plots of reduced rate of 0.24kg + 2.4kg a.i.ha$^{-1}$ and recommended rate (Table 1).

**Table 1.** Leaf stages of weed at application time of one-shot herbicide

| Application rate of PT* (kg + kg ha$^{-1}$) | One-shot herbicide* | |
|---|---|---|
| | Barnyard grass | Bulrush |
| 0.16 kg + 1.6 kg | 2.0 | 1.5 |
| 0.24 kg + 2.4 kg | - | - |
| Recommended rate | - | - |
| Control | 2.0 | 1.5 |

-: not found. *: see text

Efficacy of reduced application rate of PT

In the plot of 0.16 kg + 1.6 kg a.i.$ha^{-1}$, barnyard grass dry weight was suppressed by 92.3%, but bulrush was not suppressed, compared with the control. All weeds were controlled in the plots of 0.24 kg + 2.4 kg a.i.$ha^{-1}$ and recommended rate (Table 2). Efficacy of reduced application of PT was considered as adequate to suppress weeds during the drained period except for bulrush in the plot of 0.16 kg + 1.6 kg a.i.$ha^{-1}$. At application time of one-shot herbicide, existence of barnyard grass, bulrush and *Monochoria* were observed in control plots (Table 2).

**Table 2.** Effect of reduced application rates of PT at 15 days after treatment before sequential application of one-shot herbicide*

| Application rate of PT* (kg + kg ha-1) | | Weed species | | | | |
|---|---|---|---|---|---|---|
| | | Barnyard grass | False Pimpernel | Bulrush | *Monochoria* | *Cyperus spp* |
| 0.16 kg + 1.6 kg | No. | 62 | 0 | 6 | 0 | 0 |
| | D.W. | 0.1 | 0 | 0.02 | 0 | 0 |
| 0.24 kg + 2.4 kg | No. | 0 | 0 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 0 | 0 | 0 |
| Recommended rate | No. | 0 | 0 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 0 | 0 | 0 |
| Control | No. | 446 | 0 | 6 | 4 | 0 |
| | D.W. | 1.3 | 0 | 0.02 | - | 0 |

No. : Number of plant $m^{-2}$ D.W. : Dry weight($gm^{-2}$) - : below 0.01g * : see text

Efficacy of sequential application of one-shot herbicide

All weeds that survived PT applications of 0.16 kg + 1.6 kg and 0.24 kg + 2.4 kg a.i.$ha^{-1}$ were suppressed by the sequential application of one-shot herbicide, as well as weeds in recommended rate. In the control plot, False pimpernel remained but total of weeds was suppressed by the one-shot herbicide to 2.8% in dry weight compared with the un-weeded plot (Table 3).

**Table 3.** Efficacy of one-shot herbicide* with reduced application rates of PT at 30 days after treatment

| Application rate of PT* (kg + kg ha-1) | | Weed species | | | | | |
|---|---|---|---|---|---|---|---|
| | | Barnyard grass | Needle spikerush | False pimpernel | Bulrush | *Monochoria* | *Cyperus spp* |
| Un-weeded | No. | 490 | 204 | 186 | 8 | 102 | 64 |
| | D.W. | 90.5 | 3.4 | 4.6 | 0.8 | 16 | 4.9 |
| 0.16 kg + 1.6 kg | No. | 0 | 0 | 0 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.24 kg + 2.4 kg | No. | 0 | 0 | 0 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 0 | 0 | 0 | 0 |
| Recommended rate | No. | 0 | 0 | 0 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 0 | 0 | 0 | 0 |
| Control | No. | 0 | 0 | 220 | 0 | 0 | 0 |
| | D.W. | 0 | 0 | 3.4 | 0 | 0 | 0 |

No. : Number of plant $m^{-2}$ D.W. : Dry weight($gm^{-2}$) - : below 0.01g * : see text

Un-weeded was not applied herbicide at all.

Injury to rice seedlings

In the plot of 0.16 kg + 1.6 kg a.i.$ha^{-1}$, injury in rice seedlings caused by PT was not observed, while it was observed slightly at 0.24 kg + 2.4 kg a.i.$ha^{-1}$ and recommended rate (Table 4). Precipitation (50 mm) was recorded during the drainage period. However, aggravation caused by the rain was not observed.

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**Table 4.** Visual evaluation of herbicidal injury of rice

| Application rate of PT* (kg + kg ha$^{-1}$) | PT | One-shot herbicide* |
|---|---|---|
| 0.16 kg + 1.6 kg | none | none |
| 0.24 kg + 2.4 kg | slight | none |
| Recommended rate | slight | none |
| Control | none | none |

*: see text

## DISCUSSION

With reduced application of PT at 0.16 kg + 1.6 kg a.i.ha$^{-1}$, sufficient herbicidal efficacy was obtained with sequential application of one-shot herbicide. Injury to rice seedlings was also not observed regardless of drained or saturated soil surface at PT application time. In the plot of 0.16 kg + 1.6 kg a.i.ha$^{-1}$, bulrush survived until the application time of the one-shot herbicide, but it was controlled by successive application of the one-shot herbicide. As for false pimpernel, sequential application with reduced application of PT had equivalent efficacy to the sequential application with recommended rate of PT (Table 3).

Consequently, herbicidal efficacy of PT reduced from the rate of registered recommendation, preventing herbicidal injury to rice seedlings could be confirmed both in greenhouse and actual farmers' fields of direct seeded rice in a wet condition. This method would contribute to labor saving and cost reducing in the direct seeded rice cultivation in Akita Prefecture. Further investigations on combination of sequential application of herbicide and methods to accelerate emergence of rice, are necessary to improve weed management procedures in direct seeded rice fields in northern Japan.

## ACKNOWLEDGEMENTS

We appreciate Kumiai Chemical Industry Co., Ltd. for providing the granule herbicide of prometryn 0.80% and thiobencarb 8.0%.

## REFERENCE

Yamamoto, N. and Kikuchi, S. (2006). Weeding ability on initial weeding chemicals which can be used during dry up term after direct seeding in rice paddy. *Tohoku Agricultural Research* 59:41-42. (In Japanese)

# ROOT AND SHOOT GROWTH UNDER FLOODED SOIL IN WILD GROUNDNUT (*GLYCINE SOJA*) AS A GENETIC RESOURCE OF WATERLOGGING TOLERANCE FOR SOYBEAN (*GLYCINE MAX.*)

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**Abstract**

Water logging injury is a significant problem in soybean (*G. max* Merr.) production in Japan, because it is cultivated mostly in the converted paddy field. Wild groundnut (*G. soja* Sieb. et Zucc.), an ancestor of soybean, is growing around paddy field as a weed throughout the country. In order to evaluate waterlogging injury tolerance as a genetic resource for soybean, root and shoot growth in wild groundnut were investigated under the treatments by raised groundwater level upto soil surface for 21 days at the primary leaf and the flowering stages compared with soybean (cv. Ryuho). At the end of treatment of primary leaf stage, root length density (RLD) increased to 320%, and shoot dry matter weight per plant (DMW) decreased to 31% against no-treatment in wild groundnut, while influences on RLD and DMW were inconspicuous in soybean. It was considered that wild groundnut responded to excess water stress by increasing lateral root with root formation. At the end of treatment of flowering stage, RLD and DMW were not influenced in either species. However, the number of root nodule decreased greater in wild groundnut than in soybean, suggesting the difference in susceptibility to waterlogging injury at the flowering stage between these species. Reduction in leaf chlorophyll content by the treatment was observed continuously until maturing stage only in wild groundnut. At the maturing stage, grain yield in wild groundnut and pod number in soybean were measured to determine recovery from damages by the treatments. In wild groundnut, grain yield was equal to no treatment as well as pod number of soybean, and decreased to 15% by the treatments at the primary leaf and the flowering stages, respectively. However in soybean, pod number decreased to 54% by treatment at the flowering stage. Considering the severe damage in DMW at the end of treatment of primary leaf stage, wild groundnut showed higher ability to recover from the damage by the treatment than soybean. Consequently, it was suggested that waterlogging tolerance in wild groundnut might be related to the amount and the activity of root. Importance of root aerenchyma for waterlogging tolerance in soybean have been reported by many researchers. Therefore root development including aerenchyma formation under flooded condition should be investigated to utilize wild groundnut as a genetic resource of waterlogging tolerance for soybean.

**Key words:** genetic resource, root length density, soybean, wet injury, wild groundnut

## INTRODUCTION

In Japan, soybean (*Glycine max* Merr.) is cultivated mostly in converted paddy field where drainage is inadequate (Araki 2006), and is damaged occasionally by water logging injury. Wild groundnut (*G. soja* Sieb. et Zucc.), an ancestor of soybean, is growing as a weed throughout the country (Lee *et al.* 2008) and it has been reported that the plant develops conspicuous aerenchyma in root (Shimamura 1997) and basal part of stem (Arikado 1954) under flooded condition, resulting in high waterlogging injury tolerance. There are many reports on waterlogging injury tolerance of wild groundnut mainly from the view point of the anatomy, but there are few reports on the ecological responses. Therefore this study was conducted to evaluate waterlogging injury tolerance of wild groundnut as a genetic resource for soybean in term of the ecological characteristic.

## MATERIALS AND METHODS

Seeds of wild groundnut collected at rural area in Akita city and soybean (cv. Ryuho) were sown in plastic container (84 cm long × 63cm wide × 41cm height) filled with andosol on 31st May, 2010. After emergence, seedlings were thinned to ten plants per container. Waterlogging treatments were conducted by raising groundwater level up to soil surface from -30cm for 21 days at the primary leaf stage (W1) and the flowering stage (W2) of each species, respectively. Controls were kept at -30cm of groundwater level throughout the experiment. Root length density, shoot dry weight per plant and number of nodes on main stem were measured at the end of W1, W2 treatments and the maturing stage. Root length density ($cm/cm^3$) was calculated based on root length in soil monolith ($117cm^2$ area of base × 20cm depth), divided into 5cm interval. Other measurement included leaf area and length of main stem: end of W1, number of root nodule at the end of W2, branch number at the end of W2 and the maturing stage, leaf chlorophyll content with SPAD-502 (Minolta) at 14, 35, 55, 76, 86, 98, 119, 134 days after sowing. Grain yield of wild groundnut and pod number of soybean were measured at the maturing stage. Because grain yield of soybean could not be measured by bean bug (*Riptortus clavatus*) damage, pod number was measured at the maturing stage. The experiment was conducted with three replications.

## RESULTS

### Effect of Waterlogging Treatment on Growth and Root Length Density at the Primary Leaf Stage

At the end of primary leaf stage treatment, shoot dry weight, number of nodes on main stem and length of main stem decreased significantly in wild groundnut and number of nodes on main stem decreased in soybean. Waterlogging reduced leaf area by 55% in wild groundnut and 58% in soybean. Leaf area was not influenced by waterlogging treatment in either species (Table 1).

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**Table 1.** Effect of waterlogging treatment on growth at the primary leaf stage (W1).

| Species | Treatment | Shoot dry weight (g/plant) | Leaf area ($cm^2$/plant) | Number of nodes on main stem (/plant) | length of main stem (cm) |
|---|---|---|---|---|---|
| Wild groundnut | W1 | 0.1±0.0 *** | 25.9 ± 14.9 | 2.9±0.2 ** | 8.6 ± 0.7 *** |
| | Control | 0.3±0.0 | 58.0 ± 9.6 | 5.3±0.5 | 17.6±1.4 |
| Soybean | W1 | 1.1±0.4 | 144.5±58.0 | 3.9±0.4 ** | 12.4±1.6 |
| | Control | 1.8±0.3 | 346.1±69.7 | 5.3±0.2 | 14.1±1.2 |

Values: Average ± S.E. (n=3), t test: **; $p<0.05$, ***; $p<0.01$

At the end of primary leaf stage treatment, root length density at 5-10cm of soil depth increased significantly in wild groundnut while that of soybean was not influenced. At 0-5cm of soil depth, root length density of wild groundnut increased though significance was not obtained by fluctuation among replications. Root length density below 10cm of soil depth was not influenced in both species (Table 2).

**Table 2.** Effect of waterlogging treatment on root length density at the primary leaf stage (W1).

| Species | Treatment | Root length density ($cm/cm^3$) Soil depth (cm) 0-5 | 5-10 | 10-15 | 15-20 |
|---|---|---|---|---|---|
| Wild groundnut | W1 | 1.7±0.9 | 0.9±0.2 ** | 0.4±0.2 | 0.3±0.2 |
| | Control | 0.4±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |
| Soybean | W1 | 1.5±0.3 | 0.9±0.1 | 0.9±0.4 | 0.5±0.2 |
| | Control | 1.0±0.0 | 0.9±0.2 | 1.0±0.2 | 0.7±0.3 |

Values: Average ± S.E. (n=3), t test: **; $p<0.05$

At the end of primary leaf stage treatment, leaf chlorophyll content of both species decreased. However, this decrease recovered earlier in wild groundnut than that of soybean (Figure 1).

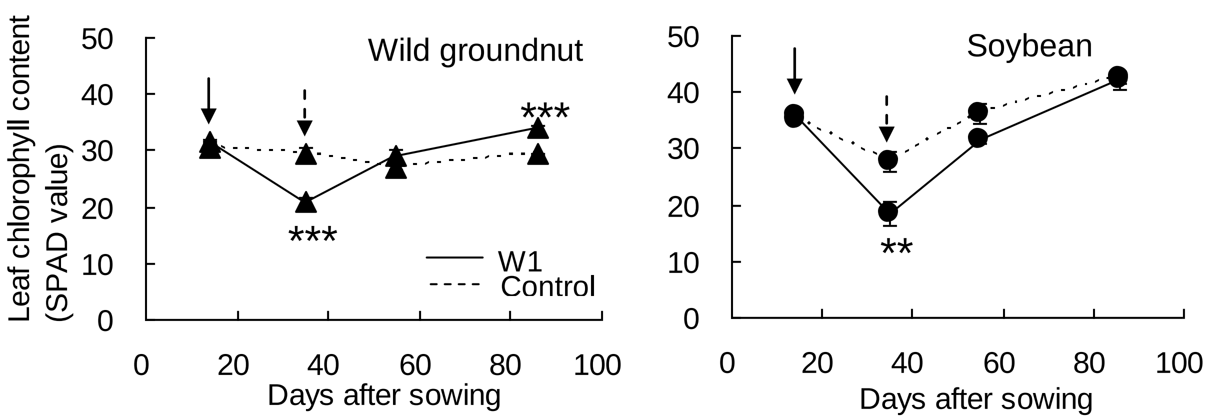


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**Figure 1.** Influence and recovery process in leaf chlorophyll content during and after waterlogging treatment at the primary leaf stage.

→: Start of treatment, - - ►: End of treatment, t test: **; p<0.05, ***; p<0.01

**Effect of Waterlogging Treatment on Growth and Root Length Density at the Flowering Stage**

At the end of flowering stage treatment, there were no influences of waterlogging on the growth in either species (Table 3).

**Table 3.** Effect of waterlogging treatment on growth at the flowering stage (W2).

| Species | treatment | Shoot dry weight (g/plant) | Number of nodes on main stem (/plant) | Branch number (/plant) |
|---|---|---|---|---|
| Wild groundnut | W2 | 39.0 ± 5.8 | 27.3±0.9 | 23.3±1.3 |
| | Control | 38.0±18.2 | 22.2±4.1 | 17.1±3.9 |
| Soybean | W2 | 18.5 ± 2.3 | 12.8±0.1 | 7.5 ± 0.6 |
| | Control | 21.7 ± 4.3 | 12.0±1.0 | 7.0 ± 0.6 |

Values: Average ± S.E. (n=3)

At the end of flowering stage treatment, root length densities were not influenced by treatments at any soil depths. Root nodule number tended to decrease both in wild groundnut and soybean (Table 4).

**Table 4**. Effect of waterlogging treatment on root length density and number of root nodule at the flowering stage (W2).

| Species | treatment | Root length density ($cm/cm^3$) Soil depth (cm) | | | | Number of root nodule (/plant) |
|---|---|---|---|---|---|---|
| | | 0-5 | 5-10 | 10-15 | 15-20 | |
| Wild groundnut | W2 | 4.2±1.5 | 4.7±1.9 | 5.4±2.3 | 5.7±2.3 | 10.5 ± 4.8 |
| | Control | 3.1±1.6 | 3.8±1.9 | 4.7±2.4 | 5.7±2.6 | 38.2±18.3 |
| Soybean | W2 | 5.2±0.8 | 5.7±1.1 | 5.5±1.9 | 4.6±1.2 | 16.3 ± 2.9 |
| | Control | 5.3±0.5 | 5.6±0.0 | 6.0±1.0 | 5.0±0.9 | 26.5 ± 3.4 |

Values: Average ± S.E. (n=3), t test: ***; p<0.01

Leaf chlorophyll content of wild groundnut decreased at the end of flowering stage treatment. Wild groundnuts plants failed to recover chlorophyll contents after the treatment, while soybean plants were recovering well after waterlogging (Figure 2).

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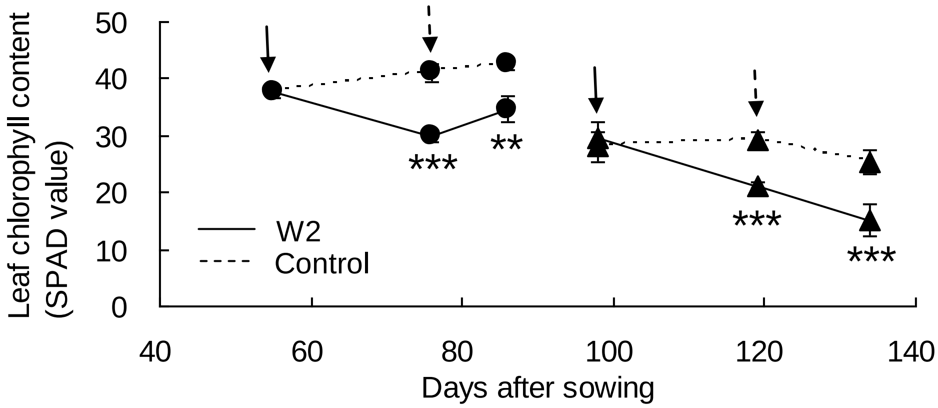


**Figure 2.** Influence and recovery process in leaf chlorophyll content during and after waterlogging treatment at the flowering stage.

▲ : Wild groundnut, ●: soybean,

⟶ : Start of treatment, - - ►: End of treatment, t test: **; p<0.05, ***; p<0.01

**Growth, Yield and Root Length Density at the Maturing Stage**

At the maturing stage, there were no significant differences in the growth of aerial parts among W1, W2 and control in either species. Significant reduction in grain yield was observed in W2 of wild groundnut. There were no significant differences in pod number as a yield component of soybean (Table 5).

**Table 5.** Growth and yield at maturing stage in wild groundnut and soybean treated with waterlogging treatment at the primary leaf stage (W1) and flowering stage (W2).

| Species | treatment | Shoot dry weight (g/plant) | Number of nodes on main stem (/plant) | Branch number (/plant) | Grain yield for wild groundnut (g/plant)<br>Pod number for soybean (/plant) |
|---|---|---|---|---|---|
| Wild groundnut | W1 | 10.3±2.1 | 29.8±1.6 | 33.6±4.5 | 2.1 ± 0.6 |
| | W2 | 7.7 ± 0.4 | 21.9±4.7 | 19.7±5.6 | 0.3 ± 0.0 * |
| | Control | 9.3 ± 1.4 | 32.1±2.1 | 33.1±3.8 | 1.6 ± 0.5 |
| Soybean | W1 | 3.9 ± 0.7 | 11.3±0.9 | 9.2 ± 1.2 | 46.1±2.9 |
| | W2 | 3.7 ± 0.5 | 12.6±0.3 | 7.7 ± 0.6 | 24.5±5.0 |
| | Control | 6.5 ± 2.3 | 13.3±0.8 | 8.4 ± 0.9 | 45.5±9.7 |

Figure: Average ± S.E. (n=3), t test: *; p<0.1

Increase in root length density at depths of 0-5cm and 5-10cm which was observed at treatment W1 was maintained till the maturing stage though the difference was not significant for either species. Furthermore, tendency of increase in root length density below 10cm of W1 was observed.

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**Table 6.** Root length density at maturing stage in wild groundnut and soybean treated with waterlogging treatment at the primary leaf stage (W1) and flowering stage (W2).

| Species | treatment | Root length density ($cm/cm^3$) Soil depth (cm) | | | |
|---|---|---|---|---|---|
| | | 0-5 | 5-10 | 10-15 | 15-20 |
| Wild groundnut | W1 | 4.0±2.1 | 3.3±0.5 | 3.8±0.8 | 4.4±1.0 |
| | W2 | 1.3±0.2 | 1.9±0.4 | 1.3±0.2 | 2.6±0.9 |
| | Control | 2.9±2.7 | 2.0±1.8 | 2.2±2.0 | 2.3±1.5 |
| Soybean | W1 | 3.8±2.5 | 2.8±0.6 | 3.1±0.7 | 2.9±0.5 |
| | W2 | 5.4±2.3 | 4.1±1.3 | 4.7±1.0 | 5.2±0.6 |
| | Control | 2.8±1.6 | 4.2±2.5 | 2.9±1.5 | 3.8±1.2 |

Values: Average ± S.E. (n=3)

## DISCUSSIONS

Aerenchyma cells develop in as a strategy of waterlogging tolerance in upland crops (Shimamura 1997). Arikado (1954) found that wild groundnut developed conspicuous aerenchyma cells in basal part of stem in response to waterlogging. In this study, in wild groundnut, root length density near soil surface increased by waterlogging treatment at the primary leaf stage. Wild groundnut showed higher ability to recover from the damages by waterlogging treatment at primary leaf stage such as decrease in dry weight and leaf chlorophyll content than soybean. This ability is considered as waterlogging tolerance in wild groundnut from the ecological viewpoint given by increase of root length density. As development of aerenchyma in root system was not investigated in this study, relationship between the amount of root and the aerenchyma formation in root system should be investigated furthermore to utilize wild groundnut as a genetic resource of waterlogging tolerance for soybean.

## REFERENCE

Araki, H. (2006). Water Uptake of Soybean (*Glycine max* L. Merr.) During Exposure to $O_2$ Deficiency and Field Level $CO_2$ Concentration in the Root zone. *Field Crop Research* 96(1):98-105.

Arikado, H. (1954). Different Responses of Soybean Plants to an Excess of Water with Special Reference to Anatomical Observations. *Proceedings of the Crop Science Society of Japan* 23(1):28-36.

Lee, J. D., J. K. Yu, Y. H. Hwang, S. Blake, Y. S. So, G. J. Lee, H. T. Nguyen and J. G. Shannon (2008). Genetic Diversity of Wild Soybean (*Glycine soja* Sieb. and Zucc.) Accessions from South Korea and Other Countries. *Crop Science* 48(2):606-616.

Shimamura, S., T. Mochizuki, J. Inouye (1997). Formation of the Lysigeneous Intercellular Space in Adventitious Roots of Leguminous Species. *Japanese Journal Crop Science* 66(2):208-213. (In Japanese with English summary)

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# RE-GROWTH FROM STEM SEGMENTS BURIED INTO PUDDLED SOIL IN *HYDROLEA ZEYLANICA* VAHL, A TROUBLESOME WEED IN THE PHILIPPINES

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## ABSTRACT

Re-growth ability from a node of stem segment of *Hydrolea zeylanica* buried into puddled soil was determined at the Philippine Rice Research Institute, Nueva Ecija in August 2008. Re-growth of the shoot was not observed from the segment with one node buried into 2 and 5 cm depth under both conditions drained and flooded with 5 cm of surface water. The segments placed on the soil surface (0cm) reproduced new shoots and both the rate of re-growth and growth of new shoots were greater in the segments under flooded than drained condition. The result suggests that burying stems by careful puddling may reduce the infestation of *H. zeylanica* , spreading rapidly as a troublesome perennial weed in rice fields in Luzon Island of the Philippines.

**Key words:** *Hydrolea zeylanica,* rice weed, re-growth, puddling, the Philippines

## INTRODUCTION

Invasive weed species such as yellow sawah lettuce (*Limnocharis flava*) and Hydrolea (*Hydrolea zeylanica*) have been recognized as new troublesome weeds spreading in the paddy fields of central and northern Luzon Island of the Philippines. Hydrolea is distributed in the tropics from India to the Philippines, growing 20-100 cm in height, in lowland rice fields as well as in marshes of ponds and banks (Harada *et al*. 1996). Though germination behavior and growth and development pattern from seeds have been reported (Fabro *et al.* 2005), biological traits on vegetative propagation is not clarified for Hydrolea because the life form of the species has been described both as annual (Pancho and Obien 1995) and perennial (Soerjani *et al*. 1987 and Harada *et al*. 1996). In order to evaluate the vegetative propagation in Hydrolea, re-growth from stem segment was investigated.

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## MATERIALS AND METHODS

### Identification of propagule

In the experimental rice fields of the Philippline Rice Research Institute (PhilRice), Munoz, Nueva Ecija, Philippines, seedlings of Hydrolea were collected to identify propagules on August 2008.

### Re-growth from stem segment under different burial conditions

Stem segments with one node at the eleventh position from the apex of shoot growing to around 30 cm in height were taken from a population infested in a fallow rice field of PhilRice on 15th August, 2008. Five segments were buried into puddled soil of Maligaya clay filled in a plastic pot of 25 cm diameter and 20 cm depth, immediately after removed from the shoot, with depth of 0 cm (soil surface), 2 cm and 5 cm, with four replications. Pots were flooded with 5 cm of water or saturated in a net house at PhilRice and re-growth from each segment was measured at 11 days after placement.

## RESULTS

### Identification of propagule

It was observed that shoots of Hydrolea developed from stem segments buried into paddy soil as shown in Figure 1. Though it did not indicate directly that the species was perennial, vegetative propagation was confirmed in Hydrolea through stem segments which might be cut and buried at plowing and puddling time in rice fields.

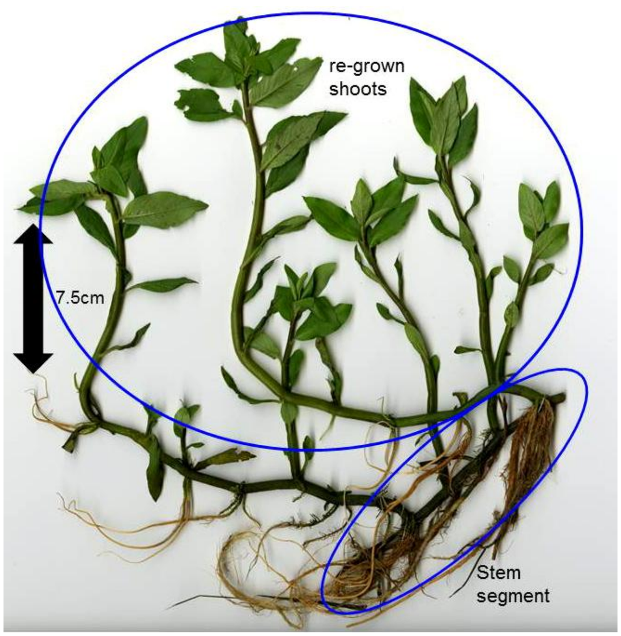


**Figure 1.** Young plant of Hydrolea collected in an experimental rice field of PhilRice.

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**Re-growth from stem segments under different burial conditions**

Air temperature during the experiment was 31.7 and 23.8 degree Celsius for daily maximum and minimum, respectively. Re-growth of shoot occurred from the node of segments placed on the soil surface (0 cm)(Figure 2), while it could not be observed from those buried in the soil at depths of 2 and 5 cm. Re-growth which was determined by new shoots started three and five days after placement, and 90 and 25 percent of stem segments had re-growth for flooded and saturated conditions, respectively. It was considered that the stem segments buried into puddled soil died because they could not be collected at 11 days after burial. Length of re-grown shoot was longer in flooded than in saturated condition (Figure 3).

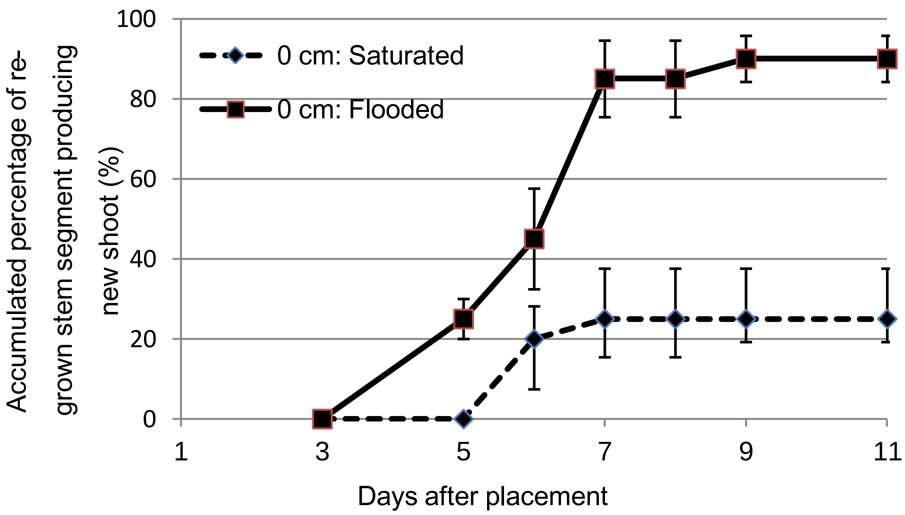


**Figure 2.** Changes in accumulated percentage of re-grown stem segments producing new shoots of Hydrolea placed on puddled soil. (Bar indicates S.E.)

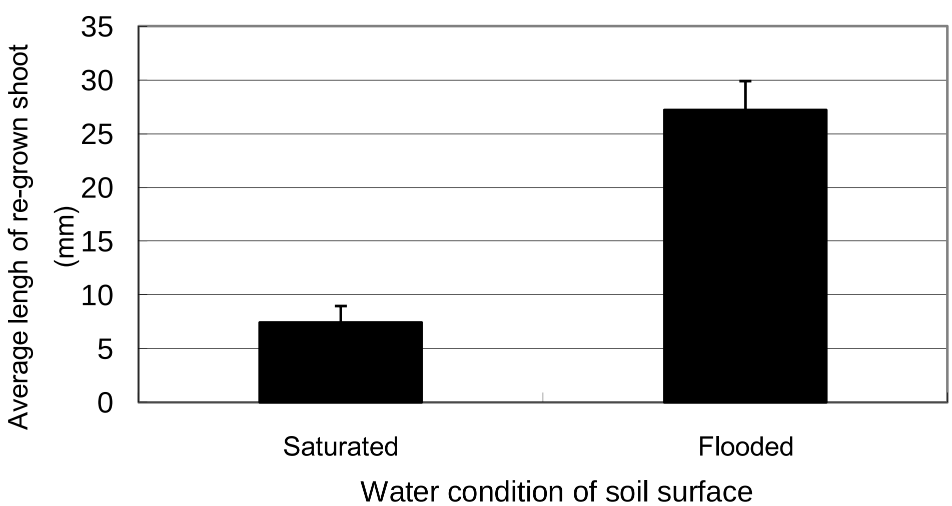


**Figure 3.** Length of re-grown shoot from stem segment placed on puddled soil (0 cm) under different water conditions at 11 days after placement (Bar indicates S.E.).

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## DISCUSSIONS

Propagation with stem segments in rice fields was observed in Hydrolea, though life form of the species requires clarification under rice cultivation in central and northern Luzon Island. Segments of wintered rhizomes of Knotgrass (*Paspalum distichum*) and its close relative (*P. distichum* var. *indutum*) could not re-grow when buried into puddled soil while approximately 80% of segments sprouted when placed on the surface of puddled soil (Okuma *et al.* 1983).

Results in this study suggest that burying stem segments by careful puddling might be effective to prevent re-growth of Hydrolea in rice fields. Flooding after placement of stem segments on the puddled soil encouraged re-growth of shoots in this study. Drainage after transplanting is practiced during the early growth stage of rice plants commonly by farmers in the regions in order to prevent damage by the apple snail (*Pomacea canaliculata*). This management practice might also be effective to suppress re-growth from stem segments placed on the surface of puddled rice fields.

Further investigations on size, position and age of segment, and texture, moisture and temperature of soil are needed to establish the effective management measures for Hydrolea in central and northern Luzon Island of the Philippines. In addition, differences in susceptibility to herbicides between seedlings from seeds and re-grown shoots from stem segments in Hydrolea should be investigated.

## ACKNOWLEDGRMENT

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## REFERENCES

Fabro, L.E., Ramirez, AHM, Bariuan, FV, Macatula, EC and Gabriel, RCL (2005). Biology and management of *H. zeylanica* (L.) VAHL. *Paper presented in the WSSP scientific session during the 36th Anniversary and Annual Convention of the Pest Management Council of the Philippines held at PhilRice, Munoz, Nueva Ecija,*.

Harada, J., Shibayama, H. and Morita, H. (1996). *Weeds in the tropics.* (AICAF, Tokyo), pp.146-147.

Okuma, M., Chikura, S. and Moriyama, Y. (1983). Ecology and control of a subspecies of *Paspalum distichum* L. “Chikugo-suzumenohie” growing in creeks in the paddy area on the lower reaches of Chikugo river in Kyushu 3.Ecological investigation on sprouting of stems. *Weed Res. Japan*28(1):31-34 (in Japanese with English summary).

Pancho, J.V. and Obien, S.R. (1995). *Manual of Ricefield Weeds in the Philippines*. (PhilRice, Nueva Ecija), pp.182-183.

Soerjani, M., Kostermans, A.J.G.H. and Tjitrosoepomo, G. (1987). *Weeds of Rice in Indonesia*. (Balai Pustaka, Jakarta). pp.310-311.

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# WEED INFESTATION AND ITS INFLUENCES ON EARLY GROWTH OF RICE (*Oryza sativa*) IN FLOODED PLAINS OF SAVANNA IN NORTHERN GHANA

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## ABSTRACT

This paper reports on collaborative research on the development of low input and sustainable rice production technology in flooded plains of lowland savanna in northern Ghana. The research aims are to identify suitable weed management methods. Weed infestation and its influence on rice plants (*Oryza sativa* c.v. Sikamo) at early growth stage were investigated in rainfed and broad-casted rice fields in Yipielgu (Y) and Zaw (Z) villages where submergence conditions were different. At March before plowing, *Paspalum scrobiculatum*, surviving the dry season, were dominant, accounting for 50 to 97 percent of dry matter weight (DMW) of weeds. At 45 days after removing weeds and broad-casting, DMW per m$^2$ was 96.1g in Z under insufficient submergence and 139.5g in Y flooded with around 5 cm depth, respectively. Dominant species were *P. scrobiculatum* and *Digitaria sangunalis*. in Z, and *Cyperus* spp., *Fimbristylis* spp. and *Acroceras zizanoides* in Y. DMW of rice plants decreased as total DMW of weed increased above 150g/m$^2$ approximately in Y, while it was not clear when total DMW was below 150g/m$^2$ in Z. Glyphosate applied before plowing or after broad-casted could not suppress weeds both in Z and Y. It is needed to determine appropriate application time and rate of non-selective herbicides for effective management of weed in rice fields in northern Ghana.

**Key words:** rainfed rice field, flooded plain, weed infestation, *Paspalum scrobiculatum*, Ghana

## INTRODUCTION

Increase in rice production is an urgent subject in Sub-saharan Africa where food shortage has become serious by increase in population. A collaborative research project on development of low input and sustainable rice production technology in flooded plains of lowland savanna in northern Ghana has been promoted to increase rice production through expanding acreage for rice, by Japan International Research Center for Agricultural Sciences (JIRCAS) and Savanna Agricultural Research Institute of Ghana SARI). Weeds are a most troublesome factor affecting stable yield of rice in flooded plains, submerged seasonally. Having knowledge of weed flora and density is important to establish effective and cost

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reducing weed management procedures in rice fields in the above environment. In this paper, the situation of weed infestation and its influence on rice plants (*Oryza sativa*) at early growth stage were investigated in rainfed and broad-casted rice fields under different submergence conditions, in northern Ghana.

## MATERIALS AND METHODS

### Locations and season of investigation

Experimental plots were established in farmer's rice fields, Z(N9.6.18, W1.9.21) (Zaw) and Y(N9.22.32, W:0.5.9) (Yipielgu) villages, in flooded plain along the White Volta river in northern Ghana. Weed removal and plowing, and sowing rice (*Oryza sativa* cv. Sikamo) at the seed rate of 1.75 g/m$^2$ took place on the 10$^{th}$, June and 1$^{st}$ July 2010, respectively. Weed species and density were measured within a quadrat of 1 m$^2$ before plowing on 30$^{th}$ and 31$^{st}$ of March (end of dry season), and at the early growth stage of rice on 16$^{th}$ and 17$^{th}$ of August 2010 (initial stage of rainy season). Number and dry matter weight of rice plants established in 1 m$^2$ were measured on 16$^{th}$ and 17$^{th}$ of August.

### Identification of weed species

Weed species collected were identified based on Johnson (1997) and a Data-Base "Plants in lowland savanna of West Africa:(http://www.jircas.affrc.go.jp/project/Ghana/home.html) compiled for the JIRCAS Project.

## RESULTS

### Weed infestation before plowing, at the end of the dry season

At the end of the dry season, seedlings from seeds and sprouts from vegetative organs of weeds were observed both in Z and Y. Five and 15 species per m$^2$ were found in Z and Y, respectively. Dry matter weight (DMW) was approximately 120 g per m$^2$, in which *Paspalum scrobiculatum* survived during the dry season accounted for 97 and 50 percent of total DMW in Z and Y, respectively. Besides *P. scrobiculatum*, weed species observed during the rice growing season in 2009 including *Acroceras zizanioides*, *Borreria filifolia*, *Fuirena umbellata* and *Melochia corchorifolia* (Morita *et al.* 2011) also emerged in the fields (Table 1).

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**Table 1.** Weed occurrence in 1 $m^2$ in Z and Y fields at the end of the dry season.

| Category and species of weed | | | Zaw field (Z) | | Yipielgu field (Y) | |
|---|---|---|---|---|---|---|
| Life form | Family or group | Botanical name | No. of plants (/$m^2$) | DMW (g/$m^2$) | No. of plants (/$m^2$) | DMW (g/$m^2$) |
| Perennial | Gramineae | *Eragrostis* spp. (survived adult) | - | - | 9.7±1.9 | 20.7±0.3 |
| | | *Eragrostis* spp. (seedling) | - | - | 9.7±1.91.0 | 9.7±1.9 |
| | | *Paspalum scrobiculatum* (survived adult) | 36.0±10.3 | 124.3±10.7 | 22.3±6.1 | 57.1±22.5 |
| | | *Paspalum scrobiculatum* (seedling) | 19.0±8.1 | 0.8±0.2 | 15.0±7.1 | 0.7±0.4 |
| | Cyperaceae | *Fuirena umbellata* | - | - | 2.3 | 5.4 |
| | Broad leaves | *Calopogonium mucunoides* (survived adult) | - | - | 7.0 | 11.5 |
| | | *Calopogonium mucunoides* (seedling) | - | - | 1.7 | 2.2 |
| | | *Ludwigia* sp. | 0.3 | 2.8 | - | - |
| Annual | Gramineae | *Acroceras zizanioides* | - | - | 144.7±108 | 5.6±2.4 |
| | | *Brachiaria* sp. | 0.3 | 0.04 | 46.3±33.3 | 3.5±2.5 |
| | | *Digitaria sp.* | 5.0 | 0.3 | 81.3 | 1.8 |
| | | *Oryza sativa* | 2.3±1.9 | 0.1±0.1 | 33.0±16.4 | 1.9±1.0 |
| | Broad leaves | *Borreria filifolia* | - | - | 2.7 | 0.2 |
| | | *Coldenia procumbens* | - | - | 1.0 | 0.6 |
| | | *Leguminosae* | | | 3.7 | 0.2 |
| | | *Melochia corchorifolia* | | | 1.3 | 0.1 |
| | | *Nelsonia canescens* | - | - | 2.0±0.5 | 2.6±1.7 |
| | | *Phyllanthus* sp. | - | - | 3.0 | 0.2 |
| | | Un-identified | 0.3 | 0.3 | - | |
| Total | | | 63.3±9.8 | 128.4±8.2 | 378.0±145.1 | 14.1±33.4 |

Collected on 30,31 March 2010, figure shows average and SE of three replications except for species occurred in one replication.

**Weed infestation at early growth stage of rice plant, at the initial stage of rainy season**

Glyphosate was applied to the experimental plots of 4 $m^2$ at the end of the dry season or immediately after sowing rice with two application rates, to evaluate the herbicidal efficacy on weeds. However, suppression to weeds could not be observed both in Z and Y by the glyphosate application. Number of species occurring was 13 and 16, number of plants was 261 and 1007 in total and DMW was 96.1 g and 139.5 g per $m^2$ for Z and Y, respectively. Dominant species in Z were Gramineous weeds such as *P. scrobiculatum* and *Digitaria* spp.. Dominant species in Y were sedges such as *Cyperus pulstulatus*, *C. halpan*, *Pycerus flavescens*., *Fimbristylis ferruginea* and *Lipocarpha sphacelata* as well as *P. scrobiculatum* and *A. zizanioides*. In addition the parasitic weed, *Rhamphicarpa fitulosa* was found in Y. Difference in the degree of submergence (Z was not flooded, Y flooded with approximately 5 cm of water) was considered a major factor affecting species composition, number of plants and biomass of weeds between the two villages.

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**Table 2.** Weed occurrence in 1 $m^2$ in Z and Y fields at the early stage of the rainy season.

| Crop and weeds | Botanical name | Zaw field (Z) | | Yipielgu field (Y) | |
|---|---|---|---|---|---|
| | | No. of plants (/$m^2$) | DMW (g/$m^2$) | No. of plants (/$m^2$) | DMW (g/$m^2$) |
| Crop | *Oryza sativa* | 67.0±27.1 | 26.4±10.7 | 61.7±12.4 | 26.4±10.7 |
| Gramineae | *Acroceras zizanioides* | - | - | 32.7±18.7 | 18.8±6.2 |
| | *Digitaria sanguinalis* | 108.0±63.2 | 24.7±12.1 | - | - |
| | *Eragrostis* spp. | 4.0±2.5 | 0.9±0.8 | 3.0±2.1 | 1.9±1.2 |
| | *Leersia hexandra* | - | - | 1.0±1.0 | 0.0±0.0 |
| | *Paspalum scrobiculatum* | 121.7±39.6 | 62.2±18.7 | 4.7±2.6 | 5.9±3.8 |
| Total of Gramineae | | 233.7±45.3 | 87.4±7.0 | 41.3±17.7 | 26.6±3.6 |
| Cyperaceae | *Cyperus pustulatus* * | - | - | 473.7±63.8 | 57.4±8.9 |
| | *Cyperus halpan* | - | - | 140.3±44.0 | 21.7±4.2 |
| | *Cyperus* sp. | 1.3±0.3 | 0.6±0.3 | - | - |
| | *Eleocharis complanata* | - | - | 5.0±5.0 | 0.2±0.2 |
| | *Fimbristylis ferruginea* + *Lipocarpha* spp. | 11.7±6.9 | 0.5±0.4 | 132.0±92.5 | 31.0±14.6 |
| | *Fuirena umbellata* | - | - | 0.7±0.7 | 0.0±0.0 |
| | *Scirpus* sp. | - | - | 0.3±0.3 | 0.0±0.0 |
| Total of Cyperaceae | | 13.0±6.7 | 1.2±0.7 | 752.0±187.0 | 110.3±33.3 |
| Broad leaves, Monocot. | *Aneilema* sp. | 6.3±1.9 | 3.4±2.1 | 2.0±1.0 | 0.2±0.1 |
| | *Burnatia enneandra* | - | - | 5.7±2.0 | 3.3±0.8 |
| Total of Monocot. broad leaves | | 6.3±1.9 | 3.4±2.1 | 7.7±3.0 | 3.5±1.2 |
| Broad leaves, Dicot. | *Borreria scaber* | 0.3±0.3 | 0.0±0.0 | - | - |
| | *Cleome viscosa* | 1.0±1.0 | 3.3±3.3 | - | - |
| | *Citrullus* sp. | 0.7±0.3 | 0.2±0.2 | - | - |
| | *Euphorbia hirta* | 0.3±0.3 | 0.0±0.0 | - | - |
| | *Leguminosae* | - | - | 0.3±0.3 | 0.0±0.0 |
| | *Ludwigia hyssopifolia* | 1.0±0.6 | 0.0±0.0 | 23.7±10.9 | 0.4±0.3 |
| | *Limnophila* sp. | - | - | 94.0±25.4 | 10.9±7.1 |
| | *Mollugo nudicaulis* | 0.7±0.7 | 0.0±0.0 | - | - |
| | *Phyllanthus* sp. | 4.3±0.7 | 0.5±0.1 | - | - |
| | *Rhamphicarpa fistulosa* | - | - | 88.3±82.4 | 5.4±4.8 |
| Total of Dicot. broad leaves | | 8.3±1.5 | 4.2±3.4 | 206.3±72.1 | 16.7±7.5 |
| Grand total | | 261.3±38.0 | 96.1±10.2 | 1007.3±157.4 | 139.5±40.9 |

Collected on 16,17 August 2010, figure shows average and SE of three replications, *: includes *Pycerus flavescence* and *Cyperus podocarpus*.

## Relationship between weed biomass and early growth of rice plant

Number of individual weed species and biomass of weeds and rice plants differed between experimental plots including those applied with glyphosate, which might be caused by position in the farmer's rice field both in Zaw and Yipielgu villages. Average value of DMW of rice plants decreased as total DMW of weeds inceased (Figure 1). In Y DMW generally exceeded approximately 150 g/m$^2$, while at Z total DMW was below 150 g/m$^2$,making it unclear what impact weed density had no rice DMW.

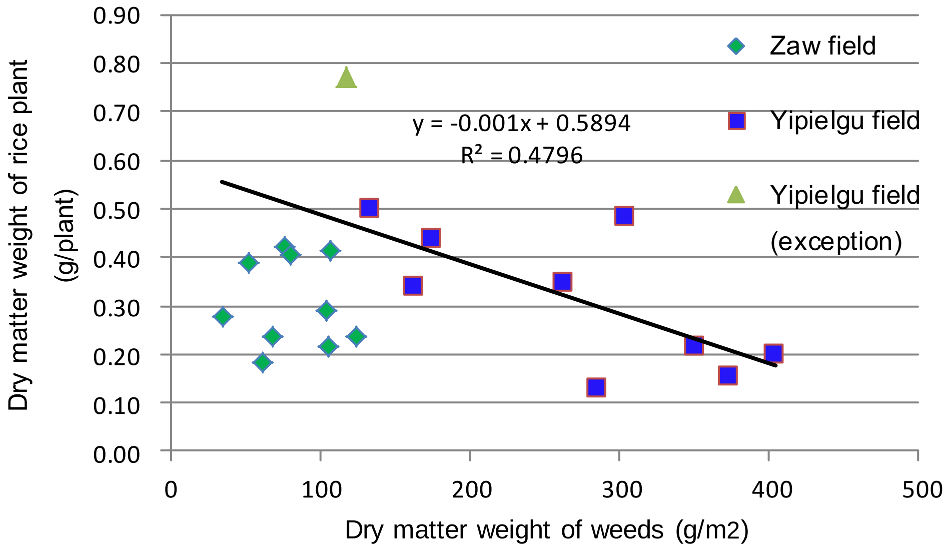


Figure 1. Relationship between total DMW of weeds and average DMW of rice plant in Z and Y fields at the early stage of the rainy season (▲: excepted from calculation for Y field).

## DISCUSSION

Results showed that weeds began to emerge at the end of the dry season, that degree of submergence affected the differences in weed infestation such as species composition, number and biomass at the early stage of rainy season, and that early growth of rice was suppressed when DMW of weeds exceeded 150 g/m$^2$. Further investigations on changes in growth habits and in responses to management procedures including herbicides with different soil moisture and submergence conditions are necessary for the development of weed management procedures for fields of lowland savanna in northern Ghana.

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## REFERENCES

Johnson, D. E. (1997). *Weeds of rice in West Africa*, (WARDA, Hong Kong).

Morita, H., Uchino, A., Inusah, Y. and Sakagami, J.I. (2011). Weed flora of rice fields in the lowland savanna of Ghana, Paper presented to Third World Rice Congress, Nov., 2010 Hanoi, Vietnam, 1-6. (http://www.ricecongress.com/extPdfs/OP09-4090-Morita-edited.pdf)

# BIOLOGY, ECOLOGY, AND CONTROL OF TROPICAL SODA APPLE (*SOLANUM VIARUM)*

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## ABSTRACT

Tropical soda apple (*Solanum viarum*) is a perennial weed that is a serious problem in many perennial grass pastures of Florida and throughout the southeastern United States. Tropical soda apple (TSA) was recently reported in Australia and could possibly be present in other Asian-Pacific Countries. Field and laboratory research in the U.S., primarily in Florida, on the biology, ecology, and control of TSA has resulted in weed management strategies that will benefit Asian-Pacific Countries that are infested with TSA or at risk for invasion by TSA. TSA is unpalatable to livestock and can infest a pasture or native area in 1-2 years resulting in lower stocking rate. At maturity, TSA is from 1 to 2 m tall; stems and leaves have broad-based white to yellowish prickles; the fruit is globular, about 2 to 3 cm in diameter and yellow when mature. Night temperature generally exerted a greater and more consistent effect than day temperature on plant growth and development. In the US, the potential ecological range for plant growth and development is greatest over latitudinal ranges of 15 to 33 degrees S, which is similar to its native South America. Throughout the year, this plant will have immature and mature fruit present that ensures large numbers of viable seeds (@40,000 per plant) with an average germination rate of 70%. Seedling emergence in Florida primarily occurs from August through March compared with April through October for other southern states. Seed in the soil can remain dormant for two or more years though germination will rapidly occur when conditions are favorable.

Tropical soda apple has been observed as a weed in agricultural land and in natural areas. The TSA seed is spread by cattle, wildlife, moving water, and anthropogenic activity (contaminated hay, grass seed, shipping cattle, and sod production). Cattle and wildlife spread TSA by consuming the fruit and spreading the seed via feces. Cattle grazing TSA infested areas should be considered a major seed dispersal vector. Tropical soda apple can tolerate a frost or freeze but the foliage will suffer cold damage with some plant mortality.

Control strategies include herbicides, biological (insects, viruses), and cultural practices. Successful (90-100%) control has been reported for the herbicide aminopyralid (Milestone), a beetle known as *Gratiana boliviana* (TSA beetle), and a virus called *Tobacco mild green mosaic tobamovirus*. An integrated weed control strategy that includes prevention (early detection, rapid response) and control is the best longterm strategy to managing TSA infested areas in the U.S. or Asian-Pacific Countries.

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## BIOLOGY AND ECOLOGY OF THE WEED

### TSA Description

At maturity TSA is from 1 to 2 m tall. Stems and leaves have broad-based prickles up to 20 mm long (Mullahey *et al.* 1993B). The prickles are more evident on the petiole and main veins than on the stems. Leaves are alternate, 10-20 cm long and 6-15 cm wide, and are shallow or sometimes deeply divided into broad pointed lobes. Both surfaces of the leaf blade contain glandular and non-glandular trichomes with the upper surface displaying a velvety sheen. The lower surface has stellate trichomes (Wunderlin *et al.* 1993). White flowers with cream-colored anthers are borne a few together and hidden beneath the leaves (Coile 1993). Fruits are globular, glabrous, about 2-3 cm in diameter and yellow when mature. The plant is readily identified by its immature fruit, which is green with white mottling like immature watermelon fruit. Each mature fruit contains about 400 light red-brown seeds that have a diameter of approximately 2.5 mm. Seeds are only moderately flattened and are found in a mucilaginous layer containing a glycoalkaloid called solasodine.

*Solanum viarum* is a species belonging to the *Solanum Acanthophora* of the prickly subgenus *Leptostemonum* (Nee 1991). This perennial shrub has been misidentified in the literature as *S. khasianum, S. aculeatissum* (Nee 1991), and *S. reflexum* (Morton 1976). Literature on TSA focuses on increasing the solsadine content (Patil and Laloraya 1981; Pingle and Dnyansagar 1980), berry yield (Reddy *et al.* 1991), and reducing number of spines (Reddy and Krishnan 1988). This plant was cultivated as a source of solasodine, a nitrogenous analogue of diosgenin, and used as a substitute for diosgenin in the synthesis of steroidal drugs. Mexico was the largest producer (750 tons/year) of diosgenin, with India using about 250 tons/year (Sahoo and Dutta 1984). Apparently, cultivation of TSA for the glycoalkaloid has significantly declined or completely stopped.

### TSA Distribution

In its native South America (Brazil, Paraguay, and Argentina), TSA is found over latitudinal ranges of 13 to 33 degrees S, occurring at low elevations in eastern Brazil to Paraguay, Uruguay, and northeastern Argentina (Nee, 1991). Potential ecological range of this weed is dependent upon photoperiod and diurnal temperatures (Patterson *et al.* 1997). A regression model was calculated to predict the percent maximum growth of TSA during each month from March through November at diverse sites throughout the US. Temperatures at sites in southern US states (latitudinal range 27 to 33 degrees S) support 30% or more of maximum growth from April through October compared to northern states (latitudinal range 33 to 40 degrees S) where that growth rate is supported only from June through September. Southern states are at greater risk for TSA invasion because of enhanced plant development and reproduction. Further north and west, growth is more seasonally restricted. Extrapolating this information to the southern hemisphere, it would appear that Asian-Pacific Countries in the latitudinal range of 15 to 33 degrees S are at greatest risk for TSA establishment and persistence. Countries south of latitude 33 degrees S are at some risk from TSA invasion but fruit production is more limited and poor seed survival through the winter season reduces the risk of re-establishment. These countries should actively scout for TSA and have an aggressive management plan to remove new plants or patches (early detection, rapid response).

In Florida, TSA is found in southern Florida, though movement north toward the Florida panhandle has been documented. The agricultural land area infested with TSA was estimated at 10,000 ha in 1990, 61,000 ha in 1992, and, according to a TSA Census of beef producers, 164,000 ha in 1993 (Mullahey *et al.* 1993A). If all land systems (natural and agricultural) were included, the area now infested with TSA in Florida would approach 300,000 ha. This exponential spread of TSA from 1990-93 in Florida should be cause for great concern among people in agriculture and those that manage natural systems. Early detection and rapid response to control plants and preventing or reducing the soil seed buildup by controlling existing plants before the plant produces fruit (i.e. seed) will slow the exponential growth that Florida experienced in the 1990's.

**TSA Biology**

Though TSA is an indeterminate plant in Florida, flowering and fruit production in Florida is concentrated from September through May (fall and winter seasons) due to favorable photoperiod and day/night temperatures (Mullahey *et al.* 1993B). Greenhouse grown plants flowered about 60 days after emergence and fruit were produced by about 105 days after emergence (Patterson *et al.* 1997). In this study, TSA produced flowers across the full range of daylengths evaluated, indicating that no naturally-occurring photoperiods in the continental US are likely to limit the ability of TSA to grow and reproduce. In field studies, flowering of TSA regrowth following mowing begins about 50-60 days after the mowing. Fruit begins to form approximately 70-90 days after mowing (Mullahey *et al.* 1996). Fruit production (i.e. seed) is similar to flowering, ensuring large numbers of viable seeds (@ 45,000 per plant). Seed germination ranges from 30-100%, though average germination of seed from mature fruit is about 70%. Consequently, during one year, a single plant could supply enough viable seed to produce 28,000-35,000 new TSA plants. Seed will not germinate inside the fruit and seed removed from the fruit need to dry (aging process) before germination occurs. Scarification increases the rate and total germination. Herbivore consumption and subsequent passage of seed in feces is the primary seed dispersal mechanism for this weed in Florida.

Seed viability is related to fruit maturity (color) and fruit diameter; the larger the fruit diameter, the higher the seed viability. However, smaller diameter, yellow fruit (i.e. mature) do contain viable seed. White seed (immature seed) germinate and these are typically present in small diameter, green fruit. Reports suggest fruit size becomes constant after 65-70 days of development (Kaul and Zutshi 1982).

Plant regeneration can also occur from the roots (Mullahey and Cornell 1994) so hand weeding of plants is successful if the root system is removed.TSA can store a high concentration (20 to 35%) of total non-structural carbohydrates (TNC) in the roots and stems (Mullahey and Cornell, 1994). TNC concentrations were higher from December to February than during the spring and summer months. Beginning in January, TNC concentrations declined in the root and were probably mobilized for plant growth and development. This also explains the rapid regrowth (i.e. 2 weeks) of TSA following a freeze or mowing event.

Literature reports indicate that seed can remain dormant for months, though the average period of dormancy was 1 month (Pingle and Dnyansagar 1979). Seed longevity studies in field soil conditions evaluating soil moisture effects on seed viability showed TSA seed remained viable over 260 days in saturated soils (-0.0 bars), while viability in drier soil (< -10 bars) was reduced up to 60% within 260 days (Mullahey, unpublished data). These results confirm field observations that TSA seed can remain viable in the soil for up to one year or longer. Preventing a soil seed-bank of TSA seed (i.e. early detection, rapid response) will greatly increase the chances of successfully removing TSA from a pasture.

Tropical soda apple contains a glycolalkaloid, solasodine, that is found in the mucilaginous layer around the seeds. Solasodine, a nitrogen analogue of diosgenin, is used in the production of steroid hormones. These steroids have been useful in the treatment of cancer, Addison's disease, rheumatic arthritis, and in the production of contraceptives. Maximum content of solasodine in TSA fruit occurred when fruits changed color from green to yellow (Kaul and Zutshi 1982). Solasodine content in TSA ranges from 4-10%, though the solasodine content decreases with the onset of post fruit maturity. Solasodine is poisonous to humans. At least 10 fruit would have to be eaten before symptoms of poisoning occurred and a lethal dose would require approximately 200 fruit (Frohne and Pfander 1983). Though the mature fruit has a sweet smell (like a plum or apple) when the berry is opened, the coated seed has a bitter taste when eaten (Mullahey, personal observation). Apparently the bitter taste does not prevent wildlife and cattle from consuming the fruit.

## TSA Ecology

TSA has been observed in pastures, sugar cane fields, vegetable fields, citrus groves, natural areas (oak hammocks, cypress heads, swamps), sod fields, ditch banks, lawns, state parks, nature preserves, landfills, and county municipal parks. It is a common weed in South America, India, the West Indies, Honduras, and Mexico. Native to northern Argentina, and southern Brazil, TSA can be expected to occur in other subtropical areas. How TSA was introduced into Florida is not known. Its introduction into North America probably resulted from seed adhering to people's shoes or escape from cultivation (Mullahey *et al.* 1993B). In Florida, it is an obligate weed mainly associated with human activities. This plant has been identified as a host for nine viruses that cause economic damage to vegetables (McGovern *et al.* 1994).

Tropical soda apple is spread by cattle, wildlife, contaminated hay, grass seed, moving water, and sod. Mature cattle and weaned calves consume the fruit and the seed pass through the animal where it is deposited onto the pasture in manure. To determine the survivability of TSA seed in the rumen, 40,000 TSA seed were fed to crossbred steers in individual crates designed for the collection of total fecal production (Brown *et al.* 1996). Greater than 90% of seed had cleared the digestive tract by 48 hours after feeding. Seed recovery continued for up to 18 days after feeding, however seed collected beyond six days after feeding did not germinate. Therefore, cattle should be held in an area that is TSA-free for at least six days before shipment.

Rapid spread of TSA is often associated with some type of soil disturbance. Discing of a field, cattle congregating around a feeder, cleaning of ditch banks, or feral hogs rooting in a field appear to provide a favorable environment for TSA germination, establishment and growth. Standing water will stress the plant, even cause plant loss, but a new plant will emerge from seed once the area begins to dry. Cypress heads will have TSA in the center of the head until the summer rains completely flood the area. Then the TSA will die back to the outer regions (drier areas). As the water in the cypress head recedes during the winter, the TSA will begin to occupy (new plants from seed) the inner regions of the cypress head. Ephemeral wetlands could be susceptible to invasion by TSA during the dry season.

Loss of carrying capacity and shade areas for cattle are two of the main production losses from TSA. A census of Florida cattlemen in South Florida was conducted to determine the level of infestation (low, medium, or high), based on ground cover, and the hectares associated with each level (Mullahey *et al.* 1994). Census results indicated a yearly production loss due to decreased carrying capacity and heat stress of $11 million per year. This figure was based on 157,018 ha infested with TSA. If we assume the same levels of infestation, but use current data on infested pasture land (405,000 ha), the economic loss would approach $29 million for Florida ranchers.

Recently, people in Florida observed a “natural decline” in older stands of TSA in pastures. Some of these pastures were heavily infested with TSA for a period of years. Suddenly, the TSA plants disappeared without any management from the rancher. Scientists suspect the decline is associated with soil pathogens and damage from insects and nematodes.This decline is not predictable and it does not occur in all pastures or areas.

**Implication of Biology and Ecology for TSA Management Strategies**

For invasive weeds, weed biology and ecology information is critically important to developing effective weed management strategies. Unfortunately, for the state of Florida, this information was not available during the period of time (1990-95) when TSA spread exponentially (4,050 ha to 202,500 ha). Early management efforts focused on chemical control with weed biology and ecology studies starting in the mid 1990’s. Knowledge about plant growth and development, fruit production, seed development, vectors of spread and habitats at risk for invasion have collectively resulted in the development of economical, effective TSA management strategies. These strategies include prevention, control, and monitoring. It is important that Asian-Pacific countries apply these strategies when TSA is initially detected. Failure to do so could allow TSA to exponentially spread as it has in Florida and thus miss the opportunity to eliminate the weed.

<u>Prevention</u>

Understanding that TSA spreads primarily from seed, ranchers need to prevent the movement of contaminated products onto the ranch. Preventing the spread of TSA seed limits the movement of this noxious weed and is a form of weed control. The seed is coated in a mucilaginous substance that enables it to “stick” to whatever it touches. Seed is transported in


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sod, hay, grass seed, and cattle. Seed will adhere to vehicles such as trucks, tractors, or mowing equipment. Ranchers should clean equipment to remove the seed before moving it from an infested pasture to another pasture or location. Hold cattle in a pasture for six days to allow time for seed to pass through the digestive tract. Confining the weed to small areas makes it easier to control. If hay or grass seed is purchased that is contaminated with TSA, refuse to buy any more hay or seed from that supplier. Monitor the areas where hay is fed to cattle for new TSA plants so these plants can be removed before they produce fruit.

Control

Control strategies for TSA include herbicides and biological control. Initial herbicide studies focused on preherbicide mowing followed by application of post-emergence herbicides such as Remedy® (1.12 kg/ha triclopyr) applied (374 L/ha) to bahiagrass pastures (Mullahey *et al.* 1993; Akanda *et al.* 1997; Mislevy *et al.* 1999). Remedy provided excellent control (90-100%) of juvenile TSA but the herbicide had two limitations: inconsistent control if plants are fruiting and no soil residual activity to control new, emerging TSA seedlings in the treated area. Additionally, the mowing and the herbicide program cost about USD $21/ha which was too expensive for most ranchers. Recent studies have shown excellent control with Milestone and this herbicide possesses superior post-emergence and/or preemergence control relative to Remedy (Ferrell *et al.* 2006). Additionally, no preherbicide mowing is required when using Milestone thus reducing the total treatment cost. Milestone® (0.08 kg/ha aminopyralid) applied (187 L/ha) in the winter months in Florida provided excellent control of TSA and this treatment increased the duration of grazing at a time of the year when nutritional requirements are higher because cows are lactating. Consistent, excellent control (95%) was observed with Milestone applied at broadcast rates ≥ 0.08 kg/ha (Ferrell *et al.* 2006) or as a spot treatment at 15-20 ml/9.46 l (Sellers *et al.* 2009). Milestone is commercially available in prepackaged mixes with other herbicides which will provide more broad spectrum weed control in pastures.

Biological control using insects collected from Brazil has been effective in controlling TSA. Preliminary surveys in Brazil and Argentina to collect insects for evaluation resulted in the collection of the leaf-feeding beetle *Gratiana boliviana* (Coleoptera: Chrysomelidae). This beetle was the first biocontrol agent introduced against TSA and it was released in Florida in the summer 2003 (Medal and Cuda 2003). The beetle quickly established at release sites in southern Florida and feeding damaged the foliage and significantly reduced fruit production. Beetles showed a dispersal ability from the release sites of 1.6 km per year. Two years after the beetle release, many of the TSA plants at one release site were replaced by desirable pasture grasses. To date, no negative, non-target effects have been observed. Studies continue looking for beetles with more cold tolerance, preference for shade, and insects that feed on TSA flowers such as the adult flower-bud weevil (*Anthonomus tenebrosus*).

A natural, biological herbicide to control TSA is currently under development and is not available for public use at this time. The product is called SolviNix LC and contains a naturally occurring virus called Tobacco mild green mosaic tobamovirus (Charudattan 2007). Based on extensive field-testing, it has been determined that nearly 100% control of TSA is possible with very small quantities of the virus (8.1 mg to 0.81 g of active ingredient per ha). The virus is best suited for spot-application with a high-pressure sprayer (5.5 to 13.8 bars) depending on the nozzle-to-target distance.


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## Education and Extension of Information

Information on preventing the spread of TSA and the adoption of integrated management strategies to control TSA evolved through education programs targeting land managers and policy makers in Florida and the southeastern US. The University of Florida, Institute of Food and Agricultural Sciences (IFAS), was the lead organization in creating and disseminating science based information on TSA. However, education was a team effort that included state government officials, federal officials, agribusiness, cattleman, public land managers, and the media. Information was disseminated using posters, brochures, information cards, PowerPoint presentations, radio spots, DVD's, websites, and trade journal articles. Information was presented during field days, producer meetings, workshops, and seminars. One significant development to educate the ranchers in Florida was the establishment of the TSA Task Force in 1993 by the Florida Commissioner of Agriculture. The task force addressed various aspects about TSA (regulatory, research, education), and its members represented the affected industries along with university officials. Officials from other state agencies throughout the southeastern US joined the task force in an effort to prevent the spread of TSA into other states. The task force was responsible for placing TSA on the Florida Noxious Weed List (1994) and the Federal Weed List (1995). The task force successfully obtained research funding for TSA projects and one project (TSA seed survivability) led to the policy of holding cattle for six days prior to shipping to avoid the spread of germinable seed. This kind of interdisciplinary approach may be most beneficial to Asian-Pacific countries that are infested with TSA.

## REFERENCES

Akanda, R. U., Mullahey, J., Dowler, C. and Shilling D. (1997). Influence of postemergence herbicides on tropical soda apple (*Solanum viarum)* and bahiagrass (*Paspalum notatum*). *Weed Tech.* 11:656-661.

Brown, W.F., Mullahey, J. and Akanda, R.U. (1996). Survivability of tropical soda apple seed in the gastro-intestinal tract of cattle. *The Florida Cattleman* (April) p.37.

Charudattan, R. (2007). A plant virus as a bioherbicide for tropical soda apple, *Solanum viarum. Outlooks on Pest Management* 167-171.

Coile, N.C. (1993). Tropical soda apple, *Solanum viarum* Dunal: the plant from hell (Solanaceae). Florida Department of Agriculture and Consumer Services Botany Circular No. 27. Gainesville, Florida Division of Plant Industry.

Ferrell, J., Mullahey, J., Langeland, K. and Kline, W. (2006). Control of tropical soda apple (*Solanum viarum*) with aminopyralid. *Weed Tech.* 20:453-457.

Frohne, D. and Pfander, H.J. (1983). *A colour atlas of poisonous plants*. pp. 215-216.

Kaul, B.L. and Zutshi, U. (1982). Cultivation of *Solanum khasianum* Clark for steroid: problems and promises. *Cultivation and utilization of medicinal aromatic plants*, p. 23-31.



ISBN Number: 978-0-9871961-0-1

McGovern, R.J., Polston, J.E. and Mullahey, J.J. (2011). *Solanum viarum*: weed reservoir of plant viruses in Florida. *Int. J. Pest Mgmt.* (In Press).

Pingle, A.R. and Dnyansagar, V.R. (1979). Induction of germination in *Solanum viarum*. *Current Science* 48(10):449-450.

Medal, J. and Cuda, J.P. (2010). Biocontrol agent released against tropical soda apple in Florida. Florida Entomologist 93(4):493-500.

Mislevy, P., Mullahey, J. and Cornell, F. (1999). Preherbicide mowing and herbicide rate on tropical soda apple (*Solanum viarum*) control. *Weed Tech.* 13:172-175.

Morton, C.V. (1976). *A revision of the Argentine species of Solanum.* Cordoba, Republica Argentina, Academia Nacional De Ciencias.

Mullahey, J.J., Cornell, J.A. and Colvin, D.L. (1993A). Tropical soda apple (*Solanum viarum*) control. *Weed Tech.* 7:723-727.

Mullahey, J.J., Nee, M., Wunderlin, R.P. and Delaney, K.R. (1993B). Tropical soda apple (*Solanum viarum):* a new weed threat in subtropical regions. *Weed Tech.* 7:783-786.

Mullahey, J.J. and Cornell, J. (1994). Biology of tropical soda apple (*Solanum viarum*) an introduced weed in Florida. *Weed Tech.* 8:465-469.

Mullahey, J.J., Mislevy, P. and Colvin, D.L. (1994). Preliminary management practices to control and prevent tropical soda apple (*Solanum viarum*). Florida Cooperative Extension Service, Agronomy Facts, SS-AGR-56.

Mullahey, J.J., Akanda, R.U. and Mislevy, P. (1996). Biology and control of tropical soda apple. *Proceedings Tropical Soda Apple Symposium*, Bartow, FL. Pp. 9-14.

Nee, M. (1991). Synopsis of Solanum Section Acanthophora: a group of interest for glycoalkaloids. p. 258-266. In J.G. Hawkes, R.N. Lester, M. Nee, N. Estrada, eds. *Solanaceae III: Taxonomy, Chemistry, Evolution.* Royal Botanic Gardens 8. Kew, Richmond, UK.

Patil, S. and Laloraya, M.M. (1981). Effect of spacing on the yield of solasodine in *Solanum viarum* Dunal: source of raw material for the synthesis of steroids. *Indian J. Plant Physiol.* 24:237-242.

Patterson, D., McGowan, M. Mullahey, J. and Westbrooks, R. (1997). Effects of temperature and photoperiod on tropical soda apple (*Solanum viarum* Dunal) and its potential range in the U.S. *Weed Science* 45:404-408.

Reddy, G.S. and Krishnan, R. (1988). Selection for berry yield and its components in ess-spiny diploid and advanced generation autotetraploids of *Solanum viarum* Dunal. *South India Hortic*. 36:131-135.



ISBN Number: 978-0-9871961-0-1

Sahoo, S. and Dutta, P.K. (1984). *Solanum viarum*, a plant for the steroid drug industry. *Indian Horticulture* 28(4):15-18.

Sellers, B., Ferrell, J. Mullahey, J. and Hogue, P. (2006). Tropical soda apple: biology, ecology and management of a noxious weed in Florida. Florida Cooperative Extension Service, EDIS, SS-AGR-77.

Wunderlin, R.P., Hansen, B.F., Delaney, K.R., Nee, M. and Mullahey J.J. (1993). *Solanum viarum* and *S. tampicense* (Solanaceae): two weedy species new to Florida and the United States. *SIDA* 15(4):605-611.



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# EARLY GROWTH OF PARTHENIUM WEED (*PARTHENIUM HYSTEROPHORUS* L.) AND CLIMATE CHANGE

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## ABSTRACT

Parthenium weed (*Parthenium hysterophorus* L.; Asteraceae), native to the tropical and subtropical Americas, is an aggressive herbaceous weed of tropical and subtropical environments. In Australia, parthenium weed occurs mainly in Queensland, where two distinct populations of the weed occur. This includes the more widespread 'Clermont' population and a less-aggressive 'Toogoolawah' population. Potential impacts of climate change on these two populations are not known. This study examined the early growth of the two Australian populations (Clermont or Toogoolawah) of parthenium weed in environmental chambers under two concentration of $CO_2$ (390 ppmv; ambient or 550 ppmv; elevated), two temperature (35/20°C; Warm or 30/15°C; Cool) and two soil moisture (field capacity; Wet or half of field capacity; Dry) regimes. The early growth (as measured by leaf production, the length of the longest leaf, the total leaf area and the plant dry weight) of both biotypes under the elevated $CO_2$, cool temperature, and wet or dry soil moisture conditions was higher from *ca.* 6% to *ca.* 305% (than the growth under ambient concentration of $CO_2$ and the same conditions of temperature and soil moisture. However, the growth rates were not significantly different when the young plants were grown under warm temperature and the same conditions of $CO_2$ concentration, soil moisture levels.

**Keywords:** *Parthenium hysterophorus*, climate change, seedling growth, soil moisture, $CO_2$ enhancement.

## INTRODUCTION

Global atmospheric carbon dioxide ($CO_2$) concentrations are predicted to rise to 550 ppmv by the middle of this present Century (Prentice *et al.* 2001 and CSIRO 2007). The stimulative effects of atmospheric $CO_2$ enrichment upon plant growth and development are expected to enhance vegetative productivity, but this will depend upon the species (Kimball *et al.* 2002, Ainsworth and Long 2005). The variations in growth response, following photosynthetic enhancement by elevated $CO_2$ concentrations, will be associated with the differential responses of the species to other growth-limiting factors, such as temperature, soil moisture and their photosynthetic efficiency , that is whether they are $C_3$ or $C_4$ plants (Kimball *et al.* 2002). In central Queensland, Australia, the average daytime summer temperature has been 30 to 32°C for many years and has been suggested that there will be a significant increase in temperature of between 2.0 to 4.5°C by 2070 (Australian Bureau of Meteorology website 2011, http://www.bom.gov.au). In addition, it is predicted that there will be a relatively small reduction in rainfall of between 10 to 20 % of the present rainfall by 2070 (Australian Bureau of Meteorology website 2011, http://www.bom.gov.au). Such a series of changes in climate will have significant impacts upon Queensland's agricultural systems. It is likely that there will be significant changes in the incidence (distribution) and abundance of certain kinds of weeds as a result of these changing climatic conditions (Houghton *et al.* 1990).

However, while there is a growing literature concerning the effects of $CO_2$ enrichment and other climate change variables upon the growth of other weed species, studies on how climate change may affect parthenium weed growth remain limited. Pandey *et al.* (2003) stated that an elevated $CO_2$ concentration (700 ppmv) would enhance the net leaf photosynthetic efficiency, the maximum photosynthetic rate, and the water use efficiency, while decreasing the light requirement for net photosynthesis, reducing stomatal conductance, and therefore the transpiration rate of parthenium weed. Thus, parthenium weed is likely to show an increasing growth rate in a climate enriched with $CO_2$ and with an increased temperature (Pandey *et al.* 2003). Moore *et al.* (1987) and Tirumala Devi and Raghavendra (1993) have described the photosynthetic pathway used by parthenium weed as being a $C_3$-$C_4$ intermediate. The upper leaves seem to use the $C_3$ photosynthetic pathway while the leaves in the middle and at the base of the plant have the typical Kranz leaf anatomy associated with $C_4$ photosynthesis (Rajendrudu and Rama Das 1990). According to Navie *et al.* (2005), parthenium weed plants show a typical $C_3$ plant response to elevated $CO_2$ (480 ppmv) becoming much taller with more biomass and a greater seed production than those grown under an ambient $CO_2$ concentration (360 ppmv), even when in competition with a $C_4$ pasture grass, buffel grass (*Cenchrus ciliaris* L.).

Parthenium weed had been introduced into Australia from North America on two separate occasions – the first was in the 1940s at Toogoolawah in south-eastern Queensland (referred to as the Toogoolawah population) and the second was in 1958 at Clermont, central Queensland (referred to as the Clermont population; Adkins and Navie 2006). It is interesting to note that plants from the Clermont population, when grown under one set of similar environmental conditions, can become much taller and significantly more massive than those from the Toogoolawah population (Navie 2002). Nevertheless, the effects of $CO_2$ enhancement, higher temperature and lower soil moisture level combination on the early growth of these two populations remain unknown.

Thus, the aims of this study are to measure the early growth (*viz.* the number of leaves, the leaf length, the leaf area and the dry biomass) of two populations of parthenium weed (i.e. Clermont and Toogoolawah) when placed under ambient or elevated $CO_2$ conditions, and when growing under 'Warm' or 'Cool' temperature conditions, in a 'Wet soil' or a 'Dry soil' in a glasshouse, and assess the effect of an elevated atmospheric $CO_2$ concentration, temperature and soil moisture content (i.e. the main climate change variables) upon parthenium weed early growth and development.

## MATERIALS AND METHODS

### Plant Material

Parthenium weed cypselas (hereafter referred to as seeds), obtained from the two Australian populations were collected from field-growing plants at Injune (S 25$^o$46'18", E 148$^o$21'06"; Clermont population) in central Queensland, and Toogoolawah (latitude 27S, longitude 152E; Toogoolawah population), in south-east Queensland. Seeds were germinated in plastic Petri dishes (9 cm diameter) on two layers of filter paper (Whatman

No. 1) moistened with 7 mL of distilled water and placed in a germination incubator (12/12 hour day/night photoperiod with a 25/20 ± 1°C, day/night thermoperiod) for 4 days. At this time healthy seedlings of uniform size were transplanted into shallow trays (20 × 25 × 6 cm; w/l/h) containing *ca.* 2.5 to 3.0 kg of a heavy clay soil obtained from Gatton, Queensland (27º33'12'' S and 152º20'21'' E). The seedlings were planted at least 5 to 7 cm apart in these trays.

**Growing Conditions**

The trays with seedlings were distributed randomly on to the surface of a bench inside one of two temperature controlled growth chambers (internal dimensions 200 x 180 x 200 cm; l/b/h; Kirby Ltd., Sydney, New South Wales). Each chamber was positioned within one of two glasshouses (one chamber per glasshouse) at the University of Queensland, Brisbane allowing the plants to receive natural sun light of *ca.* 8 to 9 sunshine hours per day for the duration of the experiment (*ca.* 800 µmol $m^{-2}$ $s^{-1}$). Within these chambers, a number of experiments were undertaken. In each experiment the transplanted seedlings were allowed to grow for 4 weeks before being harvested.

<u>Experiment 1 and 2</u>
Growth chamber (A) was set up with an elevated $CO_2$ concentration of 550 ppmv while growth chamber (B) was set up with an ambient $CO_2$ concentration of 390 ppmv for Experiment 1 and in inverse order for Experiment 2. Both chambers in two experiments were operated at a temperature regime of 30/15 ± 3$^{o}$C, day/night. These experiments were conducted from 24th July to 25th September 2009.

<u>Experiment 3 and 4</u>
Growth chamber (A) was set up with an elevated $CO_2$ concentration of 550 ppmv and growth chamber (B) was set up with an ambient $CO_2$ concentration of 390 ppmv for Experiment 3 and in inverse order for Experiment 4. Both chambers in two were operated at a temperature regime of 35/20 ± 3$^{o}$C, day/night. These experiments were conducted from 23rd March to 20th April 2010.

