Proceedings II

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17-21 March 2003
Manila, Philippines

"Weed Science, Agricultural Sustainability and GMOs"

Organized by
Weed Science