<u>$CO_2$ Enrichment</u>
To study the effect of $CO_2$ concentration on the growth of parthenium weed, the gaseous atmospheres within the two chambers were modified to create different concentrations of the gas. To do this 'Food' grade $CO_2$ gas was supplied from a 'G' size cylinder (Coregas Ltd., Brisbane, Queensland) and the concentration within the chamber monitored and modified using an ADC 2000 $CO_2$ monitor (ANRI Instruments and Controls Ltd., Melbourne, Victoria) in conjunction with a solenoid valve which when working together could modify the gas flow into the chamber.

<u>Temperature</u>
To study the effect of temperature on the growth of parthenium weed, seedlings in trays were placed in the growth chambers which were set at one of two temperature regimes (either 30/15 ± 3 °C; hereafter referred to as 'Cool' – used in Experiments 1 and 2 or 35/20 ± 3 °C, day/night; hereafter referred to as 'Warm' – used in Experiments 3 and 4).

<u>Soil Moisture</u>
To study the effect of soil moisture on the growth of parthenium weed, seedlings in trays within each chamber in each experiment were watered to create two soil moisture regimes, (i.e. field capacity, hereafter referred to as 'Wet' or half of field capacity, hereafter

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referred to as ‘Dry’) for these experiments. To determine field capacity for the soil, several kg of soil were placed into three pots and irrigated with tap water to the point of it becoming saturated. The pots were then covered at the top with section of black plastic sheet, and allowed to drain for 48 hours. The plastic sheet was then removed and three soil samples (*ca.* 300 g each) were taken from the mid position of each pot. These samples were weighed (referred to as the wet weight of soil: A) before being dried in an oven at 90 °C for 72 hours. When no further change of soil sample weigh occurred, the samples were re-weighed (referred to as the dry weight of soil: B). Field capacity (FC) was then calculated by the formula $(A – B) \times 100/B$ and half of field capacity by the formula $0.5 \times (A – B) \times 100/B$. However, for use in the experiment, the Gatton soil already contained some water. Therefore, only sufficient extra water was added to bring it up to the required level. To do this, three samples were removed from extra pots of soil, weighed (weight C), then the samples were dried in an oven at 90 °C for 72 hours. When no further change of soil weigh was observed, the samples were weighed again and recorded (weight D). Thus, the available water percentage in the starting soil was determined by $E = (C – D) \times 100$. As a result of this calculation, the amount of additional water that was needed to be added to the pots was determined for both field capacity and half of field capacity soils. At one or two day intervals during the growing period of the plants, the pots and plants were weighed, and water readded until they reached their original moisture level.

Growth Determination

At the end of the 4 week growth period, the number of new leaves that appeared on each plant was recorded as was the length of the longest leaf. Measurements were also taken on the total leaf area and the above-ground dry biomass of each plant. For leaf area, all leaves were removed and their area measured using a leaf area scanner (Paton Electronic Planimeter developed in conjunction with CSIRO, South Australia). Then the leaves and other shoot parts were put into paper bags (12 × 7 cm) and the contents dried in an oven at 90 ± 2$^{o}$C until no further weight loss occurred. This weight of contents was then taken as the dry biomass of that plant.

**Experimental Design and Statistics**

A total of 480 parthenium weed seedlings coming from each of the two populations (Clermont or Toogoolawah) were studied in the four experiments. In each experiment, each treatment had three replicate trays and each tray had 10 seedlings. This meant that the growth parameters (number of leaf per plant, leaf length, leaf area and dry biomass) were measured from 30 replicate plants, in each of the experimental conditions. Because the experiments were repeated (by swapping the chambers over), and the data from the two experiments pooled, there were in fact 60 replicates seedlings per treatment, and a total of 960 seedlings used in all the experiments. All seedling growth parameter data sets (*viz.* leaf number, leaf length, leaf area and dry biomass) were analysed by an two-way Analysis of Variance method using a general linear model procedure in Minitab, version 16 (Minitab Inc., USA).

**RESULTS**

**Leaf Production**

The production of leaves followed a linear pattern with respect to time in all cases (Figures 1 and 2). The number of leaves produced per plant by the seedlings grown under the elevated $CO_2$ concentration was significant higher (F-value = 24.33, $P < 0.05$) than those of seedlings grown under the ambient $CO_2$ concentration from *ca.* 3% to *ca.* 30%. In addition, the number of new leaves produced per plant by the seedlings grown under the Warm condition was significant higher ($P < 0.05$) than those of seedlings grown under the Cool condition from *ca.* 25% to *ca.* 67%. There was a significant difference in leaf production between the two populations under the two temperature regimes (F-value = 13.00, $P < 0.05$) with Clermont producing the most (13 leaves/plant). At the Cool temperature, the number of leaves produced per plant under the elevated $CO_2$ concentration, were significantly higher (from *ca.* 6% to *ca.* 30%) than on seedlings grown under the ambient $CO_2$ concentration (F-value = 23.35, $P = 0.0002$; Figures 1 and 2). Under the Warm temperature, the number of leaves produced per plant was significantly higher (F-value = 367.73, $P < 0.05$) than under the Cool temperature condition (Figures 1 and 2). However, no significant differences were observed between seedlings grown under different $CO_2$ concentrations or in different soil moisture levels (Figures 1 and 2).

**Leaf Length**

The length of the longest leaf also followed a linear pattern with respect to time in all cases (Figures 1 and 2). The leaf length produced by the seedlings grown under the Warm condition was significant longer (F-value = 169.44, $P < 0.05$) from *ca.* 25% to *ca.* 140% than those of seedlings grown under the Cool condition. In addition, there was a significant difference (F-value = 6.70, $P < 0.05$) in leaf length between the two biotypes grown under both temperature regimes with Clermont population producing longer leaves (from *ca.* 6% to *ca.* 39%). Under the Cool temperature, the leaf length produced overtime by all seedlings grown under the elevated $CO_2$ concentration, were significantly longer (F-value = 19.23, $P < 0.05$) from *ca.* 14% to *ca.* 89% than those on seedlings grown at the ambient $CO_2$ concentration. Under the Warm temperature conditions, the leaf length produced by seedlings grown under the elevated $CO_2$ concentration was about the same as that seen in seedlings grown under the ambient $CO_2$ concentration (Figures 1 and 2).

**Leaf Area**

Seedlings grown under the elevated $CO_2$ concentration were significantly greater (F-value = 4.61, $P = 0.0464$) from *ca.* 4% to *ca.* 264% than seedlings grown under the ambient $CO_2$ concentration, except for the Toogoolawah population (Figures 1 and 2). In addition, the leaf area per seedling grown under the Warm condition was significant greater (F-value = 73.62, $P < 0.0001$) from *ca.* 53% to *ca.* 570% than that of seedlings grown under the Cool condition.

**Plant Biomass**

The biomass of seedlings grown under the elevated $CO_2$ concentration was significantly higher (F-value = 38.40, $P < 0.0001$) from *ca.* 11% to *ca.* 305% than that of seedlings grown under the ambient $CO_2$ concentration, except for the Toogoolawah population grown under the Warm condition at field capacity (Figures 1 and 2). In addition, the biomass of seedlings grown under the Warm condition was significantly higher (F-value = 15.99, $P = 0.0009$) from *ca.* 60% to *ca.* 480% than that of seedlings grown under the Cool condition.

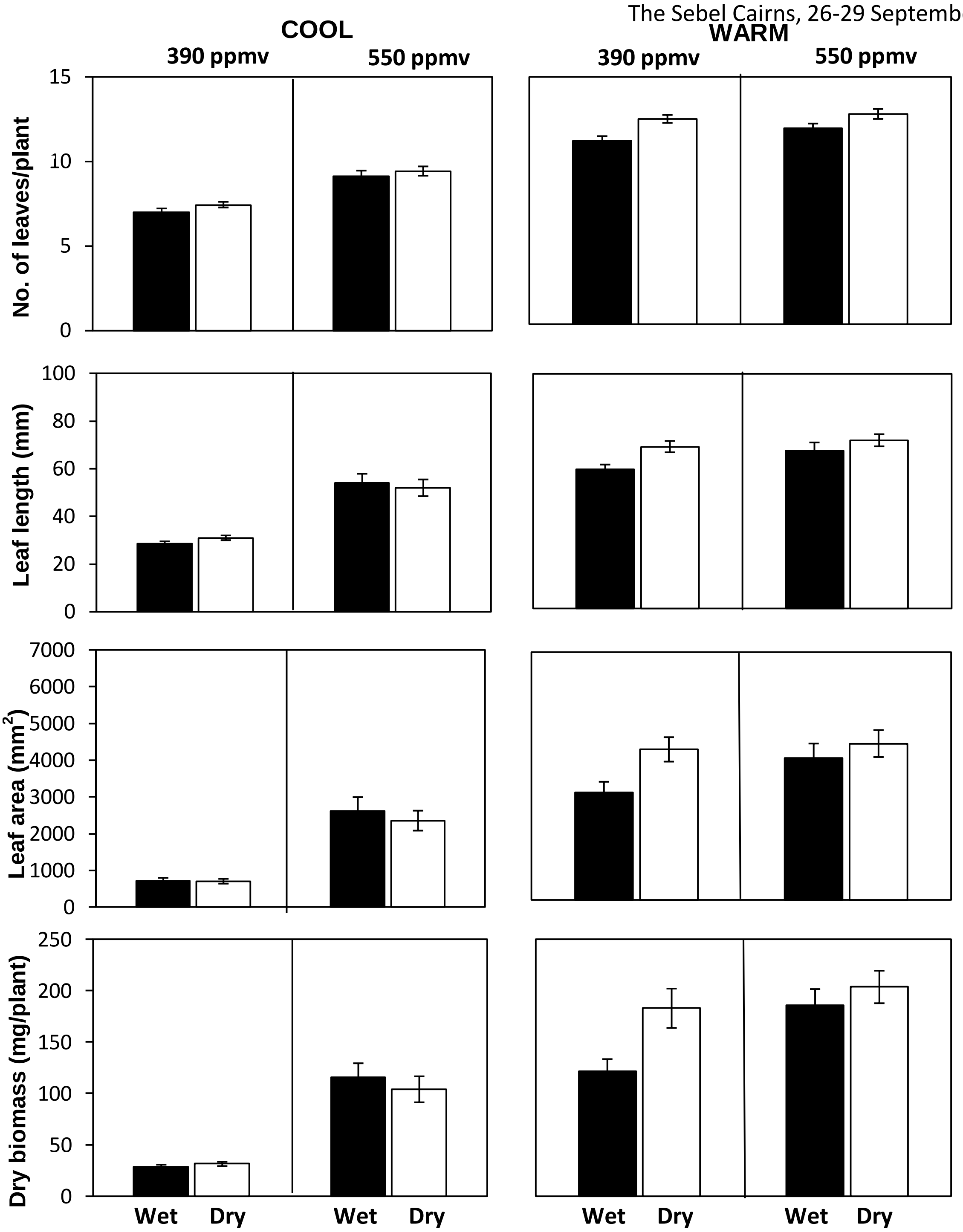


**Figure 1.** *The number of leaves per plant, the length of the longest leaf, the leaf area and the dry biomass produced by seedlings of the* ***Clermont*** *population of parthenium weed grown under a Cool temperature regime (30/15°C; day/night) or under a Warm temperature regime (35/20°C; day/night) and under an ambient $CO_2$ concentration (390 ppmv) or under an elevated $CO_2$ concentration (550 ppmv) at either of two soil moisture levels (i.e. field capacity: Wet or half of field capacity: Dry) and measured at the end of the 4 weeks study. Each treatment of the four variables had 60 replicate plants. Error bars represent two standard errors of the mean.*

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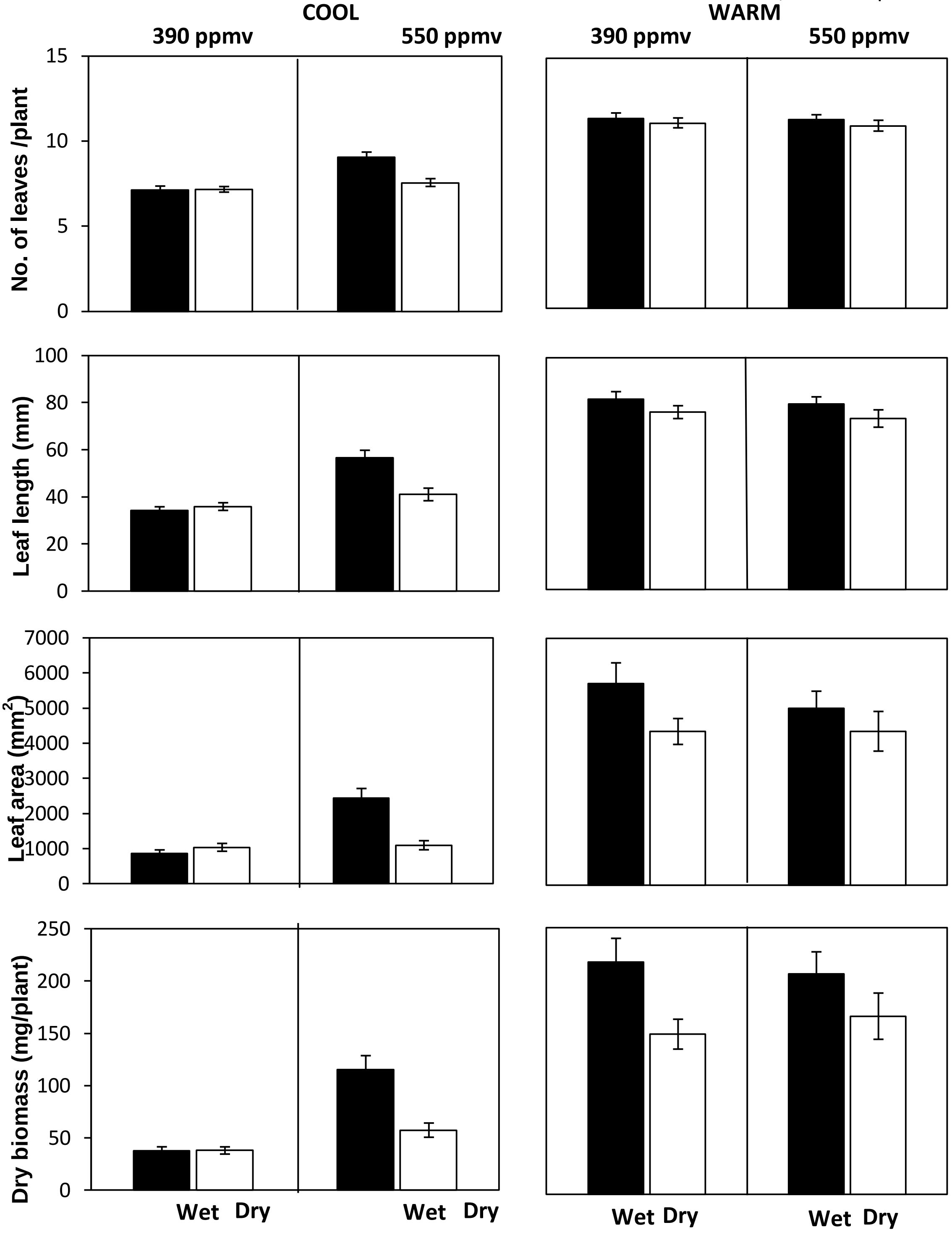


**Figure 2.** *The number of leaves per plant, the length of the longest leaf, the leaf area and the dry biomass produced by seedlings of the* ***Toogoolawah*** *biotype of parthenium weed grown under a Cool temperature regime (30/15$^o$C; day/night) or under a Warm temperature regime (35/20$^o$C; day/night) and under an ambient $CO_2$ concentration (390 ppmv) or under an elevated $CO_2$ concentration (550 ppmv) at either of two soil moisture levels (i.e. field capacity: Wet or half of field capacity: Dry) and measured at the end of the 4 weeks study. Each treatment of the four variables had 60 replicate plants. Error bars represent two standard errors of the mean.*

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## DISCUSSION

### Growth and $CO_2$ Concentrations

Under the elevated $CO_2$ concentration (550 ppmv), leaf production, leaf length and the total leaf area were all promoted as well as the overall plant growth, especially for the Clermont population (Figures 1 and 2). The results of this study are similar to those reported by Navie *et al.* (2005) who showed parthenium weed (Clermont population), grown at an elevated $CO_2$ concentration (480 ppmv), are taller and heavier than those grown under an ambient $CO_2$ concentration (360 ppmv). However, the earlier study reported increases in plant height (*ca.* 500 %) and biomass (*ca.* 950 %; Navie *et al.* 2005), much greater than those seen in the present study. One obvious difference between the studies is that the present one was only looking at early growth (young plant), whereas the earlier study was looking at the whole life of the plant. Another possibility to explain the difference in the biomass between the two studies might be the smaller variation in temperature between the day and the night used in the present study as compared to the earlier study (a 25 degree variation in the earlier study and a 15 degree variation in the present study). There is also the possibility that optimum $CO_2$ concentration for parthenium weed's growth might be closer to *ca.* 500 ppmv (earlier study) than to 550 ppmv, the concentration used in the present study.

### Growth and Temperature

According to Entz and Fowler (1991), higher temperatures accelerate the rate of plant development and reduce the length of the growing period. In this present study, parthenium weed also appeared to behave in the same manner to that seen with other species. All of the growth parameters of the weed increased under the Warm condition (35/20$^oC$ day/night; a temperature closer to what might be present under a changing climate by 2070 in central Queensland) compared to that seen under the Cool condition (30/15$^oC$ day/night; a temperature closer to the present climate in central Queensland), under both the ambient and the elevated $CO_2$ concentrations (Figures 1 and 2). This indicates that parthenium weed is likely to accelerate its growth and rate of plant development (reducing the length of its growing period) in a future, warmer environment. This means parthenium weed will become more aggressive, due to the warmer climatic conditions of the near future.

$C_3$ and $C_4$ species and changing climate: Parthenium weed is considered to be a $C_3$-$C_4$ intermediate species (Moore et al. 1987 and Tirumala Devi and Raghavendra 1993), and therefore is most likely to increase its growth in an elevated atmosphere of $CO_2$. In the present study, parthenium weed responded in a similar manner to $C_3$ plants, with the plant dry weight increasing by 11 to 305 % (depending on the population and the environmental condition). Cure and Acock (1986) reviewed many prior experiments and reported an average 28 % increase in the growth of $C_3$ species when the $CO_2$ concentration was doubled, while in $C_4$ grass species the increase was only ca. 3 to 9 %, hence an increase in the atmospheric $CO_2$ is not as beneficial to $C_4$ grass species as it is for $C_3$ species. The findings of this present study have implications for the future threat that parthenium weed may pose to grazing industry under a changing climate. It is likely that parthenium weed will be more aggressive, especially at the early growth stage which is a stage that is critically important to most plants when invading the predominantly $C_4$ pasture lands of central Queensland. Of particular concern might be its ability to compete more at the early growth stage, produce more biomass which might elevate its allelopathic potential,

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produce more seed to retain its high seed bank capacity. It may also be to compete its life cycle more rapidly but this may or may not be an advantage.

## ACKNOWLEDGEMENTS

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## REFERENCES

Adkins, S.W. and Navie, S.C. (2006). Parthenium weed: a potential major weed for agro-ecosystems in Pakistan. *Pakistan Journal Weed Science Research* 12(1-2): 19-36.

Ainsworth, E.A. and Long, S.P. (2005). What have we learned from 15 years of freeair $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. *New Phytologist* 165: 351-372.

Australian Bureau of Meteorology. (2011). Viewed on 30th March 2011 < http://www.bom.gov.au>.

CSIRO, Australia Bureau of Meteorology. (2007). Climate change in Australia: technical report 2007. CSIRO. 148pp.

Cure, J.D. and Acock, B. (1986). Crop responses to carbon dioxide doubling: A literature survey. *Agriculture Forest Meteorology* 38: 127-145.

Entz, M.H. and Fowler, D.B. (1991). Agronomic performance of winter versus spring wheat. *Agronomy Journal* 83: 527-532.

Houghton, J.T., Jenkins, G.J. and Ephraums, J.J. (1990). *Climate Change: the IPCC Scientific Assessment*. Cambridge University Press, Cambridge.

Kimball, B.A., Kobayashi, K. and Bindi, M. (2002). Responses of agricultural crops to free-air $CO_2$ enrichment. *Advances in Agronomy* 77: 293-368.

Moore, B.D., Franceschi, V.R., Cheng, S., Wu, J. and Ku, M.S.B. (1987). Photosynthetic characteristics of the $C_3$-$C_4$ intermediate *Parthenium hysterophorus*. *Plant Physiology* 85: 984-989.

Navie, S.C., McFadyen, R.E., Panetta, F.D. and Adkins, S.W. (2005). The effect of $CO_2$ enrichment on the growth of a $C_3$ weed (*Parthenium hysterophorus* L.) and its competitive interaction with a $C_4$ grass (*Cenchrus ciliaris* L.). *Plant Protection Quarterly* 20 (2): 61-66.

Navie, S.C. (2002). *The Biology of* Parthenium hysterophorous *L. in Australia*. PhD Dissertation. The School of Land Crop and Food Sciences, The University of Queensland, Australia, Brisbane.

Pandey, D. K., Palni, L. M. S., and Joshi, S. C. (2003). Growth, reproduction, and photosynthesis of ragweed parthenium (*Parthenium hysterophorus*). *Weed Science* 51(2): 191-201.

Prentice, I.C., Farquhar, G.D. and Fasham, M.J.R. (2001). The carbon cycle and atmospheric carbon dioxide. In *Climate change 2001: the scientific basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*., Eds. J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, D. Xiaosu, pp. 183-239. New York: Cambridge University Press.

Tirumala Devi, M. and Raghavendra, A.S. (1993). Photorespiration in $C_3$-$C_4$ intermediate species of *Alternanthera* and *Parthenium*: Reduced ammonia production and increased capacity of $CO_2$ refixation in the light. *Photosynthesis Research* 38: 177-184.

# PARTHENIUM WEED (*PARTHENIUM HYSTEROPHORUS* L.) IN VIETNAM

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## ABSTRACT

Originating in a region within North, Central or South America, parthenium weed (*Parthenium hysterophorus* L.), an invasive herbaceous Asteraceae weed of tropical and subtropical environments, is responsible for significant losses to rangeland and crop production and has serious effects upon human and animal health. The weed also causes serious impacts upon plant community biodiversity and the cost of its management is often very high. In Vietnam, parthenium weed has been present in the Hanoi and surrounding regions from about 1922 (Arenes *et al.* 1922).

Infestations of parthenium weed were surveyed along roadsides, in fallow land, from the north to the south of Vietnam and in more detail around the capital city, Hanoi Capital, and several protected areas (including Ba Be, Cat Ba, Xuan Son, Tam Dao, Cuc Phuong National Parks and Huong Son Protection Forest) in the north of Vietnam, to create a distribution map of the weed in Vietnam. Parthenium weed was present in many provinces in the north such as Cao Bang, Bac Kan, Son La, Thai Nguyen, Phu Tho, Vinh Phuc, Bac Ninh, Hanoi Capital, Hung Yen, Hai Duong, Hai Phong, Ha Nam, Nam Dinh, Hoa Binh and Ninh Binh. No parthenium weed was present from Ho Chi Minh City to Mekong Delta, southern region of Vietnam. However, parthenium weed was possibly present in the rest of the North and the Central region of Vietnam as well.

Several biological characteristics of the weed such as plant density and coverage, and height and seed production were measured at Cuc Phuong National Park (Ninh Binh Province) and at the Huong Son Protection Forest region (Hanoi Capital). From the measurements taken on biological characteristics of the weed, the study also indicated that the parthenium weed populations present in the two locations were similar but different to the Clermont population found in Australia. Without any valid molecular or biological comparisons between the populations in Vietnam and Australia, it would not be possible to call them as valid biotypes. Any differences in morphological or reproduction attributes between the populations in Vietnam and Australia could possibly be due to location effects. One way to eliminate this problem is to grow the two populations under identical conditions (in a glasshouse in quarantine) and compare various biological attributes. Alternatively, suitable molecular studies would also useful. Both have not yet been done.

**Keywords**: parthenium weed, *Parthenium hysterophorus*, Cuc Phuong National Park, Huong Son Protection Forest, distribution.

## REFERENCES

Arenes, J., Bonati, Danguy, P., Dop, P., Gagnepain, F., Guillaumin, Lecomte, H., Pellegrin, F. and Pitard, J. (1922-1933). Capripoliacees a Apocynacees. In Lecomte, H. and Humbert, H. *Flore Generale de L'Indo-chine*. Tome III. Paris Masson et Cie. pp: 589-590. (in French).

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# THE INVASION OF MIMOSA (*MIMOSA PIGRA* L.) ON THE DONG NAI RIVER BASIN, VIETNAM

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## ABSTRACT

Originated in the Central Americas, mimosa (*Mimosa pigra* L.), in the Fabaceae family, is now one of the worst alien invasive weeds of wetlands of tropical Africa, Asia and Australia. Mimosa causes serious impacts on the biodiversity of infested areas, and the cost of controlling is often very high (Lonsdale *et al.* 1995). In this study, we investigated the distribution, spread mode and impact of mimosa on the basin of the Dong Nai River, one of the largest and most economically important rivers of Vietnam.

The infestations of mimosa were surveyed along two banks of the main flow of Dong Nai River, La Nga River, Sai Gon River from the upper stream in Daklak, Lam Dong, Dong Nai Provinces to Ho Chi Minh City in the lower section, also along big and small roads in these provinces and cities from August, 2003 to March, 2005. The data was used to establish a map of the distribution of mimosa in Dong Nai River Basin using the Map Info program and to survey the spread mode of mimosa seeds from one location to another. In addition, local people living around Tri An Reservoir and in the buffer zone of Cat Tien National Park were interviewed about the impacts of the invasion of mimosa on their economic lives. These are negative impacts on the local farmers' agricultural activities, their fishery catch and cultivation and their crop productivity.

A map of mimosa infested areas was established, which covered the Dong Nai river and its main tributaries including the Saigon river, the Be river, the La Nga river and two large water reservoirs, the Tri An and the Dau Tieng. The invasion of mimosa on wetlands of Cat Tien National Park - the largest national park located on the Dong Nai river basin - was assessed in detail. The study also found that the transportation of sand excavated from the Dong Nai River and its tributaries for construction purposes was one of the important means of mimosa seed dispersal in the region. In addition, mimosa has reduced the beautiful appearance of the natural environment, and had a considerable negative impact on travel, sightseeing and research activities in Cat Tien National Park particularly in Bau Chim and Bau Sau Ponds. The invasion of mimosa in rice paddies has increased cultivation expenses for soil preparation, and the labor required to remove mimosa before cultivation. In some areas, this expense is so much greater than the income of agricultural products that local people fallowed (ceased to crop) part of or all of their land. It has also obstructed the aquatic traffic particularly in Tri An Reservoir, and damaged fishing-nets of fishermen living on the banks of Tri An Reservoir.

**Key words**: *Mimosa pigra*, Dong Nai River, Cat Tien National Park, Tri An Reservoir, distribution, seed dispersal.

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## REFERENCES

Lonsdale, W.M., Miller, I.L., and Forno, I.W. (1995). *Mimosa pigra* L.. In: Groves, R.H., Shepherd, R.C.H. and Richardson, R.G. eds. *The Biology of Australian weeds* (Volume 1). R.G. and F.J. Richardson, Melbourne. pp: 169-188.

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# IMPACT OF PRE- AND POST- PLANTING HERBICIDES ON GROWTH PERFORMANCE AND YIELD OF *ORYZA* GENOTYPES IN SRI LANKA

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## ABSTRACT

Herbicide application is one of the most effective methods of weed control in agricultural crops. However, herbicides can cause adverse effects on crops including rice (*Oryza* species). A preliminary greenhouse study was conducted to evaluate the herbicide tolerance in rice using five improved (BG-360, BG-352, BG-359, AT-306, AT-308), three traditional ("Machel", "Kuruluthuda", "Madathawaru") varieties of *O. sativa* and five wild rice spp. (*O. nivara, O. rufipogon*, *O. eichingeri*, *O. rhizomatis*, *O. granulata*). Broad spectrum pre-planting (Glyphosate; 360g/l Count-up®) and post-planting (Fenoxaprop-p-ethyl; 69g/l RiceStar®) herbicides were used in single (0.5 g/l, 5.0 g/l) and double dose (1.0 g/l, 10.0 g/l) concentrations respectively with control and eight treatment combinations. Seedling emergence time, plant height after two weeks and one month, whole plant biomass at maturity and productive yield were measured. Results indicated that herbicide treatments have caused a significant ($p \geq 0.05$) impact on the growth and yield of all rice genotypes at single and double doses. After treating with herbicides, traditional and wild genotypes showed comparatively a shorter germination time and better growth compared to improved varieties. Single and double doses of both herbicides indicated a significant yield loss as well as reduced biomass. Compared to AT- and BG-varieties, traditional varieties showed significantly a higher tolerance to both herbicides. The tolerance was even higher in wild genotypes. There were no comprehensive studies carried out so far on effects of pre- and post-planting herbicides on rice genotypes available in Sri Lanka. The results revealed the significance of evaluating herbicide tolerance in terms of their concentrations among cultivated rice varieties to minimize the crop and yield damage due to herbicide application.

**Keywords**: Pre- and post- planting herbicides, *Oryza* spp., Sri Lanka

## INTRODUCTION

Weeds are a source of biotic stress in crop systems that decrease the yield, increase production costs, and contribute to income risk. Therefore, weed control is an essential component of profitable crop production. Weeds can be controlled mechanically (by cultivation or hoeing), chemically (with herbicides) or by culturally (crop rotation). Most farmers rely on an amalgamation of these methods. In general, a combined approach including herbicides is more economical, and often more effective, than reliance solely on mechanical or cultural control practices (Tu *et al*., 2001).

The discovery of herbicide resistant weeds in the early 1970s generated an interest in mimicking this unintentional development for use in crop breeding. Herbicide resistant crops (HRCs) have been grown commercially since 1984, when the first triazine-resistant oilseed rape cultivar (OAC Triton) was introduced on the Canadian market (Hall *et al*., 1996).

Out of the 20 wild rice species spread throughout the world, five are found in Sri Lanka, i.e. *Oryza nivara*, *O. rufipogon*, *O. eichingeri*, *O. granulata* and *O. rhizomatis*. Studies have shown that many wild rice species exhibit herbicide tolerance (HT) (Hager *et al*., 2003). However, the HT in Sri Lankan wild rice species as well as traditionally cultivated *O*. *sativa* varieties has so far not been evaluated. Similarly the HR in developed *O. sativa* varieties has also not been evaluated so far. Evaluation of HT for broad spectrum herbicides among the Sri Lankan rice species is vital to identify the genes conferring HR. Therefore, it is worthwhile screening the Sri Lankan rice gene pool for their HR for possible incorporation in rice breeding programs.

Sri Lanka's paddy cultivation is threatened by rice weeds and these weeds have already damaged nearly 20 % of the harvest (Abeysekara *et al*., 2010). Application of herbicides is damaging when the cost and evolution of herbicide resistance in weed population is considered. On the other hand if the application of herbicide has negative effects on the rice such as growth retardation and yield loss, this problem will be more detrimental over controlling rice weeds to minimize the rice-yield loss. So far no comprehensive studies carried out to screen the effect of herbicides on rice in Sri Lanka. Since rice is the staple food for more than half of the world's population as well as in Sri Lanka, it is vital to carry out such studies because farmers are continuously applying a massive amount of herbicides on rice crops to reduce the effects of weed populations on the growth performance of rice. The present study was carried out to evaluate the effect of pre- and post- planting herbicides on growth performance and yield of Sri Lankan rice genotypes and to identify any herbicide tolerant rice varieties.

## MATERIALS AND METHODS

A greenhouse experiment was carried out to screen the effect of herbicide application on 13 Sri Lankan rice genotypes, i.e. *Oryza sativa* – developed varieties (BG-360, BG-352, BG-359, AT-306, AT-308), traditional varieties ("Machel", "Kurulutuda", "Madathawaru"), and wild species (*O. nivara*, *O. rufipogon*, *O. eichingeri*, *O. rhizomatis*, *O. granulata*). All rice varieties showed over 85% germination under normal conditions. The soil used for the study was collected from a rice field in Kalutara District where rice is regularly cultivated. The soil composition is; sand -21.3%, silt – 23.0%, clay – 55.6% and pH – 5.4. Count-up® (360 g/l glyphosate)-pre-planting and RiceStar (69 g/l fenoxaprop-p-ethyl)-post-planting herbicides were used in this study. Single and double dose concentrations of 0.5 g/l, 1.0 g/l of Count-up® and 5.0 g/l, 10.0 g/l of RiceStar® were used. There were nine different combinations of herbicide treatments as follows, subjecting 50 plants per treatment: T1 (control), T2 (No pre-:5.0 g/l post-), T3 (No pre-:10.0 g/l), T4 (0.5 g/l pre-:No Post-), T5 (0.5 g/l Pre-:5.0 g/l Post-), T6 (0.5 g/l Pre-:10.0 g/l Post-), T7 (1.0 g/l Pre-:No Post-), T8 (1.0 g/l Pre-:5/0 g/l Post-) and T9 (1.0 g/l Pre-:10.0 g/l Post-). Black polythene bags (height of 60cm and a diameter of 45cm) were used as the pots to grow the plants and the amount of soil in each bag was at the same weight. Complete Randomized Block Design (CRDB) was used in the experiment and there were ten replicates and five blocks in each treatment combination. The recommended time line of application of the herbicides specified by the herbicide manufacturers were followed in the study, i.e. pre-planting herbicide (Count-up®) should be applied seven days prior to planting/sowing and post-planting herbicide (RiceStar®) should be applied three weeks after planting/sowing. Panicles were harvested at the physiological maturity. The variables recorded were seedling emergence time (SET), plant height after two weeks (H1) and one month (H2), whole plant biomass at maturity (BM) were measured. Whole panicle weight (PW) and the weight of total de-husked seed sample (yield) of each of the plant were measured to calculate the productive yield (Y) as follows.

**Productive yield (Y) = (Seed weight/ Whole panicle weight) ×100**

Statistical analyses were carried out using SPSS v. 14.0.

## RESULTS

According to the results given in Table 1, only ten out of the 13 rice genotypes used in this study have shown successful germination on pre-planting herbicide (Count-up®) treated soil. The wild rice genotypes *O. eichingeri*, *O. rhizomatis* and *O. granulata* were not germinated. SET and the H1 were significantly ($p \geq 0.5$) affected by the herbicide Count-up® in 0.5 g/l and 1.0 g/l concentrations for all the rice genotypes. BG- and AT-varieties showed an extended germination time 2.5 and 4.0 days consecutively for the two different concentrations compared to the control (1 day). H1 and H2 were also reduced correspondingly to 12.03 cm and 29.02 cm in BG- varieties and 9.87cm and 28.06 cm in AT-varieties compared to the control (14.83 cm and 42.50 cm). However, the traditional and wild genotypes showed relatively a shorter SET and increased height compared to BG- and AT-varieties.

The growth parameters, H2, BM and yield showed an inhibitory effect by Count-up® and RiceStar®. Yield of the genotypes was reduced compared to controls. Single and double doses of both herbicides indicated a significant yield loss as well as reduced BM. Herbicide tolerance was significantly greater in AT-varieties in terms of yield/plant (5.03g) and BM (189.67g) than BG-varieties which showed an average of 3.98g yield/plant and a BM of 112.56g. Compared to AT- and BG-varieties, the traditional varieties ("Machel", "Kurulutuda", "Madathawaru") showed significantly a higher tolerance to both herbicides in terms of all the recorded parameters. The tolerance was higher in successfully germinated wild genotypes (*O*. *nivara*, *O*. *rufipogon*) compared to all other genotypes.

The percentage yield loss of the, developed varieties is nearly 30% when the plants are treated with single doses of pre- and post-planting herbicides and the treatments with double doses have shown nearly a 80% yield loss. However, the traditional and wild genotypes have shown relatively a less yield loss even under the treatments with double doses of pre- and post-planting herbicides.

The results revealed the significance of evaluating herbicide tolerance in terms of their concentrations among cultivated rice varieties to minimize the crop and yield damage due to herbicide application.

## DISCUSSION

Rice is the staple food for more than half of the world population including Sri Lanka. Herbicides have been used to control rice weeds since the introduction of herbicides as it is the easiest and effective mean of weed control. Early studies reported that herbicides have drastically reduced the yield with similar effect as weed population (Hager *et al*., 2003). However, there were no comprehensive studies carried out on effects of pre- and post- planting herbicides on rice in Sri Lanka.

The previous studies have revealed that there is a significant reduction of rice yield due to application of pre- planting herbicides (Davis *et al*., 2009). Franz *et al*.(1997) and Schuette,

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(1998) suggested that the chemical characteristics of Glyphosate such as hydrolysis half life (>35 days), soil adsorption coefficient (average over several soil pH levels) (61 g/m$^3$), anaerobic half life (22.1 days), aerobic half life (96.4 days), field dissipation days (44 days) are high and its mobility, tendency to leach in soil and volatility are very low, hence cause unfavorable effects on growth performance and yield of rice  In consistent with the previous studies, the present study has also revealed that pre- and post-planting herbicides have adverse effects on the growth performance and yield of rice genotypes available in Sri Lanka. The effects of Count-up® were significant which extended the seedling emergence time and consequently reduced the plant height at seedling stage. This has resulted in reduction of biomass of the plant and the yield. RiceStar® imposed a significant effect on plant height and biomass at mature stage and yield.

The current study proved that some of the cultivating rice varieties have nearly reduced 30% yield due to application of single dose (recommended dose of the manufacturer) of pre- and post- planting herbicides. However the same treatment has no effect on some rice weeds such as *Echinochloa* sp., *Cyperus rotundus* and *Ludwigia prostrata*. The double dose application of the pre- and post- planting herbicides has completely eradicated those weeds, but caused about 80% reduction in yield. In this study, the traditional and wild rice genotypes showed tolerance even to the double dose application of herbicides with a minimal yield loss of about 25%. These rice varieties may harbor herbicide tolerant genes which possibly will be able to incorporate in rice breeding programs.

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**Table 1**. Growth parameters and Yield data of the 13 rice genotypes used in the study

(GT- Genotype; T- Treatment; SET-Seedling emergence time; PW – Panicle weight (g); BM- Biomass (g); Y –Productive Yield; H1 – Height after 2 weeks; H2- Height after 4 weeks; NG- Not germinated; NA- Not Applicable)

| GT | T | SET | height (cm) | | yield (g) | BM | Y | % yield |
|---|---|---|---|---|---|---|---|---|
| | | | H1 | H2 | | | | |
| BG 360 | T1 | 2.0 | 12.26 | 35.26 | 3.73 | 152.43 | 92.00 | 98 |
| | T2 | 2.0 | 12.58 | 31.46 | 2.574 | 140.87 | 90.50 | 69 |
| | T3 | 2.0 | 12.11 | 22.43 | 1.082 | 112.57 | 89.85 | 29 |
| | T4 | 2.5 | 12.14 | 33.49 | 2.574 | 127.98 | 90.00 | 69 |
| | T5 | 2.5 | 12.02 | 27.48 | 2.574 | 140.47 | 90.20 | 69 |
| | T6 | 2.5 | 12.03 | 21.01 | 1.044 | 112.76 | 88.90 | 28 |
| | T7 | 4.0 | 8.05 | 21.21 | 0.933 | 113.67 | 87.50 | 25 |
| | T8 | 4.0 | 8.68 | 21.46 | 1.082 | 115.64 | 87.20 | 29 |
| | T9 | 4.0 | 7.91 | 19.33 | 0.746 | 98.25 | 85.26 | 20 |
| BG 352 | T1 | 1.0 | 13.93 | 42.50 | 5.25 | 187.56 | 90.00 | 97 |
| | T2 | 1.0 | 13.95 | 37.36 | 3.728 | 172.49 | 89.50 | 71 |
| | T3 | 1.0 | 13.90 | 32.56 | 1.628 | 152.52 | 87.85 | 31 |
| | T4 | 1.5 | 13.00 | 37.03 | 3.675 | 170.36 | 89.00 | 70 |
| | T5 | 1.0 | 13.37 | 38.27 | 3.675 | 164.89 | 88.20 | 70 |
| | T6 | 1.5 | 13.05 | 30.26 | 1.575 | 142.76 | 86.90 | 30 |
| | T7 | 2.5 | 10.05 | 32.99 | 1.523 | 135.68 | 85.50 | 29 |
| | T8 | 2.5 | 10.20 | 32.57 | 1.575 | 132.78 | 85.20 | 30 |
| | T9 | 2.5 | 9.78 | 25.44 | 1.523 | 118.84 | 85.26 | 29 |
| BG 359 | T1 | 2.0 | 11.10 | 32.86 | 2.98 | 122.89 | 95.00 | 98 |
| | T2 | 2.5 | 12.02 | 27.02 | 2.086 | 120.25 | 93.50 | 70 |
| | T3 | 2.0 | 12.11 | 22.05 | 0.894 | 118.04 | 89.75 | 30 |
| | T4 | 2.5 | 11.32 | 25.22 | 2.056 | 120.36 | 92.00 | 69 |
| | T5 | 2.5 | 11.46 | 25.35 | 2.056 | 118.02 | 93.20 | 69 |
| | T6 | 2.5 | 11.05 | 22.63 | 0.864 | 115.52 | 88.90 | 29 |
| | T7 | NG | NA | NA | NA | NA | NA | NA |
| | T8 | NG | NA | NA | NA | NA | NA | NA |
| | T9 | NG | NA | NA | NA | NA | NA | NA |
| AT 306 | T1 | 2.0 | 12.57 | 40.53 | 3.59 | 145.95 | 91.05 | 96 |
| | T2 | 2.5 | 12.52 | 37.05 | 2.621 | 142.05 | 90.50 | 73 |
| | T3 | 2.5 | 12.41 | 32.05 | 1.257 | 142.32 | 88.85 | 35 |
| | T4 | 2.0 | 11.12 | 35.89 | 2.585 | 144.09 | 90.00 | 72 |
| | T5 | 2.5 | 11.92 | 35.26 | 2.585 | 144.69 | 90.20 | 72 |
| | T6 | 2.5 | 12.03 | 30.26 | 1.149 | 130.05 | 87.90 | 32 |
| | T7 | 3.5 | 10.52 | 33.52 | 1.113 | 132.05 | 87.50 | 31 |
| | T8 | 3.5 | 10.67 | 31.45 | 1.149 | 132.25 | 87.20 | 32 |
| | T9 | 3.5 | 10.70 | 28.06 | 1.077 | 125.26 | 87.02 | 30 |
| AT 308 | T1 | 1.0 | 14.83 | 37.89 | 3.87 | 137.27 | 97.00 | 100 |
| | T2 | 1.0 | 14.52 | 35.26 | 2.903 | 137.05 | 95.50 | 75 |
| | T3 | 1.0 | 14.47 | 33.01 | 1.393 | 132.25 | 95.30 | 36 |
| | T4 | 1.5 | 13.25 | 34.25 | 2.786 | 136.25 | 95.60 | 72 |
| | T5 | 2.0 | 13.57 | 33.26 | 2.786 | 136.85 | 95.60 | 72 |
| | T6 | 1.5 | 13.20 | 33.52 | 1.238 | 130.26 | 87.90 | 32 |
| | T7 | 2.5 | 12.76 | 30.12 | 1.2 | 130.56 | 87.50 | 31 |
| | T8 | 2.5 | 12.58 | 30.59 | 1.238 | 130.02 | 87.20 | 32 |
| | T9 | 2.0 | 12.50 | 28.53 | 1.122 | 129.28 | 86.20 | 29 |
| Mada-thawaru | T1 | 1.5 | 10.05 | 29.08 | 2.87 | 209.46 | 97.0 | 97 |
| | T2 | 1.5 | 10.16 | 28.02 | 2.066 | 205.28 | 92.50 | 72 |
| | T3 | 1.5 | 10.20 | 25.02 | 1.866 | 195.28 | 92.30 | 65 |
| | T4 | 2.0 | 9.85 | 27.56 | 2.009 | 202.05 | 92.60 | 70 |
| | T5 | 2.0 | 9.67 | 28.02 | 2.066 | 202.25 | 92.60 | 72 |
| | T6 | 2.0 | 9.79 | 24.89 | 1.751 | 170.26 | 90.90 | 61 |
| | T7 | NG | NA | NA | NA | NA | NA | NA |
| | T8 | NG | NA | NA | NA | NA | NA | NA |
| | T9 | NG | NA | NA | NA | NA | NA | NA |
| *O. nivara* | T1 | 1.0 | 15.45 | 36.78 | 4.65 | 198.67 | 98.00 | 98 |
| | T2 | 1.0 | 15.44 | 36.82 | 4.092 | 199.56 | 97.50 | 88 |
| | T3 | 1.0 | 15.67 | 34.26 | 3.86 | 195.27 | 96.20 | 83 |
| | T4 | 1.0 | 15.14 | 36.02 | 3.999 | 198.25 | 97.45 | 86 |
| | T5 | 1.0 | 15.02 | 36.02 | 4.092 | 198.89 | 97.60 | 88 |
| | T6 | 1.0 | 14.98 | 34.02 | 3.813 | 196.21 | 96.50 | 82 |
| | T7 | 1.0 | 12.39 | 32.56 | 3.534 | 196.25 | 96.20 | 76 |
| | T8 | 1.0 | 12.37 | 32.01 | 3.674 | 196.28 | 96.70 | 79 |
| | T9 | 1.5 | 12.47 | 32.05 | 3.488 | 192.05 | 96.26 | 75 |
| *O. rufipogon* | T1 | 1.5 | 14.88 | 30.23 | 5.34 | 178.94 | 97.36 | 97 |
| | T2 | 1.5 | 14.99 | 30.02 | 4.646 | 177.86 | 97.20 | 87 |
| | T3 | 1.5 | 15.02 | 29.89 | 4.646 | 177.26 | 96.26 | 87 |
| | T4 | 2.0 | 14.02 | 30.00 | 4.646 | 178.05 | 96.85 | 87 |
| | T5 | 2.0 | 14.25 | 30.00 | 4.699 | 177.56 | 97.02 | 88 |
| | T6 | 1.5 | 14.16 | 29.85 | 4.539 | 175.28 | 96.56 | 85 |
| | T7 | 2.5 | 12.55 | 29.29 | 4.539 | 172.56 | 95.28 | 85 |
| | T8 | 1.5 | 12.78 | 29.25 | 4.592 | 170.29 | 96.45 | 86 |
| | T9 | 2.5 | 12.47 | 29.02 | 4.379 | 165.30 | 92.03 | 82 |
| Machel | T1 | 1.0 | 9.77 | 20.57 | 2.95 | 155.86 | 93.00 | 99 |
| | T2 | 1.0 | 9.68 | 20.01 | 2.626 | 155.56 | 91.50 | 89 |
| | T3 | 1.0 | 9.75 | 20.15 | 2.331 | 153.28 | 89.85 | 79 |
| | T4 | 1.0 | 9.70 | 20.26 | 2.508 | 155.26 | 91.00 | 85 |
| | T5 | 1.0 | 9.65 | 20.01 | 2.626 | 155.02 | 91.20 | 89 |
| | T6 | 1.0 | 9.62 | 19.02 | 2.301 | 152.69 | 88.90 | 78 |
| | T7 | 1.5 | 9.17 | 18.54 | 2.124 | 150.38 | 87.50 | 72 |
| | T8 | 1.5 | 9.02 | 18.26 | 2.095 | 152.02 | 87.20 | 71 |
| | T9 | 1.0 | 9.07 | 18.04 | 2.095 | 150.26 | 85.26 | 71 |
| Kurulu-thuda | T1 | 1.0 | 12.03 | 30.27 | 3.46 | 125.73 | 92.00 | 95 |
| | T2 | 1.0 | 12.13 | 29.75 | 2.768 | 125.02 | 90.50 | 80 |
| | T3 | 1.0 | 12.05 | 28.12 | 2.733 | 120.06 | 89.85 | 79 |
| | T4 | 1.0 | 11.99 | 29.88 | 2.768 | 125.32 | 90.00 | 80 |
| | T5 | 1.0 | 12.03 | 29.86 | 2.733 | 125.03 | 90.20 | 79 |
| | T6 | 1.0 | 12.20 | 28.01 | 2.733 | 120.32 | 88.90 | 79 |
| | T7 | 1.5 | 10.98 | 28.12 | 2.699 | 119.00 | 87.50 | 78 |
| | T8 | 1.0 | 10.95 | 27.26 | 2.699 | 119.25 | 87.20 | 78 |
| | T9 | 1.0 | 11.14 | 28.02 | 2.664 | 115.02 | 85.26 | 77 |

**O. granulata*, *O. rhizomatis* and *O. eichingeri* Did not show any successful growth under pre- planting herbicide treatments.

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## REFERENCES

Abeysekara, A.S.K., Nugaliyadde, L., Herat, H.M.S., Wickrame, U.B. and Iqbal, Y.B. (2010). Weedy rice: a threat to direct seeded rice cultivation in Sri Lanka. Rice Congress 2nd – 3rd December, Gannoruwa, Sri Lanka. pp. 17-18.

Davis, B.M., Scott, R.C., Norsworthy, J.K., and Smith, K.L. (2009). Effects of Low Rates of Glyphosate and Glufosinate on Rice, AAES Research Series 581

Franz, J.E., Mao, M.K. and Sikorski, J. A.. (1997). Glyphosate: A Unique Global Herbicide. American Chemical Society., Chap. 4:65-97

Hager, A.G., Wax, L.M., Bollero, G.A. and Stoller, E.W. (2003). Influence of Diphenylether Herbicide Application Rate and Timing on Common Water hemp (Amaranthus rudis) Control in Soybean (Glycine max), *Weed Technology* 17(1): 14-20.

Hall, J.C., Donnelly-Vanderloo, M.J. and Hume, D.J. (1996). Triazine-resistant crops: The agronomic impact and physiological consequences of chloroplast mutation. *In* Duke, S.O. ed., Herbicide-resistant crops. Agricultural, Environmental, Economic, Regulatory and technical aspects. USA, CRC Press. pp. 107-126.

Schuette J. (1998). Environmental fate of Glyphosate, Environmental Monitoring and Pest Management, Department of Pesticide Regulation, Sacramento, CA 95824-5624

Tu, M., Hurd, C. and Randall, J.M. (2001). Weed Control Methods Handbook, The Nature Conservancy, Wildland Invasive Species Team, version April 2001

# WEEDSEEKER TECHNOLOGY CAN BE USED EFFECTIVELY IN WIDE-ROW CROPS IN DRYLAND BROADACRE FARMING SYSTEMS

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## ABSTRACT

The adaptability and suitability of WeedSeeker technology for use in row crops in dryland broadacre farming systems has been determined from four field trials conducted in central Queensland in 2009/10. Inter-row post-emergence weed control efficacy and crop safety (tolerance) of glyphosate and or paraquat applied via tractor mounted shielded spray equipment fitted with detachable WeedSeeker units were measured in both chickpea and sorghum crops grown on 1 m rows. Good weed control with minimal lasting crop damage (for either crop or herbicide) supports the in-crop use of WeedSeeker technology particularly where it is used in conjunction with banded on-the-row residual pre-emergence herbicides. Economic benefits to farmers and safety to the environment can be maximised through the reduced physical amount of herbicide being applied particularly where this technology is employed in-crop as well as in the fallow where it is currently and mostly utilised. In-crop WeedSeeker use will also facilitate the cost-effective application of the more expensive herbicide products that may be required for managing herbicide resistant weeds.

**Keywords:** WeedSeeker, in-crop use, chickpea, sorghum, banded herbicide

## INTRODUCTION

WeedSeeker technology uses advanced optics (infra-red) detection units to activate spray nozzles such that herbicide is only applied to a plant target and its immediate small surrounding area. Since weeds of cropping country are not often fence to fence, the technology offers large savings in the amount of chemical applied across a total area since spray nozzles are only operating when a plant is detected (cost-effective spot spraying but on a very large scale). Currently, in Australian farming systems WeedSeeker is being utilised mostly for fallow weed management using non-selective herbicides (Jameson, 2009).

The ability to use WeedSeeker in-crop with shielded booms will facilitate zonal weed management which could be considered a component of precision agriculture. Zonal weed management means residual herbicides can be precisely banded over the crop rows at planting (using conventional spray technology) and the inter-row zones can be treated post-emergence with non-residual products via WeedSeeker but only where weeds are present. The combination of the two technologies may result in a great reduction in the amount of all

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types of herbicide used without compromising good weed control. Banding is likely to result in 50-66% reduction (row spacing and bandwidth dependent) in physical amounts of residual herbicides applied; while WeedSeeker can result in up to 90% (weed density dependent) reduction in physical amounts of knock-downs applied.

Significant herbicide reductions represent large input costs savings to growers thereby improving farm profitability. WeedSeeker will also permit the cost-effective use of the more expensive herbicide products, thus facilitating greater herbicide mode of action rotation. This potential benefit will increase in importance as more weed species develop resistance to the cheaper and more often used chemistries. The reduction in the physical amount of herbicide applied across catchments will also reduce the risks to sensitive ecological habitats within the land and seascapes (eg. the Great Barrier Reef).

Research and development trials have been conducted in central Queensland during 2009/10 to evaluate the suitability and effectiveness of shielded WeedSeeker use in wide-row sorghum and chickpea using paraquat and glyphosate for the inter-row weed control. Two research questions were addressed – (a) does WeedSeeker with shields reduce the potential damage to crop when using non-selective herbicides compared to the same herbicides applied with shields but without WeedSeeker? And (b) does shielded WeedSeeker provide sufficient control of weeds in the inter-row when using non-selective systemic and contact herbicides?

## MATERIALS AND METHODS

Four replicated randomised block trials (two chickpea and two sorghum) were undertaken in 2009 and 2010 on the DEEDI research block located at the Australian Agricultural College Emerald campus on a heavy alluvial clay soil type.

Chickpea (var. *Kyabra*) was planted in winter 2009 and 2010 on 1 m row spacing with target established populations of 300 000 plants/ha. In the 2009 trial, no residual herbicides were applied, however, in 2010 simazine (1 kg ai/ha) mixed with isoxaflutole (75 g ai/ha) was applied immediately post-planting as either a 50 cm wide band over the rows or as a blanket application (entire plot). In-crop, post-emergence herbicide treatments were applied before canopy closure and while weeds were small to medium in size. Glyphosate (900 g ai/ha) or paraquat (500 g ai/ha) were applied using a shielded boom (mounted spray hoods that hang between the crop rows) with and without WeedSeeker technology attached. The tractor mounted boom spray operating details for all (chickpea and sorghum) trials are presented in Table 1, although details for the residual herbicide applications are not provided.

Sorghum (var. *Buster*) was planted early summer (2009/10 trial) and late summer (2010 trial) on 1 m row spacing with target established populations of 50 000 plants/ha. Residual herbicide (atrazine 2 kg ai/ha) was applied as either a blanket at 21 days pre-plant or as a 50 cm band over the row immediately post-planting. Post-emergence in-crop herbicides were applied once the crop had established secondary roots and while the weeds were still small to medium in size. These post-emergence treatments included fluroxypyr (250 g ai/ha), glyphosate (900 g ai/ha), paraquat (500 g ai/ha) or glyphosate + fluroxypyr (900 g ai + 250 g ai/ha) applied using the shielded boom with and without WeedSeeker fitted.

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Weed-free (hand-chipped) and weedy controls were included in all replicates in all trials.

All trials were irrigated within a week of planting to ensure incorporation of the residual herbicides and to assist with crop and weed emergences. All crops (except the 2009 chickpea trial) were mechanically harvested using a small plot Kew header.

**Table 1.** Boom spray details for all trials undertaken.

| Year | Crop studied | Boom type | Nozzles used | Output (L/ha) |
|---|---|---|---|---|
| 2009 | chickpea | + WeedSeeker | TP6503EVS | 104 |
| | | nil WeedSeeker | DG110015 | 98 |
| 2010 | chickpea | + WeedSeeker | TP6503EVS | 173 |
| | | nil WeedSeeker | DG110015 | 81 |
| 2009/10 | sorghum | + WeedSeeker | DG8503 | 107 |
| | | nil WeedSeeker | DG110015 | 95 |
| 2010 | sorghum | + WeedSeeker | 80015EVS | 142 |
| | | nil WeedSeeker | DG110015 | 93 |

### Data Measurements

Visual weed control and crop injury ratings, as well as weed and crop biomass sampling was undertaken in all trials. However, insufficient weed pressure/density in both sorghum trials did not warrant detailed weed biomass sampling measurements. Crop yields were measured in 3 of the 4 trials. All data were subjected to analysis of variance using Genstat statistical package (11th Edition). LSD values are provided where the F probability tests were significant, unless otherwise indicated. For brevity, not all data sets are being presented.

## RESULTS AND DISCUSSION

### Impacts on the Weeds

The main weeds present in the chickpea crops included sow thistle (*Sonchus oleraceus*), African turnip weed (*Sisymbrium thellungii*), peppercress (*Lepidium* sp.) and prickly lettuce (*Lactuca serriola*). Very isolated infestations of bladder ketmia (*Hibiscus trionum*), boggabri (*Amaranthus mitchellii*), crownbeard (*Verbesina encelioides*), native jute (*Corchorus trilocularis*) and awnless barnyard grass (*Echinochloa colona*) occurred in the sorghum trials. Mean total weed densities were greater in winter (2-5 weeds/m$^2$) than in summer (0-0.5 weeds/m$^2$).

<u>Key general findings</u>

- WeedSeeker use with shields provided effective weed control in the inter-row using either glyphosate or paraquat (Tables 2, 3 and 4).
- Tendency for WeedSeeker to be slightly less effective than full spraying but the differences across the trials were not significant.
- Achieved ~90% or greater weed biomass reduction in the inter-row zone when using shielded WeedSeeker.

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- Some form of on-the-row weed control is still necessary to minimise weed-seed production across the paddock.
- While banded on-the-row residuals with shielded WeedSeeker use in the inter-rows were very effective, overall weed management was slightly better when the residuals were blanket applied (Table 2). The blanket applied residuals tended to provide some *assurance* to the overall weed control.

## Impacts on the Crops

Key general findings

- Application of glyphosate or paraquat via shields with or without WeedSeeker proved quite safe in both crops with neither herbicide being significantly more damaging than the other (Tables 2, 3 and 4).
- Any damage measured was minor (< 10% biomass reduction) and mainly transient. Greatest crop reduction was sustained in the untreated (weedy) controls and resulted from competition.
- Differences in levels of crop damage between ± WeedSeeker were not significant for either crop or herbicide, although WeedSeeker use tended to appear to be less damaging (since herbicide is being spot sprayed, there is much less exposure risk).
- Use of WeedSeeker and shields in crop is limited to the period prior to canopy closure; this will vary from crop to crop, and will be affected by row spacing. Wide-row (≥ 1 m) crops better facilitate the use of shields. High clearance equipment may be needed for applications in "taller" crops.
- In the sorghum trials, an extra shield was required in front of the external-mounted WeedSeeker units (external to the spray hood) to push and lift the overhanging crop foliage from the path of the units and hood – overhanging foliage when detected by the units triggered the spray nozzles (unnecessarily).

**Table 2.** Weed and crop biomass, visual inter-row weed control and crop damage assessments and crop yield for the 2010 chickpea trial with and without WeedSeeker (WS) use in-crop.

| Residual herbicide method# | In-crop treatment | Weed biomass ($g/m^2$) | Weed control rating (0-5 scale)* | Crop biomass ($g/m^2$) | Crop injury Rating (0-5 scale)* | Crop yield (t/ha) |
|---|---|---|---|---|---|---|
| blanket | + WS paraquat | 0 | 5 | 359 | 0.2 | 0.95 |
| blanket | nil WS paraquat | 0 | 5 | 375 | 0 | 0.86 |
| blanket | + WS glyphosate | 7.3 | 4.2 | 406 | 0 | 1.21 |
| blanket | nil WS glyphosate | 0 | 5 | 434 | 0 | 1.03 |
| banded | + WS paraquat | 22.3 | 2.5 | 288 | 0.3 | 0.94 |
| banded | nil WS paraquat | 8.8 | 3.8 | 328 | 0.3 | 1.02 |
| banded | + WS glyphosate | 28.2 | 4.2 | 313 | 1 | 1.00 |
| banded | nil WS glyphosate | 12.3 | 4.7 | 311 | 0.5 | 1.04 |
| nil | hand-chipped | 13.0 | 3.3 | 349 | 0 | 0.81 |
| nil | nil | 108.0 | 0 | 278 | 0.3 | 0.59 |
| | LSD (P = 0.05) | 22.1 | 1.7 | 109 | ns | 0.26 |

* 0 – 5 visual assessment scales: for weed control, 0 = no control and 5 = 100% kill; for crop injury, 0 = no effects and 5 = crop death; #residual herbicide = simazine + isoxaflutole (1 kg + 75 g/ha) for both methods

**Table 3.** Weed and crop biomass (actual and reductions) and crop yield pooled for main in-crop treatments only in the 2010 chickpea trial.

| Main in-crop treatment | Weed biomass (g/m2) | % weed biomass reduction | Crop biomass (g/m2) | % crop biomass reduction | Crop Yield (t/ha) |
|---|---|---|---|---|---|
| + WeedSeeker | 14.4 | 87 | 342 | 0 | 1.02 |
| nil WeedSeeker | 5.3 | 95 | 362 | 0 | 0.99 |
| LSD (P = 0.05) | 9.8* | ns* | ns | ns | ns |
| glyphosate | 11.9 | 89 | 366 | 0 | 1.07 |
| paraquat | 7.8 | 93 | 337 | 0 | 0.94 |
| LSD (P = 0.05) | ns | ns | ns | ns | ns |

*F probability value in the ANOVA was 0.06

**Table 4.** Crop yields (both trials) as well as crop biomass and inter-row weed control assessment (2010 trial only) for main in-crop treatments in sorghum. (Visual rating scale: 0 - 5, where 0 = no control and 5 = 100% kill)

| Main in-crop treatment | 2009/10 trial | 2010 trial | | |
|---|---|---|---|---|
| | Crop yield (t/ha) | Crop yield (t/ha) | Crop biomass (g/m$^2$) | Inter-row weed control rating (0-5 scale) |
| + WeedSeeker | 3.1 | 2.3 | 325 | 4.6 |
| nil WeedSeeker | 3.2 | 2.3 | 320 | 4.9 |
| LSD (P = 0.05) | ns | ns | ns | 0.2 |
| fluroxypyr | 3.6 | 2.4 | 323 | 4.6 |
| glyphosate + fluroxypyr | 2.9 | 2.2 | 316 | 4.8 |
| glyphosate | 2.9 | 2.3 | 319 | 5.0 |
| paraquat | 3.2 | 2.4 | 332 | 4.8 |
| LSD (P = 0.05) | ns | ns | ns | 0.3 |

These data (presented in all tables) demonstrate the safety and effectiveness of using shielded WeedSeeker in-crop (in sorghum and chickpea) for applications of paraquat or glyphosate to the emerged weeds within the inter-row. Overall weed management was enhanced when residual herbicide was applied at planting as a band over the crop row. While blanket applications of residuals at planting provided greater weed management assurance, their use via this method did not provide any reduction in the physical amount of residual chemical applied compared to when banded (100% of plot covered with blanket *cf*. 50% plot covered with 50 cm bands on the rows, where rows are 1 m apart). Growers will need to consider the trade-off in cost-savings versus the likely levels of weed control achievable for each situation

Since the spot spraying of glyphosate or paraquat via WeedSeeker provided very effective weed control, it can be assumed that other herbicides with knockdown activity should behave similarly if applied in the same manner. However, crop safety responses may not be the same for all herbicides. Spot spraying of the more expensive chemicals, for example, the group A grass selectives, group I broadleaf selectives, glufosinate, and amitrole, may prove to be very cost-effective using shielded WeedSeeker technology in crop, although their use may not be registered for the situation or application method. But the ability to utilise these herbicides from different modes of action groups will assist in managing resistant weeds and or avoiding development of resistance in high risk weeds.

These small scale studies have shown that WeedSeeker used with shields is adaptable and suitable (effective and safe) for use in broadacre row cropping. However, before wider on-farm adoption can occur, further development work is needed to (a) make these herbicides and uses *legal*, and (b) adapt shielded WeedSeeker technology at the larger paddock scale using commercial size booms.

## ACKNOWLEDGEMENTS

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WeedSeeker is patented spray technology owned by NTech Industries Inc. (a Trimble company), USA. The technology import and distribution licence within Australia is currently held by Crop Optics Pty Ltd, Tamworth, NSW.

## REFERENCES

Jameson, S. (2009). *Crop Optics Pty Ltd*., Pers. comm.

# INHIBITORY EFFECTS OF *MELIA AZEDARACH* L. LEAF EXTRACTS ON TWO WEED SEEDS

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## ABSTRACT

Aqueous extracts from 3 leaf stages of *M. azedarach* (young leaves, mature leaves and old leaves) were assayed at concentrations of 12.5, 25, 50 and 100 mg/mL for their effects on seed germination and seedling growth of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and wild pea (*Phaseolus lathyroides* L.). The results showed that all aqueous extracts at all concentrations inhibited seed germination and seedling growth of both bioassay species compared with control and the degree of inhibition increased with the incremental extract concentration. However, degree of inhibition was significantly different between two bioassay indicator species. All aqueous extracts more strongly inhibited seed germination and seedling growth of *P. lathyroides* than growth of *E. crus-galli*. Extracts from young leaves had higher inhibitory potential than those from the mature or old leaves. Thus, aqueous extract from young leaves was selected to efurther evaluate some physiological processes inhibited during seed germination. Aqueous extracts of young leaves (12.5 to 100 mg/mL) were used to determined their impacts upon water uptake and α-amylase activity of *E. crus-galli*. It was found that both water uptake and α-amylase activity of *E. crus-galli* were inhibited and inhibition degree increased with increasing concentration. Based on these results we conclude that young leaves of *M. azedarach* contained water soluble allelochemicals and caused inhibition of both water uptake and α-amylase activity of *E. crus-galli* during the germination process.

**Keywords:** *Melia azedarach* L., leaf extract, water uptake, α-amylase activity, weed seed inhibition

## INTRODUCTION

In recent times, studies on utilization of plants with strong allelopathic potential for reducing weed control and minimizing the dependency on synthetic herbicides have been widely published. Allelopathic activity in plants and potential of allelochemical activity for weed control can be utilized in various ways for weed management; residues used as mulch or soil incorporated, or isolated for active compounds and future development of bioactive extracts. Numerous plant residues have been suggested for useas alternative weed management agents. Establishing that allelopathic activity is actually present in the extracts of many higher plants and in many plant organs can be accomplished with bioassays under laboratory conditions.

Initial work performed with laboratory assays of allelochemicals has generally focused on seed germination and seedling growth (Vyvyan 2002). The bioassay chosen for studying the mode of action of these natural compounds is an important consideration. Water uptake and α-amylase (EC 3.2.1.1) activity is consistently linked with the germination process. Seeds begin to germinate after imbibition of an adequate moisture level and become metabolically active. Continuing water uptake activates stored hydrolytic enzymes and stimulates synthesis of new enzymes (Chong *et al*. 2002). These hydrolytic enzymes are involved in the hydrolysis and transformation of the endosperm starch into soluble sugars to provide nutrition or energy during early seed germination and seedling growth. Principal among these is α-amylase which catalyzes endohydrolysis of α-1-4 glucosidic linkages in starch and any related oligosaccharides to make oligosaccharides and glucose (Taiz & Zeiger 2006). The measurement of water uptake and α- amylase activity can be used to assess changes in germination efficiency of the seeds treated with allelochemical substances.

The present study was designed to examine allelopathic activities of *M. azedarach* extracts on germination and growth of *Echinochloa crus-galli* (L.) Beauv.) and wild pea (*Phaseolus lathyroides* L.) seedlings. Further physiological studies were to be undertaken in order to determine the mechanisms of action of this extract during seed germination.

## MATERIAL AND METHODS

### Plant Materials

The *Melia azedarach* L. plants were grown in the region of the experimental field of King Mongkut's Institute of Technology Ladkrabang, Bangkok. Young, mature and old leaves were collected during rainy season 2010. They were cleaned several times with tap water and dried in a drying oven at 45 °C for 72 h. They were then cut into 1 cm pieces, ground into powder in a blender, and sieved through a 40 mesh (420 μm) sieve.

### Aqueous Extract Bioassay

Aqueous extracts were prepared from the dried young, mature and old leaves of *M. azedarach* L. by dissolving 10 g of each powdered material in 100 mL of distilled water at 10 °C for 72 h, followed by filtration through three layers of cheesecloth to remove any debris. The supernatant was then filtered through Whatman No. 1 filter paper to a concentration of 100 g/L of dried plant material and stored in a refrigerator at 5 °C until bioassay. Dilutions of the *M. azedarach* L. extract of 12.5, 25, 50 and 100 g/L were prepared in distilled water. Seeds of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) were collected from paddy fields in the Ladkrabang district, Thailand, placed in the shade at room temperature for three months, and then incubated in a hot-air oven at 45 °C for 48 h to break their dormancy. Seeds of wild pea (*Phaseolus lathyroides* L.) were collected from an upland field production site. Twenty healthy seeds of both bioassay plants (which had imbibed for 24 h in distilled water) were placed in separate Petri dishes (9 cm in diameter) lined with two sheet of germination paper, which was moistened with 5 mL of 12.5, 25, 50 and 100 g/L of the each aqueous extract. Four replicates were maintained per treatment for each of the two bioassay species in a completely randomized manner in a growth chamber at a temperature of 25–32 °C, a 12/12 h dark/light photoperiod, and relative humidity of around 80%. Treatments with distilled water only were used as the controls. Germination was deemed to have occurred only after the

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radicle had protruded beyond the seed coat by at least the dimension of the seed at seven days after treatment. Seedling growth was measured as the root and shoot lengths at seven days after treatment. Germination (G), shoot length (SL) and root length (RL) (% of the control) was calculated as follows:

G, SL or RL (% of control) = (sample extracts/control) $\times$ 100

**Water Uptake and Assay of $\alpha$-amylase Activity**

Measurement of seed imbibition was performed using the method of Turk and Tawaha (2003). Four replicates of 100 *E. crus-galli* seeds were weighed and recorded as the original seed weight ($W_1$). These seeds were separately germinated in 7 mL of aqueous extract of young leaf of *M. azedarach* L. (0.625–10 g/L), distilled water as the control. Seed weights were recorded as the final seed weight ($W_2$) for each concentration and exposure time. Water uptake percentage was calculated from the following equation:

Water uptake (%) = $[(W_2\text{-}W_1)/W_1]$ X 100

Measurement of activity of α-amylase was performed by following the method of Bernfield (1955) and Sadasivam and Manickam (1996). After measuring water uptake, seeds (100 seeds for one determination) were homogenized with 4 mL iced-cold solution of 0.1 M $CaCl_2$ and centrifuged at 2000×g for 10 min. Supernatant was used as the enzyme extract. The $\alpha$-amylase was then assayed by measuring the rate of generation of reducing sugars from soluble starch. The reaction medium (3 mL) contained 1 mL of 1% soluble starch in acetate buffer solution at pH 5.5 and 1 mL of the enzyme. The assay medium was incubated for 15 min at 37°C. The reaction was terminated by addition of 1 mL DNS reagent (40 mM 3,5 dinitrosalicylic acid, 0.4 N NaOH and 1M K-Na tartrate), and immediately heated in a boiling water bath for 5 min. The mixture was cooled under running tap water. A total volume was made up to 7 mL with distilled water. The intensity of color was measured as absorption at 560 nm in a spectronic GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA). A standard graph was prepared using maltose, and the amount of $\alpha$-amylase present in the sample was calculated from the standard curve and expressed as $\mu$mol maltose/min/g(FW).

## RESULTS

**Effects of three leaf stages of *M. azedarach* aqueous extracts on germination and initial seedling growth of two weed species**

The allelopathic potential of aqueous extracts of the young, mature and old leaves of *M. azedarach* L. was evaluated on the germination and initial seedling growth of *E. crus-galli* and *P. lathyroides* (Figure. 1). There were significant differences in inhibition activity among aqueous extract from different leaf growth stages. The young leaf stage aqueous extract exhibited stronger inhibitory activity against *E. crus-galli* than mature leaves and old leaf extracts. Aqueous extracts of *M. azedarach* at all treatments markedly reduced the percentage seed germination of *E. crus-galli* bioassay species in various degrees compared with that of the distilled water control. *E. crus-galli* germinated at frequencies of 5%, 25%,

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50%, and 100% in aqueous young leaf exracts at concentrations of 12.5, 25, 50 and 100 g/L, respectively, but completely inhibited germination and seedling growth of *P. lathyroides*. These results indicated that the inhibitory effect of the extract varied with the weed indicator species. All extracts significantly reduced the initial seedling growth of *E. crus-galli*, except the 12.5 g/L concentration, with which there was a negligible and nonsignificant reduction of the root and shoot lengths of *E. crus-galli* bioassay species. At higher concentrations (25–100 g/L), the shoot and root lengths of of *E. crus-galli* was markedly reduced and the inhibitory effects were stronger on root length than on shoot length. We noted that only the highest concentration (50-100 g/L) completely inhibited the seedling growth of the *E. crus-galli* test species.

**Water uptake and assay of α-amylase activity**

The results showed that aqueous extract of young leaf stage of *M. azeradach* reduced water uptake and α-amylase activities of *E. crus-galli* seeds. Data further showed the differences in the percentage of water uptake between control and treated *E. crus-galli* seeds with increasing concentration application of aqueous extracts at different imbibition periods (Figure. 2). The percentage of water uptake in the control seeds exhibited marked increase with prolonged period of imbibitions. The time required for 21.96%, 25.59% and 68.70% of water uptake was 12, 24 and 48 h, respectively. With similar concentrations, the percentage of water uptake in treated seeds increased by prolongation of the imbibition period.

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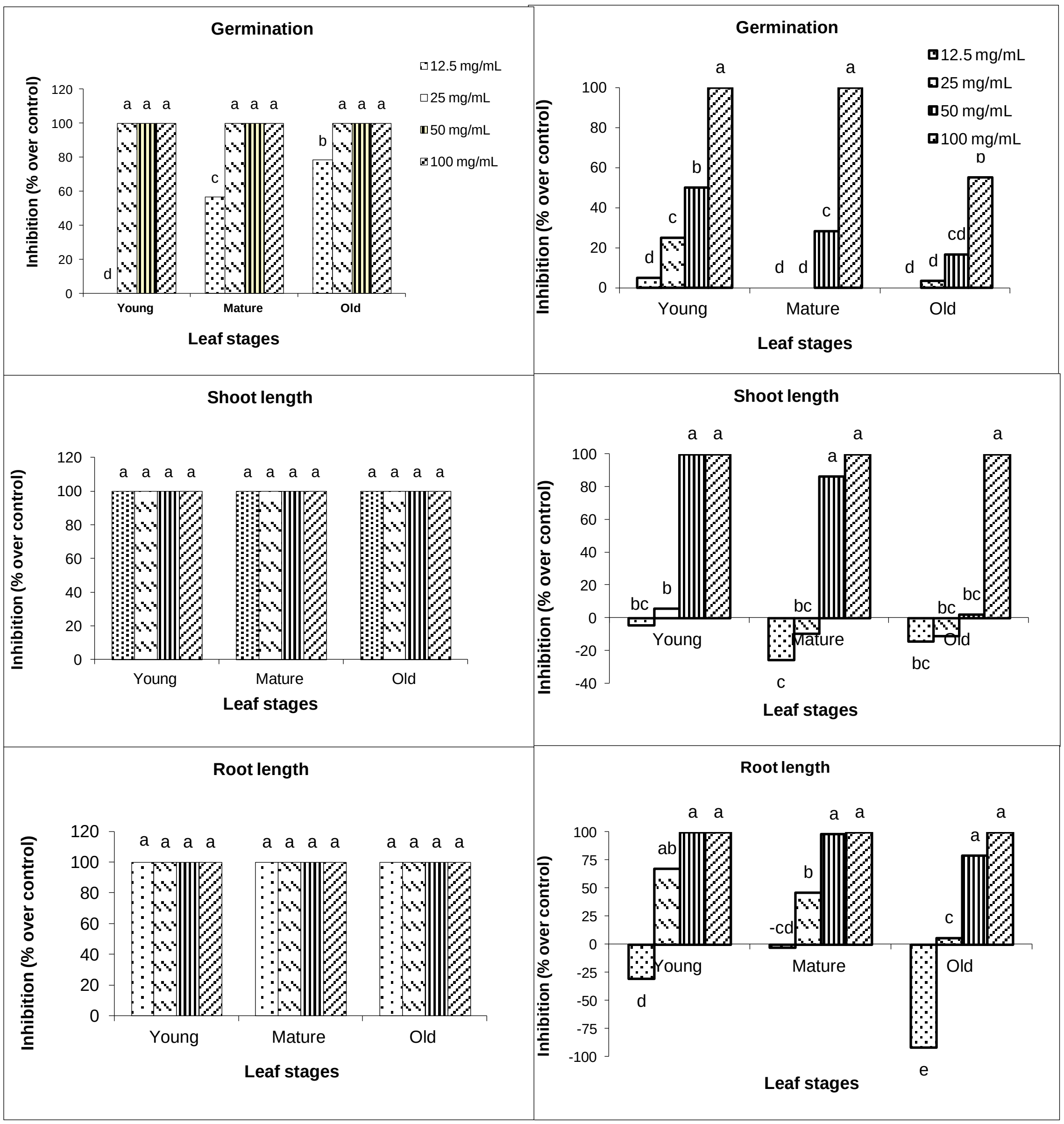


***Wild pea*** ***Barnyard grass***

**Figure 1.** Effects of aqueous extract from 3 leaf stages of *M. azeradach* L. on germination and seedling growth of wild pea and barnyard grass. The values represent treatment means. Different letters indicate significance differences ($p$<0.05) between treatments.

For all treatment concentrations, no significant differences in water uptake after 12 and 24 h imbibition were observed. After the 48 h imbibition period, the percentage of water uptake caused marked changes in seedling parameters with all concentrations evaluated. The activities of α-amylase in *E. crus-galli* seeds were also investigated and the results were observed in Figure. 2. Using similar concentrations at various periods of imbibition, α-amylase

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activity increased with prolongation of imbibition period. Application of 0.625 to 2.5 mg/mL aqueous extract of young leaf stage of *M. azeradach* had no effect on activity of α-amylase on *E. crus-galli*. Increased concentration of aqueous extract of young leaf stage of *M. azeradach* resulted in inhibition of α-amylase activitywhen seeds were imbibed in aqueous extracts of young leaf stage of *M. azeradach* at concentrations of 5 and 10 mg/mL for a period of 48 h.

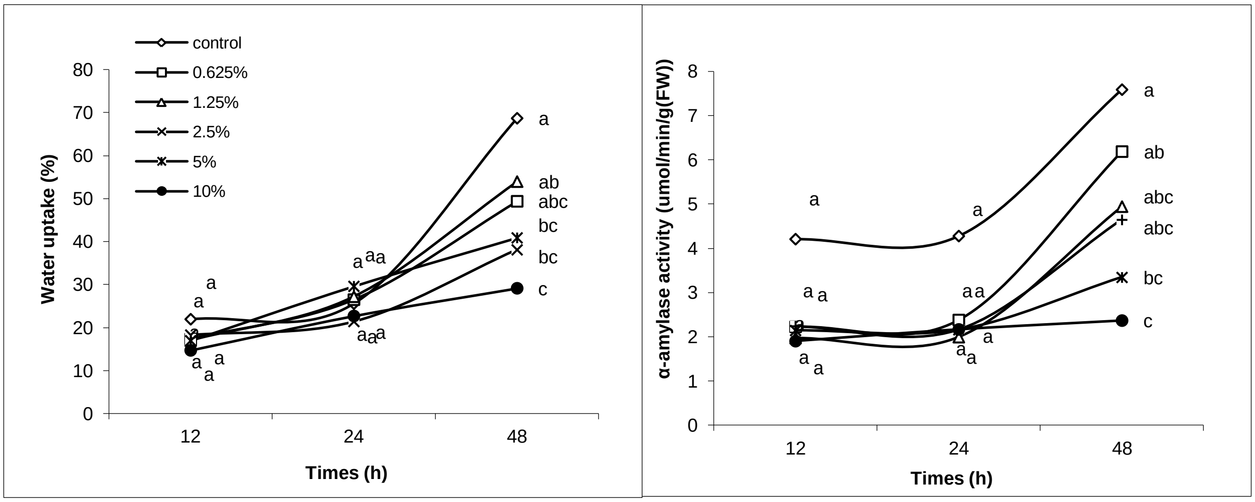


**Figure 2.** Effects of aqueous extract of young leaf stage of *M. azeradach* L. on water uptake and *α*-amylase activity of barnyard grass seeds. The values represent treatment means. Different letters indicate significance differences (*p*<0.05) between treatment times.

## DISCUSSION

A number of studies have suggested that the degree of allelopathic inhibition generally increases with increasing extract concentration (Laosinwattana *et al*. 2007; 2010)). Interestingly, the inhibitory effects on *E. crus-galli* were less than those on *P. lathyroides*. These results indicate that the inhibitory effect of the extract varied with the weed species indicator. The results of this study are congruent with the data of Lin *et al*. (2006), who reported that the inhibitory effects of Saururaceae (*Houttuynia cordata* Thunb.) varied with the weed indicator species evaluated.

In the present study, it was clearly shown that aqueous extracts from young leaf stage of *M. azeradach* inhibited *E. crus-galli* seed germination. Exposure of dry *E. crus-galli* seeds to the extract inhibited the imbibition of *E. crus-galli* seeds, compared to control seeds. This finding is in agreement with that of Han *et al*. (2008) who reported that ginger aqueous extracts, especially stem and leaf extracts, inhibited imbibition in seeds of chive and soybean. Most seeds require an adequate moisture level for activation of metabolism within seed (Chong *et al*. 2002). However, seed which exhibited inhibited water uptake may be limited in specific enzymes required for metabolism of reserved food and hence exhibit poor seed germination. The mode of negative action of aqueous extract from young leaf stage of *M. azeradach* may be associated with inhibition of the activity of α-amylase. It was shown that α-amylase activity was inhibited by the presence of allelochemicals. Kato-Noguchi and Macı´as (2005) had reported that the lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds treated by 6-methoxy-2-

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benzoxazolinone (MBOA) reduced activity of α-amylase during seed germination. The α-amylase activity catalyzes endosperm starch hydrolysis and transformation into soluble sugars and hence its utilization for providing energy during seed germination (Chong *et al*. 2002). Inversely, the suppression in $\alpha$- amylase activity as the result of exposure to aqueous extract from young leaf stage of *M. azeradach* could suggest the retardation of substrate production for respiration and consequently limited energy production. Therefore, aqueous extracts from young leaf stage of *M. azeradach* may adversely affect seed germination.

Differential sensitivity of the roots and shoot to the presence of aqueous extracts from young leaf stage of *M. azeradach* was also evident in our experiments. *E. crus-galli* radical elongation was found to be more sensitive to allelochemical exposure than was shoot growth. These results are similar to those that reported that shoot length was less sensitive to presence of phytotoxins extracted from allelopathic plants than radical length (Laosinwattana *et al*. 2010).

## CONCLUSION

The young leaves of *M. azeradach* aqueous extract had the strongest inhibitory activity against *E. crus-galli* and *P. lathyroides* seed germination and seedling growth. The exact mechanism by which germination was reduced by aqueous extracts of young leaf stage of *M. azeradach* likely involves inhibition of water uptake and also α-amylase activity. Significant growth reduction was observed in seedlings due to the toxicity of the aqueous extracts obtained from young leaf tissues of *M. azeradach.*

## ACKNOWLEDGMENTS

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## REFERENCES

Bernfeld P. (1955). Amylases α and β. In: *Method in Enzymology.* (eds. By Colowick S.P. and Kaplan N.O.). Academic Press, New York, 149-158.

Chong C., Bible B.B. and Ju H-Y. (2002). Germination and Emergence..In: *Handbook of Plant and Crop Physiology*, Second edition. (ed. by Pessarakli M.). Marcel Deckker, Inc.New York, 57-115.

Han C-M., Pan K-W., Wu N. Wang J-C. and Li W. (2008). Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive. *Scientia Horticulturae*. 116, 330–336.

Kato-Noguchi H. and Macías F.A. (2005). Effects of 6-methoxy-2-benzoxazolinone on the germination and $\alpha$-amylase activity in lettuce seeds. *Journal of Plant Physiology*. 162, 1304–1307.

Laosinwattana, C., Phuwiwat, W. and Charoenying, P. (2007). Assessment of allelopathic potential of vetivergrass (*Vetiveria* spp.) ecotypes. *Allelopathy Journal* 19 : 469-478.

Laosinwattana C., Boonleom C., Teerarak M., Thitavasanta S. and Charoenying P. (2010). Potential allelopathic effects of *Suregada multiflorum* and the influence of soil type on its residue's efficacy. *Weed Biology and Management.* 10, 153-159.

Lin, D., Sugitomo, Y., Dong, Y., Terao H. and Matsuo, M. (2006). Natural herbicidal potential of saururaceae (*Houttuynia cordata* Thunb) dried powders on paddy weeds in transplanted rice. *Crop Protection* 25 : 1126-1129.

Sadasivam S. and Manickam A.(1996). *Biochemical Methods*. New Age International (P) Ltd., New Delhi.

Taiz L. and Zeiger, E. (2006). *Plant Physiology*, fourth edition. Sinauer Associates, Massachusetts.

Teerarak M., Laosinwattana C., Charoenying P. (2010). Evaluation of allelopathic, decomposition and cytogenetic activities of *Jasminum officinale* L. f. var. *grandiflorum* (L.) Kob. on bioassay plants. *Bioresource Technology*. 101, 5677-5684.

Turk M.A. and Tawaha A.M. (2003). Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop Protection*. 22, 673–677.

Vyvyan J.R. (2002). Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron* 58, 1631-1646.

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# EFFECT OF FORMASULFURON + ISOXADIFEN-ETHYL IN COMBINATION WITH UREA FOR WEED CONTROL IN MAIZE (*ZEA MAYS* L.)

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## ABSTRACT

A field experiment was conducted at Faisalabad, Pakistan to study the effect of formasulfuron + isoxadifen-ethyl applied alone and in combination with urea on weeds and yield of spring (March-May) and autumn(August-October) maize (*Zea mays* L.). Six treatments comprised of control, manual hoeing, formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$, 1125 g a.i. ha$^{-1}$ + 1% urea, 1125 g a.i. ha$^{-1}$ + 2% urea and 1125 g a.i. ha$^{-1}$ + 3% urea solution sprayed as post – emergence (20 days after sowing). Manual hoeing, and formasulfuron + isoxadifen-ethyl @1125 g a.i. ha$^{-1}$ combined with 3% urea were the most effective treatments for controlling *Cyperus rotundus, Achyranthus aspera* and *Trianthema portulacastrum*. Maximum weed control efficiency with formasulfuron + isoxadifen-ethyl @1125 g a.i. ha$^{-1}$ combined with 3% urea was 87.00 % for *C.rotundus*, 75.19 % for *A. aspera* in spring maize. It was 82.05 % for *C. rotundus* and 89.97 % for *T. portulacastrum* in autumn maize. The maize yield from formasulfuron + isoxadifen-ethyl @1125 g a.i. ha$^{-1}$ + 3% urea treated plots was 56% higher in spring and 68% higher in autumn than those from control and was comparable to that of manual weeding. Maximum loss in grain yield due to un-weeded control plots in spring and autumn maize was 44 and 41%, respectively.

**Keywords:** Formasulfuron + Isoxadifen-Ethyl, Urea, Weeds, Maize, Grain yield

## INTRODUCTION

Maize is the third most important cereal grown in Pakistan after wheat and rice and is known as the “King of the grain crops”. The average grain yield is 3.48 t ha$^{-1}$ (Anonymous, 2006). Among the factors responsible for low yield, presence of weeds is considered to be the most important one. Weed interference in maize leads to 37 to 68% reduction in crop yield (Adigun and Lagoke, 2003). Control of weeds is, therefore, essential for obtaining good crop harvest. An increase of 31-33% in maize grain yield has been reported with adequate weed control (Maina *et al.,* 2001 and Chikoye *et al.*, 2005). Mechanical methods of weed control are useful but are getting expensive, laborious and time consuming. Moreover the acute shortage of labour available for agricultural operations means it will not be possible or economical to continue with traditional practices. Therefore in view of these limitations, chemical weed control is an important and effective alternative if properly employed. Weed control efficiency of 85-95% have been reported with herbicides in maize (Knezevic *et al*., 2003; Alister and

Kogan, 2005). Different types of pre and post-emergence herbicides are used to control weeds in maize. Post-emergence herbicides are generally absorbed through leaves. Leaf cuticle is composed of waxes and cutin that affect the herbicide absorption. The use of adjuvant in combination with herbicide enhances the herbicide retention on leaf surface and penetration through the cuticle. Urea fertilizer is an effective adjuvant which can be used along with herbicides for controlling weeds more effectively (Getmanetz *et a*l., 1991; Ssango and Balitenda, 2003; Singh and Singh, 2003 and Bunting *et al*., 2004). The present study, therefore, was conducted to determine the effect of formasulfuron + isoxadifen-ethyl alone and in combination with urea on weeds and yield of maize under field conditions.

## MATERIALS AND METHODS

The study was carried out at Agronomic Research Farm, University of Agriculture, Faisalabad, Pakistan on a sandy clay loam soil. Six treatments were studied viz. control; manual hoeing (2 hoeings); formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$; formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ + 1% urea; formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ + 2% urea and formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ + 3% urea solution. The experiment was laid out in a randomized complete block design with four replications and a net plot size measuring 7 x 3 m. Maize variety “Golden” was sown as a test crop in August and March with a single row hand drill using a seed rate of 35 kg ha$^{-1}$ in rows 75 cm apart. Plant to plant distance of 25 cm within rows was maintained by thinning extra plants twice at an early growth stage. Fertilizers were applied at the rate of 160 kg N ha$^{-1}$ and 80 kg $P_2O_5$ ha$^{-1}$. All of the P and half of the N were broadcast manually and incorporated into soil at seed-bed preparation while the remaining N was applied using the broadcast method before the second irrigation. In all, five irrigations were applied to autumn and eight to spring crop. Spray volume was used @ 300 L ha$^{-1}$. This amount was determined through calibration before spraying the herbicide. Herbicide (mixed formulation) was dissolved after preparing 3% urea solution in water. The herbicide was applied using a knapsack hand sprayer fitted with a flat fan nozzle. Hoeing was done twice using a hand hoe in the respective treatment when soil was suitably moist after the first and second irrigation.

Weeds were counted from an area of one square meter at 15 and 25 days after spray at two places selected at random in each plot with the help of a quadrate. Total dry weight of weeds was recorded by cutting at ground level from a randomly selected area of one square meter at two different places at maturity. After harvesting, weeds were cleaned and dried at room temperature and then in oven at 70$^{o}$C for 72 hours. Ten cobs were taken randomly from each plot. Number of grains from each cob was counted after shelling. To measure 100-grain weight, five samples each of 100-grains were taken randomly from the maize from each plot and weighed on an automatic electronic balance. All the cobs from each plot were separated manually and shelled with the help of a mechanical sheller and weighed to record grain yield.

The data collected was analyzed statistically using Fisher’s analysis of variance technique. Least significant difference test was applied at 5% probability level to compare treatment means (Steel *et al.*, 1997).

## RESULTS

### Effect on weeds

Weed flora at experimental site comprised of *Achyranthus aspera, Cyperus rotundus and Trianthema portulacastrum*. Application of formasulfuron + isoxadifen-ethyl alone and in combination with 1, 2 and 3% urea solution significantly reduced the density of different weeds compared with control (Table1). In spring maize, formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ + 3% urea solution performed better in controlling *C. rotundus* after manual hoeing. In autumn maize, formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ with 1 and 3% urea solution and manual hoeing were at par with one another in respect of *C. rotundus* control.

In regards to the control of broad leaf weeds i.e. *A. aspera* in spring and *T. portulacastrum* in autumn maize, formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ with 1, 2 and 3% urea solution and manual hoeing did not differ statistically. Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ with various concentrations of urea significantly reduced the total dry weight of weeds at harvest over control. Formasulfuron + isoxadifen-ethyl with 3% urea solution performed better than other treatments (Table 1).

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**Table 1.** Effect of formasulfuron + isoxadifen-ethyl alone and with urea on weed density and dry weight in maize

| | Spring maize | | | | | Autumn maize | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *C. rotundus* density $m^{-2}$ | | *A. aspera density* $m^{-2}$ | | Total dry weight ($gm^{-2}$) at harvest | *C. rotundus density* $m^{-2}$ | | *T. portulacasum* density $m^{-2}$ | | Total dry weight ($gm^{-2}$) at harvest |
| | 15 DAS | 25 DAS | 15 DAS | 25 DAS | | 15 DAS | 25 DAS | 15 DAS | 25 DAS | |
| Control | 108.00 a | 106.00 a | 3.33 a | 2.66 a | 295.90 a | 29.25 a | 10.75 a | 143.00 a | 172.00 a | 138.9 a |
| Manual hoeing | 14.00 e (87.o4) | 4.66 e (95.60) | 1.00 b (70.00) | 0.33 c (87.59) | 35.04 f (88.15) | 8.00 b (72.65) | 7.50 ab (30.23) | 24.25 b (83.04) | 32.25 b (81.25) | 55.88 b (59.77) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ | 42.33 b (60.80) | 34.67 b (63.48) | 1.66 b (50.15) | 1.33 b (50.00) | 104.20b (64.78) | 5.00 b (82.90) | 6.73 ab (37.39) | 20.75 b (85.49) | 22.50 b (86.92) | 42.92 b (69.10) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ + 1% urea | 31.67 c (70.67) | 28.33bc (73.27) | 2.00 b (39.93) | 1.00 bc (62.41) | 68.78 d (76.76) | 4.00 b (86.32) | 5.75 b (46.51) | 27.75 b (80.59) | 24.00 b (86.05) | 55.75 b (59.86) |
| Formasulfuron + isoxadifen-ethyl @ 1125 | 28.67cd | 24.00cd | 2.00 b | 1.00 bc | 77.21 c | 4.25 b | 4.00 b | 23.75 b | 26.75 b | 49.67 b |

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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| g a.i. ha$^{-1}$ + 2% urea | (73.45) | (77.35) | (39.93) | (62.41) | (73.91) | (85.47) | (62.79) | (83.39) | (84.40) | (64.24) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ +3% urea | 21.33de<br>(80.25) | 19.00 d<br>(87.00) | 1.33 b<br>(60.00) | 0.66 bc<br>(75.19) | 41.78 e<br>(85.88) | 5.25 b<br>(82.05) | 4.50 b<br>(58.14) | 19.50 b<br>(86.36) | 17.25 b<br>(89.97) | 43.48 b<br>(68.69) |
| LSD | 9.029 | 7.837 | 1.135 | 0.878 | 4.974 | 9.141 | 4.96 | 16.623 | 21.40 | 17.92 |

Means sharing the same letter did not differ significantly at 5% level of probability.

Figures in parenthesis show weed control efficiency (%).

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**Performance of Maize**

Formasulfuron + isoxadifen-ethyl alone and along with various concentrations of urea solution did not affect the number of cobs per plant significantly in the spring crop but significantly increased the cobs per plant in autumn maize (Table2). Application of formasulfuron + isoxadifen-ethyl alone and in combination with 1, 2 and 3% urea solution significantly increased the number of grains per cob and 100-grain weight over control in both seasons. There was significant variation among the different weed control treatments with respect of the number of grains per cob in spring crop but these treatments were statistically similar with respect of 100-grain weight in spring and autumn maize (Table 2).

There was a significant increase in grain yield of maize with formasulfuron + isoxadifen-ethyl either applied alone or with 1, 2 and 3% urea solution. In spring maize, manual hoeing resulted in the highest grain yield, followed by formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ + 3% urea solution. Manual hoeing and formasulfuron + isoxadifen-ethyl alone and with various concentrations of urea were statistically similar in respect of maize grain yield in case of autumn maize (Table 2).

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**Table 2.** Effect of formasulfuron + isoxadifen-ethyl alone and with urea on yield and yield components of maize

| | Spring maize | | | | Autumn maize | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of cobs per plant | No. of grains per cob | 100-grain weight (g) | Grain yield t $ha^{-1}$ | No. of cobs per plant | No. of grins per cob | 100-grain weight (g) | Grain yield t $ha^{-1}$ |
| Control | 1.00 | 281.00 e | 21.636 | 2.31 e | 0.97 b | 488.5 b | 22.08 c | 3.35 b |
| Manual hoeing | 1.06 | 480.00 a | 24.87 a | 4.12 a (98.35) | 1.12 ab | 563.3 a | 23.65 b | 4.71 a (40.47) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ | 1.00 | 360.80 b | 24.19 a | 2.88 d (24.67) | 1.12 ab | 606.5 a | 23.85 ab | 4.94 a (47.24) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ + 1 urea | 1.00 | 351.70 c | 24.94 a | 2.98 d (29.00) | 1.20 a | 599.5 a | 23.90 ab | 5.00 a (49.12) |
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ + 2% urea | 1.06 | 313.00 d | 26.11 a | 3.42 c (48.05) | 1.20 a | 592.5 a | 24.10 ab | 5.48 a (63.24) |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Formasulfuron + isoxadifen-ethyl @ 1125 g a.i. $ha^{-1}$ +3% urea | 1.06 | 362.20 b | 24.67 a | 3.62 b (56.70) | 1.25 a | 603.0 a | 24.67 a | 5.65 a (68.40) |
| LSD | NS | 6.676 | 2.362 | 0.152 | 0.172 | 44.28 | 0.979 | 0.956 |

Means sharing the same letters did not differ significantly at 5% level of probability

Figures in parenthesis show % increase over control

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## DISCUSSION

Unchecked growth of weeds throughout the crop life cycle resulted in the maximum number of weeds and weed dry weight. It is thus evident that effectiveness of pre mixed formasulfuron + isoxadifen-ethyl for weed control in maize can be enhanced by adding 3% urea solution. Several studies have reported improved weed control with herbicides when used in combination with urea solution due to improved penetration and enhanced phytotoxicity of herbicides (Borona *et al*., 2003; Ssango and Balitenda, 2003; Singh and Singh, 2003 and Bunting *et al*., 2004 ).

Decrease in yield components of maize in control treatments was due to unchecked growth of weeds, which competed with crop plants for available environmental resources. On the other hand, efficient utilization of soil and climatic resources by maize plants in the presence of relatively low weed numbers in different weed control treatments led to increased grain number and weight (Shekhawat and Gautam, 2002).

Increases in grain yield due to different treatments over control were 24.7- 78.4% and 40.5 – 68.4% in spring and autumn maize respectively. Increase in grain yield of maize as a result of better weed control with combined use of herbicide and urea was reported by Ssango and Balitenda (2003) and Singh and Singh (2003).

## CONCLUSION

It can be concluded that formasulfuron + isoxadifen-ethyl @ 1125 g a.i. ha$^{-1}$ +3% urea solution applied as post-emergence proved to be the most effective chemical weed control treatment to increase grain yield of maize.

## REFERENCES

Adigun, J.A. and S.T.O. Lagoke. 2003. Comparison of some pre-emergence herbicide mixtures for weed control in maize in the Nigerian Nothern Guinea Savanna. *J. Sustainable Agric Environ*, 5(1): 63-73.

Alister, C. and M. Kogan. 2005. Efficacy of imidazolinone herbicides applied to imidazolinone-resistant maize and their carryover effects on rotational crops. *J. Crop Prot.*, 24(4): 375-379.

Anonymous. 2006. *Economic Survey of Pakistan*. Ministry of Food and Agricultural Division (Planning unit), Government of Pakistan, Islamabad. pp. 25-27.

Borona V., V. Zadorozhny and T. Postolovskay. 2003. The influence of adjuvants on the efficacy of graminicides in soybeans and niconsulfuron in maize. *Proceedings of the 2nd Weed Conference*, Sarajevo, Bosnia and Herzogovina, 4(1):151-155.

Bunting J.A, C.L. Sprague and D.E. Riechers. 2004. Proper adjuvant selection for foramsulfuron activity. *Crop-Prot.*, 23(4):361-366.

Chikoye D, U.E. Udensi and A.F. Lum. 2005. Evaluation of a new formulation of atrazine and metolachlor mixture for weed control in maize in Nigeria. *J. Crop Prot.*, 24(11):1016-1020.

Getmanetz A.Y., S.M. Kramarev, V.P. Vittsenko, B.A. Bovykin, N.S. Tishkina and A.S. Matrosov. 1991. Chemical compatibility of ZhKU 10-34-0, KAS-28 and herbicides and their

ISBN Number: 978-0-9871961-0-1

combined use in intensive maize growing technology. *Agrokhimiya*, No. 11, 38-44; 5 ref.

Knezevic M., M. Durkic, I. Knezevic, O. Antonic and S. Jelaska. 2003. Effects of soil tillage and post-emergence weed control on weed biomass and maize yield. *Cereal Res. Communications*, 31(1-2): 177-184.

Maina J.M., E.G. Thuranira, G.N. Kibata, F.J Musembi., G. Nyanyu, J.G.N Muthamia, J.O. Okuro, I. Mutura, S. Amboga, A.N. Micheni, F. Mureithi, D. Overfield and P.J. Terry. 2001. Participatory development of weed management strategies in maize based cropping systems in Kenya. The-BCPC-Conference, *Proceed. Int. conf.* Brighton, UK, pp. 199-204.

Shekhawat P.S. and R.C. Gautam. 2002. Effect of row spacing and weed control methods on the growth attributes and grain yield of maize under tilled and untilled conditions. *Ann. Agric. Res.*, 23(4): 626-629.

Singh A.P And P.C. Singh. 2003. Effect of different weed control methods on growth and yield of rabi-sown hybrid maize cv. Hybrid 4640. *J. Living World*, 10(2): 12-15.

Ssango F. and M. Balitenda. 2003. Effect of application of which weed control options on performance of two maize varieties in Uganda. *Muarik Bulletin*, No. 6: 83-88.

Steel R.G.D., J.H. Torrie and D.A. Dicky. 1997. *Principles and Procedures of Statistics A Biometrical Approach* 3rd Ed. McGraw Hill Book International Co., Singapore. pp. 204-227.

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# SOLVING WEED PROBLEMS THE ASIAN-PACIFIC WAY: PAST AND THE FUTURE

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## ABSTRACT

In this review article we provide a brief summary of the history and achievements of the Asian-Pacific Weed Science Society (APWSS) and the contributions it has made to weed science. The vision and the objective of promoting weed science, particularly in the Asia and Pacific region, have been primarily achieved through the well attended biennial conferences, topical symposia and workshops, training courses, affiliations/collaborations, newsletters, and the establishment of an international journal. We also highlight some of the weed problems of the region, the ever changing research needs over time, the major scientific challenges for the future, and suggest ways in which the Society can further assist its members and weed science in the years to come.

**Keywords**: weed research, weed management, APWSS, regional society, herbicides.

## INTRODUCTION

The Asian-Pacific Weed Science Society (APWSS) came into existence in 1967 when some 87 delegates from 22 countries participated in the first Asian-Pacific Weed Control Interchange in Honolulu, Hawaii. The credit for facilitating this interchange must go to the East-West Center of the University of Hawaii and to the founding fathers (in particular Drs W.R. Furtick, D.L. Plucknett and R.T. Romanowski) for their vision, enthusiasm and hard work. The theme for that first Conference, held under the chairmanship of Dr Marcos Vega of Philippines, was “Weed control basic to agricultural development” and how true it remains even today. Dr Don Plucknett was elected Secretary in 1967 and was in that role for 14 years, but continued to support the Society in many ways over a longer period. The staff from the University of Hawaii and the International Rice Research Institute/University of the Philippines provided much support, ensuring the continued progress of APWSS and establishing the linkages with colleagues in the Asia-Pacific region.

The primary objective during the first meeting was to “facilitate the interchange of current weed control information and to promote research in weed science”. The desired outcomes, according to Dr Plucknett, were to identify: (a) the weed workers in the Asian-Pacific region; (b) the major weeds and weed problems; (c) the research and development needs of various countries in the region, and (d) the linkages necessary or possible in dealing with the perceived needs. A news release on 3 July 1967 by the East-West Center, after the first Conference stated “.. the Society will seek to stimulate research into how extensively weeds limit food production in the tropics, giving major attention to rice in Asia and to coconuts in the Pacific.. “.

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The objective as set out in the current Rules of APWSS is "To promote weed science, in particular in the Asian and Pacific regions, by pooling and exchanging information on all aspects of weed science". The aim of this presentation is to look back and see how well we have achieved the vision and objectives set out for APWSS, the contributions our Society has made to the Asia-Pacific region since 1967, and to discuss possible future challenges and research needs. In preparing this manuscript, we have made extensive use of the publication "APWSS: Commemorating Forty Years" (Baltazar 2007) and also consulted with a number of colleagues who have supported and contributed to the Society for many years. The information presented provides only a few examples of the activities and achievements of which there are too many to mention here. The examples included are at random and focussed more on cropping weed problems.

**Delivering On Its Objective**

The Society has delivered extensively on its prime objective of promoting weed science in the Asia-Pacific region, by pooling and exchanging information. This has been done primarily through its biennial conference held regularly in different countries. The effort has been enhanced through occasional workshops and symposia, held as necessary, which brought together many experts including some from out of the region. Two famous books viz: "The World's Worst Weeds" and "A Geographical Atlas of World Weeds" have been co-authored by APWSS founding members (Leroy Holm, Juan Pancho, Donald Plucknett and Horace Clay) with support from APWSS and the East-West Center at the University of Hawaii. The international journal Weed Biology and Management, first published in March 2001, (and now in its 11th year) is published by the Weed Science Society of Japan Inc., with support and encouragement from various national weed science societies affiliated with APWSS. The above, and some additional activities that have helped in achieving the aims of the Society, are summarised below.

<u>The Biennial Conferences</u>
Since the first meeting in 1967, a conference has been held every two years hosted by different countries in the region. The only exception was the 22nd Conference which was held in March 2010 (instead of 2009 as planned) due to some organisational difficulties. The number of participants attending each conference increased from the 87 founding members in 1967 to about 300 in the 1980's, peaking to 589 in 1989. The number of papers also increased from 50 in 1967, peaking at 223 in 1999. The quality of Conference proceedings has also improved substantially, with some Conferences producing two large volumes. The number of countries participating was lowest (8) in 2010 and the highest (27) in 1997, with some participants from outside the Asia-Pacific region.

<u>Symposia and Workshops</u>
Some examples include:

- The first symposium on integrated control of weeds was organised in 1975 as a component of the 5th Conference programme in Tokyo, Japan.
- APWSS sponsored the "International Symposium on Wild and Weedy Rices in Agro-ecosystems" which was held in Vietnam in August 1999.
- An APWSS-sponsored FAO/IWSS Expert Consultation on Improving Weed Management in Developing Countries Workshop was held in Rome in 1982 where an FAO Panel of Experts for Weed Science was appointed.
- A satellite workshop of the 18th Conference in 2001 on "Control of *Echinochloa* species" was held in May 2001, co-sponsored by APWSS at FAO.

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- The Asian-Pacific Herbicide Resistance Working Group was formed in 2001 (chaired by Dr K. Itoh) to manage herbicide resistance and search for solutions to resistance problems in the Asia-Pacific region.
- A FAO-APWSS pre-Conference sponsored workshop on “Control of Cyperaceae Weeds” was held in 2005 in Vietnam.

Training

Several organisations in the Asia-Pacific region have been active in organising training courses and seminars with assistance from APWSS. Notable among these include (i) East-West Center of the University of Hawaii, started around 1961 to increase understanding among nations through cross-cultural interchange in an academic setting; (ii) Regional Center for Tropical Biology (BIOTROP) in Indonesia, established around 1968, which has provided both intensive courses as well as long term research training courses in weed science: in the mid 1980s it established the Southeast Asian Weed Information Centre to assist the weed scientists of the region; (iii) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, which has provided training and invested considerable research effort to develop improved farming systems (including management of weeds) for the small farmers of limited means. They have shown how the cropping system itself can be manipulated to improve weed control. A couple of specific examples include (i) ‘Weed Science in the Asian Tropics’ sponsored by APWSS and Weed Science Society of Japan, held in Thailand in 1995; and (ii) the international seminar on ‘Weed Management in the North Asian Region’, co-sponsored by APWSS, held in China in 1996.

Newsletters

To enhance the communication between Society members, the idea of an APWSS Newsletter was discussed around 1973 and Philip Motooka was appointed the first Newsletter Editor. Since then an average of 4 issues have been published biennially (up to 7 issues between 1977 and 1979). Unfortunately there was a brief pause between 2005 and 2008 until Dr Nimal Chandrasena gave it a new lease of life and has kept it going since then. Former editors have included Drs Beatriz Mercado, Aurora Baltazar, John Swarbrick and Yasuhiro Yogo. These Newsletters have included news items from member countries, activities of members, developments in the industry, information on specific weeds as well as current research being conducted by weed scientists of the region.

Awards and Travel Grants

APWSS, in cooperation with the international chemical company Monsanto, established the Best Paper and Best Poster competition which ran for 20 years, from 1983 to 2003. The aim of this competition was to recognise and encourage high quality research in weed science and related fields in the region. During each Conference three Best Papers (oral) and three Best Posters were selected by a panel of judges. These high quality presentations reflected the progress of weed science in the region and highlighted the research problems prevalent at the time. The award included a plaque and a cash prize.

The Young and Deserving Scientists Travel Grant was initiated in 1987 to give partial support to members to present at the APWSS Conference. Full implementation of this Grant occurred in early 1990s and it has been in operation since then.

The first APWSS honorary life member, Y. Baron Goto, was elected in 1973. Since then many others have been added to the list. At the 20th anniversary of APWSS in 1989, a special programme was held to honour and recognise the founding members, charter members and past presidents, for their contributions to the Society. A special citation was presented to Don Plucknett who was one of the three founding fathers of APWSS in 1967.

Affiliations/Collaborations
The Weed Science Society of Indonesia was the first national society to become affiliated with APWSS around 1971. This number grew quickly to 6 by 1973 with the affiliation of the national societies of USA, Japan, India, New Zealand and Australia. With the constant encouragement and support of APWSS, more national societies were established and by 1989 there were 13 national societies represented at its Executive Committee meeting. The Weed Science Society of Vietnam and the Plant Protection Society of Sri Lanka were added to the list in 1997 and 1999 respectively. The latest, the Weed Science Society of Bangladesh, was formed in 2008. APWSS became an affiliate of the International Weed Science Society (IWSS) in 1975 and, when the first set of officers for IWSS was installed during the 1977 business meeting of APWSS in Indonesia, L.J. Matthews of New Zealand was appointed its first President and S. Matsunaka of Japan became APWSS representative. In 1987 the Weed Science Society of America (WSSA) and APWSS agreed to establish an official exchange of conference delegates to promote closer contact between the two societies. The first official WSSA delegate was Richard Schumacher and the APWSS delegate was Yuh-Lin Chen.

## An Early Start to Weed Research

The major research efforts of weed scientists in the Asia-Pacific region have revolved around: (i) identifying the major weed problems; (ii) understanding their taxonomy and biology; (iii) controlling or effectively managing them, which has often included investigating the most effective herbicide, and in later years; (iv) assessing the unintended environmental, human and off-target impacts of various control measures. Our region has kept pace with the developing technologies in weed management, although, without adequate infrastructure, the introduction and uptake of some technologies has been slower. For example, the presentations at New Zealand's National Weeds Conferences in 1948 and 1949 included papers on phenoxy herbicides and subjects such as low volume nozzles and low volume spraying of pasture weeds. Similarly K. Noda and K. Ozawa mentioned at the 3rd APWSS Conference that 2,4-D was introduced into Japan in 1950. By the early 1960s, herbicides belonging to groups such as phenoxys, amides, carbamates and dinitroanilines were introduced into many countries of the Asia-Pacific region. However, as observed by W.R. Furtick in his plenary paper at the 1st APWSS Conference, "….until 1960s unfortunately there has not been this same level of effort in the tropical and semi-tropical areas" (as in the temperate zone crops).

## The Problems of the Region

The number and diversity of weed problems are much greater in tropical agriculture than under temperate zone conditions. Weeds present a problem over a much longer period of the year or even all year round. This was echoed by the following statement from the IRRI'S 1965 Annual Report which said on page 227 "An important reason for low yields of most upland rice is the absence of effective low-cost weed control". So how true was the theme of the 1st Conference in 1967 "Weed control basic to agricultural development"? There is no doubt that the conflict between humans and weeds will continue, so long as humans modify ecosystems.

At the 2nd APWSS Conference in 1969, Leroy Holm suggested "....there are about 200,000 species of angiosperms recorded.... Some estimates suggest that 30,000 of these may

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behave as weeds. We have looked at 3000 and were able to reduce the list of weed species to 100…. *Cyperus rotundus,* a native of Asia, may be the world's worst weed. It is in all of the major crops and most of the important agricultural regions of the world….".

In rice, the most important crop of our region, *Echinochloa crus-galli* probably received the most attention on its impact and control as well as taxonomy. *Phalaris minor* became such a menace in wheat crops that it threatened the sustainability of rice–wheat cropping systems in countries like India. Control strategies for these and many other weeds in crops like rice, wheat, maize, sorghum, pulses, vegetables, sugar cane, pineapple etc. have been investigated and reported. Management of weeds in plantation crops has been reported from researchers in many countries as have pasture weeds, especially from Australian and New Zealand scientists. Aquatic weeds are distributed throughout the region and much research on their mechanical, chemical and biological control (as well as their beneficial uses) has been reported for species like *Eichhornia crassipes*, *Salvinia molesta*, and *Alternanthera philoxeroides*. The noxious weeds of non-agricultural land such as *Imperata cylindrica*, *Mimosa pigra* and *Lantana camara* have also posed considerable threats and have been the subject of many investigations. Additionally, biosecurity issues and the spread of invasive weeds like *Parthenium hysterophorus*, *Mikania micrantha*, and *Chromolaena odorata* have received much attention as they are likely to have a serious impact under predicted climate changes. It is not possible to list here even the most important weed species of the region, but some further problem weeds are mentioned in later sections.

**The Ever Changing Research Needs**

With the discovery of selective herbicides, the efforts of weed scientists accelerated towards a 'weedless' agriculture. If the efforts on chemical weed control were successful, it was predicted by D.L. Umali at the 2nd Conference held at IRRI in 1969 "….. in after years it will be a great source of pride for us to say that on these grounds was held the meeting that spelled the doom of weeds in agriculture…" The weed species, or at least some of these, by their nature, have defied most predictions and continued to evade, shift, evolve and survive.

Noda and Ozawa mentioned at the 3rd Conference in 1971 that, by the late 1960s in countries like Japan, nearly the total area of paddy fields were being treated with herbicides. The famous Green Revolution of the 1960s gave the Asia-Pacific region in particular, the short statured high yielding varieties of rice and wheat which required very high levels of weed control for best performance. This, combined with increased irrigated areas and

introduction of more effective herbicides, encouraged farmers in many countries to shift from transplanted to direct seeded rice. Unfortunately the water regimes in direct seeded rice caused a shift in weed flora to more competitive grasses and some very difficult to control broadleaf weeds. Such shifts, along with increasing costs of labour and the quest for greater economic returns, saw the introduction of many new pesticides during the last 3 decades of the 20th century. Availability of new herbicide chemistries (eg aryloxyphenoxys, sulfonylureas), with very low use rates, saw shifts from sequential applications in 1970s to one-shot treatments by late 1980s. Similar, although maybe not so elaborate, developments could be related for many other crops in the region.

Herbicides thus became an indispensable part of modern agriculture in most countries of the region. They reduced yield losses due to weed competition and provided the

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foundation for protection of yield potential. They also helped to prevent erosion caused by tillage and have had a direct impact on the exploitation of human labour in the poor countries of our region. Two major problems, (i) concerns for environment and human safety, and (ii) appearance of herbicide resistant weeds, led to the devaluation of herbicides and questioned the sustainability of this technology developed over the last 50 – 60 years. A large volume of research on environmental impacts of herbicides on the soil and water resources has been conducted in the region and efforts have been made to optimise herbicide regimes, doses and application techniques to mitigate the adverse impacts (eg. Rahman and James 2002). A gradual reduction in the efficacy (accelerated degradation) of certain herbicides as a result of their continued use has also become an issue in some cropping systems.

Herbicide resistance became a subject of discussion within East, Southeast Asia and Australasia in the early 1980s. Sulfonylurea resistant sedges, broadleaves, and 2,4-D and triazine-resistant broadleaf weeds, were among the first to appear on the scene. Propanil-resistant *E. crus-galli* and isoproturon-resistant *P. minor* were the subject of many research papers. Resistance to sulfonylurea herbicides had developed in nine annual weeds and three perennial weeds found in rice cultivated areas in Japan, and for some of these resistant biotypes, the herbicide dose for 50% mortality was found to be 100 to 1000 times higher than that for the susceptible biotypes (Itoh 2005). In Australia, the greatest number of occurrences of herbicide resistance has been for *Lolium rigidum* growing as a weed in continuous cropping rotations (usually wheat/lupins). In New Zealand, the development of resistance to phenoxy herbicides in three pasture weeds poses a special difficulty for their selective control because pastures usually contain a mixture of two sown species viz. grass and legumes (Rahman *et al.* 2001). Today weed resistance problems have achieved a dramatic magnitude and they are ever increasing all over the world.

For a majority of countries in the Asia-Pacific region, weedy rice or red rice emerged as a new and possibly the most dangerous weed. It has recently been reported as the major pest that currently threatens the rice production in Vietnam (Chin and Thi 2010). First noted in 1988 in Malaysia, it had infested many fields there by 1993 (Vaughan *et al.* 1993). It has the same scientific name as cultivated rice (*Oryza sativa*), but it has some weedy characteristics such as easy shattering and a red pericarp. Weedy rice has a short life span, is a tall plant, with weak culms and small seeds. The origin of weedy rice is not certain. It may have originated from the degradation of cultivated rice or may have come from the crossing between cultivated rice and wild rice.

Persistent reports on negative environmental impacts, concerns about human safety and the ever increasing number of herbicide resistant weeds shifted research emphasis from chemicals to integrated, ecological and biological approaches to manage weeds. So since 1990s, Integrated Weed Management (IWM) and sustainable agriculture became the subject of discussion of many presentations. It is interesting to note, however, that back in 1975 the theme of the 5th Conference held in Japan was ‘Integrated Control of Weeds’. Also starting from the 13th Conference in 1991, at least six APWSS Conferences have had the word ‘Sustainable’ included in their theme. Increased effort also went into developing botanical and biological weed control agents including mycoherbicides.

Genetically modified (GM) crops, however, resulted in a big shift towards less emphasis on herbicide research. As a consequence, investments in research to discover new modes of action have decreased precipitously. Who would have thought that by the year 2000, 66% of the soya bean production in USA would contain a gene that confers resistance to

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glyphosate. For the most important crop of our region viz. rice, commercial developments have taken place for three herbicide resistant varieties: Clearfield (imidazolinone resistant), Roundup Ready (glyphosate resistant) and Liberty Link (glufosinate resistant), with genes for resistance derived from different sources (Baki 2005).

Today, the emphasis in some countries has turned from weed control as such towards vegetation management using environmentally acceptable, yet economically effective remedies resulting from integrating management strategies. To meet these changes increased effort has gone into knowledge-based decision-support approaches such as expert systems and simulation models. Expert systems have many predictive and diagnostic applications in weed control, particularly in the development of integrated approaches to crop management. While waging a protracted war on weeds, we need to keep exploring the potential for using some of them as resources, such as food for humans and animals, raw materials for a range of products, bio-fuels, phyto-remediation and further exploiting the chemical warfare between plants (allelopathy).

**Major Future Scientific Challenges For The Region**

As mentioned in the Section above, much has happened in weed science within the Asia-Pacific region since the Society held its first conference. As examples, we have had new herbicide chemistries, herbicide safeners, application technologies, herbicide resistance in weeds, accelerated degradation of herbicides, greater concern about the effects of herbicides on the environment and safety to humans, biological herbicides, genetic engineering and the move towards developing integrated sustainable weed management strategies. Many of these problems are going to confront us for years to come and new challenges are going to arise. In future, weed scientists in our region need to promote research in the following areas (and the list is by no means exclusive or exhaustive):

- Integrated management of weedy rice, which is threatening the productivity of major staple food crops of most Asian countries
- Continued risk assessment and monitoring of existing and new herbicides
- Accelerated development of practical and economic strategies for control of herbicide resistant weeds
- Sustainable weed management strategies for GM crops to stop the potential emergence of “super weeds”
- Sustainable weed management under residue-rich conservation agriculture, such as zero and minimum tillage
- New targeted and precision application technologies and site-specific weed management for minimising herbicide dosages
- More effort on developing biological agents including natural products and mycoherbicides for emerging weed problems, particularly in the non-crop situations
- Expansion of expert systems for weed management and precision agriculture technology through greater use of GIS, GPS and remote sensing
- Utilisation of weeds, both terrestrial and aquatic species, as discussed in detail recently by Chandrasena (2008)
- Mechanisms and preventive measures against globalisation of invasive alien species such as *P. hysterophorus*, *M. micrantha* and *M. pigra,* due to increased international trade, transport and tourism
- Impact of climate change on weeds and their management strategies
- Targeted and effective extension/technology transfer to end users for a faster uptake of improved management options.

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APWSS has played an extremely important role by connecting so many people from so many parts of the world and providing a vibrant forum for the exchange of weed science information. In a speech acknowledging his special Citation for APWSS mentioned at the 12th Conference in 1989, D.L. Plucknett, the founding father, said,…."I have always been proud of what the Society has been able to achieve. I have been told that APWSS is now the largest regional Weed Science Society in the world….". We wholeheartedly echo his words regarding achievements of APWSS. The Society can further assist in the coming years, by: (i) organising/facilitating regional workshops on specific topics; (ii) supporting publication of special issues of the journal Weed Biology and Management to deal with specific areas; (iii) formulating and encouraging networks/working groups for widespread problems such as weedy rice and herbicide resistance; (iv) linking up young weed scientists with the industry and internationally recognised institutions; (v) providing advocacy for adequate education and research grants in weeds science; and (vi) being at the forefront of identifying emerging issues of weeds and creating opportunities for employment of long-term solutions to weed problems. Most of all, the Society can take an active role in making the public aware of factors that may endanger the agricultural production systems being able to keep pace with the increasing demand for food – weeds being among the biggest threat at the top of the list.

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## REFERENCES

Baki, B.B. (2005). The World's landscapes of weedy rice – an overview. *Proceedings 20th Asian-Pacific Weed Science Society Conference:* 45-48.

Baltazar, A.M. (2007). *Asian-Pacific Weed Science Society – commemorating forty years (1967-2007)*. University of the Philippines Los Banos, Laguna, Philippines. 52pp.

Chandrasena, N.R. (2008). Liabilities or assets: some Australian perspectives on weeds. In Kim, K.J., Shin, H. and Lee, I.J. (eds). *UItility of weeds and their relatives as resources*. Kyungpook National University, Daegu, Korea, pp. 9-56.

Chin, D.V., Thi, H.L. (2010). Fifty Years of Weed Research in rice in Vietnam. In Bong, B.B., Bo, N. V., Buu, B.C. (eds). *Vietnam Fifty Years of Research and Development*. Agriculture Publishing House, Ministry of Agriculture and Rural Development, Hanoi. pp. 283-292

Itoh, K., Uchino, A. and Park, T.L. (2005). An overview of herbicide resistance of sulfonylureas in Japan and Asia. *Proceedings 20th Asian-Pacific Weed Science Society Conference:* 24-34.

Rahman, A., James, T.K., Seefeldt, S. (2001). The current situation with herbicide resistant weeds in New Zealand. *Proceedings 18th Asian-Pacific Weed Science Society Conference: 500 – 508.*

Rahman, A., James, T.K. 2002. Minimising environmental contamination by selecting appropriate herbicide dose. In Kookana, R.S., Sadler, R., Sethunathan, N. and Naidu, R. (eds). *Environmental Protection and Risk Assessment of Organic Contaminants*. *Science Publishers, Inc*., NH. USA. pp. 209-224.

Vaughan, D.A., Watanabe, H., Zain, A.M., Okuno, K. (1995). Evolution and genetic diversity of Malaysian weedy rice. *Proceedings 15th Asian-Pacific Weed Science Society Conference:* 461-466.

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# POSSIBLE APPROACHES FOR ECOLOGICAL WEED MANAGEMENT IN DIRECT-SEEDED RICE IN A CHANGING WORLD

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## ABSTRACT

Labour and water scarcity are the major drivers for shifting the method of rice establishment from traditional transplanting to the direct-seeding in many nations of Asia, where about 90% of the world's rice is produced and consumed. The risk of crop yield loss due to competition from weeds by all direct-seeding methods is higher than for transplanted rice. Changes in rice establishment method as well as water, tillage and weed management practices in direct-seeded rice (DSR) lead to shifts in weed communities and evolution of adoptive traits of individual weed species which present a further challenge for effective management of weeds in DSR based cropping systems. Recently, weedy rice is posing a serious challenge to DSR farming community. Effective and economic weed management is the key for sustainable rice production to meet the increasing food demand in the changing world. The objective of this paper is to summarise available ecological weed management options for managing weeds in direct-seeded rice for making direct-seeded rice more productive, profitable and sustainable in a changing world, and to identify areas where additional research is needed to bridge the gap. Ecological management of weeds in direct-seeded rice stresses on shifting the crop-weed balance in favour of rice by adapting in an integrated manner all available cultural, physical and biological weed management strategies and judiciously using herbicides as the last resort rather than as the only resort.

**Key words:** Direct-seeded rice, Ecological weed management

## INTRODUCTION

Rice is a staple food for more than half of the word population providing 21% of global human per capita energy. About 90% of the world's rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia (FAO, 2009). In Asia, rice is commonly grown by transplanting seedlings into puddled soil (land preparation with wet tillage). However, in addition to adverse effects of puddling on soil physical properties, puddling and transplanting require large amount of water and labor, both of which are becoming increasingly scarce and expensive, making rice production less profitable. Also, the drudgery involved in transplanting—a job largely done by women—is

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of serious concern. The increase in production costs, shortage of labor, increased labor wages, decreased water availability resulted in changes such as shift in method of rice establishment from transplanting to direct-seeding. Direct-seeding (especially wet-seeding) is fast spreading in Asian countries. In addition, upland rice is mostly dry-seeded. To meet the needs of increasing population of the world, it is estimated that about 114 million tons of additional milled rice need to be produced by 2035, which is equivalent to an overall increase of 26% in the next 25 years.

Weeds are a major impediment to direct-seeded rice (DSR) production through their ability to compete for resources and their impact on product quality (Rao and Nagamani, 2007; Rao et al., 2007; Kumar and Ladha, 2011). The risk of crop yield loss due to competition from weeds by all seeding methods is higher than for transplanted rice because of the absence of the size differential between the crop and weeds and concurrent emergence of competitive weeds along with rice seedlings (Rao et al., 2007).The change in method of rice establishment in many Asian countries, in turn resulted in change in method of weed control from hand weeding to greater herbicide use (Naylor, 1994; Azmi et al., 2005; Rao et al., 2007; Weerakoon et al., 2011). The change in the method of weed control resulted in the evolution of herbicide resistance among weeds (Rao et al., 2007), shifts in dominance of associated weeds (Azmi et al., 2005; Rao et al., 2007), emergence of difficult to control weeds, such as weedy rice as major weed threats (Rao et al., 2007; Kumar and Ladha, 2011). In addition, there is overall climate change impact on rice production and weed community (Rodenburg et al., 2011). Effective and economic weed management is the key for sustainable rice production. Through the development and adoption of improved weed management technologies, improved rice productivity and production could be achieved to meet the demands of increasing population.

The objective of this paper is to summarise the current methods of weed management in DSR, bring to light factors necessitating the ecological weed management approaches in DSR, to summarise available ecological weed management options for managing weeds in DSR for making DSR more productive, profitable and sustainable in a changing world, and to identify areas where additional research is needed to bridge the gap.

## WEED MANAGEMENT METHODS CURRENTLY IN USE IN DSR

Hand weeding continues to be used in rice production either solely or as component of weed management in countries of Asia (Rao et al., 2007) and West Africa (Rodenburg et al., 2011). In Asian countries where direct-seeding became the major method of rice establishment (e.g. Korea, Malaysia, Philippines, Sri Lanka and Vietnam), the use of herbicides increased (Naylor, 1994; Azmi et al., 2005; Rao et al., 2007; Weerakoon et al., 2011). In China, around 20 million hectares of rice fields are applied with herbicides (Zhang, 2003). It was estimated that approximately 98% of U.S. rice acres are treated with herbicides and rice production would drop by 38 %, if no herbicides were used in U.S.A. (Gianessi and Reigner. 2006). In Europe and Australia, chemical control has been used since long in direct-seeded rice (Bocchi et al., 2005, Taylor, 2007). The high costs of weed control could be a major factor constraining the widespread adoption of direct-seeding. High weed-inflicted yield losses in direct-seeded rice in Asia are due to limited number of effective and affordable weed control options available to farmers. The key to the success of direct-seeded rice is the availability of efficient weed control techniques that ate economically and ecologically viable. Alternative weed management technologies are therefore much needed (Rao et al., 2007) in the changing world. Drivers necessitating the development of alternative weed management strategies such as integrated ecological weed management in DSR are described below briefly.

**Factors necessitating the ecological weed management approach in DSR in the changing world**

Ecological management of weeds in DSR stresses on shifting the crop-weed balance in favour of rice by adapting in an integrated manner the strategies that: modify the weeds' microenvironment placing stress at multiple growth stages in weeds life cycle, enhancing weed seedling mortality, to increase the competitive ability of the rice for attaining optimum rice productivity, to minimize or attempt to eliminate weed seed production and dispersal, and to accelerate weed seed bank depletion, and judiciously using herbicides as the last resort rather than as the only resort.

Major drivers for the need of ecological weed management in DSR include: a) global water scarcity; b) labor shortage and increasing labor wages; c) enhanced interest in conservation agriculture; d) shits in weed flora and development of resistance against weeds; e) emergence of new weed problems such as weedy rice (O. sativa) (Rao et al., 2007); and f) global climate change i.e. changes in atmospheric carbon dioxide (CO2), rainfall and temperature will affect weed communities (Rodenburg et al., 2011)

Thus in a changing world, it is essential to identify practical components of integrated ecological weed management strategies for effectively managing weeds in DSR in an ecologically and economically sustainable manner.

## ECOLOGICAL WEED MANAGEMENT OPTIONS FOR DSR

The principles that underline ecological weed management system are: (a) adapting the weed management options that suits to the environment of the region, including soil, water, climate and biota present at the site; (b) optimizing the use of biological and chemical/physical resources for effective weed management in DSR. Several components of ecological weed management have the ability to reduced weed density, minimise weeds competitive ability, avoid undesirable shifts in weed community towards difficult to control weeds, improve rice productivity through improving rice competitiveness against weeds with minimal adverse impact on environment in DSR ecosystem.

## NON CHEMICAL COMPONENTS OF ECOLOGICAL WEED MANAGEMENT

Non chemical components of ecological weed management involve any aspect of non-herbicidal weed management that favors the crop relative to the weed. They include:

(a) Avoiding weed seed contamination in rice seed and the use of certified seed: Sowing seed contaminated by weedy rice is likely the primary cause of invasiveness of weedy rice in rice fields (Rao et al., 2007). Rice seed soaking in herbicide solution for controlling rice seed contaminants (Rao and Moody, 1996) or the use of certified seed have proved to be an essential component in weed management (Rao et al., 2007)

b) Sanitation: Sanitation is one means of minimizing the likelihood of weed introductions and dispersal of existing weeds throughout a farm, especially herbicide-resistant weeds. All farm machinery should be washed well to remove weed seeds and propagules of perennials in attached-soils from the neighbouring weed infested fields before being moved into clean paddy fields.

c) Soil solarisation: Soil solarisation is a method of heating the soil's surface by using transparent low-density polyethylene (LDPE film) sheets placed on the soil's surface to trap solar radiation (Khan et al., 2003). The use of transparent and black LDPE sheets reduces weed growth and increases rice yield (Khan et al., 2003).

d) Weed management during fallow period: A clean fallow period is the best strategy for drawing down the seedbank. Shallow tillage coincident with weed emergence periodicity will stimulate germination of weeds, and subsequent tillage kills these seedlings. Tillage combined with *Stylosanthes guianensis* (stylo) fallow were recommended to smallholder farmers for improving upland dry-seeded rice productivity (Saito et al., 2010).

e) Cultural practices: Cultural practices such as tillage, mulching, and burning are used by certain traditional farmers to control weeds.

Tillage: The need for thorough land preparation to ensure a vigorous rice stand and to suppress weeds is well known. Tillage increases germination of seeds in the soil seed bank, reducing the seed reserves in the soil (Chauhan et al., 2009). Harrowing and puddling are done for a range of reasons including weed control (Rao et al., 2007; Satoshi et al., 2009). Precision land leveling, obtained with laser-directed equipment, has made an important contribution to weedy rice management in European rice production (Ferrero and Videtto, 2007). Level or regularly sloping fields enable appropriate water management, which limits weed growth and guarantees uniform emergence of weeds, which in turn makes herbicides more effective.

Reduced tillage and Stale seedbed: With dry direct-seeding under Zero tillage (ZT) or reduced tillage conditions, fuel and labor costs are reduced (Kumar and Ladha, 2011). A large proportion of the weed seed bank remains on or close to the soil surface after crop planting in ZT systems, which may promote greater emergence of weed species that require light to germinate (Chauhan et al., 2009). In stale seedbed weeds are encouraged to germinate prior to seeding the crop then eliminated with glyphosate (Rao et al., 2007).

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Alternating rice establishment systems from aerobic (dry-seeding) to anaerobic (water-seeding) regimes (and vice versa) combined with the use prior to seeding of a non-selective herbicide for which resistance does not yet exist in weeds of rice (such as Roundup) allows for a major reduction of: herbicide resistant weed infestations; overall herbicide use and costs (Fischer et al., 2009).

Water management: The importance of water management in weed control in DSR is well known (Rao et al., 2007). The construction of field bunds, which retains water in the paddy, has the potential to significantly increase rice production in West Africa, while also possibly reducing labor requirements for hand weeding (Becker and Johnson, 2001). Appearance of red rice (*Oryza sativa*) as the main weed problem in rice production in the southern US has led many producers to use Pin-Point (PP) irrigation, originally developed in Louisiana, to control the weed (Noldin, 2000).

Fertiliser and weeds: To achieve high rice yields, both nitrogen fertilization and weed management are essential (Begum et al., 2009). Losses from weeds progressively decreased with higher fertility levels due to the greater competitive ability of rice (Rao et al., 2007). Judicious use of fertilizers during early growth stage in combination with other weed management practices may help DSR to compete with weeds.

f) Improving rice competitiveness: One cultural weed management approach is to reduce the effects of weeds on the crop by either making weeds less competitive or by making the crop more competitive. Improving rice competitiveness against weeds would provide a low-cost and safe tool for an ecological weed management strategy. Any cultural practice that facilitates rapid rice growth and results in rice canopy covering soil surface, and shade out weeds, increase crop competitiveness. Practices that contribute DSR competitiveness include: early sowing, selection of varieties with early growth, optimal seed rates; close spacing; adequate plant population and fertilization (Rao et al., 2007).

g) Cover crops: Cover crops grown in the period between two main crops have potential as an important component of a system-oriented ecological weed management strategy (Kruidhof et al., 2008). Cover crops fill inter-crop periods in cropping systems that would otherwise be occupied by weeds and thus reduce weed pressure and labor required for weeding.

h) Intercropping: Intercropping is practiced by farmers in both developing and developed countries. Weed growth was lower in upland dry-seeded rice/cowpea intercrop than in rice sole crop due to the successful smothering effect of cowpea inter crop because of its broader leaves and early rapid growth which blocked the light from reaching the ground (Musthafa and Potty, 2001). Smother crops like Amaranthus, Indian till were found to be much effective in reducing the weed growth without affecting the productivity of aerobic rice (Umeshanaika et al., 2009).

i) Crop Rotations: It is now evident that crop rotation reduces weed growth and increases crop yield and that the practice is essential in sustainable agriculture systems (Rao et al., 2007). Increasing cropping system diversity has been advocated as a potential means of decreasing the need for intensive chemical inputs for weed control (Liebman and Staver

2001). Successful weed management requires cropping sequences that minimizes the weed seed additions and maximise weed seed depletion in DSR soil weed seed bank.

j) Mulching: The spreading of mulch on the soil surface reduces evaporation, saves water, protects from wind and water erosion, and suppresses weed growth (Singh et al., 2007). Mulching + dry land weeder at 20 DAS proved more effective in dry-seeded rice grown without herbicide use (Hussain and Gogoi, 1996).

k) Brown manuring: Brown manuring is a 'no-till' version of green manuring, using a herbicide to desiccate the intercrop (and weeds) at flowering instead of using cultivation. The plant residues are left standing. In ''Brown Manuring' practice both rice and *Sesbania* crops are seeded together and allowed to grow for 30 days. Subsequently *Sesbania* intercrop is knocked down with 2, 4-D at 500 g ha$^{-1}$ (Singh et al., 2007). This technology reduces weed population by nearly half without any adverse effect on rice yield. *Sesbania* surface mulch decomposes very fast to supply N and other recycled nutrients.

l) Mechanical weeding: Weeding by mechanical devices reduces the cost of labor and also saves time (Subudhi, 2004). The labor involved was least with the Phulbani weeder (57 person-days ha$^{-1}$), saving nearly 57% labor compared with hand weeding (127 person-days ha$^{-1}$). It also had better weed control efficiency (Subudhi, 2004). Dry-seeded rice row seeding with interrow weeding using hoes and without any herbicide achieved higher grain yield (Satoshi et al., 2009). Mnguu (2010) opined that rice farmers can use rotary weeding instead of herbicide in controlling weeds and achieve the same grain yield of wet-seeded rice.

m) Bioherbicides: Micro-organisms also are used as tools for weed management and have a range of properties that make them desirable for ecological weed management in direct-seeded rice. COLLEGO, a powder formulation of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene*, was registered in 1982 for the control of northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) in rice. The successful mycoherbicide, *Rhynchosporium alismatis* had synergistic controlling effect on *Damasonium minus* (R.Br.) Buch. when bensulfuron-methyl was applied before fungal inoculation (Jahromi et al., 2001). In Asia, the bioherbicide research is yet to reach the practical usage stage.

In addition to above methods, usage of ducks (Furuno, 2001) and water birds ((Kendig et al., 2003) was also found effective in managing weeds and hence may be used as a components of ecological weed management in DSR systems.

**Herbicide based components of ecological weed management**

Herbicides are not ruled out in ecological weed management in direct-seeded rice. However, in ecological weed management, herbicides are treated less as a blanket solution to weed management and more as a precious resource, to be used selectively in ways that complement other weed management methods. Phenoxy and sulfonylurea compounds are widely used herbicides in Malaysia, Vietnam, and Thailand to control

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broadleaf weeds and sedges in direct-seeded rice (Azmi et al., 2005). Propanil, thiobencarb, molinate, butachlor, quinclorac, acetochlor and oxadiazon have been widely used for grass weed control. Several herbicides are now available for effectively managing specific problematic weeds in direct-seeded rice and herbicides used in different methods of DSR were listed by Rao et al., (2007). Integration of herbicides with other components of ecological weed management is needed to avoid environmental, health hazards as well as the risk of evolution of herbicide resistant weeds. Effective weed control in DSR was reported by the integration of herbicides with: a) crop rotations; (b) crop competitiveness; (c) mulching; and (d) hand weeding (Singh et al., 2007, 2008; Rao et al., 2007).

Herbicides may continue to play key role in weed control of direct-seeded rice in developed countries and in majority of direct-seeded rice growing developing countries as a component of ecological weed management. It is essential, however, to evaluate the environmental and health consequences of potential technologies that are based on chemical means of weed control.

## FUTURE RESEARCH NEEDS

Ecological weed management strategies that suit to the farmers needs in different agro-ecological regions need to be developed for varying methods of direct-seeding based on the knowledge of weed ecology and biology. A few areas of research that needs emphasis include: (a) Understanding weed ecology and biology and utilising knowledge for evolving effective and economical ecological weed management strategies; (b) Continuous monitoring of weed populations to predict/identify undesirable shifts in weed communities; (c) Simple methods of decision making to enable farmers to choose right components o ecological weed management to manage weeds in their DSR fields; (d) Identifying farmers' weeds control needs and developing ecological weed management approaches to solve location specific weed problems in DSR.

No single management technique provides complete weed control and is ideal for all conditions. Simultaneous use of multiple components of ecological weed management methods in a scientific manner, instead of focusing on single method, with a common goal of shifting crop weed balance in favor of the direct-seeded rice, is essential.

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## REFERENCES

Azmi, M., Chin, D. V., Vongsaroj, P., And Johnson, D. E. (2005). Emerging Issues In Weed Management Of Direct-Seeded Rice In Malaysia, Vietnam, And Thailand. In

Toriyama, K., Heong, K. L. And Hardy, B. (Eds) *Rice Is Life: Scientific Perspectives For The 21st Century.* Irri, Philippines And Ircas, Tsukuba, Japan. Pp. 196–198.

Becker, M. and Johnson, D. E. (2001). Improved water control and crop management effects on lowland rice productivity in West Africa. *Nutrient Cycling in Agroecosystems.* 59: 119–127

Begum, M., Juraimi, A. S., Amartalingum, R., Syed Omar, S. R., and Man, A. B. (2009). Effect of *Fimbristylis miliacea* competition with MR220 rice in relation to different nitrogen levels and weed density. *Int. J. Agric. Biol.* 11: 183–187.

Bocchi, S., Callegarin, A. M., and Baldi, G. (2005). Rice production system in Italy and its sustainability, p. 39. Productio´n arroz en Italia, Asociacio´n Cultivadores de Arroz, Italy.

Chauhan, B. S. and Johnson, D. E. (2009). Influence of tillage systems on weed seedling emergence pattern in rainfed rice. *Soil & Tillage Research* 106: 15–21.

FAO-Food and Agriculture Organization. (2009). In “FAOSTAT Database”. FAO, Rome, www.faostat.fao.org.

Ferrero, A. and Vidotto, F. (2007). Weed management in European rice fields. *International Rice Commission Newsletter (FAO).* 56: 44-51.

Fischer, A. J., Moechnig, M., Mutters, R. G., Hill, J. E., Greer, C., Espino, L., and Eckert, J. W. (2009). Managing Herbicide Resistance Using Alternative Rice Stand Establishment Techniques. In *Proceedings of the California Weed Science Society*. 61: 33-34.

Furuno, T. (2001). The power of duck. Integrated rice and duck farming, first ed. Tagari Publications, Tasmania, Australia, p.17.

Gianessi, L. and Reigner, N. (2006). The Value of Herbicides in U.S. Crop Production, 2005 Update. (National Centre for Food and Agriculture Policy, Washington, DC 20036). Available at http://www.croplifefoundation.org.

Hussain, A. and Gogoi, A. K. (1996). Non-chemical weed management in upland direct-seeded rice. *Indian J. Weed Sci*. 28, 89-90.

Jahromi, F., Cother, E. and Ash, G. (2001). Weed Control in Rice Crops - Suitability of Rhynchosporium alismatis as a Mycoherbicide for Integrated Management of Damasonium minus in Rice Fields. RIRDC Publication No 01/39. RIRDC Project No UCS 7A. (RIRDC, Canberra, Australia).

Kendig, A., Williams, B. and Smith, C. W. (2003). Rice weed control. Chapter 3.7. In Smith, C.W., and Dilday, R.H. (Eds). *Rice, origin,history,technology and production*. Wiley, New Jersey, 457-472.

Khan A. R., Srivastava R. C., Ghorai A.K. and Singh, S.R. (2003). Efficient soil solarization for weed control in the rain-fed upland rice ecosystem. *Int. Agrophys.* 17: 99–103.

Kruidhof, H.M., Bastiaans, L. and Kropff, M. J. (2008). Ecological weed management by cover cropping: effects on weed growth in autumn and weed establishment in spring. *Weed Research*. 48: 492–502

Kumar, V. and Ladha, J. K. (2011). Direct seeding of rice: Recent developments and future research needs. *Adv. Agron*. 111: 297-413.

Liebman, M. and Staver, C. P. (2001). Crop diversification for weed management. In Liebman, M., Mohler, C. L., and Staver, C. P. (Eds). *Ecological Management of Agricultural Weeds*. Cambridge University Press, Cambridge, UK. pp. 322–374.

Mnguu, Y. O. (2010). Effect of crop management practices on Nitrogen (N) availability and N use efficiency in direct wet-seeded Rice. *Journal of Animal & Plant Sciences*. 6, 589-596.

Musthafa, K. and Potty, N. N. (2001). Effect of *in situ* green manuring on weeds in rice. *Journal of Tropical Agriculture* 39: 172-174.

Naylor, R. (1994). *Herbicides in Asian Rice: Transitions in Weed management.* Inst. Int. Stud., Stanford University, IRRI, Manila, Philippines.

Noldin, J. A. (2000). Red rice status and management in Americas. In Baki *et al.,(*eds.) IRRI Manila, Philippines. pp. 21-24.

Rao, A. N. and Nagamani, A. (2007). Available technologies and future research challenges for managing weeds in dry-seeded rice in India. In *Proc. of the 21st Asian Pacific Weed Sci. Society Conf.* 2 to 6th October 2007, Colombo, Sri Lanka. pp.391-400.

Rao, A. N. and Moody, K. (1996). Seed soaking in herbicide solution for controlling *Ischaemum rugosum*, a rice seed contaminant. In: Proc. of the *15th Asian Pacific Weed Science Society Conf*. 24th–28th July 1995, Tsukuba, Japan.

Rao, A. N., Mortimer, A. M.., Johnson, D. E., Sivaprasad, B. and Ladha, J. K. (2007). Weed management in direct-seeded rice. *Advances in Agronomy*. 93: 155-257.

Rodenburg, J., Meinkee, H. and Johnson, D. E. (2011). Challenges for weed management in African rice systems in a changing climate. *Journal of Agricultural Science*. Pp. 9. (Online first, doi: 10.1017/S0021859611000207)

Saito, K.; Azoma, K. and Oikeh, S. O. (2010). Combined effects of *Stylosanthes guianensis* fallow and tillage management on upland rice yield, weeds and soils in southern Benin. *Soil & Tillage Research*. 107, 57-63.

Satoshi, H., Anuchart, K., Boonrat, B., Akihiko, K., and Junko, Y. (2009). Spatial variability in the growth of direct-*seeded* rainfed lowland *rice* (Oryza sativa L.) in northeast Thailand. *Field crops research*. 111: 251-261.

Singh, S., Ladha, J. K., Gupta, R. K., Bhushan, L., Rao, A. N. 2008. Weed management in aerobic rice systems under varying establishment methods. *Crop Protection* 27, 660–671.

Singh, S., Ladha, J. K., Gupta, R. K., Bhushan, L., Rao, A. N., Shiva Prasad, B. and Singh, P. P. (2007). Evaluation of mulching, intercropping with *Sesbania* and herbicide use for weed management in dry-seeded rice (*Oryza sativa*). *Crop Protection*. 26, 518-524.

Subudhi, C. R. (2004). Evaluation of weeding devices for upland rice in the Eastern Ghats of Orissa, India. *IRRN*. 29: 79-80.

Taylor, M. (2007). New weed management options for Australian rice. *IREC Farmers' Newsletter*. 177: 41-43.

Weerakoon, W. M. W., Mutunayake, M. M. P., Bandara, C., Rao, A.N., Bhandari, D. C. and Ladha, J. K.. (2011). Direct-seeded rice culture in Sri Lanka: Lessons from farmers. *Field Crops Research*. 121: 53-63.

Zhang Z. P. (2003). Development of chemical weed control and integrated weed management in China. *Weed Biology and Management.* 3: 197–203.

# RIM, LUSO, PERTH AND THE WIZARD: A COMPLEMENTARY FAMILY OF MODELS FOR SUPPORTING WEED MANAGEMENT DECISIONS IN CROPPING SYSTEMS

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## ABSTRACT

This paper presents a number of related models developed to address different weed management questions in Australian cropping systems. The models are compared and contrasted in terms of purpose, application and design, in order to show that the models form a complementary family, with each suitable for addressing a different range of aims and applications.

**Keywords**: weed, management, model, herbicide, resistance, simulation

## INTRODUCTION

Weeds are an important factor in every agronomic system. They reduce yields through competing with the crop for nutrients, water and light, and can also contaminate harvested grain and poison stock. However, in some sense, the real problem is not the weeds growing in the crop, but the bank of weed seeds lying hidden beneath the soil. No matter how many weeds I can kill right now, there will always be more seeds waiting in the ground. The weed seedbank is a serious challenge for three main reasons: it is invisible, it is patient and it is complex. While the weeds can be seen competing with the crop above ground by the light of day, their seeds lurk hidden beneath the ground. While most cropping weeds come and go within a few months, their seeds can often wait happily for months or years until conditions are suitable. And while it may seem that the best way to control weeds is simply to kill the plants, the long-term fluctuations in weed numbers will be affected by the complex interaction of a large number of factors. These include the varying dormancy of a range of different species; competition among crops and different weed species; the effects of herbicides, tillage (or non-tillage), harvesting options and other management techniques on soil, plants and seeds; weather and environment; individual genetic variability within populations that may evolve over time under different selection pressures, affecting resistance to herbicides, dormancy and other characteristics; and even seed-eating insects and microbes!

One way to help understand, predict and manage a system that is mostly hidden beneath the soil and dependent on a large number of complex interactions that play out over a relatively long term, is to create a computational model of that system. A computational

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model can help integrate existing knowledge and hypotheses gained from observations, literature and focussed experiments and trials. By synthesising this information in a model, we can build a reasonable representation of the way things will work in a much wider range of interacting conditions and over a much longer time period than would be possible to address directly in field trials or experiments. The simulation can provide a window into the parts of the system that are usually hidden (the seedbank and the population genetics for example), and look at how they influence and are influenced by the parts of the system that directly affect us (the weeds) and the parts that we can control (management options).

Constructing a model involves making decisions and trade-offs about what underlying processes to include or not include in the model, the appropriate level of detail and realism at which to represent these processes, and what temporal and spatial scale to base the model on. There are also choices about how to enable user interaction with the model, and what kind of results should be provided by the model. In general, like all design decisions, these decisions should be made in light of a clear idea of the purpose for which the model is being built. For example, the best choices for a model intended to be a practical decision-aid tool that can help farmers and consultants manage weed populations in specific agricultural contexts and seasons are likely to be very different to the best choices for a model intended to help researchers analyse the efficacy of general long-term management strategies for reducing the risk of evolving herbicide resistance.

This paper briefly presents a number of related models developed to address different weed management questions in Australian cropping systems. The aim is to compare and contrast the models in terms of purpose, application and design, and to show that the models form a complementary family, with each suitable for addressing a different set of aims and applications.

**Resistance and Integrated Management (RIM)**

RIM (Resistance and Integrated Management or Ryegrass Integrated Management) is a computer package developed in Microsoft Excel that allows users to try out various combinations of weed treatments and observe their predicted impacts on ryegrass populations, crop yields and long-term economic outcomes (Pannell *et al*. 2004). A wide variety of chemical and non-chemical weed treatment options are included, so that as chemicals are lost to herbicide resistance, users can experiment to try to identify the next best substitute. RIM has been used extensively as part of interactive workshops with farmers, agronomists and agricultural science students, and for economic analyses of various weed control options (eg. Monjardino *et al*. 2004).

RIM works on an annual time step and represents a single paddock within a farm. A southern Australian agricultural system is represented, where there is one crop or pasture option possible each winter, and a summer fallow. The model represents an average season within an average paddock; no specific weather or soil data is required, and the effects of such specific information cannot be modelled directly. There is therefore no representation of climate variability, and the only way to make the model specific to a particular locality, farm or paddock is to change all relevant parameters (such as potential yield) within the model. The standard version of RIM represents one species only, ryegrass, although other versions have been constructed for wild radish and poppy (Monjardino et al. 2004, Torra et al 2010). The level of biological detail is relatively simple and abstract. For example, only a single seedbank pool is used, with no representation of different soil layers or seed cohorts. Four different weed plant cohorts are included. Herbicide resistance is represented in the model in a simple way; it is assumed that only a

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limited number of uses are available for each herbicide group. There is no representation of individual or population genetics or actual evolution of resistance.

The user enters their proposed crop or pasture options for 10 or 20 years and then their proposed management options within each year, by entering letters or tick marks into an Excel spreadsheet. The model automatically warns if options are not appropriate for the land use for which they have been entered. As each option is entered, the user is immediately provided with updated output including the total number of weeds and the total number of weed seed numbers in each year, the gross margin achieved for each year and the 'average' annual profit over the full 10 or 20 years (actually the nominal annuity). More detailed biological and economic results, for more detailed understanding of the main outputs, can also be viewed if desired.

**The Weed Seed Wizard (WSW)**

The development of the Weed Seed Wizard has been funded by the Cooperative Research Centre for Australian Weed Management and the Grains Research and Development Corporation, with the aim of creating a practical decision-aid tool that can help farmers and consultants manage weed populations in real agricultural contexts. Implemented in the Java programming language, the model uses detailed and specific paddock management and weather records, and simulation of important aspects of seed biology, in order to track and predict the number, ages, soil depth, dormancy levels, viability and germination of seeds in the soil. The model then predicts the amount of weeds appearing each year (Renton *et al.* 2008).

Some aspects of the underlying models within WSW are based on similar models in RIM, although WSW adds much greater temporal resolution and biological detail and realism, as it works on a daily time step. WSW represents a single paddock or section of paddock within a farm and aims to be able to represent any kind of farming system, with crops sown and harvested on any day of the year, although the current version is best suited to simulating a southern Australian agricultural system. Unlike RIM, the model requires detailed daily weather data for the period simulated, and is thus able to simulate the effects of specific variations in such data, such as season-to-season variation in the timing of the opening rains and differences in weather in different locations. There is also some representation of the effects of different soil types. WSW has been constructed so that new weed species can easily be added to the model and populations including multiple weed species can be simulated without difficulty. The accuracy of these simulations depends of course on the adequacy of the parameterisation of each species included in the model. The level of biological detail in WSW is relatively complex and realistic compared to RIM. For example, multiple seedbank cohorts are represented for different soil layers and age cohorts. Different weed plant cohorts for every separate germination date are also included. Resistance is not explicitly represented in the model at all, although it can be included by assuming a decline in efficacy of a certain herbicide over time.

The user enters all their proposed management options into WSW as 'events', specifying the date for the event. Possible management events include herbicide applications, sowing of crops, tillage, grazing and harvest. Various options can be specified for each event, such as the kill-rate achieved by a given herbicide application, although sensible defaults are provided as a guide. Management events can be provided to represent a single year or as many consecutive years as desired, and the user also specifies the start and end date of the simulation. When the user is satisfied with the sequence of events entered, the scenario is simulated and the user provided with updated summary output including the

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total density of weed seeds following each harvest, the crop harvest achieved, and the amount of potential harvest lost to weed competition. Much more detailed biological and economic results can also be viewed if desired, such as seedbank densities for different soil layers and species or weed densities for each species on each day of the simulation. The current version of the model does not provide economic outputs.

**Land Use Sequence Optimiser (LUSO)**

The development of LUSO has been funded by the Grains Research and Development Corporation with the aim of creating a bio-economic model and optimisation framework for analysing the drivers of tactical and strategic decisions regarding ‘break-crops’ and land-use sequencing within agricultural systems. Implemented in the Python programming language, the model simulates the dynamics of weed populations, plants disease loads and soil nitrogen levels over many years, together with their effects on yield and profit (Lawes and Renton 2010).

The weed model underlying LUSO is based on RIM, with the addition of plant disease and soil nitrogen modules and a number of automated bio-economic analysis tools. Like RIM, LUSO works on an annual time step and represents a single paddock within a farm. A southern Australian agricultural system is represented, with one crop or pasture option possible each winter and a summer fallow. The standard version of the model represents an average season within an average paddock; no specific weather or soil data is required, the effects of such specific information cannot be modelled directly and there is therefore no representation of climate variability. The model can be quite easily adapted to a specific locality, farm or paddock by changing all relevant parameters (such as potential yield) within the model, since the number of parameters is relatively small. Moreover, a prototype version of LUSO has been developed to account for seasonal variability and its effects on crop yield, pasture production, plant disease dynamics and weed seed set. The standard version of LUSO represents a single weed species; by default this is parameterised to represent ryegrass, although the model has been designed so that it can easily be adapted to represent any other species of interest or potentially a number of species if necessary. Like RIM, the level of biological detail is relatively simple and abstract. For example, only a single seedbank pool is used, with no representation of different soil layers or seed cohorts. Herbicide resistance can be represented in the model in simple ways, either by assuming a fixed number of available uses for a herbicide or a steady decline in the efficacy of the herbicide.

LUSO requires a list of default parameters for a number of possible land uses. A standard version of the parameter file is provided with the model and can be easily customised by the user using Microsoft Excel as an editor to address a particular question or situation of interest. The analysis framework can then be used in a number of ways. Most simply, a specific sequence of land use options can be specified and the resulting predicted profit calculated, similar to RIM. Alternatively, a number of optimisation algorithms are available, which can be used to determine the best land use sequence or sequences for the given assumptions and their corresponding predicted profits. Automated sensitivity analyses can then be conducted to determine how the optimal land use sequence and the optimal profit change as one or more model parameters vary. For example, this capability could be used to determine what average level of weed seed set control would need to be achieved in a canola crop for canola to be included in a farmer’s optimal land use sequence strategy or what increase in wheat competitiveness would be required to achieve a 10% increase in long-term profitability of the overall cropping system. The framework allows analysis of

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both strategic and tactical decisions. For example, it can be used to analyse the drivers and thresholds influencing the decision of whether or not to sacrifice short-term profit for long-term weed management benefits by green-manuring a lupin crop, or it can be used to analyse the drivers and thresholds influencing whether lupins are included in a long-term crop sequencing strategy.

**Polygenic Evolution of Resistance To Herbicides (PERTH)**

The development of PERTH has been supported by the Grains Research and Development Corporation through the Australian Herbicide Resistance Initiative. The PERTH model was created to analyse the effect of different long-term management options on the risk of evolving herbicide resistance in agricultural systems and to account for the effects of weed biology, weed ecology, population dynamics and the genetics underlying resistance. Implemented in the Python programming language, the model simulates the dynamics of weed population numbers and genetics over many years, together with their effects on crop yield (Renton *et al.* 2011).

The model of weed population dynamics underlying PERTH was originally based on RIM, although it has been extended by adding detailed representation of resistance genetics and their interaction with herbicide efficacy, and, more recently, more detailed representation of seedbank density at different soil depths and important aspects of weed biology, such as dormancy and breeding system (level of out-crossing). Like RIM, PERTH works on an annual time step and represents a single paddock within an agricultural system where there is one crop or pasture option possible each year. The standard version of the model represents an average season within an average paddock, with no representation of specific weather or climate variability. There is little need for more specificity, since the model is aimed at evaluating general long-term strategies. However, a prototype version of PERTH is being developed to account for seasonal variability and its effects on risk of herbicide resistance evolution, as affected by weed seed set and variation in crop yield, herbicide efficacy and application timing. The standard version of PERTH represents a single weed species; by default this is parameterised to represent ryegrass, although the model has been designed so that it can easily be adapted to represent any other species of interest. The level of biological detail is intermediate between the simplicity of RIM and LUSO, and the complexity of WSW. For example, the resistance genetics is represented in great detail, with each seed represented separately for this purpose. The seedbank is also divided into a number of soil layers and the weeds into four different cohorts. Cohorts are affected by management in different ways. Herbicide resistance is represented in the model in much greater detail and at a higher level of realism than the other three models. Evolution is simulated explicitly and mechanistically, emerging as a result of the interaction between population dynamics, individual resistance genetics, differential survival of individuals at different herbicide applications and genetic recombination with breeding.

PERTH requires a list of default parameters specifying weed characteristics, resistance genetics factors, and weed management options. A standard version of the parameter file is provided with the model and can be customised by the user using Microsoft Excel as an editor to address a particular question or situation of interest. The model is then run and produces a series of outputs showing the results of the simulation under the given assumptions. These graphical and tabular outputs include weed densities at critical time of the season, levels of resistance specified in various ways, crop yield as a percentage of potential of weed-free yield, and details of genetics, all given for each year of the simulation, so their dynamics can be tracked over time. One summary output is also provided, the number of years for which weed numbers remain low enough for the system to remain agronomically sustainable. Example Python scripts are also provided that can be used to determine the effects of varying different model parameters on the number of years of the systems' sustainability.

## DISCUSSION AND CONCLUSIONS

The list of weed simulation models presented in this paper is not at all intended to be exhaustive. Many other models have been developed to simulate important aspects of weeds in Australian cropping systems. These include the well-established Agricultural Production Systems Simulator (APSIM), which includes modules for simulating the growth of weeds in competition with crops (Keating *et al.* 2003), and Thornby and Walker's (2009) model that integrates APSIM with a model of weed population dynamics to predict herbicide resistance evolution in northern Australia cropping systems.

The models presented in this paper have different intended purposes, which are reflected in the different design choices. RIM and WSW have sophisticated graphical user interfaces, as they are intended to be used by a wide range of non-technical users, while LUSO and PERTH have simpler user interfaces. RIM's interface can be adapted quickly, allowing it be used easily in interactive workshops, while WSW's interface is more complex and slower to use, because it allows access to a much greater array of underlying biological and management details. The design choices reflect conscious trade-offs. For example, the focus on genetics in PERTH makes PERTH good at predicting long-term evolution of resistance, while the seed dormancy mechanisms and specific detailed weather data in WSW make WSW good at predicting temporal germination patterns under actual weather conditions. LUSO and RIM include economics while WSW and PERTH do not. The relative simplicity of LUSO makes it feasible to develop computationally intensive optimisation routines that would not be feasible for WSW. Each model is specialised for its intended purpose.

When new issues arise, it may seem attractive to try to adapt an existing model to which resources and time have already been devoted. While this may sometimes be the case, in practice it will often be more efficient to develop a new model aimed specifically at addressing this new question. As an analogy, rather than adapt your car to travel on the ocean, it may be easier to just build a boat. With a clear goal in mind, appropriate design decisions can be made. The new model will thus contain only those features and processes necessary to address the issue. Useful parts of existing models can be included and less relevant parts left out. Specific purpose models are thus kept as simple as possible, making them easier to maintain and use and minimising computational demands, while models regularly extended to address new demands tend to steadily increase in complexity and thus computational and maintenance requirements. Specialised models can also work together efficiently. For example, instead of trying to build economics into every aspect of WSW or PERTH, their output can be used to identify key parameter values for LUSO, which can then be used efficiently for economic analysis. Instead of trying to build detailed resistance genetics into WSW or include specific weather data in PERTH, WSW can be used to predict the effects of climate on germination variability, and this can then be incorporated into PERTH. If we also want predictions of crop yield variability to include in PERTH, then instead of trying to build a detailed yield prediction model into PERTH itself, we can look for an appropriate model to provide these predictions, which may be APSIM. Instead of trying to make one model that does everything, we can create a family of efficient models that specialise in simulating different aspects of weed dynamics

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and find ways to allow these models to work together to support weed management decisions.

**REFERENCES**

Keating, B.A., Carberry, P.S., Hammer, G.L., Probert, M.E., Robertson, M.J., Holzworth, D., Huth, N.I., Hargreaves, J.N.G., Meinke, H., Hochman, Z., McLean, G., Verburg, K., Snow, V., Dimes, J.P., Silburn, M., Wang, E., Brown, S., Bristow, K.L., Asseng, S., Chapman, S., McCown, R.L., Freebairn, D.M. and Smith, C.J. (2003). An overview of APSIM, a model designed for farming systems simulation. *European Journal of Agronomy* 18: 267-288.

Lawes, R. and Renton, M. (2010). The Land Use Sequence Optimiser (LUSO): A theoretical framework for analysing crop sequences in response to nitrogen, disease and weed populations. *Crop and Pasture Science* 61: 835-843.

Monjardino, M., Pannell, D.J. and Powles, S. (2004). Economic value of pasture phases in the integrated management of annual ryegrass and wild radish in a Western Australian farming system, *Australian Journal of Experimental Agriculture* 44: 265-271.

Pannell, D.J., Stewart, V., Bennett, A., Monjardino, M., Schmidt, C. and Powles, S. (2004). RIM: A bioeconomic model for integrated weed management of *Lolium rigidum* in Western Australia. *Agricultural Systems* 79: 305-325.

Renton, M., Diggle, A. and Peltzer, S. (2008). Understanding, predicting and managing weed seedbanks in agricultural systems with the Weed Seed Wizard. 16th Australian Weeds Conference. Cairns, Australia, 77-79. http://www.caws.org.au/awc/2008/awc200810771.pdf.

Renton, M., Manalil, S., Diggle, A. and Powles, S. (2011). Does cutting herbicide rates threaten the sustainability of weed management in cropping systems? *Journal of Theoretical Biology*, In Press, doi:10.1016/j.jtbi.2011.05.010

Thornby, D.F. and Walker S.R. (2009). Simulating the evolution of glyphosate resistance in grains farming in northern Australia. *Annals of Botany* 104: 747-756.

Torra, J., Cirujeda, A., Recasens, J., Taberner, A. and Powles, S. (2010). PIM (Poppy Integrated Management): a bio-economic decision support model for the management of Papaver rhoeas in rain-fed cropping systems. *Weed Research* 50: 127-139.

# GLYPHOSATE SUSCEPTIBILITY OF ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* L.) POPULATIONS IN NO-TILLAGE SYSTEMS IN URUGUAY

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## ABSTRACT

The response of annual ryegrass (*Lolium multiflorum* L.) populations to different glyphosate rates was assessed in highly infested sites of the Western agricultural area of Uruguay. Six sites with previous information regarding crop rotation history, years under no-tillage and glyphosate usage were selected for this study. Seven glyphosate rates were tested: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 kg a.i./ha and a control treatment without herbicide treatment was also included. Visual estimations at 10, 20, 30 and 40 days after the applications were conducted to determine control efficiency of the glyphosate rates. Results showed significant differences in the speed of control among different rates. No satisfactory controls were achieved at the lowest herbicide rates at any of the evaluation times. Furthermore, the increase of glyphosate rates reduced the differences on control efficiency between sites. Ten days after the herbicide treatments the control efficiency was extremely variable across sites. However, after 30 days from the herbicide treatments, rates of 4.0, 6.0, 8.0 kg a.i./ha achieved more than 90% of control efficiency at all sites. No glyphosate-resistant ryegrass populations were found in this study. The susceptibility of annual ryegrass populations was highly dependent on the phenological stage when the herbicide treatments were applied, as well as the dry matter per hectare present in each of the sites.It was observed that some ryegrass seedlings survived high glyphosate rates showing a tolerance response and suggesting the need to frequently monitor these sites in the future.

**Keywords**: chemical control, glyphosate, resistance, annual ryegrass

## INTRODUCTION

In Uruguay, crop production has traditionally been undertaken by rotating beef cattle production and crops in the same area across years. This system has been demonstrated to be environmentally sustainable as soil erosion is reduced and nutrients are recycled. However, in the last ten years the Western agricultural area of Uruguay has experienced an intensification partially associated with the high adoption of no-tillage systems together with transgenic glyphosate-resistant soybean crops. The usage of transgenic glyphosate-resistant soybean cultivars in Uruguay was accepted in 1999 and since then, the sowing area of this crop has increased dramatically (Department of Agricultural Statistics –DIEA-2010).

The strong dependence on using herbicides in no-tillage systems and the introduction of glyphosate herbicide-resistant cultivars has increased the frequency of the herbicides' treatments, imparting a high selection pressure to those weed species more tolerant to this chemical. In the short term, these systems face the risk of weed shifts to glyphosate-tolerant species and in the long term, a higher risk of occurrence of glyphosate-resistant weed biotypes (Powles 2008). Previous results have suggested shifts of the weed flora in the region, showing an increased abundance of weeds belonging to the Asteraceae family and which seeds are wind scattered (Tuesca and Puricelli 2001; Rios *et al.* 2005; Rios *et al.* 2008). Within the Poaceae family, annual species such as summer grass (*Digitaria sanguinalis* (L.) Scop) also increased their abundance. Perennial species such as Johnsongrass (*Sorghum halepense* (L.) Persoon) and purple nutsedge (*Cyperus rotundus* (L.)) have also shown an increase in their presence as they are naturally tolerant to glyphosate (Tuesca and Puricelli 2001; Rios *et al.* 2005; Vila-Aiub *et al.* 2007; Rios *et al.* 2008).

The present study aimed to evaluate the susceptibility of several annual ryegrass populations to increasing glyphosate rates during winter fallows. This species has already developed glyphosate resistance in other parts of the world (Powles *et al.* 1998; Heap 2011) and its population dynamic under Uruguayan conditions poses it as the main threat for the occurrence of glyphosate-resistant biotypes. Furthermore, annual ryegrass has been identified as the weed species with highest current presence in the country`s no-tillage systems during winter fallows (Rios *et al.* 2008). Thus, we believe it is crucial to regularly monitor the current response of the species to the most common herbicide used in the region.

## MATERIALS AND METHODS

### Study Sites

The experiments were installed in the West agricultural area of Uruguay (Lat. 33° 15' 8''S Long. 58° 1' 41''W), in departments of Paysandú, Río Negro and Soriano. This region has historically concentrated its major agricultural area and has generally adopted a no-tillage system from the 90's. Paddocks under no-tillage for a number of years and with available information regarding glyphosate applications and crop rotation were selected. From this selection, six sites at different farmers' properties were included for the study as they showed high infestation levels of annual ryegrass.

The climate is characterized by temperate conditions; from a 30 year average the annual precipitation is 1236.5 mm, occurring evenly throughout the year. However, the annual rainfall was well below this level for the surveyed year (i.e. 862.4 mm in 2010). The mean night temperatures are lower than 5.5°C in winter and above 30°C in day in summer (GRAS, National Agriculture Institute –INIA- 2011).

### Assessment of the Species Susceptibility to Glyphosate

In order to characterize the annual ryegrass populations prior to the application of the treatments, the species biomass was sampled by using five quadrats (30 × 30 cm) per site. Upon cutting, plants were partitioned into above-ground and below-ground plant material and these two classes were put into separate brown paper bags. When returning to the National Agriculture Institute (INIA) in Colonia, Uruguay, samples were carefully washed using a grid underneath to prevent losses and dried in an oven at 60 ± 5 °C for *ca.* 72 hours.

Within each of the six selected sites, the experiments were set up using a completely randomized block design with three repetitions (i.e. rectangular plots of 2 x 5 m size) and seven glyphosate rates: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 kg a.i./ha plus a control treatment without herbicide. The herbicide applications were performed between 28 August 2010 and the 17 September 2010 depending on weather conditions in each site. The glyphosate formulation contained potassium N-phosphonomethyl glycine salt at a concentration of 500 g a.i./L. Herbicide treatments were performed using $CO_2$ spraying equipment with Teejet AI 110 02 flat spray tips and 110 L/ha of water. Weather conditions, such as air humidity, wind speed and temperature were recorded at the time each of the treatment applications.

Control efficiency at each site was evaluated at 10, 20 and 30 days after the herbicide treatments using a visual estimate of the senescence rate of annual ryegrass plants. The visual scale was: very poor control (59% or less of the plants yellow); poor control (between 60 to 79% of the plants yellow); poor to satisfactory control (between 80 to 94% of the plants yellow) and excellent control (more than 95% of the plants yellow).

## Statistical Analysis

All data sets were analysed by an Analysis of Variance using a General Linear Model in S.A.S (SAS Institute Inc, Cary, NC, USA).The general linear model was set up with sites and glyphosate rates as factors for the analysis. According to normality tests a root-arcsine transformation of the data was performed into x/100.

Data were analysed using an Adjusted Sum of Squares approach with 95.0 % confidence intervals. When significant treatment effects were detected, pair wise comparisons of the means were undertaken using Tukey HSD method with 95.0 % confidence intervals.

## RESULTS

Significant differences were found in the speed of control across rates at all evaluation times (i.e. 10, 20 and 30 days after herbicide treatments). Within each site, at the three evaluation times, the amplitude of the control efficiency range decreased as the herbicide rates increased. No satisfactory control was achieved at the lowest herbicide rate at any time after the herbicide treatments. In the evaluation performed 10 days after the herbicide treatments the percentages of control that were achieved with the 0.5 kg a.i./ha glyphosate were lower than 42% (Figure 1, Table 1). Furthermore, the increase of glyphosate rates reduced the differences on control efficiency between sites (Table 1). Glyphosate rates of 1.0 and 1.5 kg a.i./ha showed equal amplitude of control efficiency ranges across sites (*ca.* 30%), as well as rates of 6.0 and 8.0. kg a.i./ha (40%). After 30 days from the herbicide treatments, rates of 4.0, 6.0, 8.0 kg a.i./ha achieved more than 90% of control efficiency at all sites (Table 1). Treatment rates of 0.5 kg a.i./ha showed poor control for all sites, except sites 5 and 6, where efficiencies of 78% and 85% were recorded, respectively. Excellent control was achieved with treatments of 1.0 kg a.i./ha for sites five and six, with efficiencies of 96% and 97% respectively. With the exception of site 1, the remaining tested sites averaged 70% control efficiency. Rates of 1.5 kg a.i./ha also achieved excellent controls for sites 1, 6 and 5 while the remaining sites ranged between 53% to 77% of control efficiency. For all rates higher than 4.0 kg a.i./ha control surpassed 90% of efficiency.

Table 1. Control efficiency ranges across the six sites for the seven herbicide rates and for the three evaluation times: 10, 20 and 30 days after herbicides treatments.

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| Evaluation time (days after herbicide treatment) | 10 | | 20 | | 30 | |
|---|---|---|---|---|---|---|
| Herbicide dose (Kg a.i./ha) | Control efficiency range across sites (%) | | | | | |
| | Mínimum | Máximum | Mínimum | Máximum | Mínimum | Máximum |
| 0.5 | 6.6 | 42 | 22 | 53 | 37 | 85 |
| 1.0 | 20 | 50 | 33 | 88 | 47 | 97 |
| 1.5 | 28 | 60 | 45 | 93 | 53 | 97 |
| 2.0 | 40 | 77 | 62 | 96 | 78 | 99 |
| 4.0 | 50 | 87 | 78 | 99 | 90 | 100 |
| 6.0 | 50 | 90 | 92 | 100 | 96 | 100 |
| 8.0 | 53 | 93 | 94 | 100 | 99 | 100 |

When relating the results on control efficiencies with the dry biomass of ryegrass populations from each site (data not presented) at the time of the herbicide treatments, those sites with higher available biomass (sites 2 and 4, with 694 y 578 g DM. $m^{-2}$ respectively) where the ones with less control efficiencies overall. Conversely, sites one, five and six, which recorded the lower biomass figures (461, 450 and 433 g DM. $m^{-2}$ respectively) achieved the best control efficiencies, even at lower glyphosate rates and as soon as 20 days after herbicide treatments. However, site three, which presented the lowest available biomass (228 g DM. $m^{-2}$) due to previous grazing, achieved the worse control efficiencies.

No clear trends were detected between the above and/or below-ground biomass of annual ryegrass plants and the obtained control efficiencies.

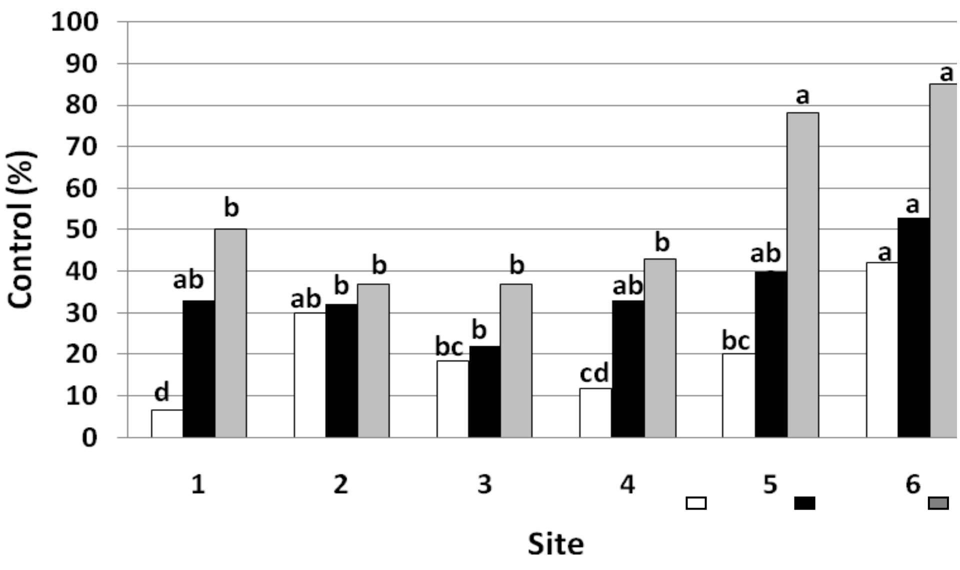


**Figure 1**. Control efficiency (%) for the six evaluated sites and 0.5 kg a.i./ha of glyphosate (lowest dose) at 10 ( ), 20 ( ) or 30 ( ) days after herbicide treatments. Different letters above bars represent significant differences across sites and between evaluation times calculated thru the Tukey HSD method with 95.0 % confidence intervals.

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## DISCUSSION

Results from the present study suggest that glyphosate rates of 1.0 kg a.i./ha and lower would provide an unsatisfactory control as various tolerant ryegrass plants survived the treatments. These circumstances would not only promote the presence of the weed but also increase the frequency of glyphosate-tolerant biotypes.

No glyphosate-resistant ryegrass populations were observed in this study. The susceptibility of annual ryegrass populations was highly dependent on the phenological stage when the herbicide treatments were performed, as well as the dry matter per hectare present in each of the sites. Even though better controls are achieved with less available biomass it is essential that sufficient above-ground plant material is exposed to the herbicide treatment. Thus grazing of fallows should be restricted previous to herbicide treatments. If this is possible to accomplish, rates of 1.5 and 2.0 kg a.i./ha combined with 20 days of fallow period might be enough to reach satisfactory control efficiencies.

It was observed that some ryegrass seedlings survived high glyphosate rates showing a tolerance response and suggesting the need to frequently monitor these sites in the future.

## REFERENCES

Department of Agricultural Statistics, Uruguay, DIEA (2010). Novedades http://www.mgap.gub.uy/portal/hgxpp001.aspx?7,5,352,O,S,0,MNU;E;27;6;MNU.

Agro-climate and Information System Group, GRAS National Agriculture Institute –INIA, Uruguay (2011) http://www.inia.org.uy/online/site/825574I1.php.

Heap, I. (2011) International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/in.asp

Powles, S.B., Lorraine-Colwill, D.F., Dellow, J.J. and Preston, C. (1998). Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Science* 46: 604-607.

Powles, S.B. (2008). Review: Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest Management Science* 64: 360-365.

Rios, A. (2005). Resistencia de malezas a herbicidas. Young, Sociedad Rural de Río Negro, INIA. *Serie de Actividades de Difusión* 408: 10.

Rios, A., Belgeri, A., Caulin, M.P. and Garcia, A. (2008) Weed Communities Related to No-Tillage Systems in Agricultural Center Area of Uruguay. National Agricultural Research Institute (INIA) Uruguay, Colonia, Uruguay. 5th Meeting, International Weed Science Congress, Vancouver, Canada (June 2008).

Tuesca, D. and Puricelli, E. (2001). Análisis de los cambios en las comunidades de malezas asociados al sistema de labranza y al uso continuo de Glifosato. In: Díaz, R. coord. *Siembra directa en el cono sur*. Montevideo. Procisur: 183-201.

Vila-Aiub, M.M., Vidal, R.A., Balvi, M.C., Gundel, P.E., Trucco, F.D. and Gersha, C.M. (2008). Review glyphosate-resistant weeds of South American cropping systems an overview. *Pest Management Science* 64: 366-371.

# RESISTANCE OF WILD OAT (*AVENA FATUA*) POPULATIONS TO ACCASE-INHIBITING HERBICIDES AND THE MOLECULAR BASIS OF RESISTANCE

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## ABSTRACT

ACCase-inhibiting herbicides [aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD)] have been used extensively since the late 1970s to control wild oat. However, continued reliance on ACCase-inhibiting herbicides has resulted in the widespread evolution of resistant wild oat populations. Dose-response studies on four resistant wild oat populations determined the level of resistance to the APP herbicides diclofop-methyl and fenoxaprop-p-ethyl, the CHD herbicides clethodim and sethoxydim, and to the phenylpyrazoline (PPZ) herbicide pinoxaden. All four resistant populations exhibited high level diclofop-methyl and fenoxaprop-p-ethyl resistance, but varied in the level of resistance to other ACCase-inhibiting herbicides, indicating either different resistance mutations or different resistance mechanisms in these populations. Mutations of the ACCase gene are known to endow target-site resistance to ACCase-inhibiting herbicides in other grass species. Therefore, we sequenced the carboxyl-transferase (CT) domain of the plastidic ACCase gene from the ACCase-inhibiting herbicides surviving individuals to identify any mutations endowing resistance. In most, but not all individuals, three known amino acid substitutions endowing resistance were identified in the resistant populations: the Ile-1781-Leu; Asp-2078-Gly; and Cys-2088-Arg. Polymerase chain reaction (PCR)-based marker analysis further confirmed the mutations are associated with the resistance in these populations. Some of the individuals in one population also contained multiple (double and triple) mutations. Evidently, these mutations in the ACCase gene endow high level of resistance in diploid grass species such as rigid ryegrass (*Lolium rigidum*) and blackgrass (*Alopecurus myosuroides* Huds). Whether the same mutations are to endow similar level of resistance in hexaploid wild oat remains to be investigated.

Keywords: *Avena* spp., ACCase-inhibiting herbicides, dose response and cross resistance patterns, ACCase gene, amino acids substitution, (d)CAPS.

## INTRODUCTION

Acetyl-CoA carboxylase (ACCase; EC 6.4.1.2), the key enzyme involved in the first step of fatty acid biosynthesis in plants, is the primary target of ACCase-inhibiting herbicides (Délye 2005). Three chemically distinct classes of herbicides; the aryloxyphenoxypropionates (APP), cyclohexanediones (CHD), and the recently developed phenylpyrazoline (PPZ) group with a single herbicide, pinoxaden (Hofer et al. 2006) are all herbicides that inhibit ACCase activity. Since the commercialization of the ACCase-inhibiting herbicides in the late 1970s, they have been extensively used to control many grass weed species. As a result of the great dependence on the ACCase-inhibiting herbicides, many grass weed species have evolved resistance to these herbicides.

Currently, resistance to a single or cross-resistance to many ACCase-inhibiting herbicides has been documented in 40 grass species in 31 countries (Heap 2011), with most ACCase resistance cases in ryegrass (*Lolium* spp.), blackgrass (*Alopecurus myosuroides* Huds) and wild oat (*Avena* spp.). Wild oat is one of the world's major grass weed species in the temperate cropping areas. Wild oat ranks only second to ryegrass as the most important herbicide-resistant weeds species globally (Heap 2011). Evolved wild oat resistance to ACCase-inhibiting herbicides was first reported in Western Australia in 1990, in a wheat field (Piper 1990). Since then, evolved resistant wild oat populations (mostly *Avena fatua* L. and *Avena sterilis* L.) have now become widespread in many countries including Australia (Heap 2011).

Target-site ACCase gene mutations and non target-site enhanced metabolism have been established in resistant wild oat populations. For the ACCase gene mutations, eight distinct resistance endowing amino acids substitution have been implicated in several grass species: the Ile-1781-Leu substitution; the Trp-1999-Cys substitution; the Trp-2027-Cys substitution; the Ile-2041-Asn/Val; the Gly-2096-Ala substitution, the Asp-2078-Gly substitution; and the Cys-2088-Arg substitution (reviewed by Délye 2005; Powles and Yu 2010). Five of them (1781, 1999, 2027, 2041 and 2078) have been reported in ACCase herbicide resistant wild oat populations (Christoffers et al. 2002; Liu et al. 2007).

A random survey across the very large Western Australia grain belt revealed that many wild oat populations have evolved resistance to ACCase herbicides (Owen and Powles 2009). Four populations with resistance to several ACCase-inhibiting herbicides were selected for further investigation in this study. The objective of the research is therefore to quantify and compare the whole-plant resistance levels and cross-resistance patterns to ACCase-inhibiting herbicides, and to identify the molecular and biochemical basis of ACCase resistance in these populations.

## MATERIALS AND METHODS

### Whole-Plant Dose Response

All experiments were conducted during the 2009 growing season (May-September) at the University of Western Australia. Four resistant wild oat (*Avena fatua* L.) populations, namely R1, R2, R3 and R4, plus a known susceptible population were used in this study. Plants were grown in pots outdoors under natural sunlight, watered and fertilized regularly. All seedlings were sprayed with selected ACCase-inhibiting herbicides at the 3-4-leaf stage.

Commercial ACCase-inhibiting herbicide formulations were used in all dose-response studies. ACCase-inhibiting herbicides: diclofop-methyl (Hoegrass®), fenoxaprop-p-ethyl (Wildcat®), clethodim (Select®), sethoxydim (Sertin®) and pinoxaden (Axial®) plus an appropriate adjuvant were applied using a custom-built, dual nozzle (TeeJet® XR11001) cabinet sprayer. All experiments were arranged in a randomized complete block design with three replications per herbicide per treatment. The herbicide rate required for 50% mortality ($LD_{50}$) and resistance index (R/S) were calculated.

Visual assessments of plant survival were made 21-day after herbicide treatment and the mean percentage of survival was calculated for each population. Shoot materials of individual plants surviving each ACCase-inhibiting herbicide at dosages that killed all the

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susceptible population plus untreated susceptible plants were sampled and stored at -80ºC for ACCase DNA sequencing and PCR-based marker analysis for ACCase resistance mutations.

### DNA Extraction and ACCase Sequencing

All genomic DNA extraction and amplification procedures for both resistant and susceptible plants followed Yu et al. (2008) and Liu et al. (2007) under our specific modified PCR conditions. The PCR product was purified from agarose gel and sequenced using a commercial sequencing service.

### PCR-based Marker Analysis

The published derived cleaved amplified polymorphic sequence (dCAPS) markers for 1781 (Kaundun and Windass 2006) and 2078 (Kaundun 2010) mutations were used in this study with modified primers and PCR conditions, whereas a CAPS marker for the 2088 mutation was developed. Restriction digestion and reaction procedures were carried out according to Yu et al. (2008).

## RESULTS

### Whole-Plant Dose Response to ACCase-inhibiting Herbicides

All four resistant populations were found to be highly resistant to diclofop-methyl. The diclofop-methyl $LD_{50}$ value for the susceptible population was 454 g ai $ha^{-1}$, whereas $LD_{50}$ values greater than 8800 g ai $ha^{-1}$ (>20-fold) were recorded in all resistant populations (Table 1). A similar result was observed in response to fenoxaprop-p-ethyl. The fenoxaprop-p-ethyl $LD_{50}$ values for the resistant populations were 9 to >14-fold greater than the susceptible population (Table 1).

For the CHD herbicide sethoxydim, the calculated $LD_{50}$ values for the resistant populations were 3 to 11 times higher than the susceptible population (Table 1). Meanwhile, the level of resistance was much less for the herbicide clethodim. The $LD_{50}$ values for the resistant populations were 3 to 7 times higher than the susceptible population (Table 1). For the herbicide pinoxaden, the $LD_{50}$ of the resistant populations were 6 to 12 times higher than the susceptible population (Table 1).

**Table 1.** The ACCase-inhibiting herbicides dose required for 50% mortality ($LD_{50}$) of the susceptible and resistant wild oat populations R1, R2, R3 and R4.

| | $LD_{50}$ (g $ha^{-1}$) | | | | |
|---|---|---|---|---|---|
| Herbicide | Susceptible | R1 | R2 | R3 | R4 |
| Diclofop-methyl | 454 | >8800 | >8800 | >8800 | >8800 |
| Fenoxaprop-p-ethyl | 21 | 191 | >308 | >308 | >308 |
| Sethoxydim | 104 | 272 | 1114 | 780 | 1121 |
| Clethodim | 4 | 9 | 12 | 11 | 24 |
| Pinoxaden | 3 | 19 | 20 | 12 | 40 |

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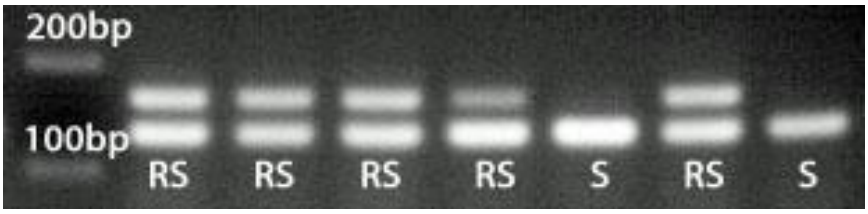
**ACCase Sequencing Identified One to Three Mutations in a Single Resistant Plant**

At least 38 individual plants surviving ACCase-inhibiting herbicides application from each resistant population were sequenced, and three known ACCase mutations were identified: Ile-1781-Leu, Asp-2078-Gly and Cys-2088-Arg in all four resistant populations. While the majority of the populations contain only a single mutation, interestingly, multiple (double and triple) mutations were identified in some individuals in the population R2. It is also noted that while the majority of the plants in populations R2 and R3 have ACCase mutations, 92% of the resistant plants in population R1 and 27% in population R4 did not contain any of the known mutations.

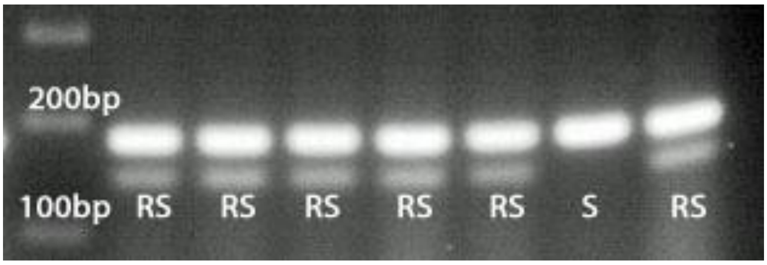
**PCR-based Marker Analysis Revealed Fixed Heterozygosity for the Mutations in Individual Resistant Plants**

ACCase sequencing revealed heterozygosity for each of the three mutations identified as double peaks (both mutant and wild type nucleotides) at the same position in the chromatograms. To confirm this nucleotide heterozygosity, three (d)CAPS markers were used to genotype sequenced individuals. Consistent with the sequencing results, only heterozygous resistant (RS) or homozygous susceptible (S) but no homozygous resistant (R) genotypes were detected in a total of 157 samples analysed (Figure 1).

(a) Mutation Ile-1781-Leu




(b) Mutation Asp-2078-Gly




(c) Mutation Cys-2088-Arg


**Figure 1.** Genomic PCR based marker analysis of the ACCase mutations shows only homozygous susceptible (S) and heterozygous resistant (RS) genotypes for the: (a) 1781, (b) 2078 and (c) 2088 mutations detected in the ACCase-inhibiting herbicides surviving plants.

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## DISCUSSION

Results from this study confirmed that cross-resistance to ACCase-inhibiting herbicides in wild oat populations in the Western Australian grain belt is evident. All four resistant populations were found to exhibit high level of resistance to diclofop-methyl and fenoxaprop-p-ethyl, moderate level to sethoxydim and pinoxaden, and low level to clethodim. Differing patterns of resistance to ACCase-inhibiting herbicides in other wild oat populations have also been reported (Bourgeois et al. 1997; Uludag et al. 2007). The variations in resistance levels and resistance patterns exhibited by these populations in this study are likely due to different mutations or different resistance mechanisms (Maneechote et al. 1997).

In this study, the Ile-1781-Leu, Asp-2078-Gly and Cys-2088-Arg mutations of the ACCase gene were identified in the four wild oat populations. One of the important findings of the current study is the identification of multiple ACCase mutations in individual plants. Thus, the role of target-site ACCase gene mutations in the polyploid wild oat species truly needs further investigation. In addition to the ACCase target site mutations, many of the resistant individuals in the populations R1 and R4 were found to have no known ACCase mutations in the CT domain. Hence it is likely that a single population can have both target-site and non target-site resistance mechanisms. Thus, further work is underway to confirm the non-target site resistance mechanisms in these populations.

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## REFERENCES

Bourgeois, L., Kenkel, N. C. and Morrison, I. N. (1997). Characterization of cross-resistance patterns in acetyl-CoA carboxylase inhibitor resistant wild oat (*Avena fatua*). *Weed Science* 45(6): 750-755.

Christoffers, M. J., Berg, M. L. and Messersmith, C. G. (2002). An isoleucine to leucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. *Genome* 45(6): 1049-1056.

Délye, C. (2005). Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Science* 53(5): 728-746.

Heap, I. (2011). International Survey of Herbicide Resistant Weeds. Available at http://www.weedscience.com.

Hofer, U., Muehlebach, M., Hole, S. and Zoschke, A. (2006). Pinoxaden-for broad spectrum grass weed management in cereal crops. *Journal of Plant Diseases and Protection* 20: 989-995.

Kaundun, S. S. (2010). An aspartate to glycine change in the carboxyl transferase domain of acetyl CoA carboxylase and non-target-site mechanism(s) confer resistance to ACCase inhibitor herbicides in a *Lolium multiflorum* population. *Pest Management Science* 66(11): 1249-1256.

Kaundun, S. S. and Windass, J. D. (2006). Derived cleaved amplified polymorphic sequence, a simple method to detect a key point mutation conferring acetyl CoA carboxylase inhibitor herbicide resistance in grass weeds. *Weed Research* 46(1): 34-39.

Liu, W., Harrison, D. K., Chalupska, D., Gornicki, P., O'Donnell, C. C., Adkins, S. W., Haselkorn, R. and Williams, R. R. (2007). Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. *Proceedings of the National Academy of Sciences* 104(9): 3627-3632.

Maneechote, C., Preston, C. and Powles, S. B. (1997). A diclofop-methyl-resistant *Avena sterilis* biotype with a herbicide-resistant acetyl-coenzyme A carboxylase and enhanced metabolism of diclofop-methyl. *Pesticide Science* 49(2): 105-114.

Owen, M. J. and Powles, S. B. (2009). Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop and Pasture Science* 60(1): 25-31.

Piper, T. J. (1990). Field trials on diclofop-methyl tolerant wild oats (*Avena fatua*). Proceedings of the 9th Australian Weeds Conference. pp. 211-215.

Powles, S. B. and Yu, Q. (2010). Evolution in Action: Plants Resistant to Herbicides. Annual Review of Plant Biology Vol. 61. Palo Alto, *Annual Reviews* 61: 317-347.

Uludag, A., Nemli, Y., Tal, A. and Rubin, B. (2007). Fenoxaprop resistance in sterile wild oat (*Avena sterilis*) in wheat fields in Turkey. *Crop Protection* 26(7): 930-935.

Yu, Q. Han, H. P. and Powles, S. B. (2008). Mutations of the ALS gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. *Pest Management Science* 64(12): 1229-1236.

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# PARTHENIUM (*PARTHENIUM HYSTEROPHORUS* L.) DISTRIBUTION AND ITS BIO-RESOURCE POTENTIAL FOR RICE PRODUCTION IN PUDUCHERRY, INDIA

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## ABSTRACT
Parthenium (*Parthenium hysterophorus* L.) is an important problematic invasive weed of India. It is known to cause severe economic, environmental, human and animal health problems. It achieved the status of weed of national importance in India. The existence of Parthenium was noticed in the Puducherry, a coastal town of South India. Hence, the distribution and its bio-resource potential were investigated on growth and yield of rice at Union Territory of Puducherry during 2008-09.

A weed survey, using list or census quadrat method was conducted to study the distribution and intensity of Parthenium in Puducherry. A field experiment was carried out to investigate the possibility of using Parthenium weed as a potential bio-resource to increase the rice (*Oryza sativa* L.) production.

The results of the weed survey indicated that the Parthenium had encroached into the majority of villages in Puducherry (87.5%) except the sea shore villages. It was also found to infest the fields of agricultural and horticultural crops like sugarcane, banana, and coconut. The results of the field experiment indicated that use of Parthenium as a nutrient bio-resource significantly increased the productivity of rice (4.57 t/ha) compared to non application (4.13 t/ha) and reduced the fertilizer application by 25%.

**Key words:** Bio-resource, Parthenium Distribution, Rice

## INTRODUCTION
Parthenium (*Parthenium hysterophorus* L.) is the major problematic weed found in India. It is found to release enormous amount of pollen grains which tend to cause allergy, asthma and dermatitis in human beings. Parthenium is an extremely prolific seeds producer (25,000 seeds/plant) which can lead to an enormous soil seed bank of 2,00,000 seeds/m$^2$ (Joshi, 1991).

Recently, its existence was observed in and around the Union Territory of Puducherry, India. Being obnoxious, Parthenium is considered unfit as cattle feed, but it is regarded as potentially useful as an important component of integrated nutrient management to improve soil health and yield potential of rice.

The present study was undertaken up to study the distribution of Parthenium in the Union Territory and also explores the possibility of utilizing Parthenium weed biomass as bio-resource for improving the productivity of rice.

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## MATERIALS AND METHODS

Weed survey was undertaken in order to study the distribution and intensity of Parthenium in and around the coastal town of Puducherry, located in southern part of India. Initially, the particulars on names of villages/hamlets, revenue villages and communes (blocks) in Puducherry was obtained from the survey department of Puducherry Union Territory.

The list or census quadrat method was adopted to collect data on distribution and intensity of weeds in each revenue village. A list quadrat of 50 x 50 cm was used to record the population of Parthenium. Clip quadrat method was adopted to collect data on biomass of Parthenium.

Also, a field experiment to study the bio-resource potential of Parthenium was conducted at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, Puducherry U.T during *kharif* (June –September) 2008 on sandy clay loam soil. The soil had pH of 7.29, low in available nitrogen (172 kg/ha) and high in available phosphorus (33 kg/ha) and potassium (373 kg/ha).

The experiment was laid out in factorial randomized block design with nine treatment combinations consisting of three levels of weed organic sources ($W_0$: no weed biomass addition, $W_1$: Parthenium @ 5 t/ha, and $W_2$: water hyacinth @ 10 t/ ha) and three levels of fertilizers level ($F_0$: 0, $F_1$: 75% and $F_2$: 100 % recommended Nitrogen, Phosphorus and Potassium (NPK)).

The recommended fertilizer dose for rice in Puducherry region is 150: 50: 50 kg NPK /ha during *kharif* season (June-September month). The weed organic sources - fresh Parthenium and water hyacinth were collected from nearby areas around the institute and incorporated into the soil 15 days before transplanting as per the treatments. The rice cultivar ADT 43 was transplanted with the spacing of 20 cm x 10 cm.

The data on rice grain and straw yield were subjected to statistical scrutiny as per the procedures given by Panse and Sukhatme (1967).

## RESULTS

### Weed Survey

The results of the weed survey indicated that the Parthenium had found encroached into majority of villages in Puducherry (87.5% of villages) except the sea shore villages of different communes.
Out of the seven communes in Puducherry, three communes *viz*., Nettapakkam, Mannadipet and Villanur were encroached by Parthenium plants. The population density was ranged from 4 to 76, 4 to 120 and 4 to 168/$m^2$, respectively. The Parthenium fresh weight ranged from 1 to 40 t/ha (Table 1). It is found in the uncared fields, grown with wide spaced crops like sugar cane, banana and coconut, respectively.

**Table1.** Intensity, population and fresh weight of Parthenium in different communes of Puducherry, India

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| Sl.No | Commune | Intensity (%) | Population range (number/ $m^2$) | Fresh weight range (t/ha) |
|---|---|---|---|---|
| 1 | Puducherry | 62.5 | 4 - 64 | 4 - 40 |
| 2 | Oulgaret | 50.0 | 4 - 68 | 4 - 22 |
| 3 | Ariyankuppam | 66.7 | 4 - 104 | 1 - 24 |
| 4 | Bahour | 85.7 | 4 - 80 | 1 - 30 |
| 5 | Nettapakkam | 100 | 4 - 76 | 1 - 30 |
| 6 | Mannadipet | 100 | 4 - 120 | 1 - 30 |
| 7 | Villianur | 100 | 4 - 168 | 1 - 30 |
| | Mean | 87.5 | | |

**Bio-resource Utilization**

Parthenium used as bio-resource resulted in rice grain and straw yields of 4.57 and 8.07 t/ha, respectively. Also, Incorporation of water hyacinth biomass @ 10 t /ha resulted in significantly higher grain and straw yields of 4.72 and 8.35 t/ha, respectively.

Among the fertilizer levels, 100 % NPK registered significantly higher grain and straw yields (4.89 and 9.31 t/ha, respectively) than non application of fertilizers (3.96 and 6.47 t/ ha). However, it was found to be on par with application of 75% NPK (4.57 and 8.44 t/ha). In general, the grain and straw yields of rice had a linear response with fertilizer levels. The interaction effects were not significant (Table 2).

## DISCUSSION

The occurrence of Parthenium was found to be high in Nettapakkam, Mannadipet and Villanur, where industrialization was more. It is due to the variable dormancy behaviour of Parthenium weed. So, Parthenium is found to grow and flower throughout the year unaffected by soil types, soil pH and other climatic conditions (Mani *et al*., 1976). However, Parthenium was less common in the coastal villages of Puducherry, where the tidal line is less than three kilometres.

The experimental results for its bio-resource potential revealed that incorporation of biomass of water hyacinth and Parthenium enhanced the grain yield of rice by 14.1 and 10.7 %, respectively over control.

**Table 2.** Effect of Parthenium weed as bio-resource on grain and straw yields (t/ha) of rice

| Treatments | Grain yield (t/ha) | Straw yield (t/ha) |
|---|---|---|
| *Weed Source* | | |
| $W_0$: No weed biomass addition | 4.13 | 7.81 |
| $W_1$: Parthenium @ 5 t/ha | 4.57 | 8.07 |
| $W_2$: Water hyacinth @ 10 t/ ha | 4.72 | 8.35 |
| S.Em. | 0.21 | 0.40 |
| C.D. (P=0.05) | 0.44 | 0.84 |
| *Fertilizer levels* | | |
| $F_0$: No fertilizer application | 3.96 | 6.47 |
| $F_1$:75% recommended NPK | 4.57 | 8.44 |
| $F_2$:100% recommended NPK | 4.89 | 9.31 |
| S.Em. | 0.21 | 0.40 |

| | | |
|---|---|---|
| C.D. (P=0.05) | 0.44 | 0.84 |
| Interaction | | |
| S.Em. | 0.36 | 0.70 |
| C.D. (P=0.05) | NS | NS |

NS: Not Significant

The additive benefits derived from Parthenium and water hyacinth may be due to its macro and micro nutrient availability to crops (Ramasamy, 1990). The availability of nutrients in soil to the rice crop resulted in better growth and yield attributes and ultimately the rice yield. Also, the problem of emergence of Parthenium was not encountered due to the continuous submergence of water under lowland conditions for rice cultivation.

Similar effects of weed biomass utilization on yield of crops were earlier reported by Son and Ramaswami (1997) and Saravanane *et al*. (2008). It was observed that incorporation of weed biomass and application of 75% fertilizer dose of rice registered grain and straw yields similar to that of application of 100% recommended fertilizer dose, facilitating the reduction of 25% of synthetic fertilizer application.

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## REFERENCES

Joshi, S. (1991). Interference effects of *Cassia uniflora* Mill. on *Parthenium hysterophorus* L. *Plant and Soil* 132: 213-218.

Mani, V. S., Gautam, K. C., Kulreshtha, G., Bhagwandas, K., Singh, R. and Pande, R. (1976). Parthenium problem.*Weed Research Newsletter* 1: 1.

Panse, V. G. and Sukhatame, P. U. (1967). Statistical methods for agricultural workers. ICAR, New Delhi.

Ramasamy, A. (1990). Yield maximization in lowland rice through integrated use of organic manures and fertilizer N under varying plant densities. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Saravanane, P., Nanjappa, H.V. and Ramachandrappa, B.K. (2008). Effect of weeds utilization as nutrient source on soil fertility and tuber yield of potato. *Mysore Journal of Agricultural Sciencs* 42 (3): 464-467.

Son, T. T. N. and Ramaswami, P. P. (1997). Bioconversion of Parthenium for sustainable agriculture. In.*Proceedings of first international conference on Parthenium management*, UAS, Dharwad, pp.119-121.

# SUSTAINABLE MANAGEMENT OF WEEDS IN RAINFED EGGPLANT IN INDIA

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## ABSTRACT

On-farm participatory experiments were conducted at Nalloor cluster comprising three villages *viz.,* Keelakuruchi, Melakuruchi and Kattumailoor in the National Agricultural Innovation Project implemented by Annamalai University in southern India. Grazing by goats in the off-season (March, April and May) was integrated with mulching of perennial crop wastes such as sugarcane trash and coconut husks in the vegetable crop of eggplant (*Solanum melongena* L.) raised during June-September. Goat grazing supplemented control of *Trianthema portulacastrum* with a weed control index of 27%, where as, mulching with sugarcane trash offered 64% control of perennial weeds such as *Cyanodan dactylon*. Integrating both goat grazing and sugarcane trash mulching compared better than conventional practices of hand-weeding and herbicide treatments, in offering a sustainable weed control and favouring crop yields. A 21% enhancement in the soil organic matter content was also achieved due to this integration.

**Keywords:** sustainable weed management, rainfed vegetable, eggplant

## INTRODUCTION

Brinjal (or) egg plant (*Solanum melongena* L.) of the family Solanaceae is one of the most popular vegetables in the households of India. It is widely distributed in the tropical and temperate zones of China, Turkey, Syria, Iraq, Japan, Indonesia, Philippines, Thailand and Jordan. India being a centre of origin it is grown in almost all the states covering 6 lakh hectares with a production of 104 lakh metric tonnes during 2008 (Anon, 2009). Statistics reveal that it is the third most important crop in the family of Solanaceae after potato and tomato (Anon, 2000). Brinjal is a source of phosphorus (47 mg/100g) Carotene (74 µg) has 12mg/100g of vitamin C, as well as possessing some medicinal properties(Shukla and Naik 1993).

Poor weed management in brinjal is the most important factor limiting the productivity which necessitates adoption of sound weed control measures at the early growth stage. The problem of weeds has been particularly more acute because of tropical condition prevailing in India. The losses on account of high weed infestation are estimated to be much more in vegetables as compared to cereals. Weeds compete with crop plants for different growth factors and significantly to the cost of farm operations (Qasem, 2003, Zaragoza 2003). Weed management is a major constraint in brinjal cultivation in India because it's a long duration crop and it is growth period coincides with the monsoon season. In India the cultural methods of weed management are widely used by the farmers these are laborious, time consuming and expensive on account of scarcity of labour particularly during the peak periods of labour demand which coincides with the field operations in the other crops. Moreover, hand weeding is not efficient because even with

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precise hoeing, weeds very often close to the crop plants are not removed. Under these circumstances the option left for managing weeds is by using herbicides or combination of both herbicides and cultural practices. Weed control in vegetables have been reported with the use of different herbicides (Patel *et al.*, 1986; Keeling *et al*., 1990). Pendimethalin applied at doses > 0.75 kg a.i./ha in some vegetable crops has not only given good control of weeds but also effectively controlled specific and dominant weed taxa with widely varying growth forms (Rapparini 1994). Herbicides treatment significantly reduced weed counts and weeds dry weight in chilli and carrot and weed nutrient uptake in chilli (Mukund *et al*. 1995; Kumar *et al.* 1995).

Repeated use of herbicide may lead to reduction in crop growth and herbicidal resistance and hence integrated approach involving organic mulching, grazing with animals like goat/sheep are some of the options for sustainable weed control. Reduction of weed biomass was observed from 23.7 to 29.5% due to grazing of goats in the off-season (Kathiresan 2010). The present investigation was undertaken to study and compare the efficacy of grazing with goat. Hand weeding, chemical control with pendimethalin @ 1 kg a.i. / ha and mulching with sugarcane trash and coir pith.

## METHODS

The experiments were carried out in holdings of small and marginal farmers participating as development partners of National Agricultural Innovation Project Implemented by Annamalai University in the rainfed cluster (Nalloor) of Cuddalore district during 2008-09 and 2009-10. Nalloor cluster comprises of three villages *viz*., Keelakurichi, Melakurichi and Kattumailoor. One among the ten treatments was imposed in every farmers holding along with an untreated control and each of these were considered as a replication. As 50 farmers participated, five replications of 10 treatments were compared in a randomized block design.

**Treatments;**

$T_1$ - Off season grazing + Un-weeded control

$T_2$ - Off season grazing + Hand weeding

$T_3$ - Off season grazing + Pendimethalin @1kg a.i./ha

$T_4$ - Off season grazing + Sugarcane trash mulching

$T_5$ - Off season grazing + Coir pith mulching

$T_6$ - Off season fallow + Un-weeded control

$T_7$ - Off season fallow + Hand weeding

$T_8$ - Off season fallow + Pendimethalin @1kg a.i./ha

$T_9$ - Off season fallow + Sugarcane trash mulching

$T_{10}$ - Off season fallow + Coir pith mulching

Two goats were provided to the beneficiary under NAIP Project for grazing trails, these goats were allowed to graze in the offseason (March, April and May) at morning and night. Hand-weeding, chemical control with pendimethalin @ 1 kg a.i. /ha (Stomp Extra® 30%

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EC) and mulching of sugarcane trash and coir pith(also known as Coconut peat or Coir dust.) were adopted as per treatment schedule in 1000 $m^2$. Brinjal seeds were sown in a nursery and transplanted on $30^{th}$ day. The regular cultural practises were followed except weeding. Hand weeding was done thrice on $25^{th}$, $50^{th}$ and $75^{th}$ day after transplanting, for, herbicide application pendimethalin @ 1 kg a.i. /ha was sprayed as pre emergence on second day after planting. Mulching with sugarcane trash and coir pith @ 5 tonnes/ ha were spread in the fields as per treatment schedule. Observations on the weeds present, weed count, weed biomass were recorded from four quadrates of 0.25 $m^2$ area at 45 & 90 DAP and the data on 90 DAP is presented considering the space requirement. Weed Control Index (WCI) was also calculated using the formula suggested by Thakur (1994).

$$WCI = \frac{a-b}{a} \times 100$$

Where, a - weed biomass of unwedded plot

Dry matter production and fruit yield per 1000$m^2$ were recorded and converted per hectare. Soil organic matter content was calculated based on Walkley black method expressed in percent (Dipak and Abhijit 2005). The data were analysed using the analysis of variance to draw the standard error differences and ultimately the critical difference was worked out at 0.5% probability as suggested by Panse and Sukhatme (1978).

## RESULTS

The following weed species were found in the selected experimental fields of Nallor cluster: Cynodon *dactylon, Cyperus rotundus, Chenopodium album, Amaranthus viridis, Trianthema portulacastrum, Phalaris minor, Phyllanthus niruri* and *Cleome viscose, Dactyloctenium aegypticum.* Among these species the most abundant were *C.dactylon*, *T.portulacastrum* and *A.viridis*, as they occur in frequent intervals at all the trial fields. The rest of the weed flora listed were sporadic in occurrence.

Integration of farming elements like offseason grazing of goats and mulching with sugarcane trash and coir pith significantly reduced the individual weed numbers of the three predominant weed species when compared with off season fallow and conventional weed control practices like hand weeding and application of pendimethalin (Table 1)

In general, integration of offseason grazing with other super imposing treatments excelled better than the treatments imposed on off season fallow fields. Among all the treatments off season grazing + sugarcane trash mulching reduced the individual weed count of all the predominant weed floras. This treatment was on par with off season grazing + hand weeding in both the years. The weed count of these three species was reduced in the consecutive second year when goat grazing was practised in the off season.

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**Table 1.** Effect of goat grazing, mulching and chemical control on individual weed count in *S. melongena* L.

| Treatment Details | *Cynodon dactylon* / $m^2$ | | *Trianthema portulacastrum* /$m^2$ | | *Amaranthus viridis*/ $m^2$ | |
|---|---|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 |
| Off season grazing + Un-weeded control | 5.5<br>(30.1) | 5.4<br>(28.5) | 6.7<br>(45.3) | 6.7<br>(44.2) | 4.9<br>(24.3) | 4.92<br>(23.6) |
| Off season grazing + Hand weeding | 3.9<br>(15.3) | 3.9<br>(14.8) | 3.3<br>(10.4) | 3.2<br>(9.9) | 2.5<br>(6.1) | 2.4<br>(5.5) |
| Off season grazing + Pendimethalin @1kg a.i./ha | 4.3<br>(16.7) | 3.9<br>(15.0) | 5.3<br>(27.5) | 5.1<br>(26.1) | 2.6<br>(6.5) | 2.5<br>(6.0) |
| Off season grazing + Sugarcane trash mulching | 3.8<br>(14.4) | 3.7<br>(13.8) | 3.1<br>(9.4) | 3.1<br>(9.1) | 2.3<br>(5.1) | 2.3<br>(4.6) |
| Off season grazing + Coir pith mulching | 4.2<br>(17.7) | 4.1<br>(16.6) | 5.2<br>(26.4) | 5.1<br>(24.9) | 2.7<br>(6.9) | 2.6<br>(6.6) |
| Off season fallow + Un-weeded control | 9.7<br>(95.1) | 9.9<br>(98.5) | 8.1<br>(65.1) | 8.3<br>(68.2) | 6.2<br>(38.3) | 6.4<br>(40.6) |
| Off season fallow + Hand weeding | 4.8<br>(23.4) | 5.1<br>(25.6) | 5.5<br>(30.4) | 5.8<br>(33.1) | 2.9<br>(8.4) | 3.2<br>(10.0) |
| Off season fallow + Pendimethalin @1kg a.i./ha | 4.6<br>(21.5) | 4.9<br>(23.7) | 5.0<br>(25.0) | 5.2<br>(27.1) | 3.1<br>(9.3) | 3.2<br>(10.2) |
| Off season fallow + Sugarcane trash mulching | 4.7<br>(22.4) | 5.0<br>(24.7) | 8.3<br>(48.4) | 7.1<br>(50.0) | 3.3<br>(10.5) | 3.4<br>(11.2) |
| Off season fallow + Coir pith mulching | 5.01<br>(24.6) | 5.2<br>(26.8) | 7.1<br>(50.6) | 7.2<br>(52.2) | 3.5<br>(11.7) | 3.7<br>(13.1) |
| Standard Error difference. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Critical Difference(P=0.05) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

Figures in parenthesis are original values

The results of individual weed numbers of predominant weed flora reflected in total weed count (Table 2). The total weed numbers was significantly reduced in integration with offseason grazing when compared to integration with fallow fields. The least total weed count was observed in offseason grazing + sugarcane trash mulching (34.3 & 32.8 in 2008-2009 & 2009-2010 respectively). This treatment was on par with off season grazing + hand weeding. There was significant reduction in weed biomass due to integration of grazing with other conventional method. In grazing fields the weed biomass was reduced in the consecutive second year where as in fallow fields the biomass was enhanced irrespective of super imposing treatments. The least biomass observed in offseason grazing + sugarcane trash mulching was significantly higher than the next best treatment of off season grazing + coir pith mulching. The weed control index (WCI) was enhanced to the greater level of 89.1 & 89.8 in the first and second years respectively in offseason grazing+ sugarcane trash treatment. However in offseason fallow+ sugarcane trash mulching the WCI observed was much lower which was on par with off season fallow + hand weeding.

**Table 2.** Effect of goat grazing, mulching and chemical control on weed numbers, biomass and control index in *S. melongena* L.

| Treatment Details | Total weed numbers/ $m^2$ | | Weed biomass/ $m^2$ | | Weed control index | |
|---|---|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 |
| Off season grazing + Un-weeded control | 11.6 (135.4) | 11.3 (128.2) | 114.4 | 110.6 | 38.7 | 44.1 |
| Off season grazing + Hand weeding | 5.9 (35.1) | 5.7 (33.0) | 28.6 | 27.2 | 84.6 | 86.2 |
| Off season grazing + Pendimethalin @1kg a.i./ha | 6.2 (38.1) | 6.0 (36.0) | 34.2 | 30.6 | 81.6 | 84.5 |
| Off season grazing + Sugarcane trash mulching | 5.9 (34.3) | 5.7 (32.8) | 20.3 | 20.1 | 89.1 | 89.8 |
| Off season grazing + Coir pith mulching | 6.0 (36.1) | 5.8 (34.2) | 23.4 | 22.6 | 87.4 | 88.5 |
| Off season fallow + Un-weeded control | 14.5 (210.3) | 15.1 (228.2) | 186.7 | 198.2 | - | - |
| Off season fallow + Hand weeding | 7.9 (63.4) | 8.1 (65.8) | 57.3 | 59.8 | 69.2 | 69.8 |
| Off season fallow + Pendimethalin @1kg a.i./ha | 8.1 (65.1) | 8.2 (67.2) | 59.4 | 63.7 | 68.1 | 67.8 |
| Off season fallow + Sugarcane trash mulching | 8.1 (65.7) | 8.3 (68.6) | 58.4 | 60.5 | 68.6 | 69.4 |
| Off season fallow + Coir pith mulching | 8.3 (69.7) | 8.5 (72.1) | 60.8 | 62.6 | 67.4 | 68.3 |
| Standard Error difference. | 0.1 | 0.1 | 0.4 | 0.5 | 0.6 | 0.5 |
| Critical Difference(P=0.05) | 0.2 | 0.2 | 0.9 | 1.1 | 1.3 | 1.2 |

Figures in parenthesis are original values

The yield of *S.melongena* were significantly increased due to the integration of farming elements like goat grazing and organic mulching (Table 3). In general dry matter production (DMP) and fruit yield were recorded higher when off season grazing was practised with the super imposing weed control treatments. The highest DMP (250.7 kg/ha and 260.86 kg /ha during the first and second year respectively) and fruit yield (28,923 kg/ha and 29,386 kg/ha) were observed and this was significantly higher than the treatment offseason grazing + coir pith mulching which was on par with offseason grazing + hand weeding. Soil organic matter (SOM) content was enhanced due to integration of off

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grazing treatments and organic mulches. The highest SOM was recorded in offseason grazing + sugarcane trash mulching followed by coir pith integration.

**Table 3.** Effect of goat grazing, mulching and chemical control on the yield of*S. Melong ena* L. and soil organic matter

| Treatment Details | Dry matter production (kg/ha) | | Fruit yield (Kg/ha) | | Soil organic matter content (%) | |
|---|---|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 |
| Off season grazing + Un-weeded control | 175.36 | 185.36 | 26,116 | 26,876 | 0.38 | 0.40 |
| Off season grazing + Hand weeding | 210.67 | 225.86 | 27,421 | 28.248 | 0.44 | 0.45 |
| Off season grazing + Pendimethalin @1kg a.i./ha | 190.29 | 206.27 | 26,712 | 27,128 | 0.37 | 0.40 |
| Off season grazing + Sugarcane trash mulching | 250.75 | 260.86 | 28,923 | 29,386 | 0.61 | 0.63 |
| Off season grazing + Coir pith mulching | 215.63 | 228.17 | 28,075 | 28,327 | 0.58 | 0.59 |
| Off season fallow + Un-weeded control | 160.83 | 175.23 | 24,708 | 25,009 | 0.30 | 0.27 |
| Off season fallow + Hand weeding | 190.23 | 201.19 | 26,176 | 27,601 | 0.36 | 0.33 |
| Off season fallow + Pendimethalin @1kg a.i./ha | 162.37 | 176.12 | 25,123 | 26,827 | 0.32 | 0.31 |
| Off season fallow + Sugarcane trash mulching | 198.67 | 213.32 | 27,008 | 28,118 | 0.50 | 0.51 |
| Off season fallow + Coir pith mulching | 190.23 | 201.78 | 26,216 | 27,018 | 0.47 | 0.49 |
| Standard Error difference. | 2.25 | 2.57 | 345 | 317 | 0.03 | 0.03 |
| Critical Difference(P=0.05) | 4.97 | 5.23 | 762 | 701 | 0.08 | 0.08 |

## DISCUSSION

Goat grazing repeatedly in the fields during off seasons before growing an eggplant crop may have depleted the food reserves and rejuvenation potential of underground propagules of perennial weeds such as *C.dactylon* and this could have helped reducing their population to the minimum during cropping season that followed. This has been shown previously by Kathiresan (2010). Accordingly contribution of goat grazing on the control of *T. portulacastrum* and *A. viridis* is comparatively less. As a supplement to fill up this gap, mulching with crop waste such as sugar cane trash performed superior by its weed smothering effect. As a wholistic integration goat grazing + sugar cane trash mulching was excelling other conventional treatments like hand weeding or herbicides. Weed infestations are lesser in farmers holdings where in mulching is practiced using sugarcane trashes compared to those with coconut coir mulching. This is attributed to better smothering efficiency by virtue of higher magnitude of blocking sun light and aeration by continuous surfaces of trashes compared to smaller particles of coconut coir. Similar observation is reported earlier by Vijayabaskaran and Kathiresan 1993.

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## REFERENCES

Anonymous. (2000). *FAO STAT statistics Database.* FAO, Rome, Italy.

Anonymous. (2009). *N.H.M. Database.* 2008-2009.

Dipak, S. and Abhijit, H. (2005). *Physical and chemical methods in soil analysis*. New age international publishers. pp 89-90

Kathiresan, RM. (2010). Spatial and temporal integration of component enterprises in small holder farm of India for sustainability in farming and rural livelihoods, *Proceedings of 9th European IFSA symposium 4-7 July 2010,* Vienna, Austria.

Keeling, J.W., Bender, D.A., and Abernathy, J.R. (1990). Yellow nut sedge (*Cyprus esculentus*) management in transplanted onion. *Allium cepa* L. *Weed Technology* 4, 68-70.

Kumar, A., Thakral, K.K. and Pratap, P.S. (1995). Studies on Weed management Practices and nitrogen levels on weed biomass with special reference to *Trianthema portulacastrum* in chilli *Weed Abstract* 46; No. 1557.

Mukund. J., Kumarasamy. A.S., and Murthy, N.K. (1995). Effects of herbicides on weed control and seedling growth in chilli pepper nursery. *Weed Abstract*. 45; No. 4073.

Panse, V.G. and Sukhatme, P.V. (1978). *Statistical methods for Agricultural works II.* Edn. ICAR, New Delhi, India.

Patel, C.L., Patel, Z.G., and Patel, R.B. (1986). Efficiency and economics of some new herbicides for weed control in onion. *Gujarat. Agricultural University Research Journal* 12(1):14-17.

Qasem, J.R. (2003). *Weeds and their control.* University of Jordan Publications, Amman, Jordan. pp 628.

Rapparini, G. (1994). The development of mechanical methods and chemical products in the control of infestations. *Inf. Agrar.* 50(39): 111-112.

Shukla, V and Naik, L.B. (1993). Agro techniques of solanaceous vegetables. In *Advances in Horticulture Vol.5: Vegetable crops, Part 1.* K.L.Chadha and G.Kalloo (Eds), Malhotra publishing house New Delhi.P-365.

Thakur, D.R. (1994). Weed management in inter cropping system based on maize (*Zea mays*) under rainfed mid-hill conditions. *Indian J. Agron.,* 39(2); 203-206.

Vijayabaskaran, S. and Kathiresan, R.M.(1993).Integrated weed management in rice .cotton cropping system. In: *Proceedings of the International Symposium.* Indian Society of Weed Science, Hisar, vol; 3, pp.62 .64.

Zaragoza, C. (2003). Weed management in Vegetables. In: Labrada,R. (Ed), *Weed management for developing countries (Addendum I.).* FAO Document Repository. FAO Plant production and Protection Paper 120 Add.1. Agriculture Department, FAO, Rome, Italy.

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# STUDYING THE ALLELOPATHIC EFFECTS OF SHOOT AND ROOT WATER EXTRACTS OF HOARY CRESS (*CARDARIA DRABA* L.) ON GERMINATION AND SEEDLING GROWTH OF WHEAT

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## ABSTRACT

In order to evaluate the effect of water extracts of above-ground, below-ground and a mixture of above-ground and below-ground organs of hoary cress on germination and seedling growth of wheat, a factorial experiment based on completely randomized design with 4 replications was conducted at the Research Laboratory of the Faculty of Agriculture, Islamic Azad University, Birjand branch, Iran in 2008. Treatments included water extracts of different organs of hoary cress (above-ground, below-ground and an equal mixture of above-ground and below-ground) and five levels of water extract concentration including 5, 10, 20, 40 and 80% (v/v) plus a control (distilled water). Results showed that the different organs of hoary cress and the concentration of water extracts had a significant effect on germination and seedling growth wheat compared with the control. Increasing the concentration of water extract of different organs of hoary cress significantly decreased germination percentage, germination rate and also length and weight of radical and coleoptile of wheat. Estimation of three-parameter logistic model showed that the concentration required for 50% reduction of maximum germination was 18.72, 43.36 and 37.02% for aboveground, belowground and mixture of organs respectively, suggesting a stronger allelopathic impact of aboveground organs of hoary cress compared with belowground organs.

**Key words**: allelopathy, aqueous extract, hoary cress, wheat, germination

## INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. Commonly cited effects of allelopathy include reduced seed germination and seedling growth (Ferguson and Rathinasabapathi, 2003). The phytotoxic substances are called allelochemicals (Wu et al., 2001). Allelochemicals disrupt plant growth and development through changes in cell wall structure and functions and by preventing cell division, some enzyme function, seed germination, nutrient uptake, photosynthesis, protein and pigment synthesis and denaturation of DNA and RNA (Seigler, 1996).

Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates, and their derived compounds, can have allelopathic activity that varies over a growing season. In the laboratory, plant extracts and leachates are commonly screened for their effects on seed germination, with further isolation and

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identification of allelochemicals from greenhouse tests and field soil, confirming laboratory results (Ferguson and Rathinasabapathi, 2003).

Many weeds, including hoary cress (*Cardaria draba*), interfere with plants through production of allelochemicals. Hoary cress is a member of the Brassicaceae or mustard family. It is a deep-rooted perennial plant that reproduces vegetatively and by seed production. Disturbances such as grazing, cultivation, and especially irrigation can promote the colonization and spread of the plant. Hoary cress is native to the Balkan Peninsula, Armenia, Turkey, Israel, Syria, Iraq and Iran.

Qasem (1994) showed hoary cress has severe allelopathic effects on wheat and barley. In his experiment both root and shoot extracts significantly reduced wheat and barley seed germination and growth.

This experiment was conducted to evaluate the effect of shoot and root water extract of local populations of hoary cress in south Khorasan, Iran on wheat germination and seedling growth.

## MATERIALS AND METHODS

This experiment was conducted as a factorial based on randomized complete design with four replications in the research laboratory of the Islamic Azad University, Birjand branch, Iran in 2008. Factors investigated were water extracts from different plant organs and in different concentrations. Water extracts of different organs included aboveground (A), belowground (B) and an equal mixture of above- and below-ground (A+B) organs of hoary cress. Water extract concentration consisted of control (distilled water), and 5, 10, 20, 40 and 80% (v/v).

To prepare the basic solution, 20 g powder of each organ were added to 200 ml distilled water and then shaken for 24 hours. Twenty wheat seeds were placed on a whatman filter paper in a glass petri dish. Water extracts prepared from different treatments were added (5 ml) to the petri dish and the dishes placed in a germinator (25/15 °C for 12/12 hours). The observations recorded included germination percentage and rate, root and shoot length and their fresh weight. Analysis of variance was conducted with spss software. A three parameter logistic model was used to evaluate the allelopathic potential of different hoary cress organs.

## RESULTS

Increasing the hoary cress water extract (HWE) concentration reduced seed germination percentage significantly (table 1). The highest seed germination (100%) was found in the control. Germination failed in $A_{40}$, $A_{80}$, $B_{80}$ and $A+B_{80}$. Also germination rate decreased significantly with an increase in the concentration of hoary cress water extract so that treatments A5, A10 and A20 reduced germination rate by 8, 22 and 68 percent respectively.

The control treatment had the highest shoot and root length and fresh weight. Increasing the HWE concentration significantly reduced these traits.

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**Table 1.** Interaction of Hoary cress water extract concentrations and their source on some wheat germination traits

| Source of extract | Water extract concentration (%) | Germination percent | Germination rate(seed/day) | Root length (mm) | Shoot length (mm) | Root fresh weight (mg) | Shoot fresh weight (mg) |
|---|---|---|---|---|---|---|---|
| Aboveground | 0 | 100 a | 20 a | 80.4 a | 130 a | 50.6 abc | 127.33 a |
| | 5 | 98.33 ab | 18.32 ab | 53.8 bc | 121.22 a | 42.7 bcd | 90 cd |
| | 10 | 86.66 b | 15.68 cd | 43.84 cd | 72.29 de | 38.3 cde | 77.43 de |
| | 20 | 45 c | 6.5 e | 13.44 f | 39.99 f | 17.4 g | 39.43 f |
| | 40 | 0 d | 0 f | 0 g | 0 g | 0 h | 0 g |
| | 80 | 0 d | 0 f | 0 g | 0 g | 0 h | 0 g |
| Belowground | 0 | 100 a | 20 a | 81.14 a | 130.33 a | 55.66 a | 132.66 a |
| | 5 | 100 a | 20 a | 63.9 b | 102.21 b | 50.73 abc | 105 bc |
| | 10 | 100 a | 19.66 a | 45.37 cd | 91.43 bc | 42.96 bcd | 80.96 d |
| | 20 | 100 a | 19.41 a | 41.91 d | 83.22 cd | 34.43 def | 76.5 de |
| | 40 | 95 ab | 15.05 d | 24.48 e | 72.22 de | 25.53 fg | 56.36 ef |
| | 80 | 0 d | 0 f | 0 g | 0 g | 0 h | 0 g |
| Aboveground + Belowground | 0 | 100 a | 20 a | 83.62 a | 130.66 a | 59.5 a | 118.33 ab |
| | 5 | 100 a | 19.44 a | 64.53 b | 98.88 bc | 52.86 ab | 83.53 cd |
| | 10 | 98.33 ab | 18.96 ab | 51.26 cd | 83.52 cd | 41.2 bcde | 77.53 de |
| | 20 | 96.66 ab | 17.29 bc | 29.03 e | 63.34 e | 35.75 def | 55.76 ef |
| | 40 | 38.33 c | 6.15 e | 11.96 f | 41.82 f | 29.53 efg | 47.16 f |
| | 80 | 0 d | 0 f | 0 g | 0 g | 0 h | 0 g |

- Means that have similar letters are not significantly different according to LSD (α = 0.05)

## DISCUSSION

The reduction in plant seed germination percent and rate and shoot and root length and fresh weight with water extract of other plants has been reported by other researchers (Uremis et al, 2005; Yasmin et al, 1999).

Allelochemicals can lower the levels of hormones GA and IAA (Kamal and Bano, 2008). Shoot and root length reduction with increasing of HWE may be related to decreases in GA and IAA that reduce cell enlargement (Inderjit, 2002).

Estimation of three-parameter logistic model showed that the concentration required for 50% reduction of maximum germination was 18.72, 43.36 and 37.02 % for above-ground, below-ground and mixture of organs, respectively, suggesting a stronger allelopathic impact of above-ground organs of hoary cress compared with below-ground organs. Probably water extracts from above-ground parts of hoary cress contain more of the allelochemicals than water extracts from the below-ground parts.

## REFERENCES

Ferguson, J. J. and Rathinasabapathi, B. (2003). Allelopathy: How Plants Suppress Other Plants. (Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida).

Inderjit, H. (2002). Allelopathic effect of *Pluchea lanceolata* on growth and yield components of mustard (*Brassica juncea*) and its influence on selected soil properties. *Weed Biology and Management* 2: 200-204.

Kamal, J. and Bano, A. (2008). Allelopathic potential of sunflower (*Helianthus annus* L.) on soil metals and its leaves extracts on physiology of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology* 7(18): 3261-3265.

Qasem, J. R. (1994). Allelopatic effect of white top (*Lepidium draba*) on wheat and barley. *Allelopathy Journal* 1: 29-40.

Seigler, D. S. (1996). Chemistry and mechanisms of allelopathic interaction. *Agronomy Journal* 88: 876- 885.

Uremis, I., Arsalan, M.and Uludag, A. (2005). Allelopathic effect of some *brassica* species on germination and growth of cutleaf ground-cherry (*Physalis angulata L.*). *Journal of Biological Science* 5:661-665.

Wu, B. H., Pratley, J., Lemerle, D. and Haig, T. (2001). Allelopathy in wheat (*Triticum aestivum*). *Annals of Applied Biology* 139:1-9.

Yasmin, S., Saleem, B. and Irshad, A. (1999). Allelopathic effects of aqueous extract of chickpea (*Cicer arietinum*) and wheat (*Triticum aestivum*) on each others growth and quality. *International Journal of Agriculture and Biology* 1(3): 110-111.

# DISPERSAL OF POND APPLE (*ANNONA GLABRA*) BY RODENTS, AGILE WALLABIES AND FLYING FOXES

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## ABSTRACT

Pond apple is primarily water-dispersed, but native and exotic animals are also potential dispersal agents. Previous studies confirmed cassowaries and feral pigs as vectors; this study investigated the dispersal role of other species, including rodents, wallabies and flying foxes. Infrared motion-detecting cameras recorded three native rodent species consuming seeds: giant white-tailed rats (*Uromys caudimaculatis*), cane field rats (*Rattus sordidus*) and pale field rats (*Rattus tunneyi*). All appeared to destructively consume large quantities of seeds without moving them from host plants. The cameras also recorded agile wallabies (*Macropus agilis*) consuming seeds. Intact seed was found in 66% of field dung samples; viability was confirmed by germination from dung. Dung containing seed was found up to 230 m from host plants, sometimes in areas where water dispersal was not possible. The dispersal role of these animals may be significant, given their wide northern Australian distribution. Flying foxes (*Pteropus conspicillatus*) were not recorded consuming pond apple seeds in this study; anecdotal evidence suggests they consume the flesh and carry fruit short distances from host plants. Other research shows that the seed is too big for this species to swallow and disperse via gut passage. This study shows that there are many different animal interactions with pond apple, some beneficial and some detrimental to its spread.

**Keywords:** pond apple, *Annona glabra*, animal dispersal, wallabies, flying foxes

## INTRODUCTION

Pond apple (*Annona glabra* L.) is a highly invasive, small tree native to the Central Americas. It is considered to be the weed of greatest concern to the Wet Tropics Bioregion and is a declared Class 2 Weed under the Land Protection (Stock Route and Land Management) Act 2002. Pond apple is listed as one of Australia's 20 Weeds of National Significance.

Since its introduction in 1912, pond apple has invaded 2000 ha of the Wet Tropics and there is a high risk of further spread in this region and other suitable environments along Australia's eastern and northern coastline. Pond apple thrives in a wide range of habitats, particularly tidal or seasonally inundated coastal ecosystems such as melaleuca wetlands and mangrove communities. Pond apple plants form dense monocultures that exclude undergrowth and prevent regeneration of over-storey species.

Pond apple produces large amounts of fruit during the wet season, when flood events allow large-scale seed dispersal by water, both in fresh water and out to sea and then via ocean currents (Mason & Hardy, 2007; Setter *et al* 2008). The fruit is also attractive to animals, which readily consume the flesh and seed and act as vectors for dispersal. For example, studies by Setter *et al* (2002) and Westcott *et al* (2008) found cassowaries and feral pigs were capable of consuming and dispersing viable pond apple seed. It is suspected that other vertebrate species commonly found in pond apple infestations, including rodents, flying foxes and wallabies, may also consume pond apple fruit and seed. It is expected that different species will impact the seed differently: some may destroy seeds, and different gut retention and excretion rates will affect how and where other seeds are deposited.

This study aims to discover which vertebrate species consume pond apple, the impacts of their respective consumption of the seed and subsequent effects on dispersal. Animal dispersal may be significant, particularly in situations where they can disperse seed into areas where it is unlikely to be moved by water. This has obvious implications for surveillance programs.

## MATERIALS AND METHODS

This experiment was conducted in a high density pond apple infestation along the riparian zone of the Russell River near Babinda (17°15’57” S, 145°56’3” E) in Far North Queensland. The first component of this study involved recording which vertebrate species ate pond apple and, where possible, to determine their effects on the seeds.

### Photographic Documentation (Identifying Species)

Four infrared motion sensing cameras were placed within the pond apple infestation to record animal interactions with fruit on the trees and fallen on the ground. The cameras were positioned to focus on pond apple tree trunks or the ground during the 2007 fruiting season. Cameras were kept in the field for two months, with memory cards downloaded and batteries replaced on a weekly basis.

Cameras were set up again after the fruiting season had finished and most of the freely available fruit and seed had been removed naturally. This would provide a concentrated source of food for the camera to focus on and an increased chance of photographing the more discreet and small animals such as rodents and bats. To prevent consumption by larger animals (such as cassowaries and pigs), fruit and seed were placed in mesh baskets in pond apple trees or in cages on the ground at the base of pond apple trees. Fruit that was used in the cages and baskets had been collected during the fruiting season and frozen. Seeds in the cages and baskets were also inspected to see what impact that these animals had. Cameras were kept in the field for two months, with memory cards downloaded and batteries replaced on a weekly basis. Additional fruit and seed were also placed out each week.

### Field Collected Wallaby Dung – Seed Counts and Dispersal Distances

As wallabies were the only species observed to consume whole seeds, studies were conducted to understand the impact of consumption on the seeds for this species.

Dung was collected in a grassy transitional zone between a monoculture of pond apple and the mountainous ranges commonly associated World Heritage-listed tropical rainforest.

A collection of wallaby dung was taken on 14 February 2007 during the fruiting season. On this occasion a field search was conducted and any dung encountered was collected.

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An area of approximately 4.5 ha was searched and 50 dung samples were collected. Collections started close to the infestation and worked out from it until it was obvious that dung no longer contained pond apple seeds. This distance was recorded as the maximum dispersal distance. The 50 dung samples were returned to the laboratory, where each dung sample was broken apart and seeds were counted, removed and recorded.

### Viability of Pond Apple Seeds in Dung

A second field search for wallaby dung was conducted in the above location during the fruiting season of 2011. Twenty-five fresh dung samples were collected and transported to shade house facilities at the Centre for Wet Tropics Agriculture near South Johnstone for germination and viability testing. To keep conditions as natural as possible, dung was not pulled apart to count seeds prior to germination testing. Dung samples were placed individually on the surface of a commercial loamy soil mixture in 220 mm pots. Pots were watered twice daily to field capacity and monitored weekly for germination over a period of 4 months. Seeds were considered germinated if the radicle extended at least 0.5 cm beyond the seed coat. After this time the pots were taken from the shade house to the lab, where the remaining dung was dissected and seed counts and viability tests were conducted on ungerminated seeds. Viability tests involved squeezing seed to see if there was any resistance to pressure – those that felt firm were considered as potentially viable.

## RESULTS

### Photographic Documentation

Species that were photographed by the motion sensing cameras were *Macropus agilis* (agile wallaby), *Uromys causimaculatus* (giant white-tailed rat), *Rattus sordidus* (cane field rat) and *Rattus tunneyi* (pale field rat). One species of macropod and three species of rodent were documented eating pond apple seeds. The rodents consumed large amounts of seed, and visual inspection of seed after rodent consumption showed all seeds to be destroyed, with the embryos fatally damaged. Wallabies consumed seeds without visible ill-effects on the embryo, i.e. the seed found in wallaby dung was intact.

Flying foxes have often been anecdotally linked with pond apple infestations. We were unable to make any direct observations of flying fox-pond apple interactions using the cameras but the establishment of colonies within infestations has been observed.

Field Collected Wallaby Dung – Seed Counts and Dispersal Distances
Whole pond apple seeds were found in 66% of the wallaby dung sampled. The mean number of pond apple seeds per dung was 6 (range 0-42, n=50). No dung containing pond apple seeds was found at greater than 230 m.

Viability of Pond Apple Seeds in Dung
Approximately 73% of the seed in the dung samples germinated over a period of four months. There was no notable difference between the germination, viability, or time to germination of the seeds in the wallaby dung when compared to unconsumed seed from other studies. For example, Setter *et al* (2004) found 71% of seeds germinated after three months on the surface of the soil in pots in similar conditions.

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## DISCUSSION

How animals consume and potentially disperse pond apple seed is important information, as it assists in the management of this weed. Consumption by the animals in this study had both negative and positive impacts on pond apple seed.

Rodents destructively consumed large quantities of seed by damaging the embryo. Rodents are commonly known to consume hard seeds by gnawing through them, as they are not capable of consuming them whole. Destructive consumption of seeds by rodents will reduce the number of seed entering the seed bank, but it is considered that this will have a minimal impact on the overall seed bank. It has been suggested that rodents may cache seed; we did not find any evidence in this regard. However it is likely that if caching did occur, seed would not be taken very far from the parent tree.

Agile wallabies were able to consume and excrete viable seed, which should assist in the dispersal of pond apple. Although only a relatively short dispersal distance was observed in this experiment, macropods are recorded to have a mean gut retention time of 48 hours. Warner (1981) and Stirrat (2003) estimates that agile wallabies in the Northern Territory have a maximum home range of 24.6 ha. This suggests that they could potentially transport viable seed considerably further than recorded in this study. It is possible that other species of wallaby may also consume and transport seeds, and given that wallabies are far more abundant than cassowaries and may be present in a wider range of habitats, they may play a role in pond apple dispersal across a larger range of pond apple sites (Setter *et al* 2008).

As the large size of pond apple seeds precludes the ability for flying foxes to swallow them any movement would occur locally. However, the other means by which flying foxes could potentially spread seeds is by carrying off fruit in their mouths. They tend to do this with fruit that are large and often with fruit they have stolen from a tree that is patrolled by an already resident flying fox. The distance they would travel would be minimal as their intentions are to find the nearest tree to eat the fruit. (Fox, S pers.comm. 2007).

Dispersal of pond apple seed by any animal imposes the risk of moving it where water would not – such as upstream, uphill and possibly between catchments. For example, the wallaby that had dispersed seed furthest from the infestation (230 m) in this observation had gone through two barbed wire fences, across a bitumen road and uphill into the rainforest margin. This unpredictability of dispersal direction and distance by animals directly impacts on search pattern and search area for detecting new infestations. One factor leading to a reduction in the effect of animal dispersal over water dispersal is that some animal dispersal will, by its more random nature, disperse seed to places unsuitable for germination and survival, while water dispersal will largely be moving seed to suitable pond apple habitat. Improved knowledge of plant-animal interactions will generally assist weed management by enabling improvements to management strategies and protocols.

## REFERENCES

Mason, L.B. and Hardy, T. (2007). Ocean dispersal modelling for propagules of *Annona glabra*. Report prepared for the Queensland Department of Primary Industries and Fisheries by the Marine Modelling Unit of the Australian Maritime College.

Setter, M., Bradford, M. Dorney , B, Lyons, B. Mitchell, J. Setter, S. and Westcott, D. (2002). ‘ Pond apple – are the endangered cassowary and feral pig helping this weed to invade Queensland's Wet Tropics?' in H. Spafford-Jacob, Dodd J, and J.H. Moor (eds), Proceedings of the 13th Australian Weeds Conference, Plant Protection Society of Western Australia Inc., Perth.

Setter, M.J., Campbell, S. D., and Setter, S. D. (2008) Utilising knowledge of the biological and ecological traits of pond apple to improve management practices. A report to the Australian Government Department of Environment, Heritage, Water and the Arts prepared by the Queensland Department of Primary Industries and Fisheries.

Setter, S.D., Setter, M.J. and Campbell, S.D (2004). Longevity of pond apple (*Annona glabra* L.) seeds and implications for management. In Sindel, B.M and Johnson, S.B(eds), Proceedings of the 14th Australian weeds Conference, Weeds Society of New South Wales, Wagga Wagga.

Stirrat, S. C. (2003) Seasonal changes in home-range area and habitat use by the agile wallaby (*Macropus agilis*). Wildlife Research, Volume 30. pp 593-600.

Warner,A.C.I.(1981) 'The mean retention times of digestive markers in the gut of the Tammar, *Macropus eugenii*. Australian Journal of Zoology 29, 759-71.

Westcott, D.A. Setter, M. Bradford, M.G., McKeown, A. and Setter, S. (2008) 'Cassowary dispersal of the invasive pond apple in a tropical rainforest: the contribution of subordinate dispersal modes in invasion. Diversity and Distributions 14(2): 432-439.

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# POND APPLE (*ANNONA GLABRA* L.) - INVESTIGATING NOVEL MECHANICAL CONTROL OPTIONS

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## ABSTRACT

Pond apple usually occurs in swampy areas, but mechanical control may be a viable option in some locations during drier periods. Two machines, the Positrack™ and the Tracksaw™, have been trialled for initial kill rate, amount of follow-up control required, safety to field operators, cost-efficiency and selectivity (effect on native vegetation), compared to other control options. The Positrack™ is a tracked bobcat with a slasher-type attachment that cuts individual trees off near ground level and mulches them. It has no on-board herbicide application capability and requires an additional on-ground operator to apply herbicide by hand. The Tracksaw™ is a tracked mini-excavator with a chainsaw bar and spray applicator on the boom that cuts individual trees off near ground level and applies chemical immediately to the cut stump, requiring only a single operator. Initial trials were done in infestations of similar sizes and densities at the Daintree (Positrack™) and in Innisfail (Tracksaw™) in late 2009. Kill rates to date are 83% for Positrack™ mechanical, 95% for Positrack™ mechanical plus herbicide, and 78% for the Tracksaw™ combined treatment. If ongoing comparison proves either of these machines to be more cost effective, selective, and safer than traditional control methods, mechanical control methods may become more widely used.

**Keywords:** Pond apple, *Annona glabra*, mechanical, control

## INTRODUCTION

Pond apple (*Annona glabra* L.) is a small semi-deciduous exotic tree from North, Central and South America. It has invaded freshwater swamplands, creek banks, seasonally flooded areas and the upper edges of mangrove swamps along the Queensland coastline (Swarbrick and Skarratt, 1994). Pond apple is considered to be a serious threat to Australia and is listed as a Weed of National Significance (Thorp and Lynch, 2000). It was also the highest ranked weed species in a Wet Tropics Bioregion weed risk assessment (Werren, 2003).

Since its introduction into Australia in 1886 (Sugars *et al.* 2006), pond apple has invaded thousands of hectares of wetlands, riparian ecosystems and manmade landscape structures such as agricultural and domestic drainage systems. A number of control programs now exist throughout northern Queensland.

This study investigated two different machines - the Positrack™ and the Tracksaw™ - for their effectiveness as an initial control method, as well as the associated costs and follow up treatments required. Both machines are transportable, sole operator machines (however the Positrack™ requires additional operators if herbicide is to be applied),

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robust, and with the ability to maneuver amongst vegetation. They are, however, limited to operating under non boggy conditions.

The trial sites were at the Daintree (16 $^{o}$13.214 S, 145 $^{o}$26.874 E) (Positrack™) and Innisfail (17$^{o}$31'12" S, 146$^{o}$0'39" E) (Tracksaw™) with pond apple infestations of similar size and density. This paper reports on the population structure of these two pond apple infestations in North Queensland, quantifies the effectiveness of two mechanical control methods, and discusses the broader management of pond apple infestations.

## MATERIALS AND METHODS

### Positrack™

The Positrack™ is a tracked bobcat with a slasher-type attachment that cuts individual trees off near ground level and mulches them. It has no on-board herbicide application facility, requiring herbicide to be applied to the tree stumps by an on-ground operator. Therefore, the effectiveness of both the mechanical action only, and mechanical action plus herbicide application were tested.

The site in the Daintree National Park consisted of pond apple interspersed with a mixture of native tree and shrub species. The original vegetation type is dominated by a melaleuca species over-story with an extensive pandanus under-story. Other significant native species present and threatened include gardenias, melastomes, palms and orchids. Pond apple trees were present in the mid-story at a density of 6 400/ha and seedlings were interspersed within the site at a density of approximately 11 000/ha. Trees on average were 5.7 m high with a diameter of 18.7 cm at the base and 6.6 cm at chest height (n=850).

Within the site, 18 transects (20 m long x 3 m wide) were established, with all trees measured and pond apple seedlings counted. Six transects were assigned control, six Positrack™ treatment only, and six Positrack™ treatment plus herbicide application. The herbicide used was glyphosate ® (360 g $L^{-1}$) mixed with water 1:1 applied using a hand held pressure sprayer.

### Tracksaw™

The Tracksaw™ is a tracked mini-excavator with a chainsaw bar and spray applicator on the boom that cuts individual trees off near ground level and applies herbicide immediately to the cut stump via a spray nozzle. It requires only a single operator. It was only tested using the combined mechanical action plus herbicide application, as it would be unlikely to be used without herbicide.

The Innisfail site consisted of pond apple interspersed with a mixture of native species. This area of degraded native vegetation consisted of a melaleuca, hibiscus and palm over-story with pandanus under-story. The pond apple trees were the mid-story at a density of 7500/ha with seedlings being 3300/ha. Average tree height was 6.9 m with a diameter of 20.3 cm at the base and 9.4 cm at chest height (n=370).

Within the site, 12 transects (20 m long X 2 m wide) were established, with all trees measured and pond apple seedlings counted. Six transects were assigned as control and six as Tracksaw™ treatment (mechanical action and herbicide application together). The herbicide used was glyphosate ® (360 g $L^{-1}$) mixed with water 1:1.

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## Measurements

The efficacy of each treatment was measured at three monthly intervals (up to 12 months at the time of writing) as percentage of pond apple killed, amount of re-suckering, seedling recruitment and cost. Monitoring will continue until both sites are free of pond apple in order to allow for cost estimates to include follow-up control.

## RESULTS

### Positrack™

The Positrack™ was capable of moving freely around native trees to treat pond apple trees selectively. Each tree was reduced to fragments within 10 to 30 seconds, with only the shattered 15 cm high stump remaining. The largest tree killed was 60 cm in basal diameter and 8 m in height. The spinning cutter bars with associated push bar were capable of driving over and along fallen tree trunks, thus ensuring complete fragmentation. The result was a layer of mulched tree fragments with the over-story of natives remaining untouched. Under-story grasses and pandanus were effectively mowed.

As pieces of timber were being thrown from the machine it was safe to approach the cut stump only after the machine had moved on. As a consequence, there was a delay of approximately two minutes from when the stump was cut until herbicide application.

### Tracksaw™

The Tracksaw™ also treated pond apple trees selectively while maneuvering around native trees, but its maneuverability was inhibited somewhat by the articulated boom, its apparent greater mass and its low clearance compared to the Positrack™. The chainsaw bar on the end of the articulated boom reduced the machine’s ability to move and it was required to work along the face of the infestation.

The stems of the pond apple trees were cut flush to the ground with a typical chainsaw cut-stump appearance. The herbicide was applied within seconds of the stump being cut. As each tree was cut at the base, the tree was then swept to one side by the boom to facilitate forward movement. The largest tree killed had a 50 cm basal diameter and was 8 m in height.

### Comparative Effectiveness

Table 1 shows the efficacy and costs of the two machines as used in this experiment. The initial cost includes machine operator, labour for herbicide application (Positrack™ only) and herbicide costs.

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**Table 1.** Area treated, cost and efficacy of the Positrack™ and Tracksaw™ machines and pond apple regrowth 12 months after treatment application.

| | Positrack™ | Tracksaw™ |
|---|---|---|
| Area treated in two days | 1.5 ha | 0.75 ha |
| % kill machine only | 83 | - NA |
| % kill machine and herbicide | 95 | 90 |
| % stumps resuckering(herb) | 5 | 10 |
| Initial no. seedlings/ha | 11 000 | 3 300 |
| No. seedlings emerging/ha | 2 600 | 18 500 |
| Initial cost to treat / ha | $2 300 | $2 730 |

## DISCUSSION

### Machines

Both machines removed pond apple trees whilst minimally damaging native tree and shrub species. There are, however, notable differences in the landscape following treatment with the two machines. The Positrack™ creates mulch from the destroyed plants, while the Tracksaw™ leaves the cut tree in place, making it difficult to move through treated areas. The increase in the seedling density and suckering after the Tracksaw™ treatment may be attributed to the type and level of disturbance and lack of mulching. The Tracksaw™ has the advantage of being able to extend the cutting bar several metres, for example to reach the other side of a small drain or creek, or even to treat right to a drain or creek edge, which the Positrack™ could not do.

The Tracksaw™ has herbicide application integrated into its control method and thus requires only a single operator, while the Positrack™ required herbicide application by hand. As the Positrack™ stump was fragmented, it allowed for a greater volume of herbicide to be absorbed by the remaining plant tissue. This may enhance herbicide uptake compared to traditional cut stump methods, which may compensate for the greater time period between the cut and herbicide application. The Positrack™ could potentially be easily modified to apply herbicide from the one platform, thus eliminating extra labour costs and associated Workplace Health and Safety Issues.

Both machines require the soil to be relatively dry to operate, which may limit the usefulness of this tool to certain times of year or situations. This does, however, correlate to traditional control methods which are usually carried out in the drier months.

The impact on the natural environment where the two machines were tried was minimal. The native over-story remained intact, although more so in the Positrack™ site, and the under-story was quick to recover. The greatest off target damage occurred to saplings.

Follow-up treatment (i.e. monitoring and treatment of resuckering plants, missed plants and seedlings) would be considerably easier and thus less costly in the infestations treated by the Positrack™ due to the mulching of the fallen trees. Although pond apple wood is relatively soft (decomposing in a few years), the cut pond apple trunks remaining after the Tracksaw™ treatment make access and follow-up treatment more difficult and time-consuming, and therefore more costly. Pond apple tree trunks also present a safety hazard to field operators applying follow up control treatments. Suckers up to 3.5 m in height were recorded after 12 months at both sites, with these potentially reproducing within the following year.

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## Cost

Traditional methods employed for large scale pond apple control have been labour-intensive and sometimes hazardous. The methods themselves are sound, cost-effective and appropriate to some situations and cannot be totally replaced, but mechanical control may provide an additional control option in some circumstances. The time, expense and hazards (e.g. crocodiles, snakes, personal injury) associated with manually treating large areas may be reduced by mechanical control techniques.

Holloway (2004) estimates average costs per hectare for cut stump and stem injection treatment of pond apple to be $2750 and $2660 respectively. This figure, although dated, is representative of the costs of manual herbicide treatment. Associated costs of safety issues, training, staff retention etc. are not quantified. The costs of pond apple control using the Positrack™ and Tracksaw™ as described here are comparable, at $2300 and $2730 respectively, but may be associated with greatly reduced safety issues, training, and staff retention costs. The costs do not include those required for follow-up treatments, which will be necessary, and may be quite variable between the two machines, as evident by the differing amounts of resuckering and seedling recruitment recorded at 12 months.

## ACKNOWLEDGMENTS

We thank Dr. Shane Campbell for advice on experimental design and methodology, and Dr. Wayne Vogler and Dr. Dane Panetta for reviewing the manuscript. We also thank Travis Sydes (FNQROC) and the Cairns and Cassowary Coast Regional Councils for funding and technical assistance and DERM for technical assistance.

## REFERENCES

Holloway, I. (2004) Adaptive management: pond apple control in the catchments of the Russell-Mulgrave and Tully-Murray river systems. A report to the Australian Government Department of the Environment and Heritage.

Sugars, C., Charleston, K. & Doak, A. (2006) Pond apple management, control methods and case studies. (Queensland Department of Natural Resources, Mines and Water, Australia).

Swarbrick, J.T & Skarratt, D.B, (1994) The ecological requirements and potential Australian distribution of Pond apple (*Annona glabra*). A report to the Wet Tropics Management Agency.

Thorp, J.R., Lynch, R. (2000) The determination of weeds of national significance. National Weeds Strategy Executive Committee. (Department of Agriculture, Fisheries and Forestry Australia, Launceston, Tasmania, Australia).

Werren, G.L. (2003). A Bioregional perspective of weed invasion of rainforests and associated ecosystems; focus on the Wet Tropics of North-east Queensland. Proceedings of the Weeds of Rainforests and Associated Ecosystems Workshop, eds Grice, A.C & Setter, M.J. pp 9-18 (Cooperative Research Centre for Tropical Rainforest Ecology and Management, Rainforest CRC. Cairns.)

# SPREAD OF PARTHENIUM WEED AND ITS BIOLOGICAL CONTROL AGENT IN THE PUNJAB, PAKISTAN

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**ABSTRACT**

Parthenium weed is invasive in many regions worldwide. In Pakistan, parthenium weed was first reported from the Gujarat district of the Punjab Province in the 1980s. After 20 years of slow spread, it has spread rapidly in the past 10 years to many districts of the Punjab and Khyber Pukhtunkhwa Province and possibly to Sind Province. Parthenium weed is now the dominant weed in wastelands and is becoming a problematic weed in other situations such as irrigated and rainfed cropping systems, pasture lands, forests and national parks. Studies conducted prior to 2000 indicated that parthenium weed was present only in the northern districts of the Punjab. However, a survey carried out in 2009 on the distribution of parthenium weed and its biological control agent *Zygogramma bicolorata*, a leaf defoliating beetle, revealed that this weed has rapidly spread while the biological control agent is spreading in a zone behind that of the weed. The weed has now moved from the northern to the southern districts of the Punjab and is threatening the districts of Okara, Pakpattan, Sahiwal, Khanewal, Multan and Bahawalpur. The presence of parthenium weed in the southern Punjab is a potential threat to cotton and the dairy industries of this Province.

**Keywords:** Parthenium weed, *Zygogramma bicolorata*, Punjab, distribution

## INTRODUCTION

Parthenium weed (*Parthenium hysterophorus* L.) is an annual herb of the Asteraceae family, originating from the tropical Americas and now a weed of global significance in many countries around the world (Dhileepan, 2009; Figure 1). It reduces crop and pasture productivity, reduces native plant community biodiversity and negatively affects human and animal health (Nath, 1981; Shabbir & Bajwa, 2006; McFadyen, 1995). Parthenium weed was first reported from the Gujarat district of Punjab Province in 1980 (Razaq *et al*., 1994) and since then it is rapidly spreading throughout the Province of the Punjab, the Islamabad Capital Territory (ICT) and parts of the Khyber Pukhtunkhwa (KPK) Province (Shabbir, 2002; Adkins and Navie, 2006; Shabbir and Adkins, 2008). In Pakistan, parthenium weed is mainly found in wastelands, along the roadsides, and abandons fields but recently the weed is found in pasture and crop lands (Shabbir, 2002). The weed is threatening the natural and agricultural ecosystems and has been reported from number of national parks and forest reserves.The core infestations of this noxious weed are in the central and northern districts of the Punjab, including the ICT, though its exact distribution is not well documented. As yet, it has not been reported from the southern districts of the Punjab province. However, given parthenium weed's highly invasive nature and the

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changing climatic conditions of the Punjab, it is hypothesized that the weed has spread to the southern districts of this province and possibly to the Sind province.

A leaf defoliating beetle, *Zygogramma bicolorata* Pallister (Chrysomelidae: Coleoptera), was found in the forest reserves near Lahore, Pakistan (Javaid and Shabbir, 2007). *Z. bicolorata* had been tested and released as a classical biological control agent in 1980 in Queensland, Australia, where the agent has since had a significant effect on parthenium weed (Dhileepan, 2001 & 2003). The beetle was independently tested and released as a biological control agent against parthenium weed in India in 1984 (Jayanth 1987). Presumably the agent arrived in Pakistan from the release made in India. To date, there is very little data on the present distribution of the beetle in the core parthenium weed infestations of Pakistan. The main objective of this study was to record the current distribution of parthenium weed and its biological control agent, *Z. bicolorata,* in the Punjab province of Pakistan to aid in future weed management planning.

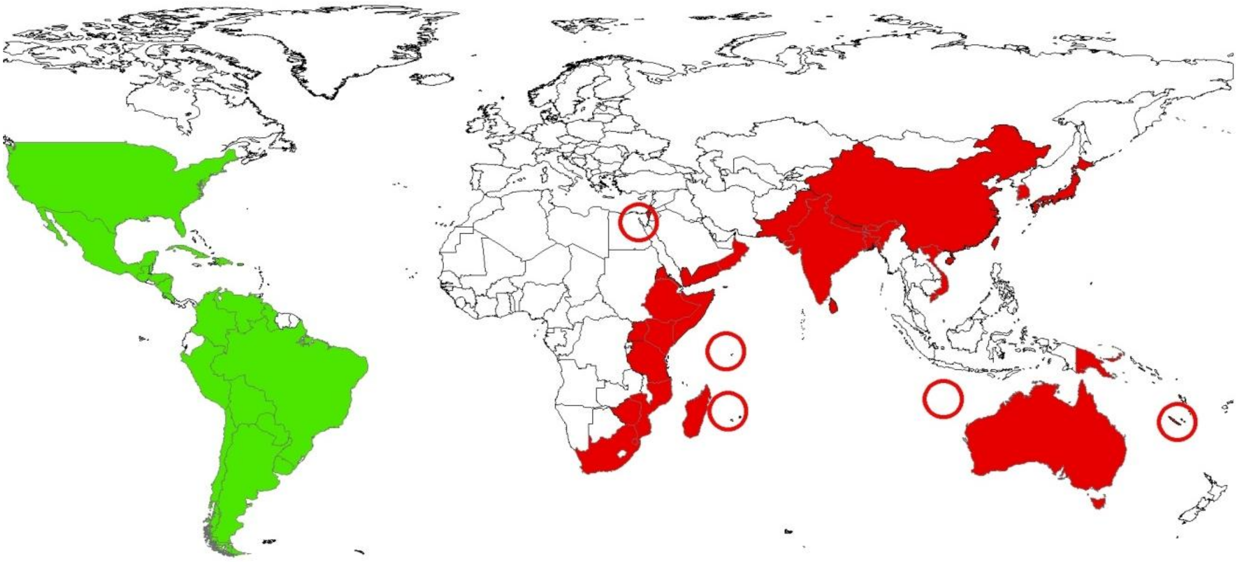

**Figure 1.** A worldwide map of parthenium weed distribution. Parthenium weed is invasive in the countries shaded or circled red; countries shaded green are considered its native range.

## MATERIALS AND METHODS

Surveys for parthenium weed and *Z. bicolorata* were carried out throughout the Punjab Province and the ICT in March-May 2009. More extensive surveys were undertaken in central Punjab. The surveys followed the major road networks out from the core infestation of parthenium weed. Wastelands and cropped areas outside of the core infestation were also surveyed. Every 10 km the presence/absence of parthenium weed and *Z. bicolorata* was recorded. If parthenium weed was present, its density per square metre was also recorded: low, 1-2 plants/m$^2$; medium, 2-4 plants/m$^2$; high, >4 plants/m$^2$. The geographical coordinates and altitudes of all the survey sites were recorded using a GPS navigator (Garmin Model – 60CSx). ArcGIS v. 9.3 software (ESRI) was used to map the distribution of parthenium weed and *Z. bicolorata* .

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## RESULTS

The survey revealed that parthenium weed is now more widespread in the Punjab province and the ICT (Table 1). Earlier studies (Shabbir 2002; Shabbir and Adkins 2008) have shown that the core infestation of parthenium weed was centred in the north eastern districts of the Punjab province, including the ICT. Current surveys show parthenium weed is moving from north eastern districts to the southern districts of the Punjab (Figure 2). The southern districts with parthenium infestations include Okara, Pakpattan, Sahiwal, Khanewal, Multan and Bahawalpur. Occurrence of parthenium weed in southern districts such as Sahiwal and Kanewal present potential threats to agriculture and the dairy industries of the country. Similarly, parthenium weed in the south (Sahiwal, Khanewal, Multan and Bahawalpur) is a threat to the cotton industry of the country.

**Table 1.** Parthenium weed density and occurrence of *Z. bicolorata* in different districts of the Punjab province and the ICT, Pakistan, as surveyed in March-May 2009.

| Survey number | Punjab province districts | Parthenium weed density[a] | *Z. bicolorata*[b] |
|---|---|---|---|
| 1 | Attock | M | + |
| 2 | Bahawalnagar | Nil | - |
| 3 | Bahawalpur | L | - |
| 4 | Bhakkar | Nil | - |
| 5 | Chakwal | L | + |
| 6 | Dera Ghazi Khan | Nil | - |
| 7 | Faisalabad | M | - |
| 8 | Gujranwala | H | + |
| 9 | Gujrat | H | + |
| 10 | Hafizabad | H | + |
| 11 | Jhang | M | - |
| 12 | Chiniot | M | - |
| 13 | Jehlum | M | + |
| 14 | Kasure | H | + |
| 15 | Khanewal | L | - |
| 16 | Khushab | M | + |
| 17 | Lahore | H | + |
| 18 | Layyah | Nil | - |
| 19 | Lodhran | Nil | - |
| 20 | Mandi Buhaudin | H | + |
| 21 | Multan | L | - |
| 22 | Muzzafargarh | Nil | - |
| 23 | Mianwali | L | - |
| 24 | Nankana | H | + |
| 25 | Narowal | H | + |
| 26 | Okara | H | - |
| 27 | Pakpatan | M | - |
| 28 | Rahim Yar Khan | Nil | - |
| 29 | Rajan Pur | Nil | - |
| 30 | Rawalpindi | H | - |

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| | | | |
|---|---|---|---|
| 31 | Sahiwal | M | - |
| 32 | Shekhupura | H | + |
| 33 | Sialkot | H | + |
| 34 | Sargodah | H | + |
| 35 | Toba Tek Singh | H | - |
| 36 | Vehari | Nil | - |

[a] L = low, M = medium, H = high, Nil = absent
[b] + = present, - = absent

Parthenium weed was growing in the north west districts of Chakwal, Mianwali and Attock but not found in the salt range of Potohar plateau. In the Kasure district, the town of Pattoki had a high density of parthenium weed. Parthenium weed was not found in most of the southern districts bordering the Baluchistan (such as DG Khan and Rajanpur) and Sind (such as Rahim Yar Khan). However, it was found near sale points of nurseries in the southern districts including Bahawalpur, a district remote from any other parthenium weed infestation

Major clusters of *Z. bicolorata* were found in the northern Punjab and in the ICT. One cluster lies close to the eastern districts (Lahore, Narowal, Gujrat and Sialkot) on the Indian border and the other is in the north west districts (Attock, Chakwal). The beetle was not recorded from the central and southern districts of the Punjab province (Figure 2).


**Figure 2.** Distribution of parthenium weed and its biological control agent *Zygogramma bicolorata* in Punjab, Pakistan as surveyed in 2009 (red circles indicate the sites where both parthenium weed and Zygogramma beetle were recorded).

## DISCUSSION

In the late 1990s, parthenium weed was reported only in the eastern districts of the Punjab province and the ICT (Shabbir, 2000). Now parthenium weed is present in 28 of the 36 districts of the Punjab province, having spread from northern to southern Punjab. It is still at a relatively low density in the Southern districts (Table 1). Parthenium weed harbours a number of insect pest and crop diseases, including the Tobacco Streak Virus (TSV) which affects important crops such as cotton (Kishun and Chand 1987; Pandey *et al* 1991; Ahmed *et al* 2001) . Parthenium weed is the principle source of TSV epidemics in Australia (Sharman *et al*., 2009). In Pakistan, TSV has caused severe yield declines in cotton crops (Ahmed *et al.,* 2003). The spread of parthenium weed into the southern cotton belt poses a new and grave threat to the cotton industry, the backbone of Pakistan's agricultural based economy.

Seed transport by vehicles is a major factor in the long distance dispersal of parthenium weed in Australia (Nuygan unpublished data). The spread of parthenium weed in the Punjab may likewise be due to frequent vehicle movement and goods transport across districts, as all districts have a network of major and minor roads. Parthenium weed is now a threat to the agriculture and pastures lands of the KPK Province (formerly North West

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Frontier Province) (Hasan, 2009), probably due to the spread of the weed from bordering districts of the Punjab.

Parthenium weed was found near plant nurseries in almost all major towns in the Punjab province, including the remote southern Bahawalpur district. Large populations of parthenium weed were also growing in the Pattoki floriculture farms of the Kasur district. Pattoki is a floriculture business hub of the country and farms here supply plants not only to all of the Punjab but also to other provinces, including the ICT. The soil used for potting plants may be contaminated with parthenium weed seeds.

The biological control agent *Z. bicolorata* is present in the northeast and north-west districts of the Punjab, as predicted by the CLIMEX model developed by Dhileepan & Senaratne (2009). Areas in KPK and some southern districts in the Punjab province also seem suitable to this beetle, but so far it has not been observed there. The current data of the distribution of parthenium weed and *Z. bicolorata* is therefore not only useful for mapping but also for further climate suitability studies to aid effective redistribution of the biological control agent in Pakistan.

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## REFERENCES

Adkins, S. W. and Navie, S. C. (2006). Parthenium weed: a potential major weed for agro-ecosystems in Pakistan. *Pakistan Journal of Weed Science Research* 12: 19-36.

Ahmed W., Butt T.B., Ihsan J. and Rehman, A. (2003). Natural occurence of tobacco streak virus in cotton in Pakistan and screening for its resistant sources. *Pakistan ournal of. Botany*. 35: 401-408.

Dhileepan, K. (2001). Effectiveness of introduced biocontrol insects on the weed *Parthenium hysterophorus* (Asteraceae) in Australia. *Bulletin of Entomological Research* 91: 167-176.

Dhileepan, K. (2003). Seasonal variation in the effectiveness of the leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) and stem-galling moth *Epiblema strenuana* (Lepidoptera: Tortricidae) as biocontrol agents on the weed *Parthenium hysterophorus* (Asteraceae). *Bulletin of Entomological Research* 93: 393-401.

Dhileepan, K. (2009). *Managing Parthenium Weed across Diverse Landscapes: Prospects and Limitations.* (Springer, Netherlands).

Dhileepan, K. and Wilmot Senaratne, K.A.D. (2009). How widespread is *Parthenium hysterophorus* and its biological control agent *Zygogramma bicolorata* in South Asia? *Weed Research* 49 (6): 557-562

Kishun, R and Chand, R (1987) 'New collateral hosts for *Pseudomonas solanacearum'*, *Indian Journal of Mycology and Plant Pathology*, 17:2 237.

Hasan, G. (2009). First annual report on biology, ecology and management of parthenium weed; a threatening alien weed for natural and agricultural ecosystems of Pakistan. (Higher Education Commission (HEC), Pakistan).

Javaid, A. and Shabbir, A. (2007). First report of biological control of *Parthenium hysterophorus* by *Zygogramma bicolorata* in Pakistan. *Pakistan Journal of Phytopathology* 18: 99-200.

Jayanth, K.P. (1987). Introduction and establishment of *Zygogramma bicolorata* on *Parthenium hysterophorus* in Bangalore, India. *Current Science* 56: 310–311.

McFadyen, R. (1995). Parthenium weed and human health in Queensland. *Australian Family Physician* 24: 1455–1459.

Nath, R. (1981). Note on the effect of Parthenium extract on seed germination and seedling growth in crops. *Indian Journal of Agricultural Sciences* 51: 601-603.

Pandey, AK, Luka, RS, Hasija, SK & Rajak, RC (1991) 'Pathogenicity of some fungi to Parthenium, an obnoxious weed in Madhya Pradesh', *Journal of Biological Control*, vol. 5, pp. 113–5.

Razaq, Z.A., A.A. Vahidy and S.I. Ali (1994). Chromosome numbers in Compositae from Pakistan. *Annals of Missouri Botanical Gardens*. 81: 800-808.

Shabbir, A. (2000). Allelopathic effect of *Parthenium hysterophorus* L. on companion weeds in Islamabad. A report submitted for the partial fulfilment of MSc degree in Botany. (Department of Biological Sciences, University of Arid Agriculture, Rawalpindi).

Shabbir, A (2002) '*Parthenium hysterophorus* L. An exotic weed threatening agricultural lands and biodiversity of Islamabad and adjoining districts', M.Phil theis, (Department of Botany University of the Punjab, Lahore Pakistan)

Shabbir, A. and Adkins, S.W. (2008). Parthenium weed: management prospects in Pakistan. In van Klinken, R.D., Osten, V.A., Panetta, F.D. and Scanlan, J.C. (eds). *Proceedings of the 16th Australian Weeds Conference*. Queensland Weeds Society, Brisbane. p. 271.

Shabbir, A. and Bajwa, R. (2006). Distribution of Parthenium weed (*Parthenium hysterophorus*) I: an alien invasive weed species threatening the biodiversity of Islamabad. *Weed Biology and Management* 6(2): 89-95.

Sharman, M., Persley, D.M. and Thomas, J.E. (2009). Distribution in Australia and seed transmission of Tobacco streak virus in *Parthenium hysterophorus*. *Plant Disease* 93: 708-712.

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# MODELING INVASION BY *ALLOCASUARINA HUEGELIANA* IN KWONGAN HEATHLAND AND ITS MANAGEMENT IMPLICATIONS

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## ABSTRACT

In most natural systems worldwide, land managers are combating the spread of invasive weeds and attempting to minimize their damaging impacts. Often invasion is linked to disturbance regime shifts, and particularly in the case of native invasives disturbance is often critical in the incursion behavior. Manipulation of disturbance regimes can provide effective control methods for managers. Modeling as an informative tool in invasive species control is common, though underutilized when applying disturbance as the management strategy. We constructed and analyzed a population model to inform management of the native invasive tree *Allocasuarina huegeliana*. Local managers believe that its spread is due to an altered fire frequency. We built a population model of *A. huegeliana* and simulated fire and fire coupled with managed removal over a range of strategies. We found that current fire frequencies likely cause high densities leading to biodiversity loss unacceptable in the local adaptive management plan. Losses could be mitigated by managed removal, a strategy most effective at high levels of effort.

**KEYWORDS:** native invasive, individual-based model, fire, sandplain heathland, rock sheoak, adaptive management, disturbance-based management

## INTRODUCTION

Invasive species have been shown to negatively impact ecosystem biodiversity levels and function through direct and indirect effects (Vitousek, D'Antonio et al. 1996; Mack and D'Antonio 1998; Parker, Simberloff et al. 1999; Valery, Fritz et al. 2009). Though the term invasion most often applies to non-natives, similar effects can be seen in the human-induced spread of some species within their own native range (Reise, Olenin et al. 2006; Valery, Fritz et al. 2008; Davis 2009). The causes of native invasion are not fully understood. However, a strong link between disturbance in a system and biological invasion has been shown in many studies (Hobbs and Huenneke 1992; D'Antonio, Dudley et al. 1999; Hierro, Villarreal et al. 2006). The theoretical link between disturbance and invasion is intuitively strong in native invasion. To control a native invasion, the underlying cause must often be treated in addition to managing the species itself (Hobbs 2000). Thus, returning a system to historical disturbance regimes – or implementing well-planned novel regimes – must be used in conjunction with more direct treatments such as chemical, manual, and biological control to manage the spread of disturbance-linked invasion in a system.

In many systems worldwide, fire is a major mechanism of disturbance and the alteration of historic fire regimes is thought to have led to major shifts in the vegetation, including invasion by non-native as well as native species (Vitousek, D'Antonio et al. 1996; Van Auken 2000). In this study, we investigated population modeling techniques to assess fire as a disturbance-based management tool for the control of a native invasive in a fire-prone Australian system. We focus on land manager concerns about invasion in kwongan heathland, found predominantly in the wheatbelt region of Western Australia.

In the past several decades, the native tree species *Allocasuarina huegeliana*, a fire sensitive tree species, has been invading in kwongan habitat where previously it was recorded as rare or absent (Main 1993; Bamford 1995). There is increasing concern that a shift to high *A. huegeliana* densities will cause a large decrease in kwongan biodiversity. We developed an individual-based model of *A. huegeliana* populations to examine the efficacy of a number of single and combined management techniques with a focus on fire as the predominant method of control. Our goal was to determine how fire frequency and regularity, and its pairing with managed removal affect *A.huegeliana* spread in kwongan. We analyzed rates of increase, spatial distribution, and the point at which acceptable thresholds of alpha diversity impacts are crossed under each treatment.

### Species

*Allocasuarina huegeliana* is a species native to Western Australia. As a reseeder species, A. huegeliana is at a potential disadvantage to resprouting kwongan species more fire tolerant when subjected to high frequencies. It has been shown to have dramatically increased seedling occurrence immediately post-fire (Yates, Hopper et al. 2003). However, there is a corresponding occurrence of heightened seedling mortality post-fire (Maher, Hobbs et al. 2010).

### Site

Kwongan is a highly diverse shrub-dominated ecosystem found in the wheatbelt of Western Australia. It is composed mainly of woody and herbaceous cover less than two meters tall. Vegetation in the kwongan has adapted to frequent fires, with many life history characteristics dependent on fire for optimal growth (Keith, Holman et al. 2007). Fire can also preserve the high diversity between canopy layers (Keith and Bradstock 1994; Keith, McCaw et al. 2002).

## MATERIALS AND METHODS

### Model

The *A. huegeliana* population model was coded in R and is an individual-based matrix model. A single cell has three possible states: absence of *A. huegeliana*, presence of *A. huegeliana* seedling, or presence of *A. huegeliana* adult individual. Timesteps are annual and include seed production, establishment, aging, and death. *Allocasuarina huegeliana* is dioecious, and we estimated 40% of the total population as female, as per field data. Once reproductive age is achieved, each female tree produces a uniform number of seeds each year. Newly produced seeds are distributed throughout the model landscape with a dispersal distance drawn randomly from a Cauchy distribution with location $x_0$ = 4 and scale $\gamma$ = 1, and the direction of dispersal randomly determined. All seeds are assumed to germinate immediately, and become seedlings of age one. Seedlings are more vulnerable to environmental mortality than fully established adults. Therefore, a high rate of seedling

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mortality is assumed for the first two years, at which point any surviving seedling becomes an adult. In this adult stage, a set mortality probability of 5% per year is assumed.

All environments experience 'good' and 'bad' years. To simulate this stochasticity, our model randomly assigns an annual seasonal suitability based on a truncated normal distribution with mean $\mu$ = 0.5 and standard deviation $\sigma$ = 0.1. Three parameters vary dependent upon seasonal suitability: seedling mortality, seed production, and reproductive age (see Figure 1).

We examined two methods of control: fire and managed removal of adult trees. Fire is simulated as homogeneous across the site. Following fire, 100% mortality of *A. huegeliana* within the burned site is assumed. Additionally,, simulated seed production in years of fire occurrence increases by a magnitude of ten to imitate the increased germination of reseeder species post-fire. Once the simulated site is burned, it is considered bare of vegetation. In accordance with higher seedling mortality rates observed in the field, increased seedling mortality of *A.huegeliana* is assumed for three years post-fire. Finally, we simulated managed removal of *A. huegeliana* adults in conjunction with fire. Adult trees are 'cut down' and experience mortality without the increased seed rain or harsher seedling environment. We assume some human error, so adult trees have a 90% chance of removal. Because of the inevitable presence of fire in the system – both wildfires and management fires for fuel load and heath regeneration purposes, we did not look at managed removal alone.

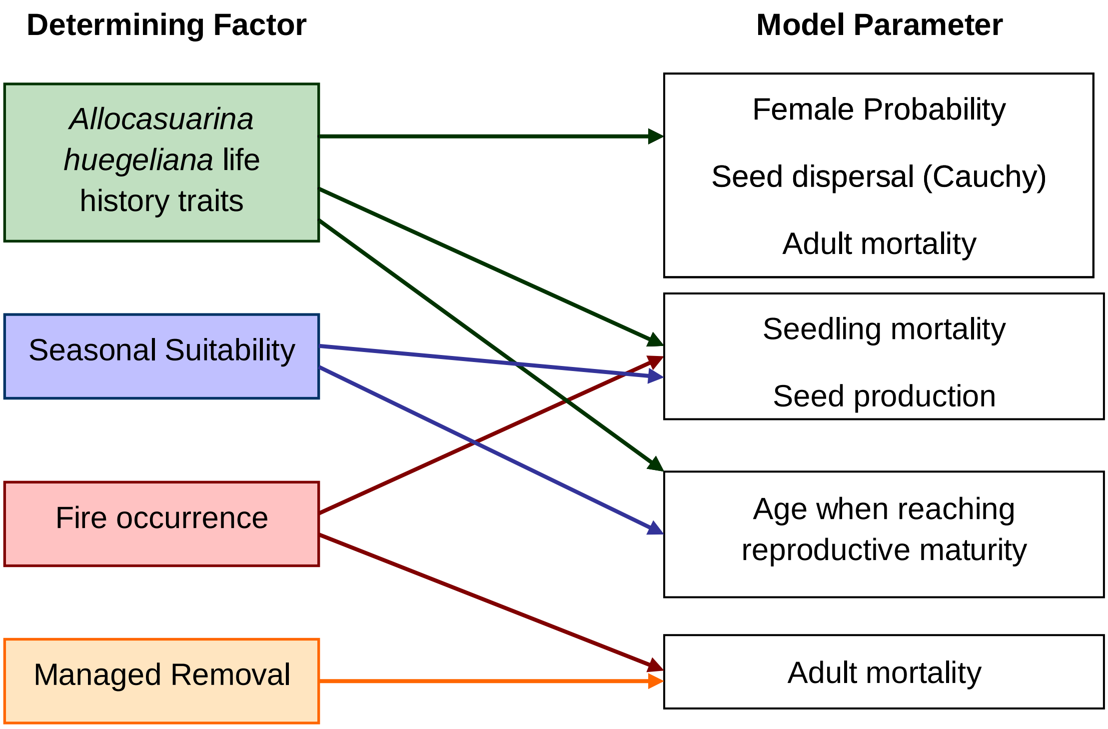


**Figure 1:** Model parameters and the factors influencing their values.

**Virtual Experiments**

A series of virtual experiments was conducted. In kwongan patches, there is generally an adjacent *A. huegeliana* seed source such as an *A. huegeliana* stand at the base of a granite outcrop. To simulate this situation in our model, 10% of the landscape was designated as a fire refuge that did not burn during the model runs. The remaining area

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represented the fire-prone heath patch and was subjected to a range of management strategies. We considered several different management regimes: regular fire occurrence, random fire occurrence, and regular fire occurrence in conjunction with managed removal. In the regular fire occurrence, we considered burn frequencies of between 10 and 80 years, in increments of 5 years. For the random pattern, fire occurred each year with a certain probability. We considered a range of probabilities corresponding to the same 10-80 year range of frequencies considered for the regular pattern. Managed removal was simulated by testing managed removal of adults every 9 years, every 18 years, and every 27 years and a range of removal areas, starting with the entire site or rather the center 75%, 50%, and 25% of the site.

**Measure of Impact**

We used species richness as our measure of impact. The estimated richness impact was based on field data collected at field sites and the species-area curve. We calculated the mean species richness for each area within a nested design and fitted this data to a species-area curve using the power function species-area relationship: $S = cA^z$ where S is species richness, A is area, c is a constant, and z is the rate at which species accumulate with increasing area. We assumed that each *A. huegeliana* individual reduced available area by the area of one cell (2.25 m * 2.25 m ≈ 5 m²). We calculated impact as the reduction of species based on the reduction of area inhabited by *A. huegeliana*. At a level of only one species, the invasion had a measurable, negative impact on the system. At five species, some of the sites dropped below 90% of the original site diversity. Therefore, we defined the thresholds of major interest as one, three, and five species loss, corresponding with densities of 134, 380, and 595 trees per ha, respectively.

**RESULTS AND DISCUSSION**

The model was able to predict population dynamics of the native invasive of concern (see Figure 2) and its response to different management tactics. The simulated populations showed many of the behaviors commonly found in invasions, such as satellite population development and density edge effects from the seed source.

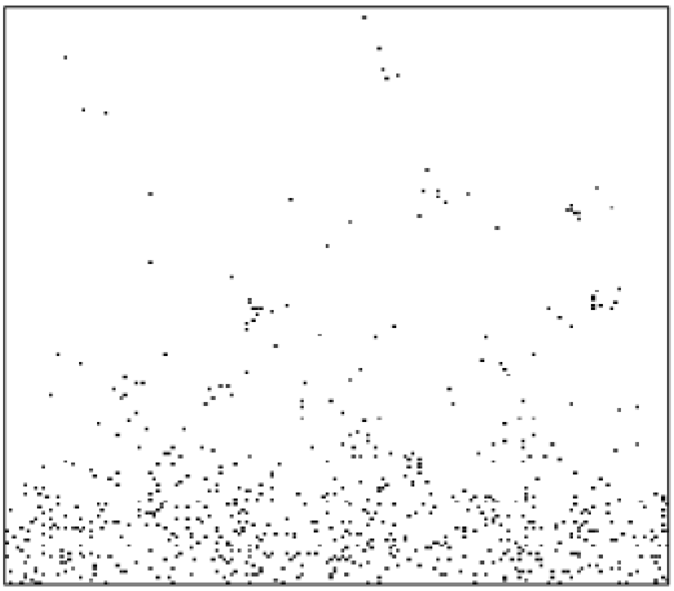

**Figure 2**: Typical output of simulation. The area depicted, totaling 20.25 ha, is both the

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simulated fire refuge (lower 10% of area) and heath patch (upper 90% of area), with white space being those cells unoccupied by *A. huegeliana*. Each black cell represents an *A. huegeliana* individual.

Changes in fire frequency made a gradual but significant difference in the increase and spread of the species post-fire. DEC states in their management plan that the purpose of *A. huegeliana* control is to maintain biodiversity levels in the kwongan heath (Beecham, Lacey et al. 2009). It is important to understand the management strategies that will effectively attain that goal. Biodiversity loss thresholds were crossed under many of the management scenarios. However, the model was able to predict levels of each strategy necessary to delay or prevent that loss, thus maintaining current biodiversity levels.

Disturbance-based management has the potential ability to not only control invasion itself but also to address the underlying cause. Our model focuses on fire, only one of the many disturbance regimes contributing to global invasive species establishment and spread (Didham, Tylianakis et al. 2007). Similar modeling applications would be effective in looking at other disturbance-based management techniques, such as hydrologic disturbance in ephemeral or tidal wetlands or grazing by native or introduced herbivores. Additionally, there are many potential further impacts of invasive species beyond our measure in the current model, including competition, nutrient shifts, allelopathy, microclimate changes or shifts in pollinator/plant interactions. We used a very conservative measure, looking only at the physical space occupied by the invader. Further research needs to be undertaken to establish ecologically sound thresholds for use in management-focused modeling.

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## REFERENCES

Bamford, M. (1995). *Exploring Wheatbelt Woodlands*. Perth, Department of Conservation and Land Management.

Beecham, B., P. Lacey, et al. (2009). Conserving the Biodiversity of the Tutanning Nature Reserve. D. o. E. a. Conservation.

D'Antonio, C., T. Dudley, et al. (1999). "*Disturbance and biological invasions: direct effects and feedbacks*." Ecosystems of the world: 413-452.

Davis, M. (2009). *Invasion Biology*, Oxford University Press.

Didham, R. K., J. M. Tylianakis, et al. (2007). "Interactive effects of habitat modification and species invasion on native species decline." *Trends in Ecology & Evolution* 22(9): 489-496.

Hierro, J. L., D. Villarreal, et al. (2006). "Disturbance facilitates invasion: The effects are stronger abroad than at home." *American Naturalist* 168(2): 144-156.

ISBN Number: 978-0-9871961-0-1

Hobbs, R. (2000). *Ecological repair following biotic invasions. Best Management Practices for Preventing and Controlling Invasive Alien Species*. G. Preston, G. Brown and E. v. Wyk. Kirstenbosch Botanical Gardens, Cape Town, South Africa, The Working for Water Program.

Hobbs, R. J. and L. F. Huenneke (1992). "Disturbance, Diversity, and Invasion - Implications for Conservations." *Conservation Biology* 6(3): 324-337.

Keith, D. A. and R. A. Bradstock (1994). "Fire and Competition in Australian Heath - a Conceptual-Model and Field Investigations." *Journal of Vegetation Science* 5(3): 347-354.

Keith, D. A., L. Holman, et al. (2007). "Plant functional types can predict decade-scale changes in fire-prone vegetation." *Journal of Ecology* 95(6): 1324-1337.

Keith, D. A., W. L. McCaw, et al. (2002). *Fire regimes in Australian heathlands and their effects on plants and animals*, Cambridge University Press, Cambridge.

Ladd, P. G. (1989). *The status of Casuarinaceae in Australian forests*. First National Conference on Australian Forest History, Canberra.

Mack, M. C. and C. M. D'Antonio (1998). "Impacts of biological invasions on disturbance regimes." *Trends in Ecology & Evolution* 13(5): 195-198.

Maher, K. A., R. J. Hobbs, et al. (2010). "Woody shrubs and herbivory influence tree encroachment in the sandplain heathlands of southwestern Australia." *Journal of Applied Ecology* 47(2): 441-450.

Main, A. R. (1993). *Landscape Reintegration: Problem Definition. Reintegrating Fragmented Ecosystems*. R. J. Hobbs and D. A. Saunders. New York, Springer-Verlag.

Parker, I. M., D. Simberloff, et al. (1999). "Impact: toward a framework for understanding the ecological effects of invaders." *Biological Invasions* 1: 3-19.

Reise, L., S. Olenin, et al. (2006). "Are aliens threatening European aquatic coastal ecosystems? ." *Helgoland Marine Research* 60(2): 77-83.

Valery, L., H. Fritz, et al. (2008). "In search of a real definition of the biological invasion phenomenon itself." *Biological Invasions* 10(8): 1345-1351.

Valery, L., H. Fritz, et al. (2009). "Ecosystem-level consequences of invasions by native species as a way to investigate relationships between evenness and ecosystem function." *Biological Invasions* 11(3): 609-617.

Van Auken, O. (2000). "Shrub invasions of North American semiarid grasslands." *Annual Review of Ecology and Systematics* 31: 197-215.

Vitousek, P. M., C. M. D'Antonio, et al. (1996). "Biological invasions as global environmental change." *American Scientist* 84(5): 468-478.

Yates, C. J., S. D. Hopper, et al. (2003). "Impact of two wildfires on endemic granite outcrop vegetation in Western Australia." Journal of Vegetation Science 14(2): 185-194.

# A COMPARATIVE PHENOLOGICAL AND GENETIC DIVERSITY ANALYSIS OF TWO INVASIVE WEEDS, CAMEL MELON (*CITRULLUS LANATUS* (Thunb.) Matsum. and Nakai var. *LANATUS*) AND PRICKLY PADDY MELON *(CUCUMIS MYRIOCARPUS* L.*)*, IN INLAND AUSTRALIA

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**ABSTRACT**

The biological attributes of two invasive weed species, prickly paddy melon and camel melon, were studied in different disturbed habitats of the Riverina region, NSW during 2010-2011. Seedlings first germinated in early to mid November 2010, once optimal soil temperatures were achieved. Flowering began in both species, generally 35 to 45 days following seedling establishment. Both species exhibited monoecious tendencies, with production of male flowers rapidly followed by production of both male and female flowers on the same vine. Both species exhibited prolific fruit production at all sites, until senescence occurred, at 150-180 days following establishment. Date of senescence varied among sites and species. Molecular genetic sequences analysis of chloroplast *(MatK)* and nuclear (*G3pdh*) genes was used to assay population genetic diversity and to verify species identity of melon species sampled from geographically diverse locations in Australia. Genetic variation within the species was not observed among the Australian populations at either of the assayed genes. This lack of genetic diversity may have resulted from a limited entry by each of the species into Australia and or sustained population bottlenecks following their entry. The absence of genetic diversity among Australian populations in both species provides some indication that future bio-control measures may be applicable across the invasive range of these species. Comparisons with sequences from overseas vouchers indentified the Australian specimens of prickly paddy and camel melon as *Cucumis myriocarpus* and *Citrullus lanatus* var. *citroides* respectively. The latter result is discordant with contemporary herbaria identifications which describe Australian camel melon as *Citrullus lanatus* var. *lanatus*.

**Keywords**: Cucurbitaceae, non-native, invasive weeds, sequence analysis, Phenology, genetic variation, molecular identification

## INTRODUCTION

Globally, invasive plants are a significant threat to biodiversity conservation (McNeely, 1997). Invasive plants are defined as non-native species that have successfully spread outside their native range and are associated with significant environmental and long-term

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sustainability issues (Richardson *et al.* 2000). Their impacts include loss of biodiversity and crop yield. Australia is currently dealing with impacts associated with invasion of over 200 significant weed invaders (www.weeds.org.au/WONS/).

There are five cucurbit species which were introduced to Australia as potential feed crops or as seed contaminants. Camel melon and prickly paddy melon are the most common (Martin *et al.* 2006), introduced into Australia in the mid 1800s, and are invasive weeds of crop plants and natural habitats (Cunningham *et al*. 1992, Parsons and Cuthbertson 2001). Although these melons are native to Africa, camel melon was most likely imported from its secondary centre of origin in India, Pakistan or Afghanistan, to use as camel fodder (Barker 1964, Parsons and Cuthbertson 2001). However, semi-arid regions of Australia have experienced serious invasive problems (Barker, 1964, PlantNET website 2011). Today, camel melon is widely distributed throughout Australia (Australian Virtual Herbarium, 2010, www.avh.rbg.vic.gov.au); globally, it is problematic in S. Europe and is a declared noxious weed in state of California, USA (Grubben 2004, USDA web site, 2011).

After introduction to Australia, both species were subsequently reported as weeds as early as 1900. In recent years they have become problem weeds in dryland cotton, sorghum, and wheat, fallow croplands, and naturalized settings (Borger and Madin 2005, Leys *et al.* 1990, Johnson *et al.* 2006). Both species are also considered to be potentially toxic to sheep and livestock (Cunningham *et al*. 1992, Parsons and Cuthbertson 2001, Mc Kenzie *et al.* 1988). In feeding trials, *Cucumis myriocarpus* fruit ingestion was reported to have caused death of cattle (Mc Kenzie *et al.* 1988). Cost of chemical control of these weeds can be high, with repeated herbicide application required for management (Rosskopf *et al.* 1999).

Genetic diversity and evolution of diversity can be important factors contributing to the success of any invasive species (Sexton *et al.* 2002, Prentis *et al*. 2008). Novel populations of introduced species often go through population bottle-necks (reductions in effective population size) during and after the introduction phase. The intensity, duration and frequency of these bottle-neck events can increase the effects of genetic drift in these populations and subsequently lead to loss of their genetic diversity. In some instances this may promote adaptations which facilitate establishment in a new environment (Prentis *et al* 2008).

Currently, little is known about the genetic diversity and identity of wild melons in Australia. A review of existing Australian references shows that authors have often confused identification and description of these two species. Globally, a detailed botanical description of *Cucumis myriocarpus* was given by Jeffrey (1975) and Kirkbride (1993), However they have been little studied in Australia, except in management trials (Felton et al. 1994, Leys et al. 1990).

Taxonomically, Jeffrey (1975) classified *Cucumis myriocarpus* into two subspecies, including *Cucumis myriocarpus* subsp. *myriocarpus* and *Cucumis myriocarpus* subsp. *leptodermis*. The subspecies are cross-compatible (Jeffrey 1975; Van Raamsdonk *et al.* 1989). These subspecies designations are used varyingly among Australian herbaria, with Queensland refering to specimens as subsp. *myriocarpus,* and NSW, ACT and VIC refering to them as subsp. *leptodermis* (ANHSIR extracts 2011). Other states do not use any sub-specific classifications. Our germplasm collection to date suggests this likely represents a single subspecies. Camel melon in Australia was described by Jacobs and Pickard (1981) as *C. lanatus* var. *lanatus*, however this sub-specific nomenclature is only used by herbaria in NSW and QLD. *C. l*. var. *lanatus* has cross compatibility with its relative, *Citrullus colocynthis* (Singh, 1978), another invasive melon naturalised across north Australia (Australian Virtual Herbarium).

By use of genetic sequence analysis, one can carefully assess variation and potential hybridisation among species and evaluate phylogeographic relationships (Dane *et al.* 2007; Dane and Lang 2004). This study uses sequence analysis to identify species and population genetic diversity in two invasive melon species, and examines their phenology in inland NSW. A sound understanding of the biology and taxonomy of camel melon and prickly paddy melon is required to assist managers in correctly identifying and managing these species.

## MATERIALS AND METHODS

Naturally occurring populations of camel and prickly paddy melon were identified in four locations within 50 km of Wagga Wagga NSW with red earth soils. Locations included: Charles Sturt University, equine paddock, Wagga campus; (ES), perennial pasture, Lake Albert, (LA); CSU horticulture irrigation site, Wagga (HA) and mixed farming site, Galore (GA). Table 1 presents information on individual experimental sites. The existing vegetation at all study sites included a variety of common broadleaf weeds and annual grasses, both native and introduced.

**Table 4.** Experimental location details

| Location | GPS coordinates | Soil type | Paddock history |
|---|---|---|---|
| **ES** | 35 4 15 S , 147 21 17E | Clay loam | Permanent grazed pasture |
| **LA** | 35 09 31 S, 147 22 33E | Red loam | Pasture followed by fallow |
| **HA** | 35 41 193 S, 139 20 35 E | Red loam | Cropping followed by fallow |
| **GA** | 34 59 45 S, 146 48 36 E | Sandy loam | Grazed pasture |

Phenological characteristics of camel melon and prickly paddy melon seedlings and plants were studied in these locations during the summer of 2011. In some sites, species range overlapped. Specifically, prickly paddy melon was studied at HA and LA sites and camel melon studied at ES and GA sites. At each site, species in question were monitored by tagging, with a minimum of 12 plants tagged per site per species.

In December-April 2011, seedlings less than 21 days of age were tagged and studied for growth and development. To prevent initial mortality, plants of both species were kept relatively weed free during their initial establishment up to 60 days after emergence (DAE) and growth analyses taken approximately every 15 days until plant senescence was noted. At each visit, data on seedling establishment, time to first flowering, and time to fruit set were noted, along with notable events including infestation by pests and pollinators. At maturity, in late March through April, six actively flowering and fruiting vines of prickly paddy melon were collected from LA and HA sites, and camel melon from ES and GA sites. Above ground plant dry weight was recorded after drying in a dehydrator at 85° C for 7 days. Leaf area was estimated with a LA meter using 5 leaves each from the terminal, middle and lower portion of each vine. Length of longest vine per sample was measured. Fruit number/vine and fruit weight were recorded. In April, 10 mature fruit were collected per each of five plants in the case of prickly melon at LA and HA, and one fruit from 10 plants, in the case of camel melon, from ES and GA locations. Sampled plants were separated by 10 m or more at each site. Seed number/fruit was obtained after fruit dissection.

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Additional germination data was collected in November 2010 on species establishment at each site. Date of first seedling emergence for each species was recorded using 1 $m^2$ grids placed randomly at locations at least 10 m apart and recording the number and approximate age of plants falling within 12 grids in each site. Data was collected on at least 5 dates in November and throughout the season as germination events occurred in association with rainfall patterns.

Genetic variation within Australian melon samples was assayed by sequencing two informative nuclear and chloroplast gene regions, previously shown as informative for detection of population genetic variation and species identification in curcurbits (Demesure *et al*. 1995; Dane *et al.* 2007). For these assays, fruit from 22 geographically distinct sites for camel melon and 16 sites for prickly paddy melon were collected across inland Australia including representative sites from NSW, VIC, SA and NT. Seed (1 seed/sample) was ground and prepared for DNA extraction, using two replicate samples per population. DNA was extracted using a Corbett 1820 extractor robot with associated extraction reagents (QIAGEN). Polymerase chain reaction (PCR) was used to amplify chloroplast *MatK* using primers 1R KIM (5' ACC CAG TCC ATC TGG AAA TCT TGG TTC) and 3F_KIM (5' CGT ACA GTA CTT TTG TGT TTA CGA G) (Ki-Joong Kim unpublished). An Intronic region within the nuclear *G3pdh* gene was targeted using primers *G3pdh* (5' CAG GCT AAT GGA AAG GGT TT) and *G3pdh*_R (5' TTG TAT CCT CCG CTT CC) (Demesure *et al*. 1995). Sample PCR's (15 µl) contained, 1.5 µl of 10X PCR Buffer, 0.9 µl of 50 µM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.3 µl each of 5 µM forward and reverse primers and 0.075 µl of Platinum® Taq DNA polymerase (Invitrogen Pty.), 9.675 µl of water and 2 µl genomic DNA. The PCR thermal profile used for both genes was: 94°C denaturing for 2 minutes, followed by 40 cycles of (94°C denaturing for 30 seconds, 52°C annealing for 30 seconds, 72°C extension for 60 seconds), 72°C extension for 7 minutes, 10°C hold. PCR's were checked for quality and size using a UV visualiser (Gel Doc Pty.) after electrophoresis through a 1.5% TAE agarose gel at 170V for 7 minutes. Quality PCR products were bi-directionally sequenced at the Australian Genomic Research Foundation (AGRF), Brisbane Australia. Sequences were aligned using SeqMan DNA star software and edited using Bioedit (Hall, 1999). Reference sequences of taxonomically identified voucher accessions were downloaded from the National Barcode of Life website (*www.ncbi.nlm.nih.gov/genbank/barcode.html*). Sample sequences were compared against references using neighbour-joining distance analysis and tree reconstruction as implemented in MEGA 4 (Tamura *et al.* 2007).

## RESULTS

As presented in Tables 2 and 3, seedling emergence of both melon species was noted from early November 2010 onwards. Time of emergence varied with species and location of study, from November 1 to 15, 2010. For both species, length of mature vines extended 2 to 3 m in length. Leaves of prickly paddy melon were smaller than those of camel melon. However, an average prickly melon produced greater leaf numbers/plant than camel melon of similar maturity. First fruit formation was observed in both species between 35 and 49 days after emergence, depending on site. Emergence was later at the Lake Albert (LA) site for prickly paddy melon. Camel melons matured several weeks earlier at the Galore site (GA). Prickly melon exhibited variation in growth and development depending on location. A comparison of both melon species with respect to total biomass production suggested that species produced similar total biomass under favourable growth conditions.

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Both prickly melon and camel melon were highly plastic with respect to their growth and potential to produce biomass at various study sites; this is reflected in terms of their capacity to produce viable fruit. We observed that camel melons produced a maximum of 14 fruits /plant, but an average plant typically produced 2-3 fruit /plant. Similarly, an average prickly melon produced upwards of 80 melons under favourable conditions, and up to 120 under optimal conditions. Camel melons typically produced between 300 to 400 seed /fruit, depending on location of production, while smaller prickly paddy melons produced between 40 to 45 seed /fruit. Total seed production/plant was greater for prickly paddy melon in comparison to camel melon at all study sites.

**Table 5.** Phenology and growth parameters assessed for camel melon in two locations (ES) and (GA) in 2010-2011.

| | **Growth parameter** | **(ES)** | **SD[1]** | **(GA)** | **SD[1]** |
|---|---|---|---|---|---|
| 1 | Time of first germination | Nov 15 | - | Nov 1 | - |
| 2 | Longest vine length (mm) | 1700 | 700 | 2850 | 900 |
| 3 | Leaf area /5 leaves ($cm^2$) | - | - | 394 | 61 |
| 4 | Duration to male flower(DAE) | 41.9 | 11.4 | 35 | 1.8 |
| 5 | Duration to female flower (DAE) | 56.7 | 14.6 | 41.7 | 1.9 |
| 6 | Duration to fruit (DAE) | 63.8 | 15.1 | 47.3 | 1.7 |
| 7 | Plant dry weight (g) | 89.1 | 18.3 | 207 | 167 |
| 8 | Fruit/plant | 2.5 | 1.4 | 2.58 | 2.19 |
| 9 | Seed /fruit | 281 | 101 | 390 | 87 |
| 10 | Dry wt. per seed (g) | 0.07 | 0.004 | 0.06 | 0.0006 |
| 11 | Male : female flower ratio | 3.1 | 3.5 | 2.6 | 2.0 |

[1] SD refers to standard deviation. SD was calculated generally for 12 plants per location.

**Table 6.** Phenology and growth parameters assessed for prickly paddy melon in two locations (HA) and (LA) in 2010-2011.

| | **Growth parameter** | **(HA)** | **SD[1]** | **(LA)** | **SD[1]** |
|---|---|---|---|---|---|
| 1 | Time of first germination | Nov 1 | - | Nov 15 | - |
| 2 | Longest vine length(mm) | 2400 | 700 | 1900 | 540 |
| 3 | Leaf area /10 leaves ($cm^2$) | 200 | 311 | 79 | 52 |
| 4 | Duration to male flower (DAE) | 33.7 | 5.2 | 39.4 | 4.3 |
| 5 | Duration to female flower (DAE) | 44.7 | 8.4 | 48.8 | 3.6 |
| 6 | Duration to fruit (DAE) | 51.6 | 9.1 | 52.3 | 2.2 |
| 7 | Plant dry weight (g) | 176 | 139 | 32 | 17 |
| 8 | Fruit /plant | 85 | 67 | 22 | 12 |
| 9 | Seed/fruit | 46 | 5 | 39 | 7 |
| 10 | Dry wt. per seed (g) | 0.020 | 0.001 | 0.022 | 0.001 |
| 11 | Male : female flower ratio | 5.9 | 3.3 | 4 | 2 |

[1] SD refers to standard deviation. SD was calculated generally for 12 plants per location.

We observed that *C. myriocarpus* exhibited a greater male/female flower ratio than camel melon. *C. myriocarpus* flowered more profusely and produced larger numbers of fruit as well. Prickly paddy melon produced an average of 80 fruits and 40 seed/fruit, while camel melon produced 3 fruit per plant, with seed number/fruit varying with location.

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Molecular studies revealed that the PCR products at *MatK* and *G3pdh* were successfully amplified from both melon species; PCR success at *MatK* was 77% and 100% at *G3pdh*. Sequence analysis at the assayed genes indicated an absence of intraspecific genetic variation in both species. *MatK* and *G3pdh* gene sequences obtained for prickly paddy melon samples showed 100% similarity to sequence accessions from *Cucumis myriocarpus* vouchers present overseas. *MatK* sequences obtained from Australian camel melons were 100% identical to those reported for overseas *C. colocynthis* accessions. *G3pdh* gene sequences for Australian camel melons were identical to a widespread *C. lanatus var citroides* genotype and differed from overseas *C. lanatus var lanatus* (cv. Crimson Sweet) and *C. colocynthis* sequence (PI- 432337) accessions by 1.34 and 1.84 %, respectively. Phylogenetic relationships at *G3pdh* reconstructed using neighbour-joining analysis indicated the Australian camel melon genotype was synonymous with *C. lanatus var citroide* accessions from Botswana, India, South Africa and the USA (Figure 1).

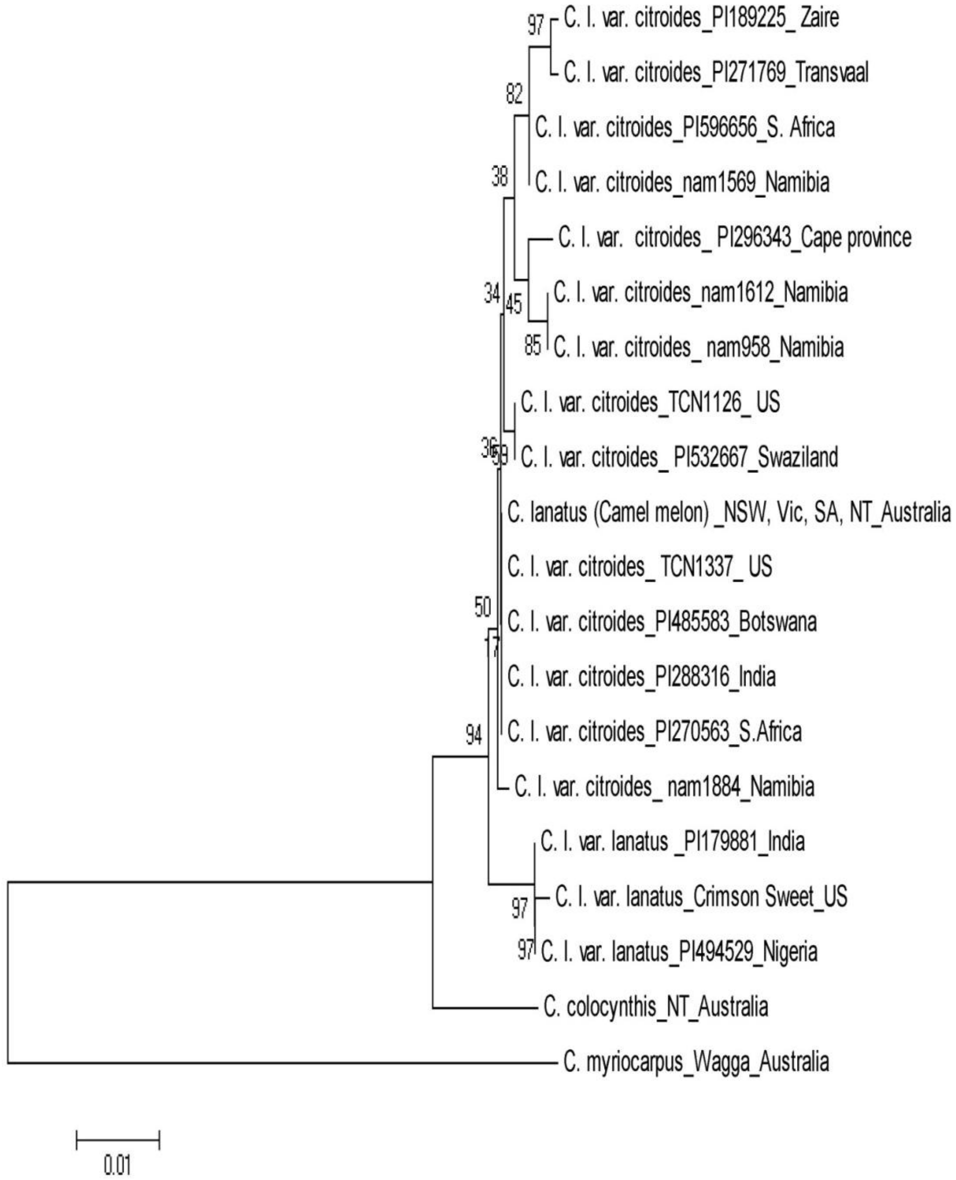


**Figure 1.** Neighbour joining tree showing genetic distance relationships among camel melon sampled from Australia and overseas melon accessions at the *G3pdh* intron -2 region. The values at nodes indicate the boot strap supports from 1000 replications. The scale bar equals one percent genetic difference.

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## DISCUSSION

Both prickly paddy and camel melon species share similarity in their growth and life history traits. They are annual vines with branches ranging from 2-4 m in length with angled, tendrillar stems. Camel and prickly paddy melon growth was initiated with germination when the paddocks received first significant rainfall, which occurred from mid November – mid January continuing until onset of low temperatures in mid April, at which time the plants senesced. Similar to Parsons and Cuthbertson (2001), we observed their monoecious growth habit, with both male and female flowers produced on the same vine. Male flowers arise in leaf axils, and female flowers are solitary in camel melon and in prickly paddy melon arise in clusters. Flowering was first noted at 5 to 7 weeks following germination, continuing until senescence. In most occasions, the male flowers were the first to open, followed by the female flowers.

Camel melon produced a larger plant with a much larger fruit than that of prickly melon, similar to Parsons and Cuthbertson (2001). We observed that prickly melon exhibited continuous staggered germination throughout the growing season. In contrast, camel melon germinated more sporadically with 3 to 4 seasonal flushes of germination observed. Camel melon evidently requires higher temperatures to flower and set fruit than prickly melon (Parsons and Cuthbertson 2001). Both melons exhibited greater male fitness, resulting in more numerous male flowers than female flowers. Protandry is a common feature of most cucurbits, with male/female flower ratio >1.

DNA sequencing was used to examine population genetic diversity and species identity of wild melon present in Australia. Our results indicated an absence of genetic diversity within and among populations of both paddy melon and camel melon; in both cases, only a single fixed genotype was observed among widespread sample locations. This contrasts with evidence of some genetic variation present within overseas melon populations at the assayed genes (Liu, 2005; Dane *et al*. 2007). It is plausible the lack of observable genetic diversity among Australian melon populations resulted from a single Australian incidence of introduction in both species. This is potentially supported by historical records that indicate the brief period of introduction of camel melon seed into Australia to provide fodder for introduced camels used for land transport (Barker 1964). Population bottlenecks following introduction to this country may also have been of sufficient size and duration to effectively eliminate all but the most common genotype available for both species. Regardless, the apparent absence of genetic diversity among Australian populations in both species provides some indication that future bio-remediation or bio-control measures may be applicable across the invasive range of these species.

Genetic analyses verified species identity of prickly paddy melon in Australia and provided evidence of a discrepancy in the nomenclature of Australian camel melon. Currently, camel melon is reported in Australia as *C. lanatus* var *lanatus* (ANHSIR extracts 2011, Leys *et al.,* 1990, *plantnet.rbgsyd.nsw.gov.au, 2011*). However, sequence analysis at *G3pdh* indicated our Australian camel melon populations are synonymous with overseas citron melons identified by authorities as *C. lanatus* var *citroides* (Liu 2005); we also observed Australian camel melon sequences differed from the overseas accessions of *C. lanatus* var *lanatus*. Additional sequencing studies with other variable gene loci and overseas voucher specimens are now underway to investigate this important taxonomic finding.

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## REFERENCES

ANHSIR extracts (2011). Australian National Herbarium, Canberra. Personal communication.

Barker, H.M. (1964). *Camels and the Outback*. London: Angus & Robertson Ltd. Retrieved from the internet on 16- 6-2011.
http://www.biology.iastate.edu/InternationalTrips/1Australia/05PAPERS/Camels.htm

Borger, J. and Madin, R. (2005). Paddy *melon and Afghan melon*. Farmnote No. 91/93 [Replaces 22/88]/2005, Department of Agriculture. Western Australia.

Cunningham, G., Mulham, W.E., Milthorpe P.L. and Leigh. J.H. (1992). *Plants of Western New Southwales.* Inkata Press, Melbourne.

Dane, F., Liu. J., and Zhang, C. (2007). Phylogeography of bitter apple, *Citrullus colocynthis*. *Gen. Res. and Crop Evol..* 54: 327- 336.

Dane, F. and Lang, P. (2004). Comparative analysis of *cp* DNA variability in wild and cultivated *Citrullus* species: implications for evolution of watermelon. *Am. J. of Bot*.91: 1922–1929.

Demesure, B., Sozi, B.N. and Petit, R.J. (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Eco.* 4: 129–131.

Felton, W. L., Wicks, G. A. &Welsby, S. M. *(*1994). A survey of fallow practices and weed floras in wheat stubble and grain sorghum in northern New South Wales. *Australian Journal of Experimental Agriculture 34(2),* 229 - 36.

Grubben, G.J.H. (2004). *Plant resources of Tropical Africa. Vegetables*. PROTA Publication, Wageningen, Netherlands. Pp. 252.

Hall, T. A. (1999). *BioEdit: a user-friendly biological sequence alignment editor and analysis*. http://www.mbio.ncsu.edu/BioEdit/bioedit.html

Jacobs, S. W. L and Pickard, J. (1981). *Pl. New S. Wales* 108.

Jeffrey, C. (1975). *Further notes on Cucurbitaceae: II*I. Some southern African taxa. *Kew Bull.* 30: 475-493.

Johnson, A., Dellow, J., and Pengilley, K. (2006). Central west weed survey, efficient weed control in central west NSW, Appendix one. DPI, NSW Pp 92-99. http//www.dpi.nsw.gov.au/agriculture/pests-weeds/weeds/publications/management/central-west

Kirkbride, J.H. (1993). *Biosystematic monograph of the genus Cucumis (Cucurbitaceae);*

*botanical identification of cucumbers.* Parkway publishers. Pp1-35.

Leys, A. R., Amor, R.L., Barnett, A. G. & Plater, B. (1990) Evaluation of herbicides for control of summer- growing weeds on fallows in south eastern Australia. *Aus. J.of Exp.Agri.* 30: 274- 279.

Liu, J. (2005). *Phylogeny and biogeography of water melon (Citrullus lanatus (Thunb.) Matsum. & Nakai) based on chloroplast, nuclear sequence and AFLP molecular marker data.* M Sc. Thesis, Auburn University, Alabama. Retrived from the internet on 16-6-2011. (http://etd.auburn.edu/etd/bitstream/handle/10415/703/LIU_JIARONG_35.pdf?sequence=)

Martin,T. G., Campbell, S. and Ground, S. (2006). Weeds of Australian rangelands. *The Rangeland J.* 28: 3- 26.

McNeely, J. (1997). IUCN (International Union for conservation of Nature and Natural Resources) alien invasive species: a framework for action. In: Conserving Vitality and Diversity: *Proceedings of the World Conservation Congress Workshop on Alien Invasive Species* (eds C.D.A. Rubec & G.O. Lee). IUCN and Environment Canada, Ottowa, pp. 3-9.

McKenzie, R.A., Newman, R.D., Rayner, A.C. and Dunster, P.J. (1988). Prickly Paddy melon (*Cucumis myriocarpus*) poisoning of cattle. *Aus. Vet. J.* 65(6): 167-70.

Parsons, W. T. and Cuthbertson, W. G. (2001). *Noxious weeds of Australia*. CSIRO publishing Victoria, 405 – 411.

Prentis, P., Dormontt, E., Wilson, A., Richardson, D. And Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Pl. Sci*. 13:288-294.

Richardson D. M., Pysek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D. & West, C. J. (2000). Naturalisation and invasion of alien plants: Concepts and definitions. *Div. and Distbs* 6: 93-107.

Rosskopf, E. N.; Charudattan, R. and Kadir, J.B. (1999). Use of plant pathogens in weed control. In Handbook of biological control: principles and applications of biological control, By T. S. Bellows, T. W. Fisher, Elsevier Science & Technology publishers. Pg. 891.

Sexton, J. P. McKay, J. K.and Sala, A. (2002). Plasticity and genetic diversity may allow salt cedar to invade cold climates in North America*.* *Ecol App*. 12: 1652–1660. [doi:10.1890/1051-0761(2002)012[1652:PAGDMA]2.0.CO;2]

Singh, A.K. (1978). Cytogenetics of semi-arid plants. III. A natural interspecific hybrid of Cucurbitaceae (*Citrullus colocynthis* Schrad x *C. vulgaris* Schrad). *Cytologia* 43: 569-576.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Bio. and Evol.* 24: 1596-1599.

Van Raamsdonk, L.W. D., Den Nijs, A. P. M., and Jongerius, M.C. (1989). Meiotic analyses of *Cucumis* hybrids and an evolutionary evaluation of the genus *Cucumis* (Cucurbitaceae). *Pl. Sys. and Evol.* 163: 133-146.

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# MOLECULAR MARKER TOOLS FOR THE IDENTIFICATION OF WEEDY *SPOROBOLUS* SPECIES IN AUSTRALIA

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## Abstract

Nine species of *Sporobolus* having overlapping morphological characteristics have been included in '*Sporobolus indicus* complex'. Five of these nine species are noxious weeds in various states of Australia. These species are major constraints to pasture production causing significant losses in the dairy and beef industries. In this study, a DNA-based molecular investigation was undertaken to help develop improved integrated management strategies for these noxious weeds. This study employed 40 *Sporobolus* seed collections coming from 14 species. Polymerase Chain Reaction (PCR) based Random Amplified Polymorphic DNA (RAPD) analysis generated a series of species-specific molecular markers that can be employed for the identification purpose. However, as the technique was highly sensitive to PCR reaction and cycling conditions, an alternative molecular approach, involving DNA sequencing of Internal Transcribed Spacers (ITS) of nuclear Ribosomal DNA (rDNA) repeat units was employed to develop a diagnostic tool for these species. The rDNA ITS sequencing approach could also be used for a phylogenetic study which revealed clear cut boundary between the weedy and the non-weedy species within the '*S. indicus* complex'. In addition to the molecular phylogenetic study, PCR-Restriction Fragment Length Polymorphism (RFLP) approach was used to develop a diagnostic tool for the weedy species. Using this approach, single restriction enzyme (*Mvn*I) was identified which could discriminate all five noxious weedy species from all others. The molecular genetic and phylogenetic information thus generated and the molecular diagnostic tool thus developed from this study can be effectively utilized for the integrated management of these noxious weeds in Australia.

**Keywords:** Internal Transcribed Spacers (ITS), Random Amplified Polymorphic DNA (RAPD), Polymerase Chain Reaction (PCR), giant rats tail grasses, molecular diagnostics

## INTRODUCTION

Weedy *Sporobolus* grasses (WSG) are the members of the '*Sporobolus indicus* complex', a cluster of nine closely related species that are to be found in Australia (Simon, 1999) and with a pantropical distribution around the globe. Five of these species [*viz*. giant rats tail grasses (GRTGs) [*S. pyramidalis* P. Beauv and *S. natalensis* (Steud.) Dur. & Schinz.] Giant Parramatta grass (GPG) [*S. fertilis* (Steud.) Clayton], Parramatta grass (PG) [*S. africanus* (Poir.) Robyns & Tourney] and American Rat's tail grass (ARTG) [*S. jacquemontii* Kunth] are considered serious weeds of pastures in many of the locations (Ensby and Betts 1998; Simon and Jacobs 1999; DPIF 2007). These five species are collectively known as the WSG (DPIF 2007).

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WSGs are now reported to infest an estimated 450,000 ha of grazing land in eastern Queensland and New South Wales. They grow in lower rainfall areas (600 mm annual rainfall areas), but are most prevalent in areas with an average annual rainfall of > 700 mm (DPIF 2007). WSG are adapted to a wide range of Australian soil types and climatic conditions and have a potential to establish in areas receiving as little as 500 mm of annual rainfall. This makes over 60 % of Queensland (108 million ha), or 30 % of Australia (223 million ha) at risk to infestation.

Seeds of the WSG can be set throughout the year but most are produced during the warmer summer season. Seed production is high with up to 85 000 seeds being produced per $m^2$ in case of *S. pyramidalis* (Vogler and Bahnisch, 2002). Most seeds that enter the soil can remain viable for up to 10 years. A soil seed bank quickly develops and sizes of up to 20,000 seeds per $m^2$ can be present. GPG is also capable of producing up to 85,000 seeds per $m^2$ each year with an initial seed viability of 90%. It has been reported that a significant proportion of its seed can remain viable for up to 10 years in the soil (Queensland Government 2011).

WSGs have been major threats to the dairy and beef industries as their infestations have dramatically reduced the economic viability of pastures leading to a lowering of land values and it has been estimated that current infestations are collectively costing the pastoral industry c. $60 million per year in terms of lost production and control costs (DPIF 2007). They become dominant grasses in many sown and native pastures and pose a serious threat to the viability of many rural industries. GPG is reported to cause losses in terms of carrying capacity and decreased production by as much as 80% (Queensland Government 2011).

WSGs being invasive weeds and have the potential to spread to larger areas of Australia via different means, and therefore an integrated weed management strategy should be developed for them. So far, several research studies, focusing on biology and mode of transmission, have been carried out on various weedy *Sporobolus* species (Andrews 1995; Vogler and Bahnisch 2002; Vogler and Bahnisch 2006) and a number of weed management strategies have been devised for their management and include the use of chemical control (Andrews 2009), mechanical and biological control (Yobo *et al.* 2009; Queensland Government 2011).

The present study aims to understand the genetic and phylogenetic relationships of the various members of the '*S. indicus* complex' and develop a robust molecular marker technique for the reliable identification of five major weedy *Sporobolus* species of Australia. Three different molecular marker techniques *viz*. 1) Polymerase Chain Reaction (PCR)-based Random Amplified Polymorphic DNA (RAPD) 2) PCR and DNA sequencing-based on Nuclear Ribosomal DNA (rDNA) Internal Transcribed Spacers (ITS) and 3) PCR-Restriction Fragment Length Polymorphism (RFLP) were used to achieve the overall objective.

## MATERIALS AND METHODS

### Plant Materials

Altogether 56 seed samples coming from 14 *Sporobolus* species were used for the entire study (Shrestha *et al*. 2003; Shrestha *et al*. 2005).

### DNA Extraction

DNA extraction was carried out from seed tissue (10-50 mg of the caryopsis) using the technique described by Edwards *et al*. (1991). DNA estimation of the various samples was carried out using a spectrophotometric method (GeneQuant II, RNA/ DNA calculator, Amarsham Pharmacia, Biotech, Australia).

### Random Amplified Polymorphic DNA (Rapd) Based Study

A Polymerase Chain Reaction (PCR)-based RAPD technique (Williams *et al*. 1990) was used for the generation of RAPD markers specific to the five major weedy species and for the study of inter and intra specific genetic diversity among the various *Sporobolus* species under study. The optimized RAPD-PCR reaction and cycling conditions used for the *Sporobolus* RAPD profiling and RAPD data analysis are those described in Shrestha *et al*. (2005).

### Phylogenetic Study Based On Nuclear rDNA ITS Sequences

Of the total 56 *Sporobolus* samples (from 14 species), 40 samples were used for ITS region sequencing. Two other species of Poaceae [*Heteropogon contortus* (L.) P. Beauv. Ex Roem & Schult and *Pennisetum alopecuroides* (L.) Spreng] were also sequenced as out group species for phylogenetic analysis.

For this phylogenetic study, same DNA samples extracted for the RAPD investigation were used. The PCR amplification of the entire ITS region, DNA sequencing, sequence analysis and phylogenetic analysis were carried out as mentioned in Shrestha *et al*. (2003).

### Diagnostic Development Using PCR-RFLP-Based Marker Technique

The consensus sequences generated for the phylogenetic analysis were subjected to web cutter program (Web cutter program 2000; http://www.firstmarket.com/cutter/cut2.html). Of the five restriction enzymes identified (*Acc*II, *Bst*UI, *Bsh*1236I, *Mvn*I and *Tha*I.) from the restriction maps that could be used for the diagnostic purpose, *Mvn*I was finally selected and used in restriction analysis involving all 14 species of *Sporobous* under study. Validation of the PCR-RFLP assay was carried out on 23 randomly and blindly selected *Sporobolus* seed samples (Shrestha *et al*. 2010)

## RESULTS

### Identification Of Weedy Species-Specific RAPD Markers

The important identification RAPD markers discovered were those of UBC $51_{490}$ for *S. pyramidalis* and *S. natalensis*, UBC $43_{310}$, UBC$43_{2100}$ and UBC$43_{2000}$ for *S. fertilis* and *S. africanus* and UBC $43_{470}$ and OPA$20_{850}$ for *S. jacquemontii*. More importantly, the DNA fingerprint profile generated by a single primer UBC43 could also be used to positively identify all five major weedy *Sporobolus* species found in Australia (Shrestha *et al.* 2005).

### Genetic Diversity and Relationship Based On RAPD Data

The RAPD similarity matrix generated using (the qualitative data) NTSYS pc program revealed the extent of the inter and intra-specific genetic diversity to be found among the various *Sporobolus* species under study (Shrestha *et al*. 2005).
In the phenograms generated from the Dice and Jaccard's coefficients, all the species of the '*S. indicus* complex' were found to group into one major cluster and the three

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Australian native species studied formed a second and distinct cluster (Shrestha *et al*. 2005).

## ITS sequence-based Molecular phylogeny of '*S. indicus* complex'

A strict consensus tree of 64 Maximally Parsimonious Trees (MPTs) separated the various species under study into two major clades, one clade comprising of the species of '*S. indicus* complex' (11 species) and another one comprising the three native species. Separation of species into these two major clades was supported by an absolute level of confidence (100 % bootstrap values). Weedy and the non-weedy species of the complex formed different clades in the cladogram (Figure1.) (Shrestha *et al*. 2003).

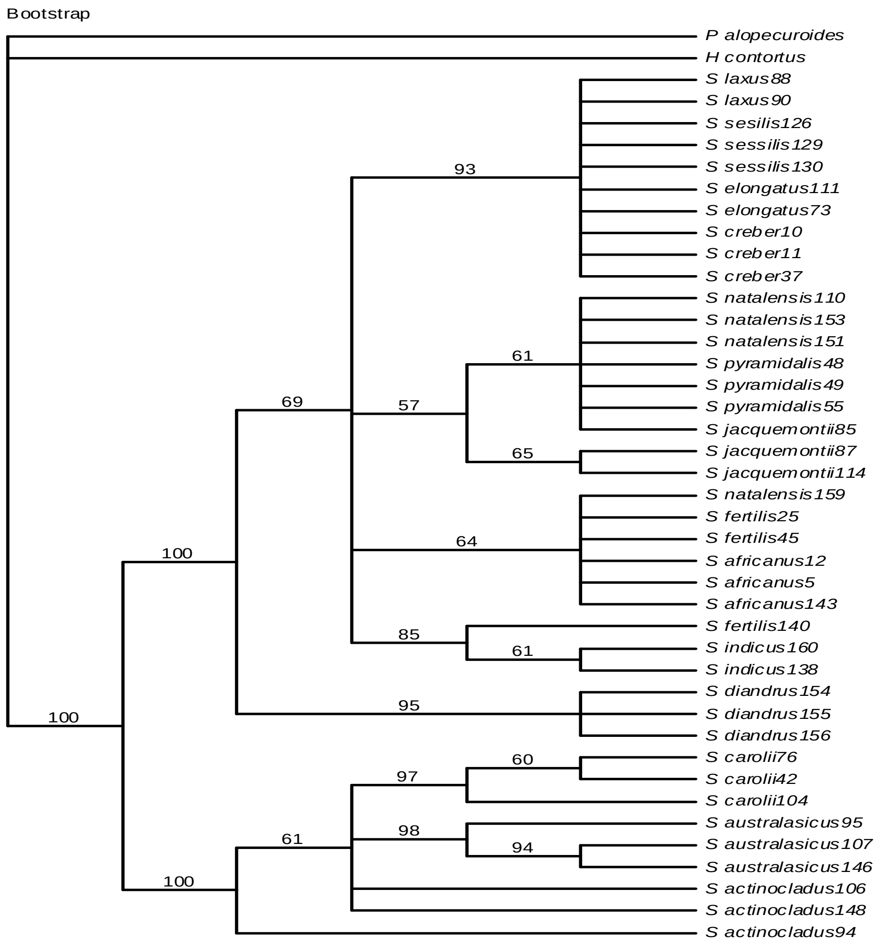


**Figure 1.** Bootstrap consensus tree generated from maximum parsimony analysis using PAUP. Bootstrapping performed with 1000 replicates. Values above nodes indicate bootstrap values.

## Molecular Diagnostic Development Based on ITS-RFLP

The ITS sequences generated for the phylogenetic study were successfully utilized for the development of a molecular tool diagnostic based on an ITS PCR-RFLP approach (Shrestha *et al*. 2010). Single restriction enzyme *Mvn*I could discriminate between the

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weedy and the non-weedy species of the complex (Plate 1). When tested on 23 randomly selected seed samples, identification was accomplished with 100 % accuracy.

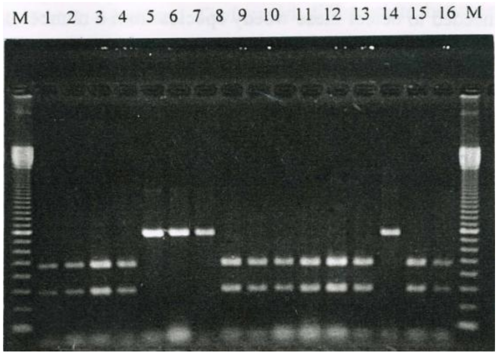


**Plate 1.** The restriction digestion pattern of the ITS fragments of 16 blindly selected samples with *Mvn*I. The digested samples were those that were members of the major weedy species. Lanes marked M, are the 100 bp ladder molecular weight markers. Note that the ITS fragment of the non-weedy species are not restricted.

## DISCUSSION

When naturally occurring protein-based and DNA-based molecular markers are interpreted as genealogical markers, they offer extraordinary power to illuminate such topics as human forensics, disease diagnostics, species diagnostics, wild life forensics, reproductive modes, population structure, intra-specific phylogeography, phylogeny, taxonomy, systematic, conservation biology etc. (Jagouix *et al*. 1994; Hocquellet *et al*. 1999; Ortiz-Diaz and Culham 2000; Li *et al*. 2002; Shrestha *et al*. 2003; Avise 2004; Shrestha *et al*. 2005; Yashuda *et al*. 2007; Shrestha *et al*. 2010).

In the present investigation, three molecular marker approaches have been employed in order to understand the genetic diversity and phylogenetic relationship pertinent to weedy *Sporobolus* species of Australia as well as to identify the five noxious weedy species from all natives. Our RAPD-based study successfully generated five weedy species-specific markers as well as DNA fingerprint profiles that can be used for a similar diagnostic purpose (Shrestha 2002; Shrestha *et al*. 2005). Fingerprint profile generated by a single primer UBC 43 could discriminate all five noxious weedy *Sporobolus* species.

Genetic diversity studies at the molecular level have great implications for formulating weed management strategies (Lopez-Martinez *et al*. 1999). The present investigation has put forth the inter and intra specific genetic diversity estimates and relationship among nine species of the complex based on RAPD qualitative data. Although carefully optimized RAPD technique have been used widely in various biological disciplines for genetic diversity studies, this technique is very sensitive to the PCR reaction and cycling conditions, and therefore its reproducibility has to be checked across laboratories using different PCR machines and reagents.

The ITS phylogenetic study revealed the '*S. indicus* complex' to be a monophyletic group, which is consistent with the study of Ortiz-Diaz and Culham (2000). The way in which species of the complex cluster, producing a monophyletic group and then sub-divide into various sub-clades is similar to that seen in the RAPD phenograms using RAPD data in which several species can be better resolved (Shrestha *et al*. 2003).

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Our ITS sequence based phylogenetic study has not only generated the inherent phylogenetic relationship within the '*S. indicus* complex' but has also resulted into a robust molecular diagnostic tool that can be employed for the positive identification of the five major WSGs. Whenever it is felt that these weeds are escaping identification (whether based on gross morphology of plants or based on seed morphology) and this has resulted in the rapid spread of these weeds in Australia, the molecular diagnostic tool developed can now be used to help prevent this.

## ACKNOWLEDGEMENTS

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## REFERENCES

Andrews, T.S. (1995). Dispersal of seeds of giant *Sporobolus* spp. After ingestion by grazing cattle. *Australian Journal of Experimental Agriculture* 35: 353-356.

Andrews, T.S. (2009). Preventing giant Parramatta grass [*Sporobolus fertilis* (Steud.) Clayton] reinfestation with reduced rates of fluropanate herbicide. *Plant Protection Quarterly* 24(3):118-119.

Avise, J.C. (2004). Molecular markers natural history and evolution. Second Edition. Sinauer Associates, Inc. Publishers Sunderland, Massachusetts.

DPIF (2007). Weedy *Sporobolus* grasses. Best Practice Manual. The State of Queensland, Department of Primary Industries and Fisheries.

Edwards, K., Johnstone, C. and Thompson, C. (1991). A simple and rapid method for preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research*, 19: 1349.

Ensby, R. and Betts, J. (1998). Giant Rat's Tail Grass identification and control. Agnote. NSW Agriculture publication, NSW.

Hocquellet, A., Toorawa, P., Bove, J.M. and Garnier, M. (1999). Detection and identification of the two *Candidatus Liberibacter* species associated with citrus Huanglongbing by PCR amplification of ribosomal protein genes of the $\beta$ operon. *Molecular and Cellular Probes* 13: 373-379.

Jagoueix, S., Bove, J.M. and Garnier, M. (1994). The phloem-limited bacterium of greening disease if citrus is a member of the α subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology* 44 (3): 379-386.

Li, X., Ding, X., Chu, B., Ding, G., Gu, S., Qian, L., Wang, Y. and Zhou, Q. (2007). Molecular authentication of *Alisma orientale* by PCR-RFLP and ARMS. *Planta Medica* 73(1): 67-70.

Lopez-Martinez, N., Salva, A.P., Finch, R.P. and de Prado, R. (1999). Molecular markers indicate intraspecific variation in the control of *Echonocloa* spp. with quinclorac. *Weed Science* 47:310-315.

Ortiz-Diaz, J. And Culham, A. (2000). Phylogenetic relationship of the genus *Sporobolus* (Poaceae: Eragrostideae) based upon nrDNA ITS sequences. In Jacobs, S. W. L. and Everett, J. (Eds.). *Grass Systematic and Evolution*. Proceedings of Third International Symposium on Grass Systematics and Evolution, 1998. CSIRO Publishing, Sydney, NSW. pp. 184-188.

Queensland Government (2011). American rat's tail grass. QLD government primary industries and Fisheries. http://www.dpi.qld.gov.au.

Queensland Government (2011). Giant Parramatta grass. QLD government primary industries and Fisheries. http://www.dpi.qld.gov.au

Shrestha, S. (2002). Molecular systematics of weedy *Sporobolus* species of Australia. Thesis submitted for the degree of Doctor of Philosophy (Ph. D.) in the School of Land and Food Sciences, University of Queensland, Australia.

Shrestha, S., Adkins, S.W., Graham, G.C. and Loch, D.S. (2003). Phylogeny of the '*Sporobolus indicus* complex' based on Internal Transcribed Spacers (ITS) sequences. *Australian Systematic Botany* 16: 165-176.

Shrestha, S., Adkins, S.W., Graham, G.C. and Loch, D.S. (2005). An identification tool for the Australian weedy *Sporobolus* species based on random amplified polymorphic DNA (RAPD) profiles. *Australian Journal of Agricultural Research* 56(2): 157-167.

Shrestha, S., Graham, G.C., Loch, D.S. and Adkins, S.W. (2010). Molecular Identification of weedy *Sporobolus* species by PCR-RFLP. *Weed Research* 50:383-387

Simon B.K. (1999). Taxonomy of the '*Sporobolus indicus* complex'. In: Proceedings of the Workshop 'Weedy *Sporobolus* Grasses, Research Update'. Gympie, 19 th November, 1999. pp. 41-47. Published by Queensland Department of Primary Industries (QDPI).

Simon, B.K. and Jacobs, S.W.L. (1999). Revision of the genus *Sporobolus* (Poaceae, Chloridoideae) in Australia. *Australian Systematic Botany*, 12: 375-448.

Vogler, W.D and Bahnisch, L.M (2002). The seed ecology of giant rats tail grass: implication for management. In 'Proceedings of the 7th Queensland weed symposium'. (The Weed Society of Queensland: Brisbane).

Vogler, W. D. and Bahnisch, L. M. (2006). Effect of growing site, moisture stress, and seed size on viability and dormancy of *Sporobolus pyramidalis* (giant rats tail grass) seed. *Australian Journal of Experimental Agriculture* 46: 1473-1479.

Webcutter program (2000). http://www.firstmarket.com/cutter/cut2.html

Williams, J.G.K, Kubelic, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA Polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531-6535.

Yasuda, K., Yano, A., Nakayama, Y and Yamaguchi, H. (2002). Molecular identification of *Echinochloa oryzicola* Vasing. and *E. crus-galli* (L.) Beauv. using a polymerase chain reaction-restriction fragment length polymorphism technique. *Weed Biology and Management* 2: 11-17.

Yobo, K.S., Liang, M.D., Palmer, W.A. and Shivas, R.G. (2009). Evaluation of *Ustilago sporoboli-indici* as a classical bio-control agent for invasive *Sporobolus* grasses in Australia. *Biological control* 50:7-12.

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# BIOSECURITY PREPAREDNESS FOR QUEENSLAND'S PRIMARY INDUSTRIES, PUBLIC AMENITY AND ENVIRONMENT

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**Abstract (Summary)**

Biosecurity Queensland (BQ), a service of the Queensland Department of Employment, Economic Development and Innovation (DEEDI) has an Emergency Management Unit (EMU) focused on optimising BQ's preparedness for emergency and incident response. EMU aims to review, improve and develop systems to ensure BQ has appropriate emergency response capability across single or multiple responses for emergency animal diseases, plant pests or other invasive species in Queensland. Preparedness encompasses ensuring DEEDI staff are aware of their commitments in an emergency response, based on sustaining a relevant structure, focused skill enhancement, policy development, use of appropriate technologies and remaining contemporary in emergency management practices. The EMU provides specialist consultancy advice to BQ management, its three programs and external stakeholders regarding tactical procedures to respond effectively to incursions.

Nationally, EMU develops and maintains incident and emergency management systems in conjunction with the Australian Department of Agriculture, Fisheries and Forestry (DAFF), Animal Health Australia, Plant Health Australia and the national institutional committees. EMU officers establish, enhance and maintain effective relationships within DEEDI, other State and Commonwealth government departments, non-government agencies and industry. EMU manages emergency preparedness training and exercises at regional and state levels while contributing to national emergency preparedness through national committee support (e.g. the Biosecurity Emergency Preparedness Working Group).

The four phases of an emergency response (investigation, alert, operations and stand-down) have different emphases in regard to policy reference. Pivotal reference documents for an emergency pest plant response include the National Environmental Biosecurity Response Agreement (NEBRA) and the National Weed Incursion Plan. The Queensland Biosecurity Emergency Operations Manual and specifically tailored standard operating procedures are managed within a document quality management system providing essential and quickly accessed guidance for an emergency plant response.

**Keywords:** Biosecurity, Emergency, Preparedness, Response, Weed, Incursion.

## Introduction and History

On 12 December 2006, BQ became a business group of the Department of Primary Industries and Fisheries (DPI&F). The mandate was to build a quality unit within the Department to focus on innovative ways to manage escalating biosecurity risks.

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Contemporary risk assessment for exotic weed species such as Heartleaf hempvine (*Mikania cordata*) and Spiked pepper (*Piper aduncum*) are high priority as well as plant and animal disease, but not disease in human health unless vector cross-transmission is proven. The Biosecurity Queensland Control Centre (BQCC) has been established to manage the continuum from incident and emergency preparedness, through field level incident and emergency response, to long-term eradication program projects. The EMU forms a functional arm of the BQCC that is charged with ensuring BQ's preparedness for emergency and incident response. EMU operates within the BQCC to set down systematic unified processes that coordinate all aspects of incident and emergency preparedness that have statewide application and fall within the BQ portfolio e.g. developing a process for handling a report of a suspect exotic species (Figure 5).

An effective emergency response requires skilled specialists to establish and maintain response policy and operations. A first response unit (FRU) is seen as an integral part of effective and efficient emergency response. The FRU is intended to be a specialist, trained group of staff drawn from across Queensland that can deploy quickly to anywhere within the state to establish a functioning, structured, organised and operational control centre using the system(s) adopted or developed by the EMU (e.g. setting a standard functional structure for a Local Pest Control Centre). These systems will be compatible with or link to systems across all emergency operations centres. Once the emergency operations centre is established and functioning, the FRU withdraws their function and commences handover to other responders, to ensure continuity of the response. They will, as required, re-enter and exit the response at appropriate intervals (Cozens 2008).

The Chief Biosecurity Officer (CBO) will decide if an emergency or incident exists. Upon declaration of an emergency, responders are tasked with completing activities guided by processes outlined in the Queensland Biosecurity Emergency Operational Manual (BEOM). The main elements are command, coordination and control with foundation laid in military process ((MPAT) 2009) and expanded in the Australasian Inter-service Incident Management System (AIIMS) that provides a system for calculating the size of a response by determining the level of response required (Australian Fire Authority Council 2004). If an emergency response is declared, the FRU will be activated by the relevant General Manager (GM)/Director, after consultation with affected GM's, Directors, CBO and/or Managing Director. This same trigger also automatically activates supporting elements for the FRU such as transition to emergency response structure and management, emergency HR conditions including role based email. Should other states/territories be at risk of incursion from a high risk exotic plant, cost sharing funding guidelines are provided in the National Environmental Biosecurity Response Agreement (Department of Agriculture Fisheries and Forestry 2011). FRU officers receive necessary inductions by regional staff and may work alongside regional staff, NRM groups and local government to assist in staffing the emergency operations centre. Authorised FRU officers use approved plans to utilize systems/tools developed or adopted by the EMU to commence operations.

## Methods Incorporated in Operational Plans

The core work of the EMU is centred on preparedness applicable to all biosecurity incidents and emergencies that fall within the BQ portfolio. Other business groups, such as Fisheries are incorporated into BQ emergency management arrangements. EMU activities focus on providing outputs that have principally a state-wide application ranging from policy development, coordinating activities to developing and delivering activities such as training. The EMU may take responsibility for regional needs where it is seen that the

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activity has a multi-region or state-wide application. Where a preparedness activity is specific to one region only or is species specific as determined by risk assessment, it may fall outside of the EMU responsibility and remain a regional program responsibility. An operational plan for the EMU assists in defining work activities and differentiation from

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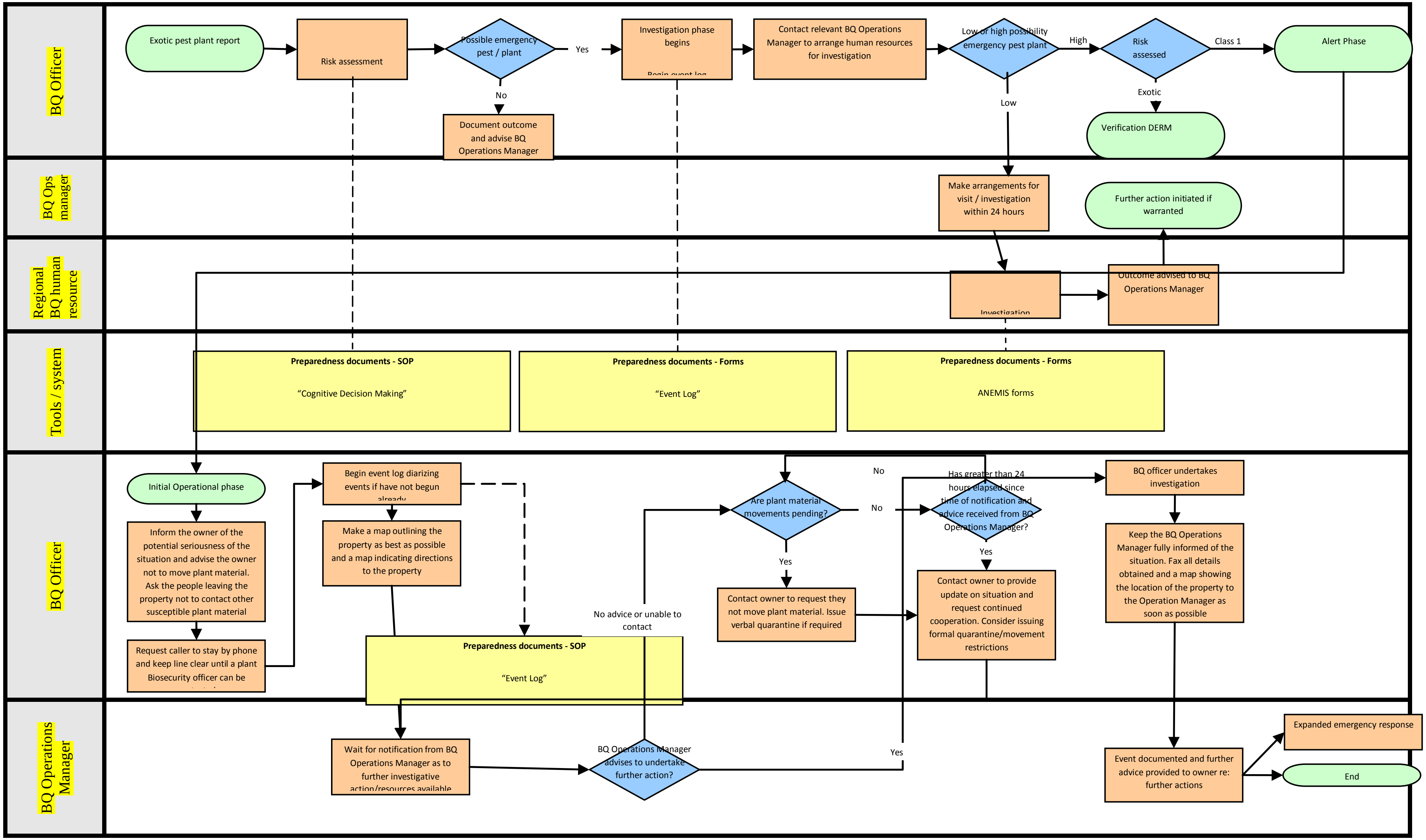


**Figure 5.** Report, investigation, alert and initial operations phases following notification of a suspect exotic pest plant. Oval shapes are start/finish/connection of process; Rectangle shapes are Processes; Diamonds shapes are decision points.

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regional program activities. Regional management, operations management and the EMU will work cooperatively to ensure incident and emergency preparedness operations are resourced and tasked appropriately to ensure delivery of desired outputs.

Individual programs and sub-programs will have defined activities and priorities for lower priority incident preparedness relevant to their business area. They are taken into account when developing the EMU operational plan and continual development of the BEOM. The Manager EMU negotiates EMU activities with the various Program areas ensuring that activities complement the needs of all Program areas.

The EMU will coordinate preparedness at all levels ensuring that readiness is optimised and that systems being used or developed for an EOC (Emergency Operations Centre) are applicable and consistent with other EOC's. Strategy, policy development, an understanding of emergency management principles, command and control and effective communications and reporting are key components addressed at the State Control Head Quarters (SCHQ) level through to Local Pest Control Centre's (LPCC's) (Cozens 2008).

The activities of national institutional committees provide an improved working framework for EMU. The Australian Government provides national policy leadership and direction, working with state and territory governments through the Natural Resource Management Ministerial Council. The focus of work in developing Intergovernmental Agreement on Biosecurity has enhanced opportunities for information sharing for preparedness and response (Hinder 2010). Government Departments have membership on the Australian Weed Committee that has major input into the Australian Weed strategy and indirectly into the Queensland Biosecurity Strategy 2009–14.

The National Biosecurity Management Consultative Committee (NBMCC) upon emergency declaration (as occurred for *Nassella tenuissima*), advises the National Biosecurity Management Group, the peak national decision-making forum, on various matters concerning the outbreak and any resulting national biosecurity incident response, and effectively coordinates the technical aspects of national biosecurity incident responses. The National Biosecurity Committee develops policy that may influence direction for the NBMCC. Moreover, Exotic Plant incursions that are limited or more easily controlled (e.g. *Stevia ovata*) are handled through The Consultative Committee on Exotic Plant Incursions that reports through the Australian Weed Committee to the Natural Resource Policies and Programs Committee (NRPPC) that is the equivalent of NBMG on less urgent incursion matters.

**National Weed Incursion Plan (NWIP)**

The NWIP is based on current national response plans such as PLANTPLAN and AUSVETPLAN. The key objectives are to prevent the introduction and establishment of new weed incursions into Australia on public and private land. Support for other agencies involved in Biosecurity is prescribed. A system of surveillance is operational to enhance the reporting of new weed incursions in Australia. Comprehensive risk assessment of exotic weed species to determine the appropriate type of response to each category of new weed incursion and the potential impact on agricultural, environmental and social values of each incursion of high-risk weeds is stipulated. EMU and BQ programs are evaluating, recording and reporting on the type and number of new responses to high-risk incursions and the effectiveness of on-ground management and eradication programs using After Action Reviews and Response Implementation Plans (Morton J. 2008).

**Queensland Pest Plant Emergency Responses**

Siam weed

The National Siam Weed Eradication Program commenced in 1995 to eradicate Siam weed (*Chromolaena odorata*) from Queensland. The nationally coordinated program is managed and operated by the Queensland Government. Siam weed is a plant exotic to Australia and has the potential to impact on the environment and other primary industries.

Mexican feather grass

Mexican Feather Grass (Tussock Grass) (*Nassella tenuissima*) was distributed in areas of Australia as a hardy visually appealing ornamental. Incursion responses were initiated in a number of Australian jurisdictions including Queensland. This was the first Queensland pest plant response where formal aspects of EMU preparedness were incorporated.

**Queensland Pest Plant Responses**

Four tropical weeds

This nationally coordinated program is managed and operated by the Queensland Government. The program involves extensive community engagement to identify infested areas, targeted weed surveys, weed control and research components. The National Four Tropical Weeds Eradication Program is a five year program to eradicate Miconia (*Miconia calvescens, M. racemosa, and M. nervosa*), Mikania vine (*Mikania micrantha*), Limnocharis (*Limnocharis flava*) and Koster's curse (*Clidemia hirta*).

Chilean needle grass and other *Nassella* species.

Chilean needle grass, (*Nassella neesiana*), was discovered in Queensland on a roadside south of Felton on the Darling Downs in 1998. Subsequent inspection revealed it in local areas with the largest expanse at the Clifton showgrounds and along the banks of the Condamine River. In 2004, the first cooperative work was conducted by the Clifton Shire Council and Queensland Government. Seasonal management strategies currently continue. Soil seed density studies show an improving situation in response to sustained management intervention. Chilean needle grass can be more invasive than serrated tussock, (*Nassella trichotoma*), when growing as a component of grasslands dominated by kangaroo grass (*Themeda triandra*). This gives perspective to it's invasiveness and persistence. It's management may be better handled by yearly sustained intervention rather than one off high resource emergency response.

**Planning for the Future in EMU**

EMU milestones and activity outputs are described and reviewed in the EMU operational plan. The EMU operational plan is developed from State Biosecurity program operational plans to ensure that the outputs of the EMU are in alignment with State needs. Where appropriate, the plan will be supplemented with emergency preparedness activities that may be strategic or beneficial to multiple/all BQ business groups and complement National endeavours. Regional needs for emergency preparedness shall be fulfilled from the regional operational plan but where expertise or resources are required from the EMU, the Manager EMU shall evaluate the need for EMU involvement on a case-by-case basis.

Activities identified for action will require negotiated prioritization. Where appropriate, activities will be benchmarked against milestones and projected outcomes. The outputs shall be measured against the EMU operational plan that is derived from a number of sources, namely: operational plans, program business plans, performance standards,

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incident and emergency response debriefs, after action reviews, continued development of the BEOM (Emergency Management Group 2010) and exercises to test emergency response activities/systems. Where additional work is required to further improve systems, a cost/benefit and risk analysis will ensure that further additional activity will cease when diminishing returns can no longer warrant the cost or mitigate the risk. Main factors considered in developing the EMU operational plan are:

1. Review the compilation of feedback material received by EMU of all the activities from operational plans, performance standards, action plans, incident and emergency response debriefs from BQ programs and subprograms relevant to incident and emergency preparedness
2. Identify incident and emergency preparedness gaps across the BQ programs
3. Identify synergies and differences so that structure provides optimal productive outputs.
4. Prioritise preparedness activities based on a risk management approach
5. Deliver priorities to the GM BQCC for risk analysis and resource allocation by EMU
6. Review invasive species risks and re-prioritise as required so standard operating procedures and work instructions address likely scenarios.

**Challenges for the EMU**

With any coordinating initiative, there are a number of challenges facing the unit. These include, but are not limited to:

- The unit competing for resources with other priority work with a shared vision for emergency preparedness improvement.
- The value of incident and emergency preparedness activities is difficult to quantify when there are no emergencies. (This will be overcome by measuring them against systems similar to the Emergency Plant Pest Response Deed, NEBRA or milestones recommended in the Australian Weeds Strategy.
- Incident and emergency preparedness activities can often be 'put off' until resources are made available to progress them. Often this does not eventuate leading to a critical resource gap that becomes evident only during a response.
- Reporting structure reorganisation e.g. FRU development and expansion.
- Staff seeing emergency preparedness as an additional workload. This may necessitate reviewing business priorities to determine where resources should be allocated within EMU. Furthermore, sustaining the EMU will lead to overall staff 'savings' as the state coordinated approach will serve to reduce duplication and streamline incident and emergency preparedness activities across all regions particularly if a business continuity plan is enacted.
- A belief that the unit may become the default response unit for whole of DEEDI emergencies, not just BQ emergencies.
- The success of the EMU and FRU will depend on whole of BQ support – i.e. specific input will be required from other BQ programs to provide an optimal EMU and FRU.
- Invasive Plants and Animals Program interaction with EMU and emergency operations management on considering new science development. Revising operational procedures is vital when bearing in mind response operational structure and the type of

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control options considered with respect to standard operating procedure development and it's effectiveness on the target species e.g. new understanding of the effects of herbicide treatment on seed viability may influence the size of surveillance buffer zones on reassessment following initial control (Patane *et al.* 2009).

After Action Review documentation, suggesting system fixes, and Implementation Plans are necessary to refine and improve response systems following an emergency incident. The EMU drives these processes in cooperation with the responsible BQ program.

Points that can be overlooked when initiating an emergency response include acting without referencing guidance documentation; initiating the response with too lean a structure (Sparkes 2009); accepting volunteers who have no training in emergency management; not appointing "champions" to lead specialist areas such as tracing and surveillance; not having a comprehensive induction package that contains conditions of employment including flexibility in working hours and the ability to claim overtime; incomplete staff training issues; poor and non-dynamic aims and objectives and not considering relevant topics in the incident action plan; incomplete or not updated MOUs between the states when considering sharing training staff/resources and use of the FRU.

In recent times, emergency responses have been improved with enhanced business operational procedures such as acquiring an expert to examine the response and make recommendations for improvement. Scope for future further emergency preparedness enhancement will be considering such concepts as a Queensland Biosecurity reserve; increasing the duty statements of some Biosecurity Officers to be permanent members of regionally based Emergency Response Teams to immediately cover key roles for specific programs; FRU members to take over formal roles within 3-5 days; memorandum of understanding to be kept contemporary between other states and Queensland due to the continual interaction on emergency response and discussion on systems development (both software and emergency systems development) other states have trialed and experienced under emergency conditions. Furthermore, negotiation could occur on policy and systems that interstate counterparts are prepared to share in preparatory management e.g. entry to Control Centers with photographic ID, bar code readers to record entry/exit times, recording systems for storing staff personal details and skills registry software. EMU could negotiate with other States with incentives in the form of BQ's Computerized Permit System (CPS) or the blueprint for the improved RAD system (Restricted Area Movement and Security Assessment Database) (Duff 2011).

## Training Enhancement

A large component of EMU business is ensuring appropriate DEEDI staff are trained in all aspects of emergency management. There have been 17 training events for the year ending May 31, 2011 and 43 training events since late 2008 equating to a total of 701 training seats. This equates to 398 individual staff that have been trained by the EMU since its formation. Identifying opportunities for cooperative training (i.e. training NSW and Queensland staff together where possible) is resource efficient, resulting in exposure to experiences and knowledge from a larger pool of specialist expertise. Consideration could be given to the possibility of joint training exercises on the NSW and Queensland border e.g. The Northern Rivers area of New South Wales. Furthermore, consideration can be given to the opportunities in utilizing interstate training staff to deliver/co-deliver specific specialized training. Experienced NSW staff could greatly assist EMU with the design and delivery of training in the logistics and planning areas because of parallel experience on different invasive species (Duff 2011).

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## Prioritisation of Tasks by the EMU

The EMU approaches task prioritisation using a risk management approach to outline the risk (failure likelihood) and the level of resources required to lower that risk to an acceptable level. The EMU and GM BQCC cooperatively work together to allocate their resources to the risks identified or to refer the risk to specialist BQ programs for guidance and alternate management strategies when EMU resources are insufficient to reduce the identified risk. Priorities consider current structures and systems and the resources required to adapt or modify these systems so that multiple programs, subprograms and business groups may be able to utilize them (Cozens 2008).

The development of a feasibility study to determine the prospects of utilizing a Biosecurity reserve is a major initiative for 2011. Better use of existing networks such as NRM groups, catchment groups and LandCare would underpin such a concept.

## References

Australian Fire Authority Council. (2004). *The Australasian Inter-service Incident Management System*, A Management System for any Emergency. AFAC Limited, East Melbourne. pp. 15-9.

Cozens, M. (2008). Biosecurity Queensland Control Centre Emergency Management Unit Development Plan, Department of Employment Economic Development and Innovation, Queensland Primary Industries and Fisheries. Brisbane.

Department of Agriculture Fisheries and Forestry. (2011). The National Environmental Biosecurity Response Agreement, National Biosecurity Committee, Canberra.

Emergency Management Group. (2010). Queensland Biosecurity Emergency Operations Manual, Department of Employment Economic Development and Innovation, Biosecurity Queensland. Brisbane.

Hinder, N. (2010). Update on Biosecurity Reforms: Intergovernmental Agreement on Biosecurity and new legislation, http://www.planthealthaustralia.com.au/index.cfm?objectid=B78D4BCC. Department of Agriculture, Fisheries and Forestry, Australian Government. Canberra.

Morton J. (2008). National Weed Incursion Plan-Preparedness and response guidelines for Weed Managers, Department of Employment Economic Development and Innovation, Biosecurity Queensland. Brisbane.

(MPAT), Multinational Planning Augmentation Team (2009). 'Military Decision Making Process ~ Multinational ~ (MDMP-M)'. http://www.mnfsop.com (Headquarters, U.S. Pacific Command, Box 64049 (J722), Camp H. M. Smith Honolulu, Hawaii 96861- 4049).

Patane, K.A., Setter, S. and Graham, M. (2009). Effect of foliar herbicides on the germination and viability of Siam weed (*Chromolaena odorata*) seeds located on plants at the time of application. *Plant Protection Quarterly* 24 (4): 138-40.

ISBN Number: 978-0-9871961-0-1

Sparkes, E. (2009). Mexican Feather Grass Emergency Response Implementation Plan. Biosecurity Queensland. Department of Employment, Economic Development and Innovation, Brisbane.

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# COMPETITIVE ABILITY OF WHEAT CULTIVARS AGAINST COMPLEX WEED FLORA IN ZERO-TILL PLANTING

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## ABSTRACT

Continuous and indiscriminate use of herbicides may lead to many problems such as resistance in weeds, residue in crop and soil, pollution hazards, health hazards to non-target organisms. In wheat crop, farmers can select a competitive cultivar to smoother weeds. Two year field studies were conducted at agricultural research farm of Banaras Hindu University to evaluate competitive attributes of wheat cultivars against complex weed population in wheat grown under zero-till. Variety K8027 which had taller plant and higher leaf area index was found most competitive against weed when compared with other varieties viz.K9107, HUW234, HUW468 and NW1014. Tank mix application of isoproturon + 2,4-D sodium salt at 1.0 + 0.5 kg/ha as post-emergence treatment was most effective in suppressing of weeds in all the varieties.

**Keywords:** Competitive ability, Wheat, Cultivars, Weeds, Zero-till

## INTRODUCTION

Rice-wheat (RW) is the most important cropping system for food security in South Asia. In India, it contributes 26% of total cereal production and 60% of total calorie intake. The area under rice-wheat system is static and the productivity and sustainability of the system is threatened because of the inefficiency of current production practices, shortage of resources, such as water and labour and socioeconomic changes (Ladha *et al., 2003*). In recent decade, wheat establishment method has shifted from conventional multi-till to reduced zero-till system. Zero tillage minimizes the loss on account of delayed sowing as it advances the wheat sowing by 10-15 days and also saves the time and cost involved in field preparation (Sen *et al.,* 2002). Weeds continue to be a serious threat to wheat productivity despite several decades of modern weed control practices realizing much of weed suppression through herbicides use. The development of herbicide-resistant weeds and weed population shifts continue to challenge the herbicide based weed management systems (Malik *et al*., 1998).

Modern wheat cultivars are nearly always evaluated for yields in weed free environments, so very little is known about their competitive interaction with weeds. The competitive ability of a crop against weeds can be measured in two ways: (a) the ability of the crop to maintain yield in the presence of weeds, and (b) the ability of the crops to suppress weeds and weed seed production. Several researchers (Blackshaw 1994; Challaiah *et al.* 1986; Bussan *et al.* 1997; Wicks *et al.* 2004) reported that use of taller cultivars reduce weed biomass and seed production more than shorter cultivars. Similarly other crop traits (flag leaf length and orientation, overall leaf area and canopy structure) have been identified to provide competitive advantage to crop over weeds (Lemerle *et al.,* 1996; Seavers and

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Wright ,1999). The objective of our research was to identify the traits associated with commonly grown wheat cultivars that confer competitiveness against complex weed flora in zero-till wheat.

## MATERIALS AND METHODS

Two year field experiments were conducted at agricultural research farm of Banaras Hindu University, Varanasi, India during 2002-03 and 2003-04 winter seasons in sandy clay loam soil to evaluate the effect of five wheat cultivars and five weed control treatments on wheat yield and related weeds. Five wheat cultivars were K9107, HUW234, HUW468, NW1014and K8027, while the weed control treatments were sulfosufuron @ 25 g a.i./ha, metribuzin @ 210 g a.i./ha, isoproturon 750 g a.i. + 2,4-D sodium salt 500 g a.i./ha (tank mix), weed free and unweeded check. The experimental design was a split plot design with four replication, cultivars were arranged in main plots and weed control treatments were assigned to sub-plots. All the herbicides were applied as post-emergence at 30 days after sowing (DAS) using spray volume of 500 l/ha by foot sprayer fitted with flat fan nozzle.

Wheat cultivars were sown at row space of 20 cm by opening slits with zero-till drill machine using a constant seed rate of 100 kg/ha. The crop received an uniform rate of 40 kg N, 60 kg $P_2O_5$ and 60 kg $K_2O$ /ha at sowing and 80 kg N/ha after first irrigation. The crop was given three irrigations at 21, 75 and 90 DAS during both the years. Weeds were counted 60 days after sowing from quadrate (0.5 x 0.5 m) randomly placed at four places in each plot. Dry weight of weeds was recorded after complete drying at 70º C for 48 hours in hot air oven. At harvest, a plant sample of one square meter from each plot was taken to determine number of ear head, number of grain/ear head and 1000 grains weight. Grain yield per plot was recorded by harvesting net plot area of each treatment. For comparison between means, L.S.D. test at 5% level was used.

## RESULTS AND DISCUSSION

### Effect of Treatments on Weeds

The dominant weeds in the unweeded plot at 60 days after wheat sowing were *Phalaris minor* Retz., *Cyperus rotundus* L., *Rumex dentatus* L., *Anagallis arvensis* L.,*Chenopodium album* L.,and *Melilotus indica*(L.) All and *Cynodon dactylon* (L.) Pers. Wheat cultivars caused mark variation in relative dominance of weeds in weedy check plots (Table 1). Cultivars NW 1014, HUW 234 and HUW 468 had higher relative density of *P.minor, R. dentatus* and *C. rotundus*. Whereas, *C.rotundus* was dominant weed in K9107 and K8027. Concerning the effect of weed control treatments on weeds, data in Table 1 indicated that tank mix application of isoproturon + 2,4-D at post-emergence reduced population of both grass and broadleaf weeds, but none of the herbicide treatment was effective in controlling *C.rotundus* . Herbicide sulfosulfuron was most effective against *P.minor* and metribuzin was least effective against the weeds.

**Table 1**: Effect of wheat cultivars and weed control treatments on relative composition (%) of weeds at 60 days from sowing.

| Treatments | *P.minor* | *C.dactylon* | *C.rotundus* | *R.denticulate* | *A.arvensis* | *C.album* | *M.indica* |
|---|---|---|---|---|---|---|---|
| HUW 234 | 9.70 | 8.3 | 10.8 | 23.9 | 17.5 | 9.6 | 6.6 |
| HUW 468 | 8.0 | 7.3 | 10.1 | 19.4 | 16.0 | 7.9 | 5.8 |
| K 9107 | 7.2 | 6.6 | 9.5 | 17.3 | 14.7 | 7.7 | 4.9 |
| K 8027 | 6.1 | 5.4 | 8.4 | 15.2 | 12.5 | 7.6 | 4.3 |
| NW 1014 | 10.0 | 9.3 | 12.3 | 26.0 | 20.5 | 10.1 | 7.5 |
| Unweeded | 18.8 | 10.2 | 14.4 | 33.4 | 5.3 | 14.9 | 0.5 |
| Sulfosulfuron | 6.2 | 12.8 | 14.3 | 28.7 | 23.3 | 8.9 | 7.2 |
| Metribuzin | 9.2 | 10.9 | 10.9 | 20.6 | 21.8 | 9.2 | 6.8 |
| Isoproturon+2,4-D | 7.9 | 10.1 | 11.1 | 14.8 | 18.3 | 8.1 | 6.2 |

Results in Table 2 revealed that different cultivars and weed control treatments significantly influenced density of different weed species at 60 days after wheat sowing. Cultivar K8027 was significantly most suppressive to different weed species, while NW 1014 was least effective in controlling weeds. Application of sulfosulfuron was significantly superior to metribuzin and isoproturon+2,4-D in reducing density of P.minor, whereas, the population of broad leaf weeds and C. rotundus was minimum in isoproturon + 2,4-D traeatment. The lowest dry weight of weeds was recorded in cultivar K8027 and greatest in NW 1014. Data in Table 2 indicated that weeds dry weight was significantly less in herbicide mixture of isoproturon + 2,4-D when compared with individual herbicide sulfosulfuron and metribuzin.

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**Table 2**: Effect of wheat cultivars and weed control treatments on weeds density (no./m$^2$) and weeds dry weight at 60 days after sowing.

| Treatment | *P. minor* | | Broad leaf weeds | | *C. rotundus* | | Weeds dry weight (g/m$^2$) | |
|---|---|---|---|---|---|---|---|---|
| | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 |
| HUW 234 | 7.20 | 5.70 | 16.50 | 15.30 | 4.33 | 3.30 | 34.90 | 31.10 |
| HUW 468 | 6.70 | 4.20 | 17.80 | 15.90 | 4.38 | 3.34 | 35.70 | 32.70 |
| K 9107 | 6.40 | 3.60 | 15.80 | 13.90 | 4.10 | 3.00 | 33.30 | 29.50 |
| K 8027 | 6.20 | 5.10 | 15.20 | 12.90 | 3.90 | 2.80 | 31.70 | 28.60 |
| NW 1014 | 7.50 | 6.30 | 19.20 | 18.10 | 4.60 | 3.50 | 36.90 | 34.30 |
| LSD (P=0.05) | 0.44 | 0.42 | 1.70 | 1.62 | 0.23 | 0.22 | 2.42 | 2.30 |
| Weed control treatments | | | | | | | | |
| Un weeded | 17.28 | 15.30 | 33.40 | 30.70 | 10.30 | 7.10 | 73.90 | 68.30 |
| Weed Free | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Sulfosulfuron | 4.70 | 3.80 | 18.90 | 16.90 | 4.10 | 3.60 | 34.60 | 30.90 |
| Metribuzin | 6.5 | 5.20 | 17.40 | 15.50 | 3.80 | 2.90 | 33.40 | 29.80 |
| Isoproturon + 2,4-D | 5.5 | 4.30 | 14.70 | 12.90 | 3.20 | 2.50 | 30.60 | 27.20 |
| LSD (P=0.05) | 0.34 | 0.32 | 0.95 | 0.90 | 0.12 | 0.11 | 2.12 | 2.01 |

## Effect of Treatments on Growth of Crop

The data on plant height, tiller number/meter row, leaf area index and crop dry weight revealed that cultivar K8027 had significantly higher values of these parameters than other cultivars of wheat (Table 3). Tank mix application of isoproturon+2,4-D recorded significantly taller plants, more number of tillers, higher leaf area index and greater dry matter accumulation by crop than individual treatment of sulfosulfuron and metribuzin.

## Effect of Treatments on Grain Yield and Yield Attributes

The data pertaining to yield contributing characters viz. head/m2, grains/head and 1000, grains weight (Table 4) indicated that cultivar K8027 had significantly greater number of heads/m2, number of grains/heads and 1000-grain weight than HUW234,HUW 468 and NW1014 (Table 4).

Amongst weed control treatments, herbicide mixture of isoproturon + 2,4-D recorded significantly maximum number of heads, grains/head and 1000-grain weight of crop than individual application of herbicide sulfosulfuron and metribuzin. It is evident from the data in Table 4 that cultivars K8027 produced numerically higher grain yield than K9107 and HUW 234, but was significantly superior to HUW468 and NW1014 while NW1014 had minimum grain yield. The herbicide treatment isoproturon + 2,4-D recorded significantly higher grain yield than sulfosulfuron or metribuzin (Table 4). However, both the herbicides produced significantly higher grain yield than unweeded treatment.

**Table 3**: Effect of wheat cultivars and weed control treatments on growth parameters of wheat at 60 days from sowing.

| Treatment | Plant height (cm) | | No. of tillers per running meter | | Late area index | | Crop Dry Weight (g/m2) | |
|---|---|---|---|---|---|---|---|---|
| | 60 DAS | | 60 DAS | | 60 DAS | | 60DAS | |
| | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 |
| HUW 234 | 59.22 | 62.39 | 69.58 | 71.53 | 3.15 | 3.46 | 364.7 | 411.2 |
| HUW 468 | 55.23 | 58.11 | 67.11 | 68.81 | 3.08 | 3.39 | 354.9 | 400.4 |
| K 9107 | 63.62 | 67.03 | 72.08 | 74.29 | 3.22 | 3.54 | 372.2 | 419.4 |
| K 8027 | 66.70 | 70.33 | 74.09 | 76.49 | 3.39 | 3.73 | 388.9 | 437.8 |
| NW 1014 | 53.79 | 55.52 | 63.94 | 65.30 | 3.05 | 3.35 | 345.6 | 390.1 |
| LSD (P=0.05) | 5.40 | 5.93 | 4.29 | 4.79 | 0.19 | 0.21 | 22.4 | 34.8 |
| Weed control treatments | | | | | | | | |
| Un weeded | 50.73 | 52.65 | 64.67 | 66.32 | 2.90 | 3.19 | 342.1 | 386.2 |
| Weed Free | 67.94 | 71.76 | 73.63 | 75.17 | 3.39 | 3.72 | 389.7 | 438.7 |
| Sulfosulfuron | 56.56 | 59.26 | 67.09 | 68.99 | 3.09 | 3.40 | 353.4 | 398.7 |
| Metribuzin | 58.99 | 61.92 | 69.36 | 71.49 | 3.19 | 3.51 | 366.7 | 413.4 |
| Isoproturon + 2,4-D | 64.34 | 67.81 | 72.05 | 74.45 | 3.32 | 3.65 | 374.4 | 421.8 |
| LSD (P=0.05) | 3.90 | 4.29 | 3.68 | 3.96 | 0.14 | 0.16 | 14.1 | 15.5 |

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**Table 4**: Yield and yield attributes of wheat crop as affected by cultivars and weed control treatments.

| **Treatment** | Ear head/ $m^2$ | | Grains/ ear head | | 1000, grains weight(g) | | Grain yield (Kg/ha) | |
|---|---|---|---|---|---|---|---|---|
| | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 | 2002-03 | 2003-04 |
| HUW 234 | 270.70 | 287.60 | 43.44 | 43.90 | 40.10 | 40.90 | 3176 | 3548 |
| HUW 468 | 256.30 | 278.86 | 42.80 | 43.30 | 38.30 | 39.02 | 2865 | 3220 |
| K 9107 | 275.90 | 294.40 | 44.50 | 46.90 | 43.30 | 44.10 | 3281 | 3658 |
| K 8027 | 278.10 | 305.10 | 47.50 | 47.90 | 44.80 | 45.70 | 3352 | 3733 |
| NW 1014 | 245.10 | 272.36 | 42.20 | 42.60 | 37.10 | 37.90 | 2738 | 3085 |
| LSD (P=0.05) | 19.87 | 20.56 | 3.70 | 3.73 | 2.70 | 2.64 | 233 | 239 |
| Weed control treatments | | | | | | | | |
| Un weeded | 243.20 | 264.20 | 38.80 | 39.10 | 38.66 | 39.44 | 1849 | 2149 |
| Weed Free | 276.90 | 307.60 | 48.20 | 48.70 | 42.40 | 43.30 | 3823 | 4229 |
| Sulfosulfuron | 261.10 | 284.70 | 43.70 | 44.10 | 40.10 | 40.80 | 2966 | 3326 |
| Metribuzin | 270.10 | 279.30 | 45.10 | 45.50 | 40.50 | 41.30 | 3229 | 3604 |
| Isoproturon + 2,4-D | 274.90 | 301.60 | 46.60 | 47.10 | 41.96 | 42.80 | 3545 | 3937 |
| LSD (P=0.05) | 10.93 | 14.26 | 2.90 | 2.87 | 2.06 | 2.08 | 124 | 133 |

## DISCUSSION

The different characteristics of a good competitive cultivars are that it must have rapid germination, initial quick growth, tillerring capacity and leaf area (Lemerle et al., 1996; Challaiah *et al.*1983;). The difference in the ability of cultivars K8027 to suppress weed growth more than other might be due to taller plants, high leaf area index,and light interception tillering capacity and vegetative growth habit (Seavers and Wright, 1999; Dhima *et al*.2008). Presence of these attributes in cultivar K8027 conferred better competitiveness against most weeds infested the crop than cultivars with shorter plants, less tillers and leaf area index. An effective herbicide is one that control complex weed community. The herbicide mixture of isoproturon + 2, 4-D had ability to control dominant grass as well as broadleaf weeds, while sulfosulfuron and metribuzin could control only grass weeds. The lower weed growth in isoproturon + 2,4-D treatment was due to broad spectrum weed control than other herbicides. The significant differences in growth attributes of cultivars can be attributed to their genetic makeup. Coleman and Gill (2002) also reported significant genetic correlations for grain yield, grain yield loss and ryegrass dry matter with agronomic and morphological traits on the wheat lines.

The variation in yield and yield attributes of cultivars can be attributed to their differential weed suppression ability that led to dry matter accumulation and its translocation for the formation of yield attributes. Cultivar K8027 which had taller plants, more tillers and leaf area index also accumulated higher dry matter that was finally used for production of agronomic characters determining crop yield.

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## REFERENCES

Blackshaw, R.E. (1994). Differential competitive ability of winter wheat cultivars against downy brome. *Agronomy Journals*, 86: 649-654.

Bussan, A.J., O.C. Burnside, J.H. Orf, E.A. Ristau and K.J. Puettmann, (1997). Field evaluation of soybean (*Glycine max*) genotype for weed competitiveness. Weed Sci., 45: 31-37.

Challaiah, R.E., O.C. Burnside, G.A. Wicks, V.A. Johnson, 1986. Competition between winter wheat (*Triticum aestivum*) cultivars and downy brome (*Bromus tectorum*). Weed Sci., 34: 689-693.

Coleman, R. K. and Gill, G. S. (2002). Identifying traits conferring weed competitiveness in wheat in Australia. In Proc.of International workshop on herbicide resistance management and zero tillage in rice wheat cropping system. CCS Haryana Agricultural University, Hisar, India, March 4-6, pp.41-46.

Dhima,K., I Vasilakoglou, A. Lithourgidis, E. Mecolari, R. Keco, X. Agolli and Eleftherhorinos (2008). Phytotoxicity of 10 winter barley varieties and their competitive ability against common poppy and ivy-leaved apeedwell. *Experrimental Agric*., 44: 385-397.

Ladha, J.K., Pathak, H., Padre, A.T., Dawe, D., Gupta, R.K.(2003). Productivity trends in intensive rice- wheat cropping system in Asia. In: Ladha, J.K., et. Al (Eds.) Improving the Productivity and Sustainability of Rice-wheat Systems: Issues and Impacts. ASA Spec. Publ. 65, ASA, CSSA and SSSA, Madison, WI, P 45-76.

Lamerle, D., B. Verbeek and S.N. Coombe, (1995). Losses in grain yield in winter crops from *Lolium rigidium L.* competition against weeds. Weed Res., 35: 500-509.

Malik RK, Gill G and Hobbs PR. (Eds.) (1998). Herbicide Resistance- A major issue for sustaining wheat productivity in Rice wheat cropping system in the Indo- Gangetic Plains. *Rice- Wheat Consortium Paper Series 3*. New Delhi, India.

Seavers, G.P. and K.J. Wright, (1999). Crop canopy development and structure influence weed suppression. *Weed Res.,* 39: 319-328.

Sen, A., Sharma, S.N., Singh, R.K.and Pandey,M.D. (2002).Effect of different tillage system on performance of wheat. In: Proc. of International workshop on herbicide resistance management and zero tillage in rice wheat cropping system. CCS Haryana Agricultural University, Hisar, India, March 4-6, pp. 115-116.

Wicks, G.A., P.T. Nordquist, P.s. Baenziger, R.N. Klein, R.H. Hammons and J.E. Watking, (2004) Winter wheat cultivar characteristics affect annual weed suppression. Weed Tech., 18: 988-998.

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# INTEGRATED MANAGEMENT OF SILVERLEAF NIGHTSHADE

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## ABSTRACT

Silverleaf nightshade is a deep-rooted summer perennial weed of southern Australia. Chemical and physical control tactics used for the past half century have not always been successful due to the resilience of the root system. Multi-year experiments established near Culcairn, NSW and Leeton, NSW showed that herbicides can reduce annual stem regrowth by up to 90%, depending upon the herbicide used and the time of application. Herbicide treatments containing the active ingredient picloram were the most effective, particularly if applied annually in summer and autumn. Competition from the perennial sub-tropical pasture species finger grass and digit grass at a field site at Wellington, NSW provided 94% suppression of silverleaf nightshade after two seasons.

**Keywords:** silverleaf nightshade, herbicides, pastures, IWM

## INTRODUCTION

Summer perennial weeds are a major cost to animal production in SE Australian mixed farming systems. Predicted climate change towards warmer, moister summers is expected to increase the spread and impact of summer weeds on pasture systems. Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a typical example of an intractable, deep-rooted, summer perennial weed which significantly impacts on livestock productivity and health.

Silverleaf nightshade infests 140,000 hectares in SE Australia, with the potential to infest 398 million hectares, with nearly 95% of the infested areas affecting pasture lands (Kwong 2006).

Silverleaf nightshade is currently classified as a noxious weed state-wide in South Australia and Victoria, and in one third of the local control authority regions of New South Wales. The spread, persistence and intractable nature of silverleaf nightshade is attributable to the presence of both a seed bank and a root bank. The seed bank is estimated to last for at least six years, and the extensive root system is thought to persist for longer, resulting in a need for long term management to reduce or eliminate a population once established.

The economic impact of silverleaf nightshade comprises direct control costs, production loss (yield and hay value), reduced land value, and environmental degradation. A survey of 254 land managers in SE Australia estimated that average total farm impact of silverleaf nightshade was $1730 per year in direct control costs and $7786 in lost production (McLaren *et al.* 2004). Silverleaf nightshade can cause cereal yield reduction of up to 70%

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(Heap and Carter 1999) due to the depletion of soil moisture and nutrients during the previous summer as well as in-crop competition.

Additionally, the presence of silverleaf nightshade can reduce land values of both infested and nearby properties, with the potential to reduce land value by 25%.

The lack of cost effective control tactics makes silverleaf nightshade management extremely difficult, with management limited to a few unreliable and expensive residual herbicides (Kidston *et al.* 2007, Ensbey 2009). A survey of 229 growers across SE Australia (McLaren, unpublished), identified that 84% growers needed more information and a package of actions that can be used to develop successful management plans for effective silverleaf nightshade control. This study investigated several options for silverleaf nightshade control.

## Methods and Materials

### Pasture Competition

A study was conducted to determine whether summer active pastures provide competition to reduce the growth of silverleaf nightshade under field conditions. A field site was established in 2008 near Wellington (S: 32 31 57.34, E: 148 48 23.75), New South Wales. A complete randomised design with three replicates was used for eight pastures; Lucerne (*Medicago sativa* cv. Aurora), finger grass (*Digitaria milanjiana* cv. Strickland), digit grass (*Digitaria eriantha* spp. *Eriantha*), Rhodes grass (*Chloris gayana* cv. Katambora), phalaris (*Phalaris aquatica* cv. Sirolan), chicory (*Chicorium intybus* cv. Puna) and bambatsi panic (*Panicum coloratum* var. *makarikariense*), and sub-clover (*Trifolium subterraneum*) as a control. Pasture biomass and composition data were collected quarterly.

### Herbicides

Field experiments were established in December 2006 at Culcairn (S: 35° 35’ 36.51”, E: 147° 10’ 5.28”) and Leeton (S: 34° 25’ 0.61”, E: 146° 22’ 10.57”), New South Wales. Treatments based on current registered herbicides, current reported practice and research reports were examined at each site in a randomised complete design with three replicates (Table 1). Treatments were applied annually using a shielded 4 m boom fitted with Lechler IDK 120-015 low pressure air induction nozzles operated at 250 kPa to provide 100 L/ha spray volume. Uptake spray oil at 1% v/v was included as a standard adjuvant.

Data on stem density, physiological maturity and mortality were collected just prior to and 4-6 weeks after each herbicide application to determine within season effectiveness of the herbicide treatments. Emergence data were recorded at the start of each season to determine between season effectiveness of the herbicide treatments.

## RESULTS

### Pastures

After two seasons, Strickland finger grass and digit grass were the most competitive, producing 10.8 and 9.6 t/ha biomass, respectively. The chicory pasture did not establish well and did not provide silverleaf nightshade control (Table 2). Phalaris and Rhodes grass did not provide significantly higher levels of control compared to the annual pasture. All

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other pastures significantly reduced silverleaf nightshade stem densities over time (P<0.05).

**Table 1.** Long term herbicide control of silverleaf nightshade stem emergence.

| Herbicide | Application Rate | Leeton: Dec 2008 Density (stems/m$^2$ ± s.e.) | Leeton: Stem Reduction 2006-08 (%) | Culcairn: Dec 2008 Density (stems/m$^2$ ± s.e.) | Culcairn: Stem Reduction 2006-08 (%) |
|---|---|---|---|---|---|
| Untreated control | | 15.3 ± 1.7 | 53 | 6 ± 3.3 | -38 |
| Roundup Powermax ® | 1080g a.i./ha glyphosate | 7.5 ± 1.5 | -32 | 2 ± 0.6 | -62 |
| Roundup Powermax fb Roundup Powermax # | 1080g a.i./ha glyphosate | 9.2 ± 3.2 | -20 | 3 ± 0.3 | -7 |
| Amicide 625 ® | 937.5g a.i./ha 2,4-D amine | 13.5 ± 2.9 | 12 | 5.3 ± 1.8 | -1 |
| Roundup Powermax + Amicide 625 | 937.5g a.i./ha 2,4-D amine + 1080g a.i./ha glyphosate | 5 ± 2 | -61 | 7.3 ± 3.1 | 0 |
| Starane 200 ® | 200g a.i./ha fluroxypyr | 7.5 ± 0.6 | -18 | 1.7 ± 0.7 | -61 |
| Tordon 75-D ® fb Tordon 75-D # | 900g a.i./ha 2,4-D + 225g a.i./ha picloram | 4.8 ± 1.4 | -34 | 0.8 ± 0.4 | -90 |
| Grazon Extra ® fb Grazon Extra # | 900g a.i./ha triclopyr + 300g a.i./ha picloram + 24g a.i./ha aminopyralid | 3.8 ± 1.5 | -60 | 1.3 ± 0.2 | -72 |
| Atrazine 500 ® | 2000g a.i./ha atrazine | 8.5 ± 1.4 | 142 | 0.8 ± 0.3 | -79 |
| Tordon 75-D | 900g a.i./ha 2,4-D + 225g a.i./ha picloram | 7.8 ± 1.2 | -39 | 1.2 ± 0.4 | -62 |
| *l.s.d. (0.05)* | | *5.9* | *123.6* | *n.s.* | *n.s.* |

# fb - denotes the same treatment applied in summer and again in autumn.

**Table 2.** Effective pastures for silverleaf nightshade control at Wellington, NSW.

| Pasture | Silverleaf nightshade Density (stems/m$^2$) 2008 | Silverleaf nightshade Density (stems/m$^2$) 2010 | Percentage reduction (%) |
|---|---|---|---|
| Annual pasture | 13.9 | 5.3 | 56.0 |
| Lucerne | 9.3 | 1.8 | 81.7 |
| Digit Grass | 13.1 | 0.9 | 94.0 |
| Rhodes Grass | 10.1 | 2.7 | 74.3 |
| Phalaris | 20.5 | 3.7 | 76.3 |
| Chicory | 9.3 | 7.3 | 21.7 |
| Bambatsi Panic | 14.4 | 2.4 | 86.3 |
| Finger Grass | 12.0 | 0.8 | 94.0 |
| *l.s.d. (0.05)* | *9.9* | *3.8* | *25.0* |

## Herbicides

Silverleaf nightshade densities in December 2008 at the Leeton site were significantly different (P<0.01) as a result of two seasons of herbicide experiments (Table 2). Silverleaf nightshade control at the Culcairn field site were less conclusive (P=0.07), although some similar trends were evident. Annual ground cover (estimated 2-3 t/ha across the site) accumulated at the Culcairn site prior to winter annual weed control in 2008 which may have contributed to the lower stem emergence observed, whereas the Leeton site had minimal ground cover between seasons.

Two applications of either Tordon 75-D or Grazon Extra provided the greatest decrease in silverleaf nightshade density. Starane 200 and Roundup PowerMax, used once or twice per season, also provided suppression of silverleaf nightshade emergence after two seasons.

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Atrazine 500 provided contrasting results between the two sites. It is speculated that differences in soil type, rainfall and general ground cover contributed to the observed differences. Similar levels of control as observed at Culcairn have also been noted at a District Agronomist's demonstration site near Ungarie. However, as the level of control achievable is not consistent it would difficult to recommend this treatment.

## DISCUSSION

Current management practices focus on limiting seed production through herbicide application during the early reproductive phases in mid-summer. These practices typify the conventional approaches that have been successfully applied to annual weeds that rely upon seed banks for population survival. However, successful management programs for perennial weeds need to also include practices that reduce the root bank.

The presence of an active pasture can reduce stem numbers present in mid to late summer. As well as a direct reduction in stem density, competitive pastures can also lead to a reduced seed production and root vigour. Additionally, if stem emergence can be manipulated using competition, the phenological maturity of the population may be more uniform and allow for more timely application of herbicides.

Management of the silverleaf nightshade root bank is critical for achieving long term control. However, the cost and time associated with multiple herbicide applications per season may inhibit adoption, particularly as it takes many years to effectively manage the root bank. The density of infestations can result in them being either time consuming to individually spot-spray or uneconomic to treat with a broadacre boom application.

While herbicides have been used for control in the past (Cuthbertson *et al.* 1976, Lemerle 1982), long term control or eradication has been difficult to achieve in the field. A range of herbicides are useful for controlling seed set with a single mid season application, while picloram based products were the most effective of the autumn applied treatments for root bank control. However, picloram based products are typically five times more expensive to use than traditional herbicide choices ($95-100 and $15-20, respectively), even at the rates examined in these field experiments, which may make this strategy uneconomical.

Integrated management packages need to be tailored to suit each infestation. The use of residual herbicides has the inherent risk of impacting upon other components of the system. The residual picloram herbicides that have proven effective for controlling the root bank will also reduce the capacity of producers to establish and maintain broadleaf pasture and crop species in the following winter and spring, therefore their use must be carefully planned to fit within the projected use patterns. Herbicides for seed set control need to be selected that will not impact of perennial pasture production over summer, while the choice of autumn applied herbicides for root bank control needs to be made with consideration given to the proposed winter pasture or cropping practices.

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## REFERENCES

Cuthbertson, E., Leys, A. and McMaster, G. (1976). s*ilverleaf nightshade* – a potential threat to agriculture. *Agricultural Gazette of New South Wales* 87: 11-13.

Ensbey, R. (2009). *Noxious and environmental weed control handbook, a guide to weed control in non-crop, aquatic and bushland situations*. 4th Edition, (NSW Department of Industry and Investment, Orange).

Heap, J. and Carter, R. (1999). The Biology of Australian Weeds. 35. *Solanum elaeagnifolium* Cav. *Plant Protection Quarterly* 14: 2-12.

Kidston, J., Thompson, R. and Johnson, A. (2007). 'Primefact 237: Silverleaf Nightshade'. (New South Wales Department of Primary Industries, Orange).

Kwong, R. (2006). Feasibility of biological control of solanaceous weeds of temperate Australia silverleaf nightshade, *Solanum elaeagnifolium* Cav. and prairie ground cherry, *Physalis viscosa* L. (Final Report, Meat and Livestock Australia, Sydney).

Lemerle, D. (1982). Chemical control of *silverleaf nightshade* using a ropewick applicator. In M.J.T. Norman (ed). *Proceedings of the 2nd Australian Agronomy Conference*, Australian Society of Agronomy, Wagga Wagga. p. 84.

McLaren, D., Morfe, T., Honan, I. and Holtkamp, R. (2004). Distribution, economic impact and attitudes towards silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia. In Sindel, B.M. and Johnson, S.B. (eds). *Proceedings of the 14th Australian Weeds Conference*, Weed Society of New South Wales, Wagga Wagga. p 701.

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# FINDING AND FIXING GLYPHOSATE-RESISTANCE RISK IN DIVERSE SUB-TROPICAL AUSTRALIAN FARMING SYSTEMS

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## ABSTRACT

Glyphosate-resistant weed populations are now present in sub-tropical Australian cropping systems and seem likely to become more common. To support the industry's approach to this problem, we developed a risk assessment framework for glyphosate resistance in these cropping systems that combines factors for weed species risk with weed control practices. We used the framework to assess a range of regionally important weeds currently controlled with glyphosate. Several species were identified as being at high risk, including both grasses and broadleaf species active in summer and winter, but other regionally important weeds were found to be at relatively low risk.

The risk assessment framework was used to build an online toolkit that is now used by growers to assess resistance risk for individual fields, and to investigate the value of possible practice change. We used the online toolkit to generate a database of risk scores and management practices and analysed nearly 40 responses. Glyphosate resistance risks were found to be highest on average in fallows and non-irrigated glyphosate-resistant cotton crops, but risks and practices were found to vary substantially within and between common crops and fallows. So, while there are crops and farming practices that the broadacre farming industry across the region should regard as being high risk, individuals' own resistance risks are likely to be significantly different from the average for some phases of their rotation, and should be analysed and responded to separately.

**Keywords**: glyphosate, resistance, risk assessment, farming systems

## INTRODUCTION

Since 2008, predictions made about the imminent arrival of glyphosate-resistant weeds in Australia's sub-tropical cropping systems (such as in Thornby and Walker 2009) have materialised. There are now more than 20 resistant populations of summer grasses in the region (Preston 2011), so while confirmed resistant populations are still uncommon, their prevalence is increasing rapidly (Heap 2011).

Nevertheless, robust weed management systems have been predicted to be of value in maintaining glyphosate susceptibility (Werth *et al*. 2008). In order to arm the region's cotton and grain growers with better information, we developed and published an online tool (Thornby *et al*. 2010; www.dpi.qld.gov.au/26_16653.htm) for the assessment of glyphosate resistance risk at an individual field level. This tool assesses two sources of resistance risk; that due to weed characteristics ('species risk'), and that due to management-driven selection pressure ('management risk'). The tool can be used to assess either a current, past, or speculative situation.

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The region's farming systems are highly variable (Osten *et al*. 2007), with a number of major and minor crops grown in rotations that are frequently changed in response to the rainfall in any particular season. These rotations rely heavily on glyphosate for weed control in fallows and for pre-planting preparation, but may also incorporate varying types and intensities of other weed control. This variability means that industry-wide assessments of and approaches to glyphosate resistance risk may not fit (to a varying extent) some farms, and proposed strategies and tactics may not be equally applicable for different years or sequences of years that may be substantially drier or wetter than average.

There is also an important distinction between species risk and species prevalence. Across the north-eastern subtropical area of Australia, weed floras differ according to local soil, climate, and farming system conditions. If we can reasonably assume that it is most useful to drive resistance prevention strategies around the weeds that are at highest risk, an assessment of individual circumstances must be made, as the most frequently controlled weeds on any farm or field may not be the ones at highest risk of evolving resistance to glyphosate.

To investigate these issues, we used a database of responses to our online risk assessment tool to measure variability in glyphosate resistance risks within the region and to analyse potential differences between prevalent species and species that are at high risk of resistance.

## METHODS

We collected responses to the online risk assessment tool by having an automated email sent for collection into a database. The email contains all of the user's responses to the tool including: weed presence or absence for 30 regionally important species; the user's crop rotation which may be of up to five years; glyphosate and non-glyphosate herbicide use; tillage frequency; the frequency and effectiveness of actions taken specifically to control survivors of glyphosate applications; and calculated risk assessment scores for rotational phases (crops or fallows) and seasons (as per Thornby *et al*. 2010).

We compared species risk scores as used in the tool with two sources of information on species prevalence: reported species prevalence rates within the tool, and data from a series of on-ground weed surveys collated by Werth *et al*. (2010).

## RESULTS

At the time of writing there had been 39 legitimate responses to the online risk assessment tool. The responses to the tool reflected the variability of farming systems in the region. Responses were grouped into rotations that were either identical or varied by only one crop or fallow from others in the same group, and under this analysis there were 28 different rotations in the dataset. No crops or fallows were reported by all growers. Cotton was the most common crop: 67% of growers reported including some form of cotton cropping (irrigated- or non-irrigated crops that were either glyphosate resistant or conventional). The next three most common phases were winter fallow (59%); wheat (54%); and summer fallow (51%).

**Weed Prevalence and Resistance Risks**

All 30 weed species included in the tool (Thornby *et al*. 2010) were selected by more than one respondent as being targets for control in the field being assessed. Frequency of selection of individual species varied between 15 and 54 %. Only one species, flaxleaf fleabane (*Conyza bonariensis*), was present in the majority of fields (Table 1).

Weed prevalence data of the top 10 weeds are compared with the results of field surveys reviewed by Werth *et al*. (2010) in Table 1. Of the 15 most-reported weeds in the online tool dataset, six did not appear in the top 15 weeds in previous field surveys. Results most closely reflected the 2009 surveys with a few exceptions: notably, substantially increased reported prevalence of turnip weed (*Rapistrum rugosum*) and pigweed (*Portulaca oleracea*), and reduced reported prevalence of peach vine (*Ipomoea lonchophylla*).

**Table 1.** Frequency of reporting of weed presence in an online tool and previous field surveys in north-eastern Australia, and predicted resistance risk scores

| Species | Rank of frequency of reporting (% of respondents) | Rank in 2001 surveys[1] | Rank in 2009 surveys[2] | Glyphosate resistance risk score[3] |
|---|---|---|---|---|
| *Conyza bonariensis* | 1 (54 %) | 14 | 2 | 8.15 |
| *Portulaca oleracea* | 2 (46 %) | 3 | 7 | 1.85 |
| *Hibiscus trionum* | 3 (44 %) | 1 | 1 | 1.11 |
| *Echinochloa spp.* | 3 (44 %) | 5 | 7 | 6.85 |
| *Rapistrum rugosum* | 5 (41 %) | >15 | >15 | 1.85 |
| *Sonchus oleraceus* | 5 (41 %) | 7 | 3 | 6.85 |
| *Tribulus terrestris* | 7 (38 %) | 4 | 6 | 1.11 |
| *Ipomoea plebeia* | 7 (38 %) | >15 | >15 | 1.85 |
| *Avena spp.* | 9 (36 %) | >15 | 15 | 3.52 |
| *Sisymbrium thellungii* | 9 (36 %) | >15 | >15 | 1.85 |
| *Polygonum aviculare* | 11 (33 %) | >15 | >15 | 2.59 |
| *Malva parviflora* | 11 (33 %) | >15 | >15 | 1.11 |
| *Amaranthus macrocarpus* | 11 (33 %) | 5 | 11 | 1.85 |
| *Lolium rigidum* | 14 (31 %) | >15 | >15 | 6.11 |
| *Ipomoea lonchophylla* | 15 (28 %) | 4 | 5 | 1.11 |

1. Mean rank across two 2001 surveys summarised in Werth *et al.* (2010)

2. Mean rank in 2009 follow-up surveys from the same fields as in 2001 surveys (Werth *et al*. 2010)

3. Species risk scores calculated as reported in Thornby *et al* (2010)

Glyphosate resistance risk scores for each species used in the risk assessment process are also shown in Table 1. There is no correlation between estimated species risk level and their frequency of reporting as species being controlled during use of the tool (coefficient of correlation = -0.31).

**Management vVariability**

The wide differences in strategies used to manage weeds in the region are illustrated by the range of management risk scores reported in the online tool. A full explanation of the

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risk assessment protocol is available in Thornby *et al*. (2010), but in brief the management risk scores are obtained by subtracting mitigating factors (uses of non-glyphosate herbicides, follow-up activities etc.) from the number of glyphosate sprays per phase. On average, summer fallow and non-irrigated glyphosate-resistant cotton were under the most risky management, but individuals' scores varied substantially both between and within phases, as shown in Table 2.

**Table 2.** Risk score means and ranges for all phases reported in the online tool

| Phase | Mean risk score | Risk score range | CV |
|---|---|---|---|
| Summer fallow | 1.49 | 5.00 | 1.02 |
| Non-irrigated GR cotton | 1.37 | 2.92 | 0.91 |
| Irrigated GR cotton | 1.05 | 2.82 | 1.17 |
| Winter fallows | 0.85 | 4.00 | 1.26 |
| Other winter crops | 0.45 | 2.97 | 1.98 |
| Sorghum | 0.38 | 3.92 | 2.82 |
| Other summer crops | 0.21 | 0.85 | 2.00 |
| Barley | 0.21 | 1.64 | 2.83 |
| Wheat | 0.13 | 1.79 | 3.29 |
| Irrigated cotton | 0 | 0 | 0 |
| Summer average | 0.86 | 3.92 | 1.28 |
| Winter average | 0.34 | 2.13 | 1.93 |

*CV – coefficient of variance, GR – glyphosate resistant

In the case of non-glyphosate-resistant irrigated cotton crops, all of the respondents recorded using enough non-glyphosate tactics in the crop to reduce risk to nil (n=4). Similarly in the case of 'other summer crops', risks were uniformly low to moderate, ranging from zero to 0.85. In all other crops and fallows, some (but not all) respondents reported using high risk, glyphosate-dominant practices (ranges 1.64-5.00), and at least one respondent in each phase reported using enough non-glyphosate tactics to record a nil risk score. Overall summer and winter scores (the weighted contribution of all winter or summer phases in each respondent's rotation) showed that while summer practices were on average more risky than winter practices, there were individual scores at both low and very high risk levels for both seasons.

## DISCUSSION

The information gained through the online tool to date indicates that the north-eastern Australian cotton and grains farming industries face particular challenges in preventing and managing glyphosate resistance. These challenges are directly related to the high variability in cropping and weed management practices present in the region, as confirmed by the data on crop frequency and rotations. In particular, the variability in crop rotations used in the region indicates that best management strategies and recommendations for practice change are not likely to be 'one size fits all'.

Similarly, the variation in levels of risk predicted for individuals' management practices in common crops and fallows is high. High range and CV scores show that there are, for nearly all phases reported in the tool, some growers who operate at a low risk. These growers incorporate several tactics into an integrated weed management strategy. Other growers manage the same phases with a high risk, relying entirely or substantially on glyphosate. Table 1 demonstrates that the weeds commonly managed in the region are a mixture of high- and low-risk species. The high risk species include both summer- and winter-dominant species. Growers, therefore, would be best served by identifying their at-risk weeds and driving their glyphosate-resistance prevention strategies around those, rather than assuming their most prevalent weeds are also those at risk of resistance.

While national and regional risk reduction strategies are valuable and broadly applicable, it is clear that high variability in this region means individuals must use those strategies in a targeted way. In most cases, there are potential practice changes that could be made that would reduce the likelihood of a glyphosate-resistant weed population developing in any individual field, but the nature and best timing of those practice changes appear to be highly individual. Growers with already low-risk winter fallows, for example, may not achieve meaningful risk reduction by making practice changes in that phase, however convenient it may be. This would particularly be the case if their species at highest intrinsic risk appeared in the summer months.

Overall the challenging, variable nature of the region demonstrates the value of a tool such as this – one that allows growers to estimate their individual risk and that is detailed enough to investigate the value of practice changes specifically designed to fit local weed floras, risks, and rotations.

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## REFERENCES

Heap, I. (2011). The International Survey of Herbicide Resistant Weeds. Online. Internet. May 10, 2011 . Available www.weedscience.com.

Osten, V., Walker, S., Storrie, A., Widderick, M., Moylan, P., Robinson, G. and Galea, K. (2007). Survey of weed flora and management relative to cropping practices in the north-eastern grain region of Australia. *Australian Journal of Experimental Agriculture*, 47:57-70.

Preston C (2011) Australian Glyphosate Resistance Register. Published on-line www.glyphosateresistance.org.au (Adelaide Australia).

Thornby, D. and Walker, S. (2009). Simulating the evolution of glyphosate resistance in grains farming in northern Australia. *Annals of Botany*, 104(4):747-756.

Thornby, D., Werth, J., Curr, M., and Walker, S. (2010). Risks and RATs: assessing glyphosate resistance risk in paddocks in north-eastern Australia. In Zydenbos, S.M. (ed). *Proceedings of the17th Australasian Weeds Conference*, New Zealand Plant Protection Society pp 246-249.

Werth, J., Thornby, D., Walker, S., Charles, G. and McDonald, C. (2010). Species shift and resistance: challenges for Australian cotton systems. In Zydenbos, S.M. (ed). *Proceedings of the17th Australasian Weeds Conference*, New Zealand Plant Protection Society pp 20-23.

ISBN Number: 978-0-9871961-0-1

Werth, J.A., Preston, C.P., Taylor, I.N., Charles, G.W., Roberts, G.N. and Baker, J. (2008). Managing the risk of glyphosate resistance in Australian glyphosate-resistant cotton production systems. *Pest Management Science*, 64: 417-421.

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# ALLELOPATHIC POTENTIAL OF *BRASSICA JUNCEA* (L.) CZERN. VAR. ENSABI

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## ABSTRACT

The wild species of Malaysian brassica [*Brassica juncea* (L.) Czern. var. Ensabi] with its pungent and slightly bitter aromatic taste has been planted by Malays and natives in the Malaysian states of Sabah and Sarawak. Some allelochemicals persent in this plant extract may, directly, prevent or promote germination and seedling growth when environmental conditions are conductive to growth and establishment. The crude ethanol extracts were prepared using extract concentrations of 10.8, 14.28, 18 and 30 $gL^{-1}$ of leaf, stem and root materials. The extracts were tested with the widely used radish seed and barnyard grass seed. Radish seed germination was inhibited at all concentrations of ethanol extraction from leaves. The inhibition of root and shoot growth was also observed in the barnyard grass and radish seeds. Both species were susceptible to allelopathy by extracts isolated from leaf, stem and root of *Brassica juncea* (L.) Czern. var. Ensabi and also their rate of germination, root length and shoot length in were decreased upon the application of both type of extractions. Results shown both species were susceptible to effect of extracts isolated from leaf, stem and root of ensabi and may utilization that allelopathy control the timing of barnyard grass germination and seedling establishment.

Keywords: Allelopathy, *Brassica juncea, Echinochloa crus-galli,* seed germination.

## Introduction

The current trend in agriculture production is to find a biological solution to reduce the perceived hazardous impacts from herbicides and insecticides (Khanh *et al*. 2005). The allelopathic properties of plants may act as a biological weed control mechanism in the agro-ecosystems and are effective tools to help resolve this critical issue (Xuan *et al*. 2005).

Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests. Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, insecticides, and other pesticides, reducing environmental soil pollution and diminishing autotoxicity hazards. There is a great demand for compounds with selective toxicity that can be readily degraded by either the plant or by the soil microorganisms which provide new strategies for maintaining and increasing agricultural production in the future (Faravani *et al.* 2008).

Research has shown that allelopathic effects can reduce seed germination and seedling growth. Like synthetic herbicides, there is no common mode of action or physiological target site for all allelochemicals. Allelochemical concentrations in the producer plant may also vary with time and the plant tissue. For example, foliar and leaf litter leachates of eucalyptus species are more toxic than bark leachates to some food crops (Iqbal 2005).

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Several examples have shown the potential of allelochemicals as herbicides and these phytotoxic compounds from plants are used in the production of new herbicides. Phytotoxic compounds from plants and microorganisms represent a wide range of chemistry and mechanism of action that have potential in the design and development of new herbicides (Duke 1987; Khalid 2002).

## Material and Methods

A laboratory study was conducted to assess the allelopathic potential of *B. juncea* var. Ensabi as a natural herbicide on radish (*Raphanus sativus* L.) and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) respectively as indicator plant and weed species to see if *B. juncea* var. Ensabi could exhibit any differences in the inhibition of barnyard grass growth and development. Plants of *B. juncea* var. Ensabi were harvested at a vegetative stage from Rimba Ilmu, University of Malaya, Kuala Lumpur (3$^{o}$ 8' N; 101$^{o}$ 42' E), Malaysia and were immediately washed with tap water to remove soil or other adhered material. Then fresh plant parts (leaf, stem and root) of ensabi were dried at room temperature. These parts were milled and homogenized. About 500 g of dried leaves, stems and roots of *B. juncea* var. Ensabi were subjected separately for extraction using ethanol as solvent in the Soxhlet extraction apparatus. The filtrate was concentrated under reduced pressure using the rotary evaporator (Aziz 2007).

Thirty radish and barnyard grass seeds were sown in 9 cm petri dishes lined with filter paper and 10 ml of each extract was applied separately. Treatment with distilled water was used as a control. The dishes were transferred to a growth chamber (set at 25 °C, 4000 lux, lit time: 09:00–17:00 h). Five replicates were maintained per concentration and plant part in a completely randomized manner in a growth chamber.

Germination, shoot and root lengths and dry weights were determined after 7 days for all treatments. The inhibitory magnitude of each plant part was averaged from their inhibitory levels on germination, root length, plant height and dry weight of the indicator plant.

## Results and Discussion

Effect of different ethanol extracts on radish seed germination:

Results showed all ethanol extracts obtained from the leaves, stems and roots of *Brassica juncea* var. Ensabi significantly ($p$<0.05) inhibited the germination of radish seed. The germination inhibition was highest (100%) at ethanol extract concentration of 30 g/L. Increase in concentration of the extracts led to a significant ($p$<0.05) decrease in final germination from 91% in the control to the lowest value (0.0%) with the application of highest ethanol extract concentration (Table 1).

Results revealed the increasing concentration of root, stem and leaf extracts significantly decreased the rate of germination from 24.3 to 0.0 seeds/day under control andwith the application of 30 g/L of ethanol extract respectively (Table 1). The highest mean period to final germination was observed at the concentration of ethanol extract of 18.0 g/L.

Ethanol extract concentration of 18.0 g/L inhibited the root length (83.7%) and shoot length (79%) of radish compared with the control. The extracts impeded the germination and caused abnormal root elongation and seedling abnormality. Analysis of variance indicated that the ethanol extract did not influence significantly ($p$<0.05) the total seedling dry matter.

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Effect of different ethanol extracts on barnyard grass seed germination:

Results showed that the germination of barnyard grass seed was inhibited significantly ($p$<0.05) when treated with the ethanol extract of the leaves or stem of *Brassica juncea* var. Ensabi (Table 2). Final germination percentage of barnyard grass decreased significantly ($p$<0.05) from 87.5 % with the application of distilled water (control) to 0.0 % with application of 30 gL$^{-1}$ ethanol extract of plant parts (Table 2).

Results indicated that the mean germination rate was 7.1 seeds /day in the control and significantly decreased with increasing concentrations of root and shoot ethanol extracts. The application of 10.8 g/L ethanol extract reduced the germination rate to 4.7 seeds /day while ethanol extract concentrations of 14.3 g/L had no significant effect ($p$<0.05). Table 2 shows that the mean period of final germination increased in response to application of ethanol extract but the differences were not significant at $p$<0.05. The same results were reported on allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination (Jefferson and Pennacchio 2003). Mean period of final germination with control was 4.1 days, which increased to 7.3 days with the application of 18 g/L plant parts extract.

Results revealed that increasing concentrations of *Brassica juncea* var. Ensabi plant parts ethanol extract significantly reduced root and shoot lengths of barnyard grass (Table 2). The root systems, especially root tips of barnyard grass, were stunted, swollen and shortened (92.0%) by ethanol extract at 18 g/L concentration.

## Discussion

Both root and shoot elongation of germinated seedlings was inhibited by the ethanol extracts. The ethanol extract of *Brassica juncea* var. Ensabi was even more potent and inhibited radish seeds.

Allelopathy can affect many aspects of plant ecology including occurrence, growth, plant succession, the structure of plant communities, dominance, diversity and plant productivity. Plants that germinate at slower rates are often smaller. This may seriously influence their chances of competing with neighbouring plants for resources such as water, especially in arid or semi-arid regions (Faravani 2008).

The allelochemicals present in *Brassica juncea* var. Ensabi extracts might have inhibited or stunted the growth of roots and shoots of germinates by at least two mechanisms, through the existence of phenolic compounds. These were identified and separated from *Brassica juncea* var. Ensabi extracts (Dafaalla 2004) and appear to have inhibited root elongation and cell division completely, with the result that the thickness of seedling roots was enlarged abnormally. It is thought that only the transverse growth of the root was maintained while longitudinal growth was greatly inhibited by the extracts and the phenolics (Chon *et al.* 2002). These phenomena may account for the results obtained in this study with the extracts of *Brassica juncea* var. Ensabi.

This research suggests that *B. juncea* var. Ensabi plant extracts significantly affected root growth and morphological differentiation of susceptible plants. Thus, the result on growing plants will be a reduction of plant biomass in the presence of either autotoxic or allelopathic compounds. These results may have value in enabling weed control based on these natural plant extracts. We have demonstrated that allelochemicals are produced in the shoot, root and leaf of *Brassica juncea* var. Ensabi. Such chemicals are both species-specific and concentration-dependent and these characteristics may influence the density

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and the composition of individual plant communities. Allelochemicals may directly prevent or promote germination when environmental conditions are conducive to growth and establishment, therefore, influencing the number of plants of each species in a community (Jefferson and Pennacchio 2003).

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# IMPACT OF SHADING AND DEFOLIATION ON VULPIA (*VULPIA* SPP.)

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## ABSTRACT

*Vulpia* species (*Vulpia bromoides*, *Vulpia myuros*) are annual grass weeds prevalent in southern Australian, dryland pastures. Vulpia provides poor quality forage and its seeds damage hides, carcasses and skins. Knowledge on how shading affects vulpia growth and survival can be used to develop grazing strategies aimed at vulpia suppression. A pot experiment was established to assess the impact of shading on vulpia growth by adjusting sward height. The swards (phalaris (*Phalaris aquatica*) and vulpia) were maintained at 3, 6, 12 or 24 cm to simulate continuous grazing at different grazing pressures, or defoliated to 2 cm once the sward reached 30 cm, to simulate rotational grazing. All treatments were compared to the control (vulpia without any shading / surrounding sward). Solar radiation and temperature at the soil surface, plus vulpia tiller numbers, panicle numbers and biomass were measured. There was a decline in solar radiation as sward height increased. Mean radiation levels in the simulated rotation were intermediate to the 12 and 24 cm treatments. However, the increase in shading in these treatments did not reduce vulpia panicle production. In contrast, vulpia panicles per plant were lower in 3 cm and 6 cm swards than in the control (16, 19 and 77 panicles per plant respectively). The most effective treatment was the rotation, or simulated grazing, which reduced tiller and biomass per plant when compared to the shorter swards (3 and 6 cm) and panicles per plant when compared to the control. A rotationally grazed system, with a combination of severe shading and defoliation, could suppress vulpia to a greater extent than in continuously grazed pastures, where solar radiation levels are higher and defoliation is less severe.

**Keywords:** vulpia, shading, defoliation, grazing management, grass weed, weed suppression.

## INTRODUCTION

*Vulpia bromoides* and *Vulpia myuros* are annual grasses which are prevalent in temperate pastures of Australia. These species have similar geographical distributions, co-occur in pastures and are collectively known as ‘vulpia’ (Dowling 1996). Vulpia provides poor quality feed and its seeds damage hides, carcasses and skins (Code 1996). Additionally, its allelopathic residues can impede germination and establishment of cereal crops and legumes (An *et al.* 2007; An *et al.* 1996).

Knowledge of vulpia ecology can be used to develop management strategies which favour sown perennial species and suppress vulpia. While research has been undertaken on vulpia germination, seedling establishment and interactions with moisture and nutrients, (e.g., Dillon & Forcella 1984; Dowling 1996; Ozanne *et al.* 1969; Scott & Blair 1987) less is

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known regarding the impact of shading on vulpia. Shading alone or in combination with severe defoliation can lead to plant mortality and reduce dry matter yield (Goldberg & Werner 1983; Wong & Stur 1996). Shading (low light intensity and low red/far red ratio) reduced ryegrass (*Lolium perenne*) vegetative and reproductive tiller production (Bahmani *et al*. 2000). When compared to undefoliated plants, defoliation of phalaris (*Phalaris aquatica*) to 6 cm doubled the percentage of light reaching emerging subterranean clover (*Trifolium subterraneum*) seedlings and increased their seedling weight (Dear *et al*. 1998). Pasture species such as phalaris have a large stature and leaf area and are easily able to overtop and shade vulpia when sward biomass is allowed to accumulate.

Shading can be manipulated in pastures by altering pasture biomass and height, which in turn is dependent on the grazing frequency and intensity. In continuously and intensively grazed pastures, there is less opportunity for sward biomass to accumulate, while in rotationally grazed pastures, sward biomass accumulates before the sward is defoliated to low levels. Under rotational grazing there is the potential for greater shading of vulpia.

To assess the impact of shading on vulpia growth and survival, a controlled microsward experiment was established. Microswards comprising potted phalaris plants surrounding a central potted vulpia plant were subjected to different defoliation regimes to simulate a range of defoliation intensities under continuous and rotational grazing. The hypothesis tested was that the regime with the greatest shading would be the most effective in suppressing vulpia growth and survival.

## METHODS

### Microsward Preparation

Vulpia seed (*V. bromoides*) was collected from a property near Vasey, Western Victoria in January, 2002. Vulpia seeds were sown on 10 July 2002 in potting mix in trays and kept in a glasshouse at ambient conditions. Six weeks later, individual seedlings were transplanted into 1.5 L (9 x 9 cm x 18 cm deep) pots containing potting mix. Phalaris (cv. Australian) seeds were sown (2 per pot) on 15 February 2002. Ten weeks after sowing, plants were transplanted into 1.5 L pots. To ensure that moisture and nutrients were not limiting, plants were grown on a capillary bed which provided sub-irrigation and slow release fertiliser was added as required according to label recommendations.

On 21 August 2002, a microsward was created by placing a pot of vulpia in the central cell of a 3 x 3 lattice and placing phalaris pots in the eight surrounding cells. Microswards were placed on the capillary bed. By this stage vulpia plants had between three and four leaves and phalaris plants were up to 30 cm in height. Keeping vulpia and phalaris in separate pots assured that root competition was not a confounding factor and that results could be more readily attributed to the different defoliation and solar radiation levels.

### Experimental design and treatments

The study was a randomised, complete block design with ten replicates of each treatment. There were four treatments in which the microswards were ‘continuously’ defoliated to 3 cm, 6 cm, 12 cm and 24 cm on day one and every seven days thereafter, until the final harvest on 11 December 2002. The increase in plant height during regrowth ranged from 5 to 10 cm per week. A fifth ‘rotation’ treatment was applied in which the microsward grew to approximately 30 cm in height and was then defoliated to 2 cm. Two defoliation events were possible under this regime, on 4 September and 20 November. There was a sixth

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control treatment in which vulpia was grown as a single potted plant, without any surrounding phalaris pots (i.e., no shading or defoliation).

### Measurements

At the final harvest, the numbers of tillers and panicles for each vulpia plant were counted. Additionally, vulpia plants were defoliated to ground level and the foliage oven dried for 72 hrs, at 70°C, to obtain dry matter estimates. Air temperature (°C) and solar radiation ($W/m^2$), both at the soil surface, were logged continuously, once an hour, for one replicate of all treatments. To obtain measurements, probes were placed on the soil surface of a central pot which had been filled with the potting mix but did not contain a vulpia plant. These data were used to obtain the average daily temperature and solar radiation over the experimental period. To convert $W/m^2$ to $MJ/m^2/day$, a daylength of 13 hours was assumed, which was the average day length over the experimental period, based on sunrise and sunset times.

Vulpia tiller and panicle number per plant and plant biomass were analysed with residual maximum likelihood function (REML). Data did not require transformation to normalise the variance. Temperature and solar radiation data were averaged over the experimental period (21 August to 11 December 2002). All analyses were performed using the statistical package GenStat 5.42 (GenStat 2000).

## RESULTS

All vulpia plants survived in all treatments, including the control.

**Table 1.** Effect of continuous defoliation to 3 cm, 6 cm, 12 cm, 24 cm, and a simulated rotation treatment, on vulpia tiller number, panicle number, and plant biomass at the final harvest in December 2002.

| Variable | 3 cm | 6 cm | 12 cm | 24 cm | Rotation | Control | P value |
|---|---|---|---|---|---|---|---|
| Tillers/plant | $524_{a}$ | $485_{abc}$ | $491_{ab}$ | $300_{cd}$ | $17_{d}$ | $420_{bc}$ | <0.001 |
| Panicles/plant | $15.6_{b}$ | $18.6_{b}$ | $37.1_{ab}$ | $43.1_{ab}$ | $17.1_{b}$ | $76.6_{a}$ | <0.001 |
| Plant biomass (g) | $6.6_{b}$ | $8.9_{b}$ | $9.7_{ab}$ | $7.2_{b}$ | $2.4_{c}$ | $13.6_{a}$ | <0.001 |

Means within the same row with the same letter are not significantly different (P<0.05).

When compared with the control, there were more vulpia tillers per plant in the 3 cm treatment and fewer tillers in the rotation treatment (Table 1). There were also fewer vulpia panicles in the 3 cm, 6 cm and rotation treatments than in the control treatment. Finally, vulpia biomass was less in the 3 cm, 6 cm, 24 cm and rotation treatment than the control. Greatest suppression of vulpia (panicle number, tiller number and plant biomass) occurred in the rotation treatment.

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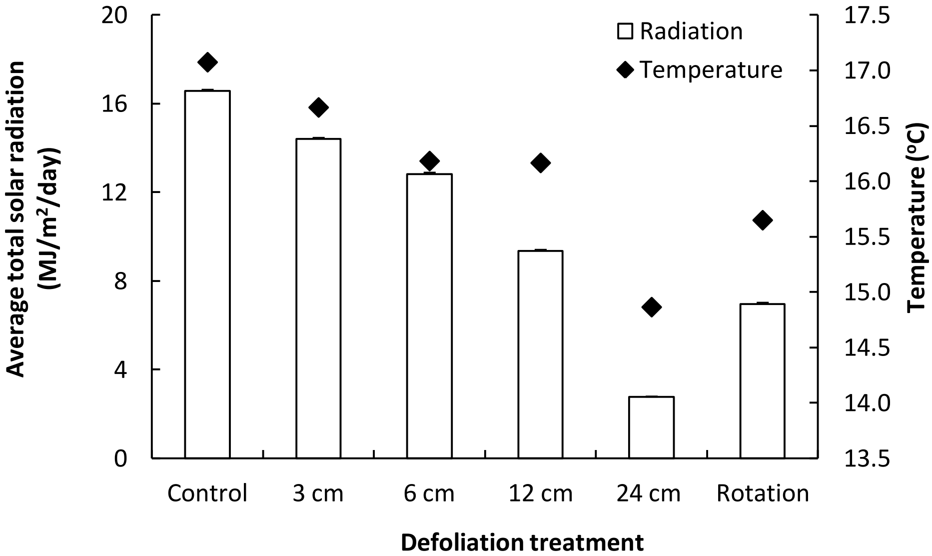


**Figure 1.** Average daily solar radiation (MJ/m$^2$/day) and average temperature (°C) at the soil surface, averaged over the duration of the experiment for the control, 3 cm, 6 cm, 12 cm, 24 cm and rotation treatments.

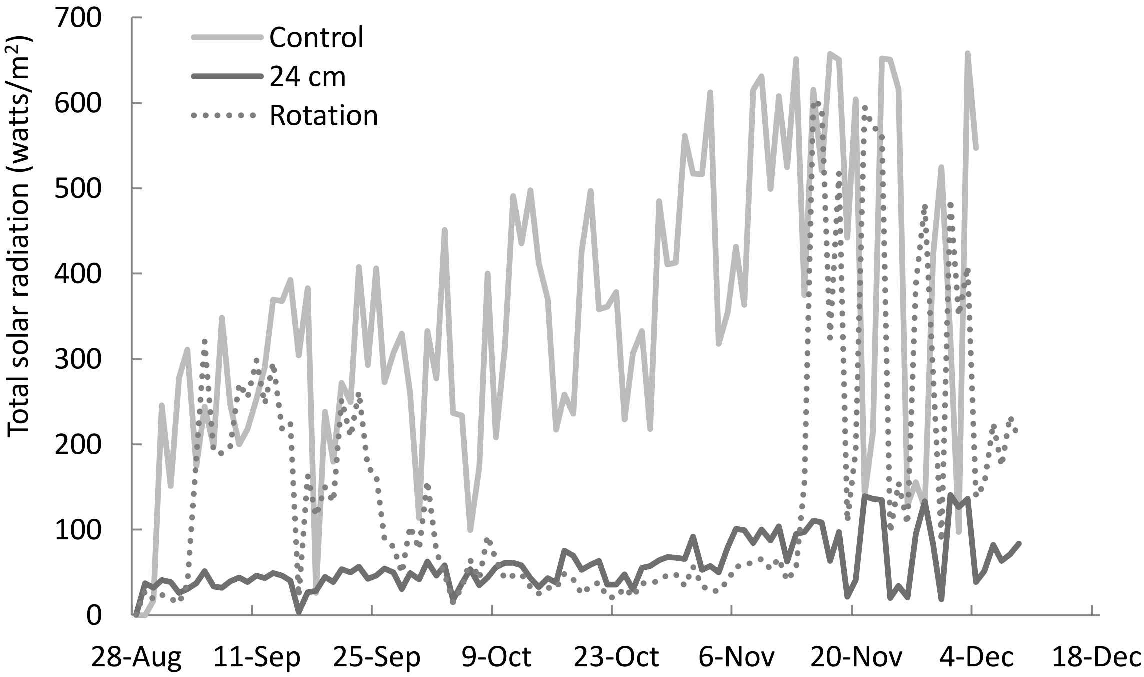


**Figure 2.** Average daily solar radiation (MJ/m$^2$/day) at the soil surface, for the control, 24 cm and rotation treatments.

Increasing defoliation height resulted in less solar radiation reaching the soil surface (Figures 1 and 2). When compared to the control, only 17% of average total solar radiation reached the soil surface in the 24 cm treatment. Solar radiation fluctuated in the rotation treatment according to stage of regrowth and dropped to radiation levels similar to in the 24 cm treatment well before the defoliation event on 20 November (Figure 2). Soil surface temperature also declined with increasing defoliation height and average temperature was lowest in the 24 cm treatment (Figure 1).

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## DISCUSSION

Vulpia growth and panicle production declined with increasing levels of shading. However, at the higher levels of shading (12 cm, 24 cm) such as would occur in some continuously grazed pastures with low grazing intensity, tiller and panicle production were no different to that of the control. Average temperatures declined with increased shading, and were also lower in the 24 cm treatment (≈ 15 $^{o}$C) than in the control (≈ 17$^{o}$C). This equated to a 12% reduction in temperature, which was much less than the 83% reduction in solar radiation. Solar radiation would appear to be more important than temperature in determining vulpia growth in this study.

In contrast, minimal shading with severe defoliation (3 cm treatment) resulted in the lowest plant biomass and fewer panicles, but more tillers. This is consistent with field studies. For example, in New South Wales temperate pastures, intense grazing of vulpia increased tiller production (Dowling & Kemp 1997).

A combination of severe defoliation and severe shading (rotation treatment) led to reductions in all three parameters: smaller plants with fewer panicles and fewer tillers. While reducing vulpia growth will eventually limit its ability to produce seed, preventing or severely suppressing panicle production is essential to eliminate vulpia from pastures in subsequent years.

Results from this microsward study are consistent with Grant and Rumball (1971), who found that barley grass abundance was less in rotationally than continuously grazed pastures. They are also in agreement with a Western Victorian field study in which vulpia was less prevalent, etiolated in appearance and subject to greater mortality in rotationally stocked than continuously stocked pastures (Tozer *et al.* 2008; Tozer *et al.* 2009). Pasture biomass accumulation was much greater in the rotationally stocked treatment (mean 3680 kg DM/ha/yr) than continuously stocked treatment (2120 kg DM/ha/year) in that field experiment (Chapman *et al*. 2003), which would have led to significantly greater shading of weeds occurring at the ground surface in the rotationally grazed pastures.

Interestingly, the combination of severe shading and severe defoliation was insufficient to kill vulpia plants in this study. There are at least two possible reasons for this. Firstly, water and nutrients were not limiting factors in this study. This is in contrast to the situation encountered in pastures, where plants are often subject to multiple stresses, including nutrient deficiencies, water deficits and shading or extreme heat. They are also subjected to defoliation and damage through trampling, dung deposition and insect damage. It is most likely that the combination of a number of these stresses, rather than shading and defoliation alone, causes vulpia mortality in temperate Australian pastures. Secondly, vulpia plants were six weeks old when treatments were imposed, and they may have been more susceptible to shading at an earlier stage. For example, mortality of *Vulpia ciliata* was particularly high during seedling emergence before the radicle had extended 2 mm (Carey & Watkinson 1993).

## CONCLUSION

While shading or severe defoliation suppresses vulpia growth and/or panicle production, the greatest suppression occurs with a combination of shading and severe defoliation. However, these two combined stresses are insufficient to kill vulpia when moisture and nutrients are not limiting plant growth. Grazing strategies that allow the accumulation of

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pasture biomass to increase shading of vulpia followed by severe defoliation have potential to suppress vulpia in perennial pastures.

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## REFERENCES

An, M.; Pratley, J.E.; Haig, T. (1996). Field application of vulpia phytotoxicity management: A case study. In: *Proceedings of the 8th Australian Agronomy Conference*, p. 616.

An, M.; Pratley, J.; Wu, H.W.; Liu, D.L. (2007). Deterioration of pasture quality due to allelopathy: the Australian experience. *Allelopathy Journal* 19: 37-47.

Bahmani, I.; Varlet Grancher, C.; Hazard, L.; Matthew, C.; Betin, M.; Langlais, A.; Lemaire, G.; Thom, E.R. (2000). Post-flowering tillering in contrasting light environments of two New Zealand perennial ryegrass cultivars with different perennation strategies. *Grass and Forage Science* 55: 367-371.

Carey, P.D.; Watkinson, A.R. (1993). The dispersal and fates of seeds of the winter annual grass *Vulpia ciliata*. *Journal of Ecology* 81: 759-767.

Chapman, D.F.; McCaskill, M.R.; Quigley, P.E.; Thompson, A.N.; Graham, J.F.; Borg, D.; Lamb, J.; Kearney, G.; Saul, G.R.; Clark, S.G. (2003). Effects of grazing method and fertiliser inputs on the productivity and sustainability of phalaris-based pastures in western Victoria. *Australian Journal of Experimental Agriculture* 43: 785-798.

Code, G.R. (1996). Why vulpia is a problem in Australian agriculture. *Plant Protection Quarterly* 11: 202- 204.

Dear, B.S.; Cocks, P.S.; Wolfe, E.C.; Collins, D.P. (1998). Established perennial grasses reduce the growth of emerging subterranean clover seedlings through competition for water, light, and nutrients. *Australian Journal of Agricultural Research* 49: 41-51.

Dillon, S.P.; Forcella, F. (1984). Germination, emergence, vegetative growth and flowering of two silvergrasses, *Vulpia bromoides* (L.) S. F. Gray and *Vulpia myuros* (L.) C. C. Gmel. *Australian Journal of Botany* 32: 165-175.

Dowling, P.M. (1996). The ecology of vulpia. *Plant Protection Quarterly* 11: 204-206.

Dowling, P.M.; Kemp, D.R. (1997). Annual grass weeds. In: *Technical Bulletin 47. Grazing management of temperate pastures: literature reviews and grazing guidelines for major species*. Eds. FitzGerald, R. D.; Lodge, G. M. NSW Agriculture, Orange. pp. 23-26.

GenStat (2000). *GenStat for Windows*. Release 4.2. Fifth Edition. VSN International Ltd. Oxford.

Goldberg, D.E.; Werner, P.A. (1983). The effects of size of opening in vegetation and litter cover on seedling establishment of goldenrods (*Solidago* spp.). *Oecologia* 60: 149-155.

Grant, D.A.; Rumball, P.J. (1971). The ecology and control of barley grass *Hordeum* spp.: a review. *Proceedings New Zealand Weed and Pest Control Conference* 24: 63-69.

Ozanne, P.G.; Keay, J.; Biddiscombe, E.F. (1969). The comparative applied phosphate requirements of eight annual pasture species. *Journal of Soil Science* 20: 809-818.

Scott, J.M.; Blair, G.J. (1987). Competition from *Vulpia myuros* (L.) C. C. Gmelin in pastures, and its control by coating seeds with herbicides. *Australian Journal of Experimental Agriculture* 27: 367-375.

Tozer, K.N.; Chapman, D.F.; Quigley, P.E.; Dowling, P.M.; Cousens, R.D.; Kearney, G.A. (2008). Effect of grazing, gap dynamics, and inter-specific seedling competition on growth and survival of *Vulpia* spp. and *Hordeum murinum* ssp. *leporinum*. *Australian Journal of Agricultural Research* 59: 646-655.

Tozer, K.N.; Chapman, D.F.; Quigley, P.E.; Dowling, P.M.; Cousens, R.D.; Kearney, G.A. (2009). Integrated management of vulpia in dryland perennial pastures of southern Australia. *Crop and Pasture Science 60*: 32-42.

Wong, C.C.; Stur, W.W. (1996). Persistence of tropical forage grasses in shaded environments. *Journal of Agricultural Science* 126: 151- 159.

# GLYPHOSATE RESISTANCE – THE RISKS AND IMPACTS FOR CROPPING IN THE SUB-TROPICAL REGION OF AUSTRALIA

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## ABSTRACT

Glyphosate has played a pivotal role in allowing widespread adoption of conservation cropping over the last two decades in Australia, as this herbicide provides reliable and broad-spectrum weed control in fallows and crop establishment. However, populations of five weed species have evolved glyphosate resistance in the sub-tropical cropping region of Australia. Modelling and field history have shown resistance becomes a noticeable problem after 15+ years of repeated use of glyphosate in reduced or zero-tilled fields with few or no alternative herbicides used and where seed-set on glyphosate-sprayed survivors is not prevented. Subsequently, poorer fallow weed control results in reduced stored soil moisture and increased weed seed-banks, resulting in lower crop yields. Glyphosate-resistant weeds can be controlled with other knockdowns, the double knock tactic (sequential application of glyphosate followed by another herbicide) and residual herbicides, although these can be more expensive and less robust than glyphosate. Once developed, glyphosate resistance will last for many years, as up to 5% of seed can remain viable for more than two years in the soil without new seed replenishment. Regular field monitoring and testing of glyphosate sprayed survivors for resistance are recommended. As well, growers can estimate the risk for developing glyphosate resistance for their current practices and crop sequences with the newly available Risk Assessment Tool, and thus adjust their practices to minimise risks for developing glyphosate-resistant weeds.

## Background

Glyphosate is the world's most widely applied non-selective herbicide, and has been used to control a broad spectrum of weeds in perennial crops, urban and industrial areas, prior to sowing annual crops, and post-emergent in transgenic annual crops since 1974 (Powles 2008). It is used extensively for fallow weed control in grain and cotton rotations in the sub-tropical cropping region on Australia, allowing the move towards conservation cropping systems with reduced or zero tillage practices. Evolution of glyphosate resistance in weeds was thought to be highly unlikely, until the world's first case of glyphosate resistance was detected in annual ryegrass (*Lolium rigidum*) in Australia (Pratley *et al.* 1996).

Herbicide resistance is an evolutionary event resulting from intense herbicide selection over genetically diverse weed populations (McGillion and Storrie 2006). It is defined as the inherited ability of an individual plant to survive a herbicide application that would kill a normal population of the same species. Herbicide resistance does not equate to poor performance of a herbicide, and resistant weeds can often survive application of herbicide at rates much higher than the recommended rate. Glyphosate resistance is naturally present in weed populations but at extremely low frequencies. However, intensive

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selection pressure with regular glyphosate spraying causes the susceptible individuals to be killed and the rare resistant individuals to survive, produce seed and over time gradually dominate the population.

Glyphosate resistance has been documented in five species in Australia (Preston 2011). Currently, there are 133 confirmed resistant populations of annual ryegrass in various cropping, horticulture and non-agricultural situations. In the sub-tropical cropping region, there are also 18 sites with glyphosate-resistant awnless barnyard grass (*Echinochloa colona*), first reported in 2007. In addition, glyphosate resistance was found in liverseed grass (*Urochloa panicoides*) in 2008, windmill grass (*Chloris truncata*) in 2010 and flaxleaf fleabane (*Conyza bonariensis*) in 2011. All of the glyphosate-resistant weeds in this sub-tropical cropping region were found in chemical fallows. This paper reviews and presents new research findings on the risks and impacts of glyphosate resistance in this region, and outlines strategies for resistant weeds.

## Glyphosate Resistance – The Risks

The majority of glyphosate-resistant grasses (awnless barnyard grass, liverseed grass, windmill grass) were found in fields with a long history of winter grain / summer fallow rotation (Preston 2011), and one glyphosate-resistant awnless barnyard grass population was found in a summer fallow / transgenic cotton rotation (Werth *et al*. 2010). The flaxleaf fleabane populations were in cropping areas, in which reduced or zero-tillage was practised for many years (Walker et al. 2011). The common factors consistently associated with these resistant populations are intensive use of glyphosate, few or no other effective herbicides used, and few or no tillage operations. Whilst the number of confirmed glyphosate resistant populations is relatively low, considerably more populations could be identified in the near future. Modelling predicted fields with 15+ years of zero-tilled, glyphosate-based summer fallows are at high risk of having a problem with glyphosate resistance (Thornby and Walker 2009; Figure 1). As these practices have been extensively used in this region over the last two decades, large areas are at risk.

## Glyphosate Resistance – The Impacts

The obvious impact of glyphosate resistance is this valuable herbicide is no longer effective in controlling these weeds in fallows and pre-plant knockdown. This is shown in our recent glyphosate dose response experiment confirming eight flaxleaf fleabane populations were resistant (Figure 2). Options for control of these resistant weeds include applying alternative knockdowns such as paraquat, using the double-knock tactic (sequential application of glyphosate followed by another herbicide) and / or residual herbicides (Widderick *et al.* 2010). The potential disadvantages of non-glyphosate alternatives are increased cost, reduced robustness, restrictions with re-cropping intervals for residual herbicides, and loss of the advantages associated with conservation cropping if using tillage. If not applied correctly, poorer weed control in the preceding fallows and prior to sowing will result in reduced stored soil moisture and increased competition with crops, which could then reduce crop yields. More surviving weeds will greatly increase the weed seed-bank, and thus create more problems in following fallows and crops, again potentially impacting on yield. Grasses can produce 2000-12,000 seeds per plant and fleabane up to 100,000 seeds per plant. Glyphosate resistance also puts the new technology of herbicide tolerant crops, Roundup Ready® cotton and canola, in jeopardy.

Glyphosate resistance is permanent in weed populations as long as resistant seeds remain viable in the soil (McGillion and Storrie 2006). Even with cessation of the use of

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glyphosate, the ratio of resistant to susceptible individuals will remain the same – only the total number of weeds present can be reduced assuming there is no fitness penalty associated with this trait. Seed persistence of grasses and fleabane is relatively short in zero-tilled systems. However, approximately 5% of grass seed remain viable for longer than two years in the top 5 cm of soil, and this increases to 20-25% remaining viable for over two years if seeds are buried to 10 cm (Walker *et al.* 2010). Even if the seed-bank is reduced to very low numbers, the return to exclusive use of glyphosate without follow-up actions on survivors will result in a major resistance problem within a very short period. Thus, cropping can be adversely affected by glyphosate resistance for many years.

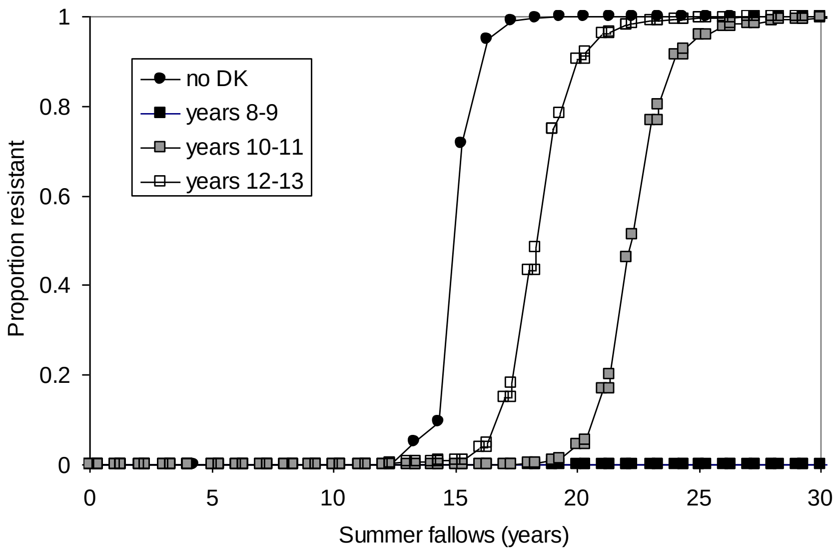


**Figure 1**. Simulation of glyphosate resistance evolution in awnless barnyard grass in fallows with weed control relying only on glyphosate (circle) or after introduction of the double-knock (DK) tactic (square) following different lengths of glyphosate only use.

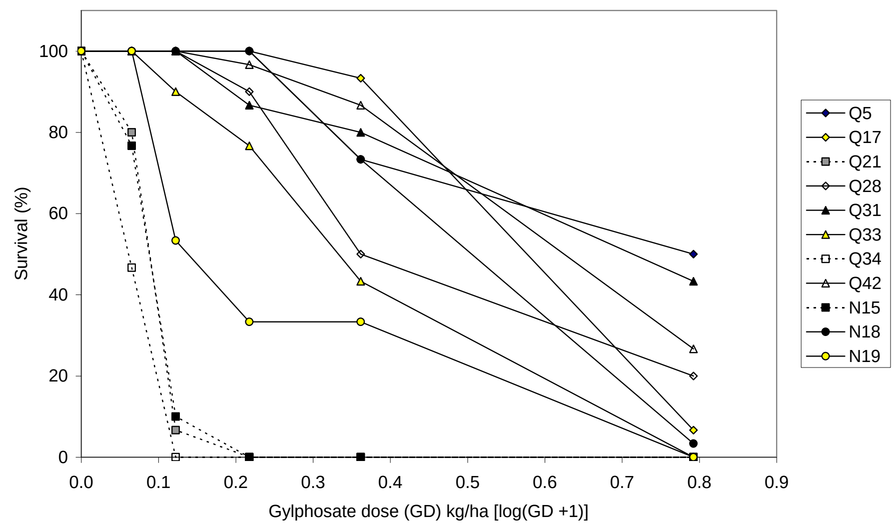


**Figure 2.** Survival of flaxleaf fleabane seedlings of three susceptible (dotted lines) and eight glyphosate-resistant (solid lines) populations following application of glyphosate at 0, 0.25, 0.5, 1, 2, and 8 times the registered rate of 0.65 kg/ha in a pot experiment.

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**Glyphosate Resistance – Avoidance And Management**

The risk of glyphosate resistance is reduced, and weed management can be markedly improved, if the principles of good crop agronomy and integrated weed management (IWM) are adopted (McGilion and Storrie 2006). This involves developing and implementing a strategy, where a range of chemical and nonchemical tactics target all parts of the weeds' lifecycles – depleting the seed-bank, effectively controlling seedlings, stopping seed-set on sprayed survivors and avoiding introduction of new weed seeds. An important component of the strategy is the double-knock tactic (Figure 1), which is highly effective in controlling glyphosate-susceptible and resistant species. To maintain glyphosate as a highly effective and reliable fallow weed control tactic, the risk for evolution of resistance needs to be reduced. This can be achieved by introducing preventive tactics, of which the number and extent needed is determined by the risk level. Fortunately, growers can now determine the risk level for their weeds and current practices using a simple on-line Risk Assessment Tool (Thornby 2010). Growers should consider having their weeds tested for glyphosate resistance if (a) their fields are assessed at moderate or high risk for glyphosate resistance, and/or (b) there are survivors of a glyphosate application. As glyphosate resistance appears initially in a few isolated, healthy plants, surrounded by dead plants of the same weed species, diligent monitoring of fields after treatment with glyphosate is important. It is essential to prevent these plants from setting seed, as within a few years glyphosate-resistant weeds will infest the whole field.

In conclusion, implementing an IWM strategy, undertaking a simple risk assessment, and routine monitoring will prevent development of glyphosate resistance. However, failure to adopt these preventive actions now is likely to markedly reduce cropping viability in the sub-tropical region of Australia in the near future.

**REFERENCES**

McGillion, T. and Storrie, A. (eds) (2006). *Integrated weed management in Australian cropping systems – a training manual for farm advisors*. Cooperative Research Centre for Australian Weed Management, Adelaide.

Powles, S. (2008). Evolved glyphosate resistant weeds around the world: lessons to be learnt. *Pest Management Science* 64: 360-365.

Pratley, J., Baines, P., Eberbach, P., Incerti, M. and Broster, J. (1996). Glyphosate resistance in annual ryegrass. *Proceedings 1996 Annual Conference of the Grasslands Society of New South Wales*, (eds J Virgona, D Michalk). Wagga Wagga, p. 126.

Preston, C. (2011). Australian Glyphosate Resistance Register. Published on-line www.glyphosateresistance.org.au (Adelaide).

Thornby, D. and Walker, S. (2009). Simulating the evolution of glyphosate resistance in grains farming in northern Australia. *Annals of Botany* 104: 747-756.

Thornby, D. (2010). Glyphosate resistance toolkit. Published on-line http://www.dpi.qld.gov.au/asp/glyphosate-resistance-toolkit.htm (Brisbane).

Walker, S.R., Bell, K., Robinson, G. and Widderick, M. (2011). Flaxleaf fleabane (*Conyza bonariensis*) populations have developed glyphosate resistance in north-east Australian cropping fields. *Crop Protection* 31: 311-317.

ISBN Number: 978-0-9871961-0-1

Walker, S.R., Wu, H. and Bell, K. (2010). Emergence and seed persistence of *Echinochloa colona*, *Urochloa panicoides* and *Hibiscus trionum* in the sub-tropical environment of north-eastern Australia. *Plant Protection Quarterly* 25: 127-132.

Werth, J., Thornby, D., Walker, S., Charles, G. and McDonald, C. (2010). Species shift and resistance: Challenges for Australian cotton systems. *Proceedings of 17th Australasian Weeds Conference*, Christchurch, pp. 20-23.

# A DECADE OF GLYPHOSATE-RESISTANT COTTON IN AUSTRALIA: WHAT HAS CHANGED?

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## ABSTRACT

Glyphosate-resistant cotton has been widely adopted in the Australian cotton industry since 2000. Weed management practices are now heavily reliant on glyphosate. Even before the introduction of glyphosate-resistant cotton varieties, glyphosate was increasingly being used. To evaluate any relevant weed issues resulting from this reliance upon glyphosate, we re-surveyed 50 fields previously surveyed in 2001. Results showed a species shift, with *Conyza bonariensis* and *Sonchus oleraceus* dominating the flora in cotton systems in 2010-2011. Other glyphosate tolerant species, such as *Convolvulus* sp., also increased in certain fields. *Hibiscus trionum*, *Ipomea lonchophylla* and *Echinochloa colona* were common in the majority of fields. In 2009, this survey resulted in identification of the first glyphosate resistant weed in Australian cotton systems, *E. colona.* This population exhibits 3-4 fold resistance to glyphosate. The presence of a glyphosate resistant grass has the potential to increase weed control costs in crop by $40 to $90/ha/yr as well as increasing fallow costs by up to $70/ha/yr. Research was undertaken to determine options for control of *C. bonariensis* and *E. colona* in cotton systems, using herbicide alternatives to glyphosate. This paper describes weed management challenges for the Australian cotton industry and explores chemical management options for glyphosate tolerant and resistant weeds.

**Keywords:** Glyphosate resistance, species shift, weed survey, Echinochloa colona.

## Introduction

Weed management practices in the Australian cotton industry have changed from systems based around tillage and residual herbicides, to minimum or zero-tillage systems, based on use of glyphosate and permanent beds in irrigated systems (Charles *et al*. 2004). Even before the introduction of glyphosate-resistant varieties in 2000 glyphosate was becoming commonly used for pre-plant knockdown applications, and shielded applications within the crop. In the fallow, glyphosate is heavily relied upon and has largely replaced tillage, particularly in non-irrigated cotton systems.

Previous surveys conducted in cotton-based farming systems (Charles *et al*. 2004, Walker *et al*. 2005) had already shown some shift towards glyphosate-tolerant, small seeded species favoured by frequent glyphosate use and little or no tillage. The introduction of glyphosate-tolerant cotton has created even more reliance on glyphosate.

We revisited fields that had previously been surveyed by Charles *et al*. (2004) and Walker *et al*. (2005) to identify any further changes to the weed spectrum since the introduction of glyphosate-resistant cotton. Glyphosate resistance has evolved in five species in the sub-

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tropical cropping region of north-east Australia - *Lolium rigidum* Gaud., *Echinochloa colona* (L.) Link, *Urochloa panicoides* P. Beauv., *Conyza bonariensis* (L.) Cronquist and *Chloris truncata* R. Br. The threat of glyphosate resistance became a reality in 2009 with a resistant *E. colona* population being identified in a glyphosate-resistant cotton rotation. The presence of a glyphosate resistant grass has the potential to increase in-crop weed control by $40 to $90/ha/yr as well as increasing fallow costs by up to $70/ha/yr (Werth and Thornby 2010). Experiments to further evaluate the resistant population and to determine the efficacy of glyphosate alternatives in cotton systems were undertaken.

## MATERIALS AND METHODS

### Field Surveys

Fifty fields were selected that had been previously surveyed, 26 from Walker *et al*. (2005) in non-irrigated cotton systems and 24 from Charles *et al*. (2004) in irrigated cotton systems. The fields were located in the Darling Downs and McIntyre valleys in Queensland, and the Gwydir and Lower Namoi valleys in New South Wales. Two surveys were done, the first at the start of the summer cropping season (November-December 2010) and the second at the end of the same season (February-March 2011). Surveys were performed in a similar manner to Walker *et al*. (2005). Transects with quadrats approximately 50 m apart were surveyed so that a total of 20 quadrats per field were surveyed. Quadrats were 10 m × 1 m. The presence and density of each weed species were noted in each quadrat with species density rated using the scale 0-3: 0 = no weeds 10/m$^2$; 1 = 1-9 weeds 10/m$^2$; 2 = 10-100 weeds 10/m$^2$; 3 = >100 weeds 10/m$^2$.

### *E. colona* trials

A dose response experiment was undertaken at the location of the resistant *E. colona* infestation. Glyphosate rates were 0, 45, 90, 180, 360, 720 and 1440 g ae glyphosate/ha with a spray volume of 75 L ha$^{-1}$. Two seedling sizes were present in field at the time of application and were measured at approximately 4 leaf stage and 3 tiller stage. Survival counts were taken 21 days after treatment (DAT).. At another suspected resistant *E. colona* site near Dalby (where we are currently characterising this population for resistance) a range of residual herbicides were tested for their effect on *E. colona* emergence. These included metolachlor (2 L/ha), pendimethalin (3.3 L/ha), norflurazon (1 kg/ha), prometryn + fluometuron (Convoy® 2.9 kg/ha) and diuron (2 kg/ha). Emergence counts were taken in 5 x 0.5 m$^2$ quadrats 10 days after each rainfall.

## RESULTS AND DISCUSSION

### Field surveys

A major change was observed in weed population dynamics since the earlier surveys were conducted in 2005; this was the considerable increase in *C. bonariensis*. In the surveys conducted by Walker et al. (2005) (Table 1) *C. bonariensis* was ranked at 14$^{th}$ in prevalence in field sites evaluated, compared to 1$^{st}$ in 2010. *C. bonariensis* did not rank in the top 20 in surveys conducted by Charles *et al.* (2004) and in 2010 was also ranked 1$^{st}$ (Table 2). *C. bonariensis* is particularly adapted to no-till systems based on glyphosate. It has long been considered difficult-to-control, and now has been confirmed resistant to glyphosate. It only emerges from the top 0.5 cm of soil, and thrives in a no-till system due to its ability to survive glyphosate based herbicide applications, and its capacity to produce over 100 000 seeds per plant (Wu *et al*. 2007).

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The reduction in tillage and subsequent reliance on glyphosate in irrigated cotton systems was noted when comparing species present in 2010-2011 with surveys conducted by Charles *et al*. (2004). *C. bonariensis* prevalence increased dramatically, particularly in the non-cotton phases of the rotation where virtually no tillage is performed. *Sonchus oleraceus* was ranked 13$^{th}$ in the fields surveyed by Charles *et al*. (2004), and increased to 3$^{rd}$ in 2008 and 2$^{nd}$ in 2010. It was also ranked highly in the Walker *et al*. (2005) survey. Like *C. bonariensis*, S. oleraceus is a small seeded asteraceae that is favoured by no-till cropping systems as it virtually only germinates from the soil surface. It has the ability to germinate year round, making it a difficult weed to get season long control.

**Table 1.** Comparison of top 10 weeds present in fields surveyed by Walker *et al.* (2005) and Charles *et al*. (2004) with the 2010-11 surveys, including presence and mean density rating of weeds at the start and end of the 2010-11 season.

| Rank | Weed Species | | Start of season | | End of season | |
|---|---|---|---|---|---|---|
| | 2001 | 2011 | % fields present | Density rating | % fields present | Density rating |
| | Fields previously surveyed by Walker et al. (2005) | | | | | |
| 1 | *Hibiscus trionum* | *Conyza bonariensis**+ | 88 | 0.6 | 68 | 0.4 |
| 2 | Sonchus oleraceus+ | Sonchus oleraceus+ | 76 | 0.7 | 63 | 0.4 |
| 3 | Portulaca oleracea | Hibiscus trionum | 59 | 0.2 | 74 | 0.3 |
| 4 | Tribulus spp. | Echinochloa colona | 47 | 0.5 | 37 | 0.6 |
| 5 | Amaranthus macrocarpus* | Convolvulus sp*+ | 41 | 0.2 | 21 | 0.2 |
| 6 | Echinochloa spp. | Ipomea lonchophylla*+ | 29 | 0.2 | 32 | 0.3 |
| 7 | Ipomea lonchophylla*+ | Chamaesyce drummondii | 24 | 0.1 | 21 | 0.2 |
| 8 | Convolvulus erubescens*+ | Phalaris paradoxa | 24 | 0.3 | - | - |
| 9 | Chamaesyce drummondii | Rhyncosia minima | 24 | 0.1 | 32 | 0.3 |
| 10 | Urochloa panicoides | Sisymbrium thellungii | 24 | *0.1* | 5 | 0.2 |
| | Fields previously surveyed by Charles et al. (2004) | | | | | |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | *Ipomea lonchophylla**+ | *Conyza bonariensis**+ | 80 | 0.2 | 59 | 0.3 |
| 2 | Hibiscus trionum | Sonchus oleraceus+ | 60 | 0.2 | 55 | 1.0 |
| 3 | Cyperus rotundus+ | Ipomea lonchophylla*+ | 53 | 0.4 | 41 | 0.3 |
| 4 | Echinochloa colona | Convolvulus sp.*+ | 47 | 0.1 | 23 | 0.3 |
| 5 | Rhyncosia minima*+ | Amaranthus macrocarpus* | 40 | 0.1 | 14 | 0.1 |
| 6 | Cullen cinereum*+ | Hibiscus trionum | 40 | 0.4 | 50 | 0.5 |
| 7 | Gossypium hirsutum* | Chamaesyce drummondii | 33 | 0.4 | 18 | 0.2 |
| 8 | Physalis sp | Cullen sp* | 33 | 0.1 | 9 | 0.2 |
| 9 | Datura ferox | Echinochloa colona | 33 | 0.2 | 27 | 0.4 |
| 10 | Neptunia gracilis*+ | Medicargo polymorpha | 20 | 0.3 | - | - |

*Not on Roundup Ready® herbicide label
+Weeds that have a naturally high level of tolerance to glyphosate (Charles et al. 2004)

**_E. colona_ trials**

The field testing of the potentially resistant QBG4 population revealed differences in plant growth only between the two plant sizes of 4 leaf and 3 tillers at lower rates (Figure 1A). At the highest rate of 1440 g glyphosate/ha, both seedling sizes had approximately 1.5% survival. This rate is equivalent to 1.44 kg/ha of Roundup Ready® Herbicide (690 g ae glyphosate/kg formulation) which is registered for use in glyphosate-resistant (Roundup Ready Flex®) cotton. This population exhibited a 3-4 fold resistance to glyphosate (Werth *et al*. 2010), so higher rates of glyphosate may provide control of small plants under ideal conditions. However, if plant size increases and/or conditions aren't favourable, the highest rate of Roundup Ready® herbicide allowed in Roundup Ready Flex® cotton (1.5 kg/ha) will provide less effective control. Therefore applications withheld until later in the season in order to control multiple cohorts with the one application may increase the risk of poor control on resistant plants in crop, particularly if there are larger plants present.

The residual herbicides evaluated were able to minimise *E. colona* emergence (Figure 1B), even in a season with higher than average rainfall. Higher rainfall did influence persistence of herbicides such as Metolachlor, Diuron, and Prometryn + Fluometuron, all of which had similar *E. colona* emergences to the untreated at 65 DAT . Pre-emergence residual and post-emergent herbicides from different mode of action groups are still vitally important tools in a glyphosate-resistant cotton production system. Tillage in the form of inter-row cultivation, bed preparation and management of irrigation furrows is suggested for inclusion in an IWM program to ensure the sustainability of glyphosate in cotton systems.

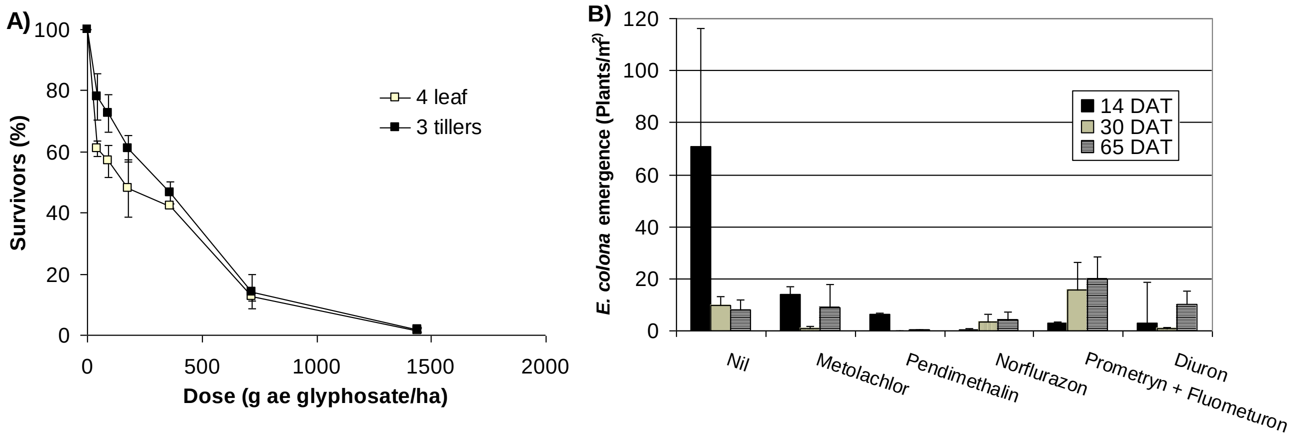


**Figure 1.** A) Dose response of the "resistant" QBG4 *E. colona* population to glyphosate in the field. B) Effect of residual herbicides on *E. colona* emergence in the field.

The Australian cotton industry faces real challenges with the ever growing problem of glyphosate resistant *C. bonariensis* and *E. colona*. The value of glyphosate-resistant technology to the cotton industry has not diminished, as glyphosate is still an important herbicide for managing a broad spectrum of weeds within crop that were much harder to manage before its introduction. However, the findings of our survey of weed infested cotton fields suggest that alternatives to glyphosate are still incorporated as part of an integrated weed management (IWM) program.

## REFERENCES

Charles, G., Taylor, I. and Roberts, G. (2004). The impact of the cotton farming system on weed succession: implications for herbicide resistance and adoption of an integrated weed management approach. *Proceedings of the 14th Australian Weeds Conference*, Wagga Wagga NSW, 410-413.

Walker, S.R., Taylor, I.N., Milne, G., Osten, V.A., Hoque, Z. and Farquharson, R.J. (2005). A survey of management and economic impact of weeds in dryland cotton cropping systems of subtropical Australia. *Australian Journal of Experimental Agriculture* 45, 79-91.

Werth, J., Thornby, D., Walker, S., Charles, G. and McDonald, C. (2010). Species shift and resistance: Challenges for Australian cotton systems. *Proceedings of 17th Australasian Weeds Conference*, Christchurch NZ, 20-23.

Werth, J and Thornby, D. (2010). Herbicide resistance – prevention is better than cure. *Proceedings of the 15th Australian Cotton Conference*, Broadbeach, Qld. http://www.australiancottonconference.com.au/resources.

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# PRODUCTION OF BIOACTIVE NAPTHOQUINONES BY ROOTS OF PATERSON'S CURSE (*ECHIUM PLANTAGINEUM*) – IMPLICATIONS FOR INVASION SUCCESS?

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## ABSTRACT

Paterson's curse roots were studied with respect to their ability to produce coloured secondary products, napthoquinones, in living roots. Young roots produced large quantities of red coloured anthro- or napthoquinones in outer layers of root periderm. In contrast, mature or aged roots exhibited blackened periderm containing accumulations of dark coloured secondary products, possibly due to oxidation or polymerization of these compounds over time. Ethanolic extracts of young root periderm tissues were bright red or pink in colour and contained several unusual napthoquinones, including acetylshikonin, and 1,3 dihydroxy -3- methylanthraquinone, as detected by LC/MS and GC/MS analyses. Mature or aged root extracts were colourless in appearance, and contained 1,3 dihydroxy-3-methylanthraquinone and other likely related constituents. Production of napthoquinones and colour of root extracts was clearly influenced by location of harvest of Paterson's curse, time of harvest and age of root tissue. Both young and aged root extracts exhibited strong inhibition of root growth of annual ryegrass, with young root extracts showing greatest phytotoxicity. Shikonin at 1 mg/ml was also phytotoxic to annual ryegrass growth, with increasing phytotoxicity noted with increasing shikonin concentration. The role of napthoquinones in plant invasion and their interactions in the plant rhizosphere require further elucidation, as novel napthoquinones exhibit potent antimicrobial, fungitoxic, and phytotoxic activity due to their impact on electron transport and cellular respiration processes.

**Keywords**: bioactive secondary products, periderm, rhizosphere, allelochemicals, antifungal, antimicrobial

## INTRODUCTION

Although our understanding of the physiology and metabolism of roots has improved in the last decade, the processes mediated by roots in the rhizosphere, including root exudation and deposition, are not yet well understood (Walker *et al.* 2003). In addition to the provision of mechanical support and transport of solutes, roots also synthesize and secrete a multitude of metabolites over time (Bertin *et al*. 2003, Brigham *et al*. 1999). Roots can release these metabolites over time, and they may play roles in defense and rhizosphere signaling (Uren, 2000; Watt and Weston 2009). Secondary products from root exudates and leachates can also influence soil microbial dynamics in the rhizosphere (Mathesius and Watt 2010). They also repel herbivores and pathogens, stimulate

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symbiotic relationships, alter soil properties, and inhibit the growth of competing plants (Bertin *et al.* 2003, Nardi *et al.* 2000, Walker *et al.* 2003).

In terms of ecological interactions, the roots of one plant can compete in the rhizosphere with their neighbours for space, water, nutrients, gases and organic materials that serve as metabolic substrates (McCully 2005, Ryan and Delhaize 2001). When roots are under stress, they can react by releasing small molecular weight compounds that are involved in plant defense. These negative forms of communication have received relatively little attention in the literature (Weston and Duke 2003, Watt and Weston 2009).

It has been reported that invasive plants use allelochemicals as novel chemical weapons to increase the plant's ability to interfere with its neighbours. This hypothesis was described initially by Callaway and Aschehoug (2000) as one mechanism influencing exotic plant invasion. Although these interactions are difficult to quantify and their impacts in the field are not well established, many invasive plants appear to utilise allelopathy or root exudation as a means to further compete and increase invasive interference (Douglass *et al*. 2011).

Paterson's curse (*Echium plantagineum*) is an introduced invasive weed species naturalized across Australia (Piggin 1982). It was originally introduced as a companion plant in the mid 1800's and later spread as an accidental contaminant of pasture seed and hay. Originally a native of Portugal or Spain, Paterson's curse has now been successfully naturalized over 30 million hectares of grazing land in Australia (Grigulis *et al.* 2001, Piggin 1982). In its native range, it occurs in mixtures with other forbs with similar growth habits and morphological traits, rendering it less competitive. However, it is now estimated to cost the Australian wool and meat industries over A$125 million per year (Carter 2009), due to reductions in pasture quality as well as direct toxicity to livestock. Paterson's curse produces pyrollizidine alkaloids that cause liver, kidney and lung damage, and eventual death in horses, sheep and cattle (Peterson and Jago 1984). Consumption may have serious impacts upon wool quality and weight in subsequent years (Pratley 1991, Rast 2006). Management of this weed has been sporadic and appears to be dependent upon successful spread of biocontrol agents and optimal environmental conditions (Cowie, 2006; Scott and Kenneally, 1981).

To date, little attention has been focused on the root system of Paterson's curse and below ground interactions. The plant exists either as an annual or biennial (Grigulis *et al*. 2001), and can survive long periods of drought due to a deep taproot. We have also observed, as widely reported, that the plant germinates throughout the winter months in NSW, forming flat rosettes which persist for months or longer, supported by a deep tap root and smaller lateral roots. Upon collection, we noted the strikingly dark colouration of the taproot and sometimes its lateral roots. Thus, we performed a series of microscopic and chemical evaluations and herein, report on our observations.

## MATERIALS AND METHODS

### Microscopy

Paterson's curse plants were collected from field sites in Coolamon and Wagga Wagga NSW from 2008-2011. At each location, 10-20 plants were collected in June and July (mid-winter) and again in August and September (early spring), once flowering had commenced. Plants in each location grew in mixed pasture settings of annual and

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perennial grasses, along with common weeds. Both sites were characterised by alluvial soils with primarily Devonian granite composition, classifed as red earth soils with a loamy sand base and low percentage of clay. After removal, plant roots were washed thoroughly in tap water and blotted dry before processing. Plants were observed microscopically in 2008-2011 using a standard dissecting microscope (Zeiss Corporation, Jena FRG) or an optical bright field light microscope with fluorescent imaging (Nikon A1 confocal microscope, Nikon Australia, Lidcombe NSW 2141). Roots were dissected using a scalpel blade and trimmed to form thin cross sections of tap roots as well as lateral roots. Longitudinal sections of tap and lateral roots were also evaluated.

Chemical extraction

In 2008-2011, roots of Paterson's curse plants were collected before and after flowering as described above. After washing, thin peridermal peels of both tap and lateral roots were collected by removing periderm using a sharp scalpel blade. Root periderm could be distinguished from underlying tissues due to their colour which was blackened or bright red/violet in most cases. Older tap roots were darkened in appearance and were separated from younger tap and lateral root tissues which were red/violet in colour. At each sampling, 2 g fresh weight of older tap roots, younger tap roots and lateral roots were collected and processed separately from each location. After weighing, root samples were extracted in 100% ethanol (2 g tissue/5 ml ethanol). Young roots were extracted a second time to further remove coloured constituents. All extracts were filtered through 0.2 µm syringe filters.

Chromatography

In 2008-2010, extracts were subjected to thin layer chromatography (TLC) using silica gel preparative plates treated with fluorescent detector developed in 6:1 chloroform:methanol. In 2010-2011, extracts were further subjected to evaluation using gas chromatography coupled to mass spectrometer equipped with a 30 m Agilent DB 5 column (GC/MS; Agilent 6890N benchtop, standard conditions) and also liquid chromatography/mass spectrometry (LC/MS) (Agilent Triple Quad MS system / UPLC). Mobile phase for all LC/MS analyses was a gradient of 50% acetonitrile:water to 100% acetonitrile over 15 minutes at 1ml/minute on an mRP 18 column (Agilent, 0.5 x 100 mm) under positive and negative ionization conditions. Standards were obtained for comparison/validation and included shikonin (0.1mg/ml) and acetylshikonin (0.1 mg/ml) (Sigma Aldrich Pty, Sydney Australia).

Chemoassay

Small glass petri dishes were used to assess the inhibitory activity of young, mature and old root extracts, as well as shikonin, upon radical elongation of annual ryegrass seedlings. Annual ryegrass seed was collected locally in 2010. Young, mature and aged, darkened root extracts were obtained by collecting Paterson's curse in May 2011 in Coolamon NSW and preparing 2 g of fresh washed roots as described above. Roots were placed in 5 ml of ethanol and each extract was filtered using a 2 µm filter to remove debris and particulates. Filtered extracts at 0.5 ml quantities were placed on filter paper (Whatman 10) placed in glass petri dishes. Ethanol was allowed to evaporate for 1 hour. Ten ryegrass seeds were placed in each dish, followed by the addition of 0.5 ml of distilled deionized water. Treatments were replicated three times for a total of 30 seedlings measured per treatment. Dishes were stored at 24°C temperature for 5 days and radical elongation was measured and recorded. Seedling growth inhibition was assessed in comparison to the untreated control, as a percentage of radical length inhibition.

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## RESULTS

In 2008 and 2009, Riverina NSW experienced significant drought. Collected plants in 2008-2009 were generally smaller with roots darkened in appearance, yielding highly coloured extracts. In 2010 and 2011, moisture availability in soils was improved due to rainfall, and plant growth was more luxuriant, but roots and extracts less coloured. In 2008 and 2009, young root extracts were pink rather than red, indicating limited extraction of coloured constituents (Table 1). Mature root extracts were uncoloured in each year and season of collection. As plants matured, roots produced red extracts after flowering (August/September) in comparison to root extracts collected in June/July.

Upon closer inspection of roots under a dissecting microscope, the peridermal layers of root tissues were coloured, with underlying tissues white or cream coloured. The peridermal layer was black or brown in oldest roots, with younger taproots and laterals exhibiting red coloured periderm, due to the presence of secondary products. In 2008-2009, this layer was bright red in younger root tissues. Upon closer examination, the periderm consisted of several layers of highly coloured cells, with each cell exhibiting reddened vacuoles or vesicles with red cellular constituents. In the oldest portion of the root, periderm layers had begun to disintegrate, exhibiting cellular extrusion and sloughing. These cells contained black constituents. Extracts of young roots were pink or bright red, depending on concentration of coloured constituents. Extracts from the oldest roots remained colourless, despite the darkened appearance of root peels; dark constituents were insoluble in ethanol. Young root extracts contained multiple fluorescent constituents and a high concentration of two to three napthoquinones, depending on the sample. Analysis by GC/MS showed 20 major and minor constituents, including napthoquinones.

LC/MS also confirmed presence of napthoquinones. Under negative ionization mode, several compounds with molecular masses of 204 to 383 were detected. Paterson's curse, a member of the Boraginaceae, produces napthoquinones similar to those of the related *Lithospermum* spp. (Brigham *et al.* 1999). Root extracts of Paterson's curse contained compounds which co-eluted with shikonin standards, suggesting their presence in the extracts. Additional GC/MS study revealed 1,8 dihydroxy-3-methylanthraquinone, a napthoquinone with a molecular weight of 383. Extracts of younger roots also contained anthraquinones. LC/MS analysis of coloured extracts revealed the presence of small amounts of shikonin (molecular weight 288), and larger amounts of acetylshikonin (330) and a related compound (356), along with unknowns. Aged roots did not contain shikonin but other anthraquinones were present. GC/MS analysis of these samples revealed the presence of 1,8 dihydroxy-3-methylanthraquinone.

Young root extracts proved highly inhibitory to seedling root growth of annual ryegrass in petri dish assays (70% inhibition), and mature and aged root extracts were also inhibitory. Shikonin also exhibited phytotoxicity at the concentration of 1mg/ml (50% inhibition), and 0.1 and 0.01 mg/ml treatments still produced observable inhibition. A single wash with ethanol removed the majority of inhibitors, as a second subsequent extraction resulted in limited inhibitory activity (17%).

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**Table 1**. Level of total shikonin derivatives in extracts of young Paterson's curse roots in ethanol, as influenced by season and year, by colourimetric evaluation of 2 g fresh tissue/ 5 ml ethanol. Dark red colour indicates high concentration of total shikonins whereas colourless solutions indicate low levels of shikonins. NA=not available.

| Year | June/July collection | August/September collection |
|---|---|---|
| 2008 | pink | dark red |
| 2009 | deep pink | dark red |
| 2010 | colourless | deep pink |
| 2011 | pink | NA |

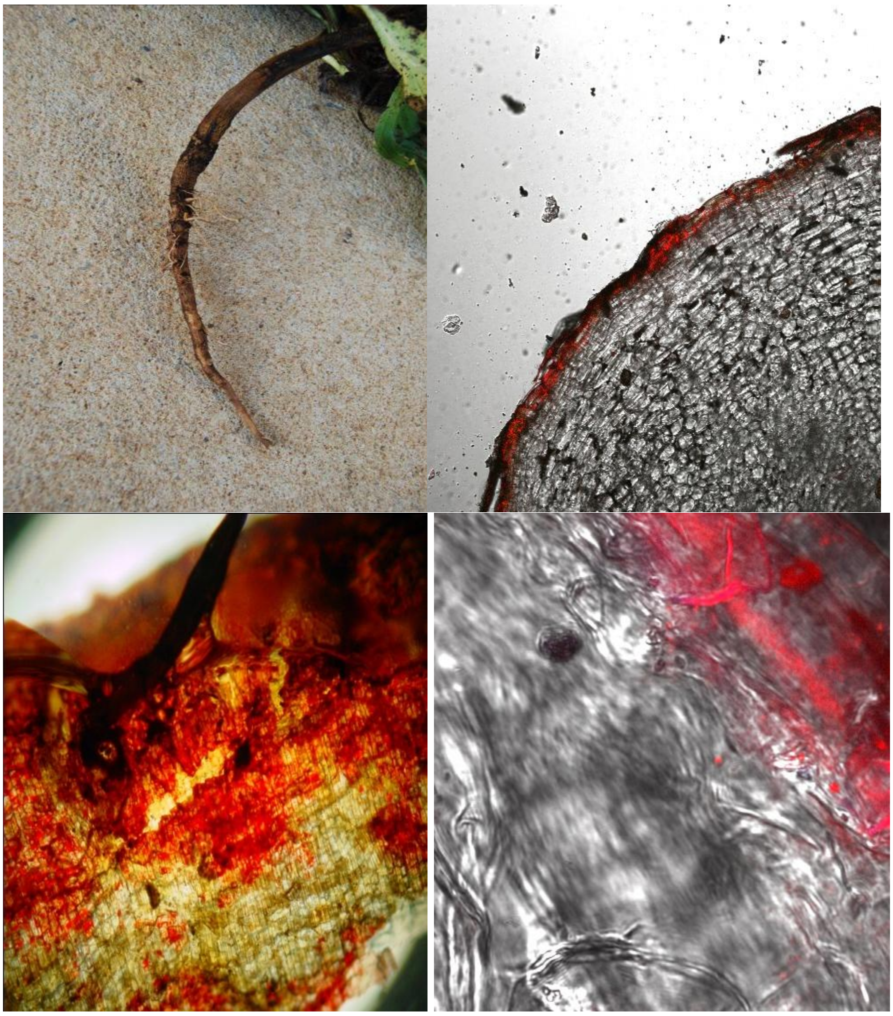

**Figure 1.** Clockwise from top left. Paterson's curse taproot showing characteristic darkened or reddish appearance of root periderm in 2009. Root periderm cross section from young Paterson's curse root, collected in 2010 and viewed with confocal microscopy, 40X. Root periderm cross section showing close up of periderm cell and red coloured constituents with confocal microscopy, 100X. Light microscopy of Paterson's curse root periderm cross section, 80X.

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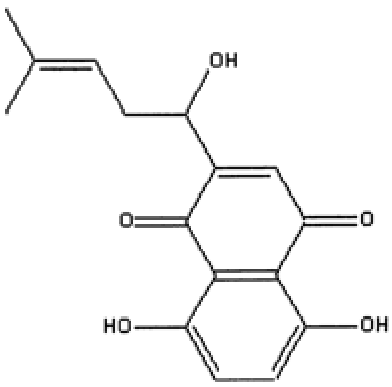


**Figure 2.** Structure of shikonin, a napthoquinone with a molecular weight of 288.3, and molecular formula of $C_{16}H_{16}O_5$.

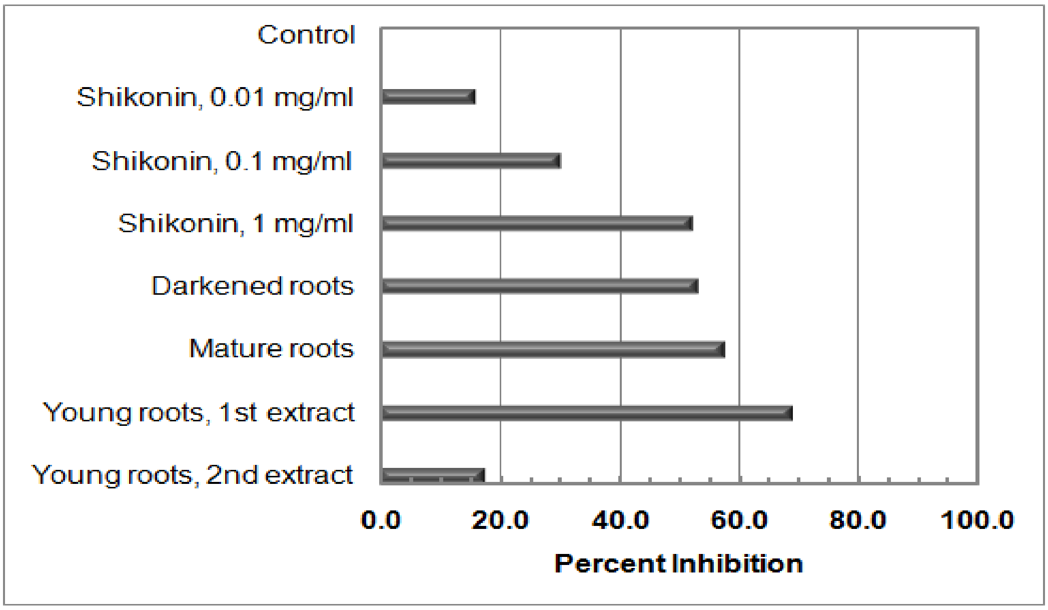


**Figure 3.** Percent inhibition of annual ryegrass radical elongation, in comparison to the control, by extracts of young roots, mature roots and aged, darkened roots (2 g fresh weight root tissue/5 ml ethanol) and shikonin at three concentrations. Observations based on a mean of 30 seedlings/treatment.

## DISCUSSION

Based on review of the published literature, the production of red coloured secondary products in plants or animals is rare with only a few uniquely bright red in colour, similar to those in Paterson's curse roots. Besides anthocyanins, haeme or haemoglobin-like molecules, there exist anthraquinones or napthoquinones which are red and, upon oxidation, purple in colour. These napthoquinones are produced by roots of several Boraginaceae. The production of red-coloured constituents, shikonin derivatives or napthoquinones is reported in cultures or living roots of *Lithospermum erythrorhizon,* a member of the Boraginaceae (Tabata and Fujita 1985). Shikonin is a red precursor to many related active napthoquinones, and its production can be induced in root cultures, by stressful conditions or pathogenic fungi (Brigham *et al*. 1999; Tabata and Fujita 1985).

Napthoquinones are used as dyes, colourants or as medicinal products. Some related naptho- or furanoquinones have been reported to be cytotoxic, anti-inflammatory and

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antimicrobial (Brigham *et al.* 1999). Napthoquinones such as juglone function as allelochemicals and inhibit plant growth, and have also been shown to inhibit electron transport or respiration (Binder *et al*. 1989). Shikonin and its derivatives were shown to be strongly elicited in *Lithospermum* root cultures by the presence of fungi such as *Rhizoctonia solani*, *Pythium aphanidermatum* and *Nectria hematococca* (Brigham *et al.* 1999). Root cultures contained several napthoquinones with strong antimicrobial activity against cultured soil microbes. *In situ* these compounds are produced and released by living root hairs of *L. erythrorhizon*, which turn red as compounds accumulate (Brigham *et al.* 1999; Tabata and Fujita 1985).

We observed that red napthoquinones are produced by living Paterson's curse roots, and localised in the periderm of young roots. Upon aging, the periderm becomes blackened and coloured constituents are insoluble in ethanol. The root extracts of young roots are also highly inhibitory to seedling growth, in assays with annual ryegrass, a common competitor in NSW pastures. Young root extracts were inhibitory at 100 µg/ml of napthoquinones or less, judging by colour and concentration of these in extracts, which were later subjected to LC/MS and GC/MS. Napthoquinones present included acetyl shikonin, shikonin and 1,8 dihydroxy-3-methylanthraquinone. Environment and genotype also influenced production of these constituents. Extracts of aged roots were also inhibitory and contained napthoquinones but were uncoloured. Additional studies will further elucidate structures of active constituents in extracts of both young and old roots.

Both GC and LC/MS studies indicated the presence of shikonin derivatives, similar to those produced by *Lithospermum* spp. (Brigham *et al*. 1999; Tabata and Yashita 1985). In *Lithospermum* spp., napthoquinones and shikonin derivatives were produced in association with the endoplasmic reticulum and later transported to the cell membrane where they bind to cell wall constituents, turning the entire cell a reddish colour (Tabata and Fujita 1985). We noted the same pattern of distribution in younger roots of Paterson's curse. In contrast to *Lithospermum* spp. however, these compounds are evidently produced in the periderm of Paterson's curse but not in living root hairs as is the case in *Lithospermum* spp. (Brigham *et al*. 1999). Root extracts contain not only shikonin, but several related compounds, including acetylshikonin and another napthoquinone, identified based on comparative analysis with published reports elucidating generation of molecular ions and fragmentation patterns using LC-MS (Brigham *et al*. 1999; Tabata and Fujita 1985). Shikonin production is strongly affected by stress, including stress induced by light, temperature and pathogen exposure plus chemical stressors, such as proteins (Brigham *et al*. 1999; Dixon and Pava 1995). We observed site and seasonal variation in production of napthoquinones. Plants collected in midwinter versus spring produced significant levels of extractable napthoquinones, with increased concentration noted over time.

Napthoquinones are novel secondary products with potent antimicrobial, cytotoxic and antifungal properties. This project has demonstrated existence of napthoquinones in Paterson's curse roots and root extracts. Extracts exhibited strong phytotoxicity to annual ryegrass seedlings. Their specific role in contributing to invasive success of Paterson's curse in Australia remains to be further elucidated.

**REFERENCES**

Bertin, C., Yang, X.H. and Weston, L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* 256: 67-83.

Binder, R.G., Benson, M.E. and Flath, R.A. (1989). Eight 1,4 napthoquinones from *Juglans*. *Phytochemistry* 28: 2799-2801.

Brigham, L.A., Michaels, P.J. and Flores, H.E. (1999). Cell-specific production and antimicrobial activity of napthoquinones in roots of *Lithospermum erythrorhizon*. *Plant Physiology* 119: 417-428.

Burton, J. and Dowling, P. (2004). Pasture management for weed control – a grazier's guide to controlling weeds in southern Australian improved pastures. NSW Agriculture, Orange.

Callaway, R.M. and Aschehoug, C.A. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290: 521-523.

Carter, J. (2009). Paterson's curse. Fact Sheet. CSIRO Black Mountain Laboratories. Plant Industries, pp. 1-2.

Cowie, M. (2006). Successful biological control of Paterson's curse. Proc. Grassland Soc. NSW. Waging war on weeds. 21: 59-64.

Dixon, R.A. and Pava, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell* 7:1085-1097.

Douglass, C.H., Weston, L.A. and Wolfe, D. (2011). Phytotoxicity and potential allelopathy in pale (*Cynanchum rossicum*) and black swallowwort (*Cynanchum nigrum*). *Invasive Plant Sci. and Management* 4: 133-141.

Mathesius, U. and Watt, M. (2011). Rhizosphere signals for plant-microbe interactions: implications for field-grown plants. In U. Luttge et al. (eds). Progress in Botany 72: 125-161. Springer-Verlag Berlin.

McCully, M. (2005). The rhizosphere: the key functional unit in plant/soil/microbial interactions in the field. Implications for the understanding of allelopathic effects. Proc. 4th World Congress on Allelopathy, pp1-8.

Nardi, S., Concheri, G., Pizzeghello, D., Sturaro, A., Rella, R. and Parvoli, G. (2000). Soil organic matter mobilization by root exudates. *Chemosphere* 5: 653-658.

Peterson, J.E. and Jago, M.V. (1984). Toxicity of *Echium plantagineum* (Paterson's curse). II. Pyrrolizidine alkaloid poisoning in rats. *Aust. J. Agric. Res.* 35: 305-315.

Piggin, C.M. (1982). The biology of Australian weeds:8. *Echium plantagineum*. L. *J. Austral. Inst. Agric. Sci.* 48: 3-16.

Pratley, J. (1991). Pasture management. In D.J. Cottle (ed). *Australia sheep and wool handbook*. Inkata Press, Melbourne Australia, p. 279.

Rast, L. (2006). Problems in livestock grazing weeds. *Proc. Grassland Soc. NSW* 21:91-96.

Ryan, P.R. and Delhaize, E. (2001). Function and mechanism of organic anion exudation from plant roots. *Ann. Rev. Plant Phys. Mol. Biol.* 52: 527-560.

ISBN Number: 978-0-9871961-0-1

Scott, J.K. and Kenneally, K.F. (1981). The distribution of Boragineaceae in Western Australia in relation to the biological control of *Echium plantagineum* L. *West. Austral. Herb. Res. Notes* 5:81-109.

Tabata, M. and Fujita, Y. (1985). Production of shikonin by plant cell cultures. In M. Zaitlin, P. Day and A. Hollaender (eds). *Biotechnology in Plant Science.* Academic Press, San Diego, CA, pp. 207-218.

Uren, N.C. (2000). Types, amounts, and possible functions of compounds released into the rhizosphere by soil grown plants. In R. Pinton, Z. Varanini and P. Nannipieri (eds). *The Rhizosphere: Biochemistry and Organic Substances at the Plant-Soil Interface.* Marcel Dekker, Inc. New York, NY, USA. pp. 19-40.

Walker, T.S., Bais, H.P., Grotewold, E. and Vivanco, J.M. (2003). Root exudation and rhizosphere biology. *Plant Phys.* 132: 44-51.

Watt, M. and Weston, L.A. (2009). Specialised root adaptations: cell-specific developmental and physiological diversity. *Plant and Soil* 322: 39-47.

Weston, L. A. and Duke, S.O. (2003). Weed and crop allelopathy. *Critical Rev. Plant Sci.* 22: 367-389.

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# FLAXLEAF FLEABANE (*CONYZA BONARIENSIS*) – STRATEGIC SOLUTIONS USING BEST MANAGEMENT PRACTICE

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## Abstract

Flaxleaf fleabane (*Conyza bonariensis*) is the most difficult to control weed of cropping in the sub-tropical grain region of Australia and threatens sustainable farming practices. Research was designed to develop a range of tactics which target all aspects of the weed's lifecycle to minimise its impact on productivity and to rapidly deplete its seed bank. Tactics have been identified for the effective management of fleabane, which infests all parts of the rotation including winter and summer fallows, wheat and sorghum. In fallow, the 'double-knock' tactic of sequential applications of herbicides from different modes of action has proven highly effective (up to 100% control) and stops seed set. Additionally, the application of residual herbicides in fallow and preceding crops has provided complete control of fleabane for up to six months. Further in-crop control was achieved with a combination of selective herbicides and crop competition. Seed bank persistence of fleabane is very short for seed on the soil surface (94% loss in two years). Thus, best management practice using selective herbicides, knock-down mixes, residuals, double-knock and crop competition can minimise this weed problem within a short period. Attention to control in adjacent areas will prevent new infestations.

**Keywords:** flaxleaf fleabane, fleabane, management

## The Problem

Flaxleaf fleabane is a major weed of cropping in southern Queensland and northern New South Wales in Australia. The weed is also becoming widespread in temperate southern and western farming systems of Australia. It can be a very difficult weed to control and is thought to have increased for several reasons, namely the:

- Increased adoption of no-till farming;
- Heavy reliance on glyphosate for fallow weed control;
- Reduction in use of residual herbicides in crop; and
- Introduction of wide row spacing in sorghum.

Fleabane has a deep tap root that can greatly reduce stored water supplies in fallow and can compete with crops, reducing yields (Wu *et al.* 2010). Some Australian populations of the weed are resistant to glyphosate (Walker *et al.* 2011) resulting from this herbicide being relied upon repeatedly and almost exclusively for fleabane control in fallows.

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The germination of fleabane is light and temperature dependant requiring a temperature of between 10-25°C (optimal 20°C) for germination (Wu *et al.* 2007). Seedlings of fleabane emerge mostly from the top 1 cm of soil and no seedlings emerge from a depth of 2 cm or below (Wu *et al.* 2007). A larger emergence in the field is often associated with crop stubble and parts of the topography that enable increased and longer periods of moisture.

In southern Queensland, fleabane emerges predominantly in autumn, early winter and spring (Wu *et al.* 2007). However, limited emergence can occur if mild conditions take place in winter and early summer. With this in mind, fleabane is mostly a problem in winter crops and fallows but can also pose problems for summer crops.

Fleabane plants that emerge during autumn often grow very slowly above-ground through winter, but their tap root system continues to grow. While such weeds appear small in size, they can be quite old (two or three months) and very difficult to control. Fleabane that emerges in spring will grow more quickly and their size more accurately reflects their age. Fleabane is most easily controlled when it is small and young. Once elongation begins, there are few effective options available.

Fleabane is a prolific seed producer with fleabane growing in southern Queensland producing up to 120 000 seeds per mature plant (Wu *et al.* 2007). Fleabane seeds possess a pappus, which aids in its dispersal by wind.

The majority of fleabane seeds in the soil lose their viability within 12-18 months. However a small percentage can persist for several years and the quantity is influenced by burial depth. A pot study showed that after three years of burial 1, 10 and 8% of viable seed remained at depths of 0-2, 5 and 10 cm, respectively (Wu *et al.* 2007).

**Fallow Solutions**

Fallow control of fleabane with a single herbicide application has been very inconsistent (Wu *et al.* 2008). Although good results have been obtained with specific treatments in some situations, no consistent robust option has been identified to cover a wide range of situations. As a result the industry has needed to look at techniques such as the 'double-knock' and residual herbicides.

**Double-knock approaches**

A 'double-knock' is the use of two different weed control strategies within a short period of time. Although double-knocks can include cultivation or other non-herbicide tactics, in this paper the term double-knock refers to the use of two herbicide applications with different mode of action, generally within a period of five to ten days.

The double-knock tactic is designed to control survivors of the first application, thereby reducing seed set and the risk for herbicide resistance. The most consistent and widely adopted double-knock for fleabane is a mix of glyphosate + 2,4-D as the first application, or knock, followed by a paraquat or paraquat + diquat (Spray.Seed®) based option as the second-knock.

Figure 1 clearly shows the value of the second knock in a heavily infested fleabane site (280 plants/m$^2$) with an average increase in control of almost 30%. The inclusion of a second knock of Spray.Seed® resulted in excellent levels of control of fleabane at the two earlier stages. However, at three months old when plants are elongating, the double-knock approach only resulted in about 90% control.

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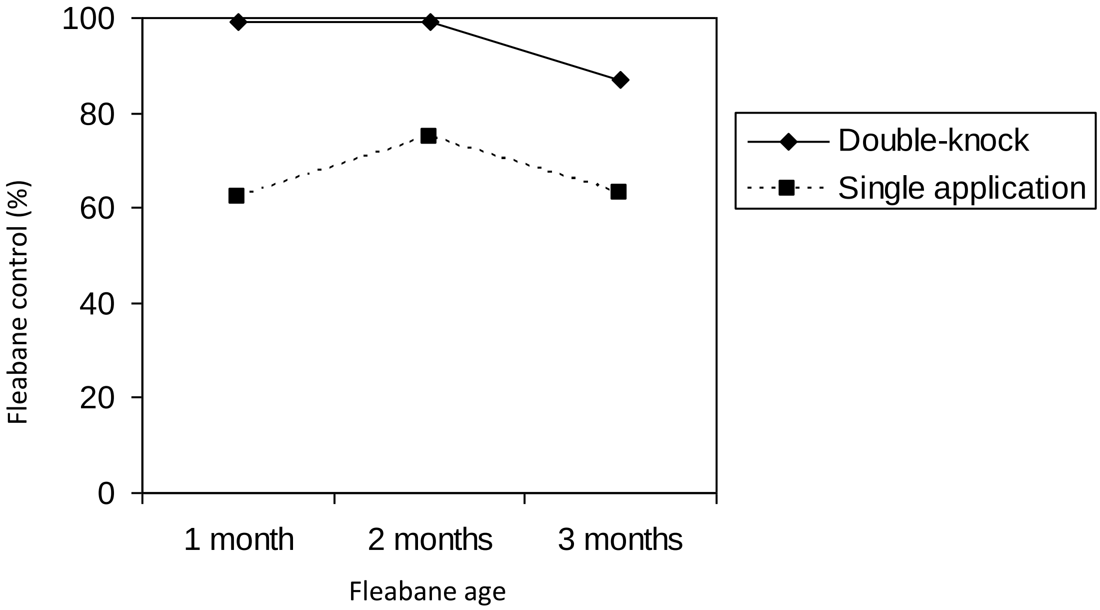


**Figure 1.** Fleabane control (%) assessed through visual biomass reduction at 42 days after treatment. Double-knock treatment - Roundup® CT @ 1.5 L/ha + Surpass® 475 @ 1 L/ha followed by Spray.Seed @ 2 L/ha; single application treatment - Roundup CT @ 1.5 L/ha + Surpass 475 @ 1 L/ha. LSD for treatments = 8.9 (P<0.001).

An additional range of single and double-knock treatments have been evaluated for fallow control of fleabane in southern Queensland and northern NSW on dense populations of 550 and 200 plants/m$^2$ respectively. The evaluation took place in 2010 and examined the impact of fleabane age on herbicide efficacy.

Results confirmed the double-knock approach as more consistently reliable than single applications especially on older fleabane (Table 2). To maintain the efficacy of the double-knock treatment on larger fleabane, the rate of herbicide applied needs to be increased.

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**Table 2.** Average visual biomass reduction (% of untreated) of fleabane 42 days after knockdown treatment in southern Queensland and northern NSW. Values in parentheses are the range of control achieved. Fleabane size at one month ≤ 5 cm and at three month > 10 cm and elongating.

| Herbicide (first knock fb second knock) (L/ha) | | Fleabane age | |
|---|---|---|---|
| | | 1 month | 3 months* |
| Roundup CT + Surpass 300 | 1.5 + 1.5 | 88 *(86-90)* | 73 *(70-75)* |
| Roundup CT + Tordon®75D | 1.5 + 0.7 | 97 *(84-99)* | 94 *(90-97)* |
| Roundup CT + Surpass 300 fb Spray.Seed | 1.5 + 1.5 fb 2.0 | 94 *(91-96)* | 94 *(91-97)* |
| Roundup CT + Tordon 75D fb Spray.Seed | 1.5 + 0.7 fb 2.0 | 99 *(97-100)* | 99 *(98-100)* |
| Roundup CT + Surpass 300 fb Alliance® | 1.5+ 1.5 fb 2.0 | 94 *(90-97)* | 92 *(89-94)* |
| Amicide®625 fb Spray.Seed | 1.5 fb 2.0 | 97 *(97-97)* | 86 *(75-97)* |

1st knock applied at 75 L/ha, 2nd knock applied at 105 L/ha

fb = followed by – second knock 7 days after first knock

* - herbicide rate was increased: Surpass 300 - 2.0L, Tordon 75D - 1.0L, Spray.Seed - 2.4L, Alliance - 2.4L.

**Residual herbicides**

The incomplete control with knockdown sprays, together with an increasing prevalence of resistance to glyphosate and other knockdown herbicides in fleabane and other weed species, has resulted in the reintroduction of residual herbicides into cropping systems. Well-timed residual application could be very effective in controlling a number of emergence flushes but will impact on crop rotation options.

A residual herbicide trial in 2009 on a dense infestation (~170 plants/m$^2$) of predominantly small fleabane (≤5 cm diameter) compared first knocks of Roundup CT @ 1.5 L/ha + Surpass 475 @ 1 L/ha or Roundup CT @ 1.5 L/ha + Tordon 75 D @ 0.7 L/ha, and second knocks (7 days after 1st knock) of Spray.Seed 1.6 L alone or in mixture with a residual herbicide (Atrazine, Diuron®, Glean® or Balance®).

One hundred percent knockdown control was obtained from all double-knock treatments. Residual control was then assessed through to January 2010 by measuring cumulative emergence. A clean-up spray of Roundup CT + Surpass was applied after each weed emergence count.

When no second knock with residual product was added, there was an average emergence of 16 plants/m$^2$ following Surpass and 2 plants/m$^2$ following Tordon. The picloram component in Tordon products has short-term residual effect and in this case reduced fleabane emergence. Good residual control (94-100%) was obtained from all residual products over a 6 month period following application. Less than 1 plant/m$^2$ emerged in plots treated with Surpass and a residual, while less than 0.1 plants/m$^2$ emerged in plots treated with Tordon and a residual. Atrazine and Balance performed best, irrespective of the first knock used.

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There are three key positions within sub-tropical cropping systems where residuals may be of benefit in managing fleabane:

a) Use of a residual in autumn/ winter during the winter fallow;
b) Use of a pre-plant or in-crop residual within the winter cropping program; and
c) Use of a pre-plant or in-crop residual within the summer cropping program.

## In-Crop Solutions

### Crop competition

Fleabane density and seed head production can be substantially reduced using crop competition even in the absence of herbicides. A field trial in southern Queensland investigated the impact of different winter cereal crop species at a range of different row spacing and crop densities on fleabane density and seed head production. For wheat, fleabane numbers decreased with increasing crop population and narrower row spacing (Figure 2). On average, weed density decreased by 28% as crop population increased from 50 to 100 plants/m$^2$ and by 44% as row spacing decreased from 50 to 25 cm. Seed production was reduced from an average 1250 seed heads/plant in wheat grown at 50 cm row spacing and density of 75 plants/m$^2$ to 120 seed heads/plant at 25 cm row spacing and 100 plants/m$^2$.

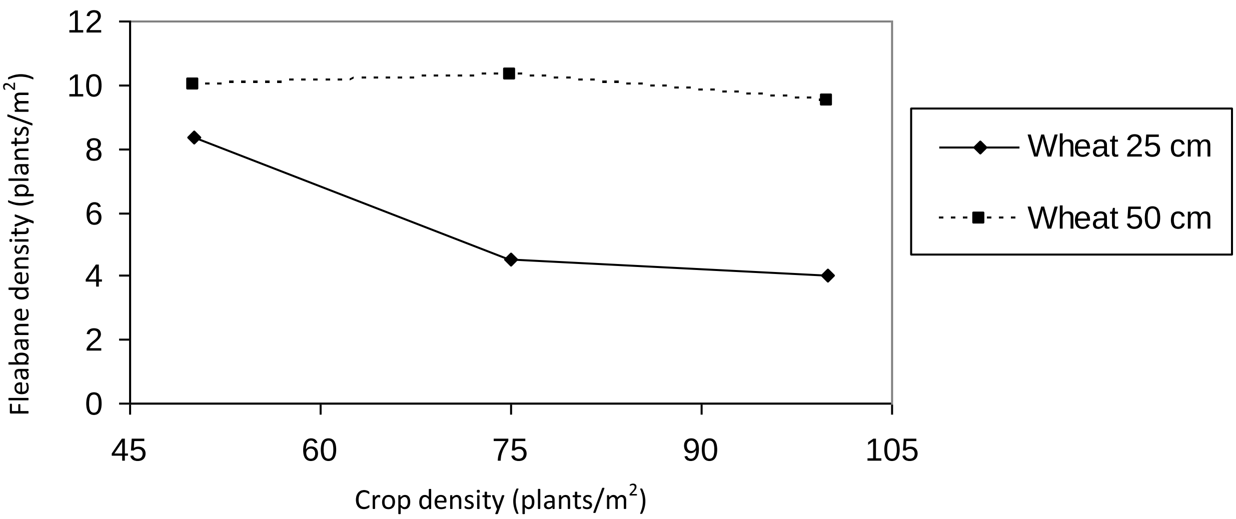


**Figure 2.** Fleabane density (/m$^2$) in wheat of different row spacing and plant density. LSD for row spacing = 4.038 (P = 0.038).

### In-crop herbicides

There are currently no in-crop herbicides registered for the control of fleabane in the sub-tropical grains region of Australia. However, there is a suite of herbicides commonly used in-crop that can be effective on young fleabane.

In a 2010 trial in southern Queensland, in-crop herbicides were applied in wheat at two different times two weeks apart. The density of fleabane at the site ranged from 12 – 72

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plants/m$^2$ and there was a range of weed sizes at each time of spraying. At the first spray the majority of fleabane were small (<5 cm) and at the latter spray there were more plants that were >10 cm. The results (Table 3) show that there are a range of treatments which provided >85% control when applied to young fleabane. A delay of two weeks in application resulted in an average 25% reduction in control.

**Table 3.** Fleabane biomass reduction (% of untreated) four weeks after in-crop herbicide treatment applied two weeks apart. LSD for time of spraying = 9 (P = 0.009).

| Herbicide | Rate (/ha) | Biomass reduction (%) | |
|---|---|---|---|
| | | First time of spraying | Second time of spraying |
| Amicide 625 | 1.2 L | 94 | 91 |
| Tordon 75D + 24D | 0.3 L + 0.375 L | 90 | 28 |
| Tordon 242 + Ally | 1 L + 5 g | 89 | 68 |
| Starane Advance | 0.6 L | 89 | 11 |
| Ally + MCPA LVE | 5 g + 0.75 L | 85 | 55 |
| Starane + MCPA | 0.6 L + 0.75 L | 77 | 59 |
| Hotshot + MCPA LVE | 0.75 L + 0.75 L | 70 | 45 |
| Ally | 5 g | 65 | 31 |
| Tordon 242 | 1 L | 62 | 87 |
| Tordon 75D | 0.3 L | 56 | 54 |
| ***MEAN*** | | ***78*** | ***53*** |

No treatment provided one hundred percent control of fleabane and there were new flushes of emergence after herbicide application. Products containing picloram (Tordon 242 and 75D) reduced subsequent emergences by more than 50%.

**Integrated Weed Management**

No single control tactic is one hundred percent effective on flaxleaf fleabane. Successful control of fleabane will only be achieved by using a combination of management tools as part of an integrated weed management (IWM) strategy. In this paper we have identified both chemical and non-chemical tactics that are effective in controlling fleabane and can be used as part of an IWM approach.

For maximum control of fleabane in fallow, double-knocks or residual herbicides should be used. Both tactics provide the most robust management of this key northern weed and should be part of a fleabane management strategy. These two tactics should be used in combination in paddocks or areas where fleabane is most prevalent.

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For maximum control of fleabane in crop, selective herbicides should be applied in combination with growing a competitive crop. Effective control of fleabane in non-cropping areas adjacent to cropping land is also important to stop new incursions.

An IWM strategy that includes chemical and non-chemical tactics, such as crop competition, for controlling seedlings and preventing seed production on survivors will result in substantially fewer fleabane problems and a reduced risk of herbicide resistance.

## References

Walker, S., Bell, K., Robinson, G. and Widderick, M. (2011). Flaxleaf fleabane (*Conyza bonariensis*) populations have developed glyphosate resistance in north-east Australian cropping fields. *Crop Protection* 30: 311-317.

Wu, H., Walker, S. and Robinson, G. (2008). Chemical control of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist) in winter fallows. *Plant Protection Quarterly* 23: 162-165.

Wu, H., Walker, S., Rollin, M.J., Tan, D.K.Y., Robinson, G. and Werth, J. (2007). Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist). *Weed Biology and Management* 7: 192-199.

Wu, H., Walker, S., Robinson, G. and Coombes, N. (2010) Control of Flaxleaf Fleabane (*Conyza bonariensis*) in Wheat and Sorghum. *Weed Technology* 24, 102-107.

# IMPACTS OF SOWING PRACTICES ON WHEAT INJURIES CAUSED BY SOIL APPLIED HERBICIDES

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## ABSTRACT

Experiments were conducted in 2007 and 2008 to determine the impact of row spacing, sowing speed and soil incorporation method on wheat damage caused by a number of soil applied herbicides. Wheat tolerance varied with herbicides, with trifluralin causing the most damage, followed by pendimethalin and triasulfuron. The narrow row spacing (17.8 cm) and the higher travelling speed (10 km/h) caused greater damage than the wide row spacing (22.9 cm) and the lower speed (6 km/h), respectively. Soil incorporation methods also affected wheat tolerance to herbicides, with star harrow treatment causing the most damage and the press wheels the least.

**Keywords:** Wheat damage, soil incorporation, residual herbicides.

## INTRODUCTION

Changes toward conservation farming systems have resulted in heavy reliance on herbicides for weed management due to the absence of cultivation, which consequently has given rise to concerns about the rapid development of herbicide resistance and herbicide residues in the environment (Maurizio and Pimentel 2000; Broster *et al*. 2011). In addition, poor efficacy of soil-applied herbicides has often been reported in no-till farming systems due to the lack of soil incorporation (Chauhan *et al*. 2006).

Residual herbicides on one hand need to be activated by soil incorporation to improve their control efficacy on weeds, while on the other hand, the incorporated herbicides can also potentially cause substantial injuries to crops. Complete crop failures have often been reported due to the inappropriate use of soil applied herbicides. Maximising the control efficacy of soil incorporated herbicides and mininising their negative impact on crop safety represent a real challenge for conservation farmers.

There have been limited studies on crop damage due to soil applied herbicides (Soltani *et al*. 2006; Sikkema *et al*. 2007). However little information is available on the impact of sowing practices on crop safety due to herbicides. In order to understand the crop tolerance to soil applied herbicides incorporated before sowing, the research was conducted to determine the impact of row spacing, sowing speed and soil incorporation method on wheat damage caused by a number of commonly used residual herbicides.

## MATERIALS AND METHODS

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Experiments were conducted in 2007 and 2008 at the research station of Wagga Wagga Agricultural Institute. Both years received less rainfall than the long-term average (Figure 1). Wheat (cv Diamondbird) was sowed at 60 kg/ha on 24th May 2007 and 24th May 2008. DAP superphosphate (18N:20P:0K:2S, Incitec Pivot) was applied at 135 kg/ha at sowing.

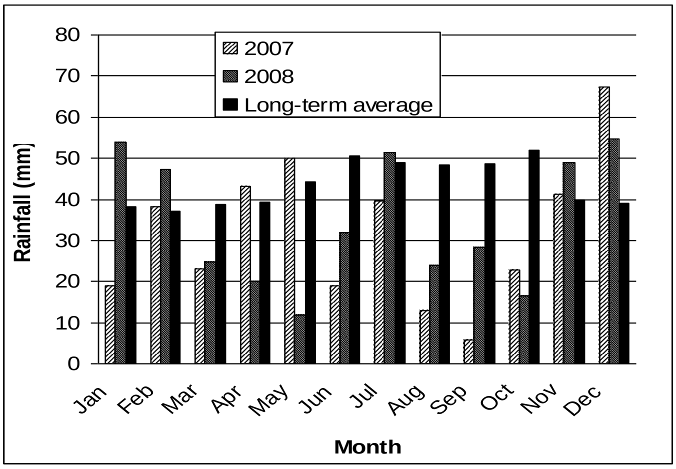


**Figure 1.** Rainfall received during the experimental period.

A strip-plot design (Ryan 2006) with three replicates was used in both experiments with four herbicide treatments (trifluralin, pendimethalin triasulfuron and a nil-treated control), two row spacings (17.8 cm and 22.9 cm), three incorporation methods (Flexi Coil, Press wheels and Star Harrows) and two sowing speeds (6 and 10 km/hr). For the 17.8cm row spacing, each plot had 8 rows of wheat so that the plot size was about 1.43 m × 10 m. For the 22.9cm row spacing, the plot had 6 rows and a size of 1.35 m × 10 m. Triflurx (480g/L trifluralin) and Stomp 330EC (330 g/L pendimethalin) were applied at 3L/ha and Logran 750 (750g/kg triasulfuron) was applied at 70 g/ha.

The residual herbicides were applied prior to sowing with a tractor-mounted compressed-air-pressurised sprayer at a volume of 100 L/ha with TurboJet 02 flat spray nozzles at 150kpa.

GreenSeeker® (NTech, USA) was used to assess the crop damage due to herbicides on 26 July 2007 and 4 August 2008, respectively. There were 30 readings of NDVI (normalised difference vegetation index) taken for each plot. Actual crop emergence was also counted at the same time in the middle 4 x 1 m row of each plot in 2007.

## RESULTS AND DISCUSSION

### Relationship Between Greenseeker Reading and Crop Density

Crop damage by herbicides has been traditionally assessed by counting the crop emergence after application. However this method is time-consuming, in particular when there are large numbers of plots to be assessed. Research data has shown that the GreenSeeker reading was highly correlated with the actual crop emergence count, with a $R^2$ of 0.782 in the 17.8 cm row spacing plot and 0.704 in the 23 cm row spacing plot (Figure 2). Such close correlation indicates that GreenSeeker could be an efficient alternative to the traditional counting method in assessing crop damage due to herbicides.

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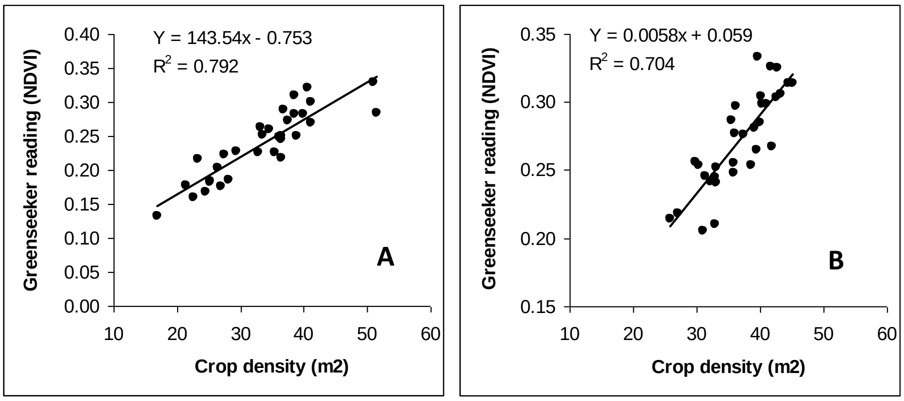


**Figure 2.** Correlation between Greenseeker reading and the crop density at row spacing of 17.8 cm (**A**) and 22.9 cm (**B**). NDVI stands for the normalised difference vegetation index.

### Effect of Row Spacing on Herbicide Crop Damage

Crop safety highly depended on the herbicide used. Trifluralin caused the most damage to wheat at the row spacing of 17.8 cm, followed by pendimethalin and triasulfuron (Figure 3). The herbicide damage to wheat varied with the different rainfall patterns received in 2007 and 2008. The total amount of rainfall in 2007 and 2008 was only about 73% and 79% of the long-term average (526 mm) (Figure 1). In 2007, it had good soil moisture at sowing but it was then followed by an extended dry period between August and October, resulting in the complete crop failure. In 2008, the dry condition was experienced throughout the whole growing season, resulting in poor crop establishment and growth. The wet conditions at sowing in 2007 improved the mobility of applied residual herbicides in the soil, resulting in greater crop damage when compared to that in 2008. The dry condition in 2008 limited the mobility of applied herbicides and consequently little differences were found between the herbicide treatments.

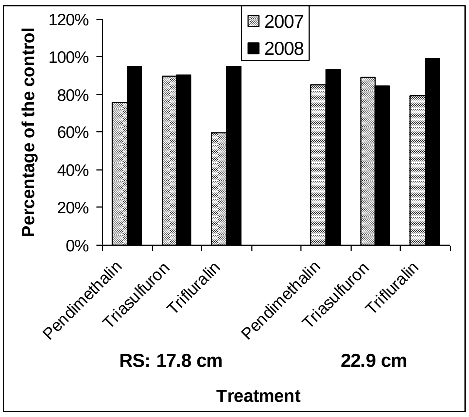


**Figure 3.** Impact of herbicides on crop safety at row spacing of 17.8 cm and 22.9 cm. (RS: row spacing at 17.8 cm and 22.9 cm, respectively).

Data from the 2007 experiment also showed that the narrow spacing at 17.8 cm resulted in higher crop damage as compared to the wide row spacing of 22.9 cm (Figure 3). A wider

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row spacing allows soil throw onto inter rows, reducing the risk of back-filling the herbicide-treated soil into the seeding furrows.

## Effect of Soil Incorporation Method on Herbicide Crop Damage

No difference in crop safety was found in the 2008 experiment irrespective to the herbicide and the incorporation method as a result of limited rainfall. However, crop damage was evidenced in the 2007 experiment (Figure 4). Except for the triasulfuron treatment, the crop damage by pendimethalin and trifluralin was the highest in the star harrow treatment (62%), followed by flexi coil (65%) and press wheels (76%), which was consistent with the level of soil disturbance created by these three covering devices. Although the use of star harrows and flexi coil could provide better soil incorporation to improve herbicide efficacy, these covering devices could drag the herbicide-treated soil back into the seeding furrows. Haskins (2010) also reported that knife points and press wheels system caused the least damage to the crop due to herbicides.

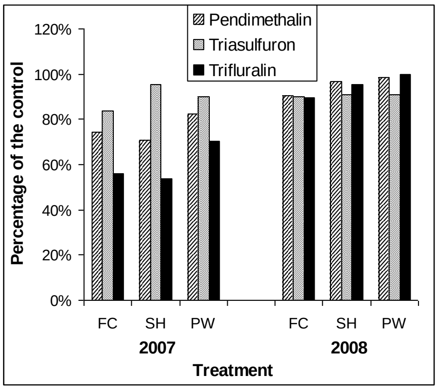


**Figure 4.** Impact of herbicide incorporation methods on crop safety. (FC, SH and PW stand for flexi coil, star harrows and press wheels, respectively).

## Effect of Sowing Speed on Herbicide Crop Damage

Sowing speed also affected the crop damage due to herbicides (Figure 5). Regardless the incorporation methods, the higher travelling speed at 10 km/ha caused higher herbicide damage to wheat (70%) than the speed at 6 km/ha (80%). Higher speed has been reported to cause greater soil throw into adjacent seeding furrow (Dempster 2011).

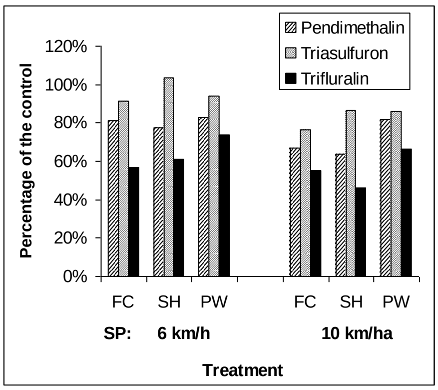


**Figure 5.** Impact of sowing speed on crop safety at the row spacing of 17.8 cm in the 2007 experiment. (SP: sowing speed at 6 and 10 km/h, respectively).

Crop damage due to soil applied herbicide is a complex issue. It depends on a range of factors, including the prevailing rainfall pattern in particular around sowing season, seeding systems, covering devices, sowing speed, row spacing and the mobility of a given herbicide in the soil. In the presence of residual pre-emergent herbicides, disc seeder tends to cause greater crop damage relative to tyne seeder, particularly in a wet season (Haskins 2010). The aggressive soil incorporation by star harrow and flexi coil tends to cause greater crop damage due to herbicides. Higher sowing speed could cause excessive throw of herbicide-treated soil into the adjacent seeding furrow, increasing the risk of herbicide damage.

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**REFERENCES**

Broster, J.C., Koetz, E. A. and Wu, H. (2011). Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. *Plant Protection Quarterly* 26: 22-28.

Chauhan, B.S., Gill, G. and Preston, C. (2006). Tillage systems affect trifluralin bioavailability in soil. *Weed Science* 54: 941-947.

Dempster, J. (2011). Effect of seeder type and sowing speed on pre-emergent herbicide incorporation. http://www.faceygroup.asn.au/PDF's/WANTFA%20-%20Jade%20Dempster.pdf. Accessed 25 May 2011.

Haskins, B. (2010). Residual herbicides at sowing using disc and tyne no till seeding equipment. IREC Farmers' Newsletter – Large Area No. 183: Spring 2010. http://irec.org.au/farmer_f/pdf_183/Pre_emergent_herbicide_management_for_no_till.pdf.

Maurizio, G.P. and Pimentel, D. (2000). Environmental risks of pesticides versus genetic engineering for agricultural pest control. *Journal of Agricultural and Environmental Ethics* 12: 279-303.

Ryan, T.P. (2006). *Modern experimental design*. John Wiley & Sons, Inc., Hoboken, New Jersey.

Sikkema, P.H., Shropshire, C. and Soltani, N. (2007). Dry bean response to preemergence-applied KIH-485. *Weed Technology* 21: 230-234.

Soltani, N., Robinson, D.E., Shropshire, C. and Sikkema, P.H. (2006). Otebo bean (*Phaseolus vulgaris*) sensitivity to pre-emergence herbicides. *Crop Protection* 25: 476-479.

# WEED MANAGEMENT PERSPECTIVES FOR INDIA IN THE CHANGING AGRICULTURE SCENARIO IN THE COUNTRY

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## ABSTRACT

India is the fourth largest economy in the world. Agriculture continues to be the most important sector of Indian economy providing employment and livelihood to nearly 70% of the total population. It has done well all through to feed the growing population. About 2.5 mt of additional food grains are required annually in the next 10 years to meet the demand of the growing population. This is a huge challenge as it has to come from shrinking (both in quality and quantity) land and water resources, adverse climatic and market forces, poor infrastructure, etc. The government is putting lot of thrust on agriculture in its 11$^{th}$ five year plan (2007-12), recognizing that agriculture growth is key to achieve the target of 9.0% growth in total GDP. The government is responding to these challenges by increasing the investment, launching several developmental schemes and providing policy support. Many of the schemes are pro-poor and pro-small holders aimed at achieving inclusive growth. One such scheme, the National Rural Employment Guarantee scheme which guarantees employment/wages to one adult in a household for 100 days in a year, has benefited millions of rural workforce. However, this has also made the labor expensive and unavailable for agricultural operations including weeding. The paper discusses the challenges and opportunities of weed management in the light of changing agricultural scenario in the country.

**Keywords**: weeds, weed management, herbicide resistant crops, resource conservation technologies, India

## INTRODUCTION

Agriculture continues to be the most important sector of Indian economy, directly involving over 60% of the country’s population, despite a progressive decrease in its share of total national GDP. The share has progressively decreased over the years from 48% in 1950s to the current 17%. The decreasing contribution signifies the increasing contribution by the other sectors particularly the services and manufacturing sector. Indian agriculture has by and large performed very well all through. From a net food importer until 1970s, to the present level of self sufficiency and a net exporter in certain commodities, is no mean achievement. The food production has quadrupled in the last 50 years from a mere 51 mt in 1950 to 232 mt in 2011. This has more than matched the three times increase in the human population from 0.36 billion to 1.02 billion during the same period. We can also boast of the huge quantity of buffer stock food grains, anywhere in the range of 40-50 mt, to tide over any adverse conditions.

The country is aiming to achieve an overall growth in the economy at over 8-9% during the 11$^{th}$ Five Year Plan period (2007-12). In order to achieve this, the agriculture sector has to grow at 4% or more. However, India’s quest to achieve this target in agriculture has remained elusive since mid 1990's. In fact the growth was less than 1.0% during 2007-08 and 2009-10. Thanks to good harvest (with 232 mt) in 2010-11, a growth rate of 5.4% has been recorded.

The changes in productivity had a great social impact on the farming community. With increased production, income from the farms improved and with greater money to spend, new needs for farm inputs, milling, processing and marketing services occurred and spurred the local economy to growth. While the per capita consumption of food grains has shown a decline in the recent past, the demand for meat, milk, chicken, fish, eggs, fruits and vegetables is on the increase. Despite these achievements, the access to food and nutritional security, particularly in the rural areas, is cause for concern. The general well being of the people can be gauged by the fact that based on Human Development Index, the country is ranked at 122 position (of total 172 countries) during 2010.

The challenges are many. The country, with only 2.3 percent of the world's total land area, has to ensure the food security for 17.5 percent of the world's population amidst declining natural resources, erratic monsoons, climate change crisis, energy crisis, loss of bio-diversity, decreasing land holding sizes, weak extension machinery, lesser productivity from the farmlands, rising input costs, inadequate storage infrastructure and high post harvest losses, all of which ultimately pulls down the returns to the farmer-producer. There is thus a general lack of interest among the farming community, especially rural youth, in engaging with agriculture as a livelihood option and most of them are migrating to the urban areas in search of a better standard of living.

It is in this context that we have to plan to revive the agricultural sector in the country. Agriculture has to shed its old ways and embrace new ones to meet the enormous challenges it faces today. The development of innovative products, processes and concepts by entrepreneurs and private players and their integration into existing agricultural systems is the pathway to true inclusive agricultural growth. An environmentally sustainable agricultural growth is a prerequisite for economic development in general and rural development in particular.

The government is responding to these challenges by increasing the investment, launching several developmental schemes and providing policy support. Through most of these schemes the government aims to achieve inclusive growth. Many are beginning to show a positive impact. The one scheme which is much talked about is the National Rural Employment Guarantee scheme. The scheme, which was passed through an Act of the parliament, aims at enhancing the livelihood security of people in rural areas by guaranteeing 100 days of wage-employment in a year to at least one person in a family living below the poverty line. This flagship program, which cost the exchequer nearly Rs 400 billion (USD 9 billion) annually, has proved very successful and has benefited a vast number of the rural workforce (40 million households in 2010). However on the flip side, this has come under serous criticism as it has led to some negative impact on the society. Nepotism, corruption and poor implementation in some instances has resulted in misuse of funds. The flow of this 'easy money' has made people lazy and it has been alleged that people especially the youth have taken to liquor, drugs and other bad habits. However, the direct and the most significant of all effects is on the availability of the labor to perform agricultural operations. A very serous shortage of labor is reported from almost all regions including the less advanced areas. Particularly hit are the progressives states (like Punjab), which depend on migrant labor from poorer regions and plantations in the southern states, notably Kerala.

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## Weeds and Weed Management

Weeds continue to be an important constraint in crop production. Despite the good efforts made in research and extension in the field of weed science, the farmers continue to experience heavy losses in crop yield due to weed interference. The crop loss estimates are many and often confusing and misleading. A conservative estimate of about 10% loss would amount to a loss of about 25 mt of food grains, currently valued at approximately USD 13 billion. Losses of similar magnitude may occur in plantation crops, fruits, vegetables, grass lands, forestry and aquatic environment. The total economic losses will be much higher, if indirect effect of weeds on health, loss of biodiversity, nutrient depletion, grain quality etc is taken into consideration. The key issues relating to weed management are briefly discussed below.

## Shortage of Labor

Basically, the agriculture that is practiced in India is highly labor intensive. Labor accounts for 60 % of the total cost of crop production. Weeding is predominantly done by use of manual labor. Currently herbicides are used to a limited extent in wheat, rice, soybean and tea. Assuming a labor requirement of 20 man-days/ha for weeding, India requires a phenomenal 5 billion man-days of labor for weeding alone! That is close to 5 days of weeding for every citizen including the children! Hence any shortage of labor will impact the weed management substantially. The implementation of the National Rural Employment Guarantee scheme as described earlier has seriously impacted the availability of labor for agricultural operations. While the scheme is a boon to unemployed rural households, the farmers find it difficult to match the wages given by the government. There is pressure on the government to raise the wages further which is currently at Rs 100 per day (USD 2.2). Farmers are often forced to adopt crops (e.g sugarcane) and technologies which demand less labor.

Weeding has never been a priority operation for majority of the farmers due to a variety of reasons. The present situation of labor shortage and increase in wages will only worsen the situation. Severe crop losses due to delayed weeding are common. Farmers are beginning to think of alternatives and herbicides are the obvious choice for many. The use of herbicides is expected to grow in the near future.

## Mechanization

The labor shortage has also forced farmers to go for machines in a big way. The combine harvesters which were mostly used in large farms for harvesting rice and wheat are increasingly being seen in small farms. Small and medium farmers are using these big machineries on a custom hiring basis. Even a subsistence farmer finds using harvesters and tractors more economical. The draught animals which were commonly used for land preparation and inter-cultivation operations are slowly but steadily making an exit. Increased pressure on land and the high cost of rearing the animals are the principal reasons for this shift.

## Zero tillage, Direct-seeding and Conservation Tillage

A rice-wheat cropping systems occupies about 18 million ha in Asia, of which 13.5 mha are in the Indo-Gangetic Plains (IGP) of Bangladesh, India, Nepal, and Pakistan and feeds about 1.3 billion people (20% of the world population). To ensure food security in Asia, it is imperative to identify rice production systems that require less irrigation water input than

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the conventional transplanted rice. In recent years, the major emphasis in the rice-wheat system has been on alternative resource conservation technologies (RCTs) for both rice and wheat to reduce the cost of cultivation and energy consumption, to sustain productivity, and to increase the profit margin of farmers. Zero tillage in wheat crop in rice-wheat system in the north western Indo-Gangetic Plains has become popular and is practiced over 2.1 mha area. A change in rice establishment method from traditional manual transplanting of seedlings to direct-seeding has occurred in many Asian countries in the last two decades in response to rising production costs, especially for labor and water. Direct-seeded rice needs only 34% of the total labor requirement and saves 29% of the total cost of the transplanted crop. At research farms, the direct seeding of rice has been shown to do as well as transplanted rice provided the weeds are effectively controlled. The spread of the technology has been restricted amongst other factors, because of absence of effective herbicides for weed control in direct -seeded rice. Despite the demonstrated advantages of conservation tillage, the technology is yet to take off. Availability and accessibility to the proper machinery is the limiting factor. Large scale demonstrations and government support will be needed to make the technology popular.

**Herbicide Resistant Crops**

Bt-cotton is the only GM crop that has been approved for commercial cultivation in India. The technology whose introduction was opposed tooth and nail by some of the civil society organizations, has found phenomenal acceptance by the farmers. Introduced in 2002, it is presently grown over 9.0 mha (86% of the total) area, by over 5.6 million farmers, including small and medium, with a production of 5.61 mt. In the past nine years, India has greatly diversified deployment of Bt genes and genotypes which are well adapted to the different agro-ecological zones. About a thousand hybrids and one variety belonging to six different types (events) of Bt-cotton are available to the farmers. The technology has resulted in 40-60% reduction in pesticide use with 50-130% increase in farmers' profit and a significant increase in employment opportunities, particularly to women. This should be proof enough to silence the critics.

However, other crops/ traits have not been so lucky! Attempts to commercialize Bt- brinjal failed in 2009. Caught between scientists/experts on one side and the NGOs on the other side, the government chose to put the proceedings on hold. The herbicide resistant soybean, cotton and maize have been under controlled trials for a couple of years now. Given the way things are progressing, they are not expected to be approved by the government in the next 2-3 years. There is this old theory that any technology which replaces labor should not be encouraged. Unfortunately despite the description given above, a few NGOs think that labor in India is cheap and plentiful! There can not be a bigger contradiction than this. Some of the so-called guardians of the environment and the well being of the society opine that HRCs not only deprive rural poor of their livelihood, but also deprive them of the opportunity to eat nutrient-rich weeds (as green vegetables) which grow in association with crop plants, and their cattle the 'quality' fodder. They recommend 'growing' of weeds in the crop fields, ignorant of the fact that weeds compete with crops and reduce yields significantly.

Herbicide-resistant crops have the potential to improve the efficiency of weed management and facilitate adoption of resource conservation technologies (RCTs). However, several important risks associated with HRCs should be examined before their widespread adoption is permitted. The greatest risk is the potential for transfer of the gene conferring the HR trait to related wild and weedy relatives. This could lead to increased

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weediness or invasiveness. While this may not pose a danger in crops such as maize or soybean, it could be very important in rice where India, being the centre of origin for rice, has wide genetic diversity in the country.

India is planning to replace the rules under the Environment Protection Act with a Biotechnology Regulatory Authority of India (BRAI) Act. This when approved is expected to give fast-track approvals for GMOs.

**Alien Invasive Weeds**

India has been a fertile field for invasion by exotic weeds. *Parthenium hysterophorus,* water hyacinth*, Mikania micrantha, Phalaris minor* are a few of the many invasive weeds that have been introduced into the country over the years. The problem has only increased with globalization and free trade policies. The Indian government imported about 6.2 MT of wheat from 10 countries between 2006 and 2007 for public distribution and it was believed that several exotic weeds have gained entry as contaminants. A National Invasive Weed Surveillance (NIWS) Programme was launched by the government in 2008, to detect the establishment of these regulated weeds. Extensive surveys and rigorous monitoring led to detection of five quarantine weeds in several parts of the country. They are: *Cenchrus tribuloides, Solanum carolinense, Cynoglossum officinale, Ambrosia trifida* and *Viola arvensis.* Now we must do everything possible to contain their spread and try to eradicate them, if possible.

**The Role of Herbicides**

Compared globally, the pesticide market in India is small. It is about USD 1 billion, compared to USD 33 billion world market. The overall pesticide consumption is also very low at 362 g/ha, the bulk of which are insecticides (67%). The share of herbicides is nearly 20% and is growing. Although the herbicides have been in use for over 3 decades, use has increased only recently. Wheat, rice, soybean and sugarcane are the major crops with approximate share of 28, 20, 9 and 7% respectively. With the labour shortage looming large, the demand for herbicides is expected to grow substantially. The lack of awareness about herbicides amongst farmers and the technical competence of the extension personnel are going to be the major challenges for the scientific community to deal with. Extensive awareness-raising activities for famers and trainings and workshops for extension personnel will be urgently needed. With the increased use of herbicides, the issues such as herbicide residues in soil, water and food, resistance to herbicides in weeds, etc would come into prominence and we should be geared up to tackle them.

**Weed Science Research in India**

India has one of the largest national agricultural research systems (NARS) in the world, with over 100 research institutes under the administrative control of the Indian Council of Agricultural Research (ICAR) and over 50 State Agricultural Universities. India also has the distinction of having an independent national research institute dedicated to weed science research. Established in 1989 at Jabalpur in central India, the centre has been recently upgraded to Directorate of Weed Science Research (DWSR). DWSR is engaged in basic and strategic research. It also coordinates the applied and location-specific research carried out at 22 coordinating units located at different parts of the country. DWSR has been successful in bringing awareness about the importance of weeds and weed management in enhancing crop productivity and sustainability. A lot more, however, is expected from this nodal centre in the years to come.

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## CONCLUSIONS

There is a greater recognition of weed science research in India now than before. Although the advantages of herbicides were known earlier, their popularity and adoption by the farmers was limited due to a variety of reasons. The increased urbanization, higher income and more importantly the implementation of the National Rural Employment Guarantee scheme which guarantees employment/wages to one adult in a household for 100 days in a year have put tremendous pressure on availability of labor to agricultural operations including weeding. While it is a boon to the vast majority of the rural poor, it is advisable to implement the scheme during agriculturally lean periods, so that the labor supply during the peak agricultural season is not affected. Due to higher wages and unavailability of labor, herbicides may now find greater acceptance by the farmers. Herbicide industry and weed scientists have a greater responsibility in educating the farmers and the extension staff on judicious and sustainable use of herbicides. Integrating HRC technology (expected to be available sooner or later in the country) with resource conservation tillage strategies will benefit the farmers in a big way. If handled appropriately, this will address production, profitability and sustainability issues in crop production. The research related to herbicides must go beyond herbicide screening to application techniques, enhancing herbicide efficiency and integrating with ecological methods of weed management. The basic research on weed biology and ecology which has been largely ignored until now needs encouragement, as the results of such studies lay the foundation for integrated weed management strategies. Agronomists should not consider weed science as their sole domain. Scientists from related disciplines must be encouraged to undertake collaborative research. This will give greater dividends. There needs to be greater interaction with international bodies and weed scientists from around the world. Going by the past record, Indian weed scientists have greater difficulty in participation at international meetings and seminars for various reasons. However, in the era of Internet, this can be overcome, albeit to some extent. This is also the era of social networking. It is high time our weed scientists started interacting with peers residing outside the country and working on collaborative research programs. Our scientists should start taking advantage of the ICTs for building their own competence, sharing knowledge and ultimately to serve the farming community.

## ACKNOWLEDGEMENT

The information shared by several friends and colleagues is deeply appreciated. The views expressed in this paper are solely of the author and not of the organization he is affiliated to.

# MULTIPLE FUNCTIONS OF ALLELOCHEMICALS IN ECOSYSTEMS

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## ABSTRACT

Plants synthesize a broad range of secondary metabolites (allelochemicals) to defend themselves against herbivores, pathogens and their competing neighbors (allelopathy). Insect damage and pathogen infection in many plants initiates a series of signal transduction processes, which in turn induce an array of defence genes to produce more compounds which can mount resistance against subsequent infection. In our laboratory 32 plant species in South China have been investigated for allelopathy. The allelochemicals have been identified from 11 plant species. RNA interference (RNAi) was used to identify functions of specific allelochemicals in rice allelopathy and defence against pathogens. Signaling pathways regulating rice allelochemical production and role of allelopathy in biological invasion of exotic plants were determined. We also demonstrated that the root exudates of eight species of Brassicaceae strongly stimulate the hyphal growth of several ectomycorrhizal (ECM) fungi. Isothiocyanates and other related compounds degraded from indole GLSs are responsible for growth stimulation of ECM fungi. Polyphagous herbivores encounter numerous allelochemicals in their many host plants. Certain plant allelochemicals (e.g. coumarin and flavone) reduced toxicity of co-occurring compounds and insecticides to insects by inducing detoxification systems, including cytochrome P450 mono-oxygenases (P450s), which can metabolize a broad range of substances. Ecological significances of xenobiotic resistance induced by plant allelochemicals will be discussed. These results suggest that allelochemicals in plants have multiple ecological functions and mediate plant interactions with various organisms in ecosystems. Appropriate manipulation of plant allelochemicals is a promising approach to reduce human dependency on synthetic chemicals.

**Keywords** Allelochemicals, ecological function, species interaction, allelopathy, cytochrome P450 mono-oxygenases, xenobiotic resistance

# IDENTIFICATION OF SILVERLEAF NIGHTSHADE USING MICROSATELLITE MARKERS AND MICROSTRUCTURE

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## ABSTRACT

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) originated in America and is a serious summer-growing perennial weed in Australia. It is often confused with the native Solanaceae species quena (*S. esuriale* Lindl.). Both belong to the "*Leptostemonum*" subclass in the Solanaceae and are remarkably similar in their morphological traits. Correct identification is critical for the successful management of *S. elaeagnifolium*, as different biotypes could vary significantly in their response to control measures, such as herbicides and biocontrol agents. In order to improve the identification of *S. elaeagnifolium*, DNA polymorphism and microstructure of *S. elaeagnifolium* and *S. esuriale* were compared. Thirteen cross-species simple sequence repeat (SSR) primer pairs were utilized to investigate the polymorphism between *S. elaeagnifolium* and *S. esuriale*. SSR markers clearly separated the two species. Three unique SSR alleles were present in *S. esuriale* but not in *S. elaeagnifolium*, which could be used to distinguish the two species. Scanning electron microscope (SEM) examination of the microstructure of the leaf surface of these two species showed that the complex stellate trichomes on the upper leaf surface of *S. elaeagnifolium* had a deep "root" structure penetrating into the palisade mesophyll, while this structure was not found in *S. esuriale*. Combination of molecular phylogeny and SEM will considerably assist in the correct identification of *S. elaeagnifolium*.

**Key Words:** silverleaf nightshade, quena, perennial weed, SSR, SEM, trichome

## INTRODUCTION

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a deep-rooted, summer-growing perennial weed which originated in America (Heiser and Whitaker 1948; Stanton *et al.* 2009). This invasive weed infests at least 350,000 ha in Australia and with the potential to infest 398 million ha (Kwong 2006; Feuerherdt 2009).

Correct identification of silverleaf nightshade is required for selection of herbicides and biocontrol agents (Nissen *et al.* 1995; Lopez-Martinez *et al.* 1999). However, silverleaf nightshade often confused with an Australian native Solanaceae species, quena (*S. esuriale* Lindl.). The misidentification resulted in delays to control of silverleaf nightshade in South Australia (Hosking *et al.* 2000). Currently differentiation between the two species is based on morphological characteristic such as stamens length, spine density or fruit shape (Kidston *et al.* 2006). However, these morphological traits vary considerably in

Australian silverleaf nightshade populations (Stanton, *et al.* 2009). Identification based solely on morphological traits is unreliable.

Molecular markers have been widely used in *Solanum* species to delineate species and cultivars (Chimote *et al.* 2004) . For example, Chimote, *et al.* (2004) used simple sequence repeat (SSR) markers to differentiate 32 Indian cultivars of potato (*S. tuberosum*). In addition, previous studies have used molecular markers to identify the main clades and phylogenetic relationships within *Solanum* (Bohs 2005; Weese and Bohs 2007). The Australian native species quena was not included in these previous studies.

Micro-morphological parameters, such as leaf trichomes were considered as some of the most distinguishing features in *Solanum* (Roe 1971). Bean (2004) noted that silverleaf nightshade was so similar in morphology to quena that microscopic examination was usually required for identification.

In this study, micro-morphological traits and SSR markers were used to differentiate silverleaf nightshade from the Australian native *Solanum* species quena.

## MATERIAL AND METHODS

### Molecular Analysis

Silverleaf nightshade leaf samples were collected from Jarklin, Shepparton and Lake Boga (Victoria), and Corowa, Morven and Gulgong (New South Wales). Quena samples were collected from Jarklin (Victoria) and Ungarie, Wellington and Wagga Wagga (New South Wales). Genomic DNA was extracted from leaf material using the standard phenol/ chloroform method (Sambrook *et al.* 1989). The DNA samples of each species from the same location were bulked for PCR amplification. Thirteen SSR primer-pairs from other *Solanum* species were used in this study. Primer details were those mentioned in previous study of Zhu *et al.* (2011). The 5' end of the forward primer of each SSR primer-pair was tailed with M13 sequence and PCR amplification and detection of the amplification products were carried out as described by Raman *et al*. (2005). The alleles were scored in a binary form as the presence or absence (1 or 0) of bands of each SSR primer-pairs for each population and data were analysed using PAST (Hammer *et al.* 2001).

### Micro-morphological Analysis

Seven populations of silverleaf nightshade from Jarklin, Shepparton, Lake Boga, Corowa, Morven, Gulgong and Wagga Wagga and four quena populations from Jarklin, Ungarie, Wellington and Wagga Wagga were observed in this study. The 4th leaf from the shoot apex was collected from each individual. Only the adaxial surface of each leaf was examined in this study. Trichomes were removed with forceps from fresh leaves and placed on a 12 mm carbon tab (ProSciTech, Australia). A total of 195 and 74 trichomes were observed for silverleaf nightshade and quena, respectively. Images of these trichomes were obtained using a scanning electron microscope (SEM) (JEOL JCM 5000 NeoScope, Japan). The length of the "root" structure of each trichome was measured using software ImageJ (Ferreira and Rasband 2010). Data were analyzed using unpaired two sample t-test of GenStat 13.0 (Buysse *et al.* 2004).

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## RESULTS

Quena populations were clearly differentiated from silverleaf nightshade populations by the unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the Jaccard's similarity indicating the genetic variability between related species (Figure 1). In addition, three unique alleles were amplified in quena: fragment 85 bp with primer-pair EM 117, 222 bp with EM 135 and 249 bp with ESM 3. These unique alleles could be utilized to distinguish quena from silverleaf nightshade.

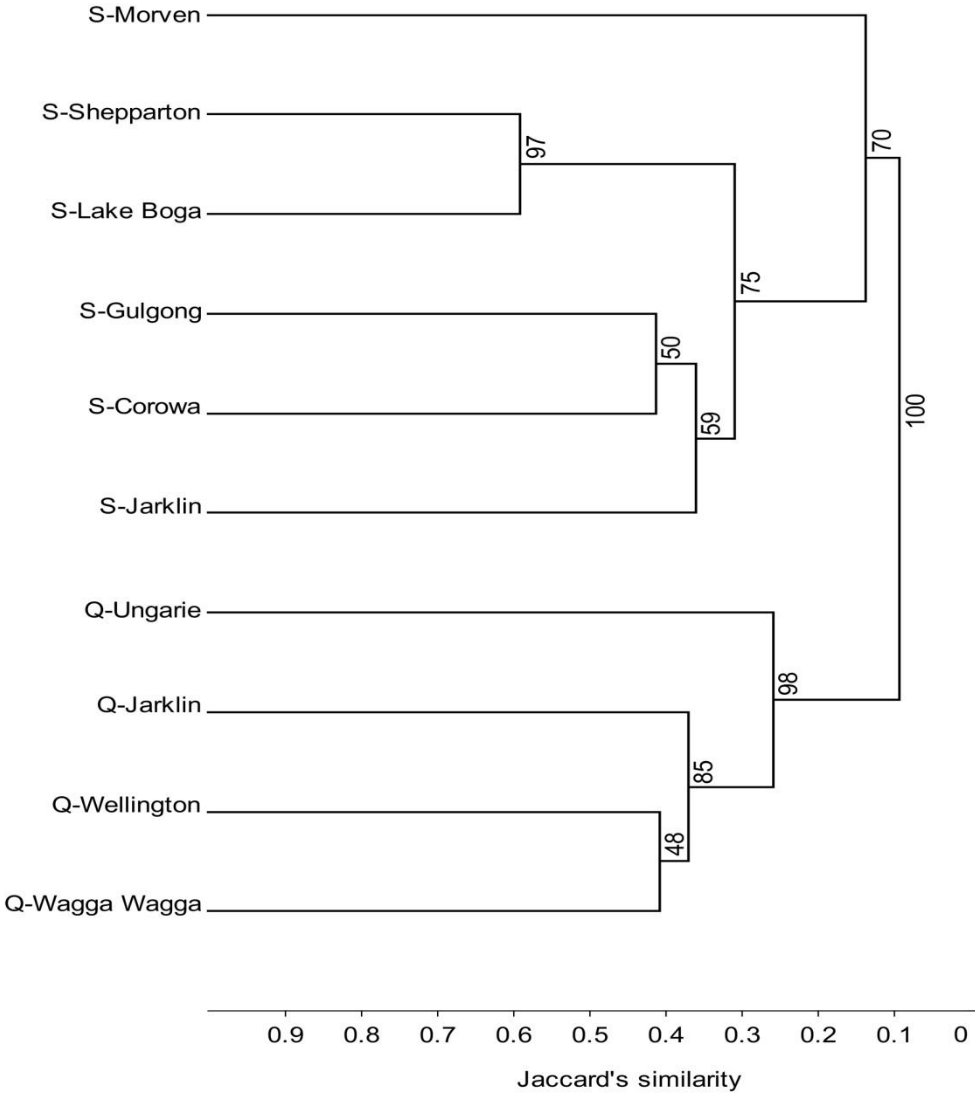


**Figure 1**. UPGMA dendrogram clearly separated quena (Q) and silverleaf nightshade (S) populations.

Significant difference on the length of trichome "root" structure ($P < 0.001$) was found between these two species (Figure 2). The average lengths of trichomes of silverleaf nightshade and quena were 134 and 33 μm, respectively.

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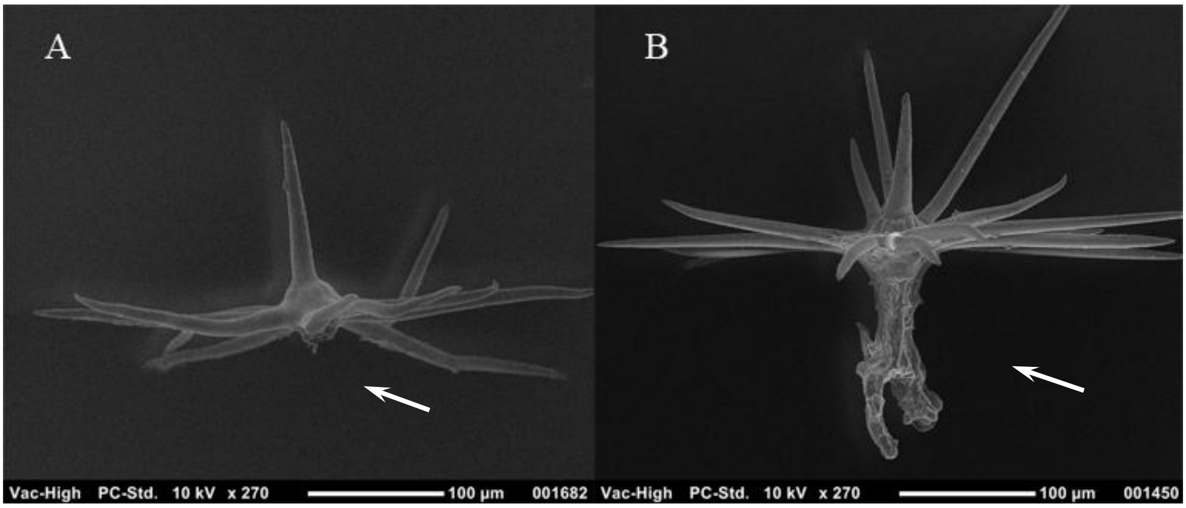


**Figure 2**. A comparison of trichome structure from adaxial leaf surface between quena and silverleaf nightshade. A: quena; B: silverleaf nightshade. Arrows indicate the root structure under epidermal level.

## DISCUSSION

Silverleaf nightshade and quena were separated at a level of about 10% similarity (Figure 1), indicating the great genetic divergence between these two species. Furthermore, the unique alleles found in quena will provide a reliable method to distinguish these two species.

This is the first report on the examined morphological characteristics of quena. Silverleaf nightshade had a much longer “root” structure of the trichome than quena, which has been highlighted by previous studies (Bruno *et al.* 1999; Christodoulakis *et al.* 2009). This “root” deeply penetrates into the palisade mesophyll making it very difficult to pull off from the leaf. The significant difference on trichome structures between quena and silverleaf nightshade found in this study can be considered diagnostic in order to discriminate these two species. This possibility of the impacts of this structure on herbicide uptake needs to be further tested.

Correct identification of silverleaf nightshade will improve the weed management. Generally, quena is easier to manage than silverleaf nightshade (Johnson *et al.* 2006), therefore correct identification will help the herbicide selection and management strategies. In addition, reliable identification is also required for biocontrol, as agent/ weed compatibility has a significant influence on agent success (Nissen*, et al.* 1995).

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## REFERENCES

Bean, A. R. (2004). The taxonomy and ecology of *Solanum* subg. *Leptostemonum* (Dunal) Bitter (Solanaceae) in Queensland and far north-eastern New South Wales. *Austrobaileya 6*(4): 639-816.

Bohs, L. (2005). Major clades in *Solanum* based in ndhF sequences In R. C. Keating, V. C. Hollowell and T. B. Croat (eds.), *A festschrift for William G. D'Arcy: the legacy of a taxonomist. Monographs in Systematic Botany from the Missouri Botanical Garden*. Missouri Botanical Garden 104. St. Louis: Missouri Botanical Garden. Vol. pp. 27-49.

Bruno, G., Cosa, M. T., and Dottori, N. (1999). Ontogenetic development of stellate trichomes in *Solanum elaeagnifolium* (Solanaceae). *Kurtziana 27*(1): 169-172.

Buysse, W., Stern, R., and Coe, R. (2004). *GenStat discovery edition for everyday use*. ICRAF Nairobi, Kenya.

Chimote, V. P., Chakrabarti, S. K., Pattanayak, D., and Naik, P. S. (2004). Semi-automated simple sequence repeat analysis reveals narrow genetic base in Indian potato cultivars. *Biologia Plantarum 48*(4): 517-522.

Christodoulakis, N. S., Lampri, P. N., and Fasseas, C. (2009). Structural and cytochemical investigation of the leaf of silverleaf nightshade (*Solanum elaeagnifolium*), a drought-resistant alien weed of the Greek flora. [Article]. *Australian Journal of Botany 57*(5): 432-438.

Ferreira, T., and Rasband, W. (2010). The ImageJ User Guide —Version 1.43. http://rsbweb.nih.gov/ij/docs/user-guide.pdf

Feuerherdt, L. (2009). Overcoming a deep rooted perennial problem - silverleaf nightshade (*Solanum elaeagnifolium*) in South Australia. *Plant Protection Quarterly 24*(3): 123-124.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for education and data analysis. *Palaeontologia Electronica 4*(1): 9.

Heiser, C. B., Jr., and Whitaker, T. W. (1948). Chromosome Number, Polyploidy, and Growth Habit in California Weeds. *American Journal of Botany 35*(3): 179-186.

Hosking, J. R., Sainty, G. R., and Jacobs, S. W. L. (2000). Certainty and uncertainty in plant identification. *The new Mexica botanist 15*: 1-8.

Johnson, A., Brooke, G., Thompson, R., Roberts, K., Hertel, K., Border, N., McNee, T., and Sullivan, P. (2006). Weed control for cropping and pastures in central west NSW. http://www.dpi.nsw.gov.au/agriculture/pests-weeds/weeds/publications/central-west

Kidston, J., Thompson, R., and Johnson, A. (2006). Silverleaf nightshade. from NSW Department of Primary Industries:

Kwong, R. (2006). *Feasibility of biological control of solanaceous weeds of temperate Australia* (Final report No. Weed 1.20). North Sydney.

Lopez-Martinez, N., Salva, A. P., Finch, R. P., Marshall, G., and De Prado, R. (1999). Molecular markers indicate intraspecific variation in the control of *Echinochloa* spp. with quinclorac. *Weed Science 47*(3): 310-315.

Nissen, S. J., Masters, R. A., Lee, D. J., and Rowe, M. L. (1995). DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. *Weed Science 43*(3): 504-513.

ISBN Number: 978-0-9871961-0-1

Raman, R., Raman, H., Johnstone, K., Lisle, C., Smith, A., Matin, P., and Allen, H. (2005). Genetic and in silico comparative mapping of the polyphenol oxidase gene in bread wheat (*Triticum aestivum* L.). *Functional & Integrative Genomics 5*(4): 185-200.

Roe, K. E. (1971). Terminology of Hairs in the Genus Solanum. *Taxon 20*(4): 501-508.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (Eds.). (1989). *Molecular cloning : a laboratory manual* (Second ed.). New York: Cold Spring Harbor Laboratory.

Stanton, R. A., Heap, J. W., Carter, R. J., and Wu, H. (2009). *Solanum elaeagnifolium* (Vol. 3). Melbourne: R. G. and F. J. Richardson.

Weese, T. L., and Bohs, L. (2007). A three-gene phylogeny of the genus *Solanum* (Solanaceae). *Systematic Botany 32*(2): 445-463.

Zhu, X. C., Wu, H. W., Raman, H., Lemerle, D., and Stanton, R. (2011). Assessment of genetic variation in silverleaf nightshade (*Solanum elaeagnifolium* Cav.) using cross-species SSR markers. *submit to weed research*.

ISBN Number: 978-0-9871961-0-1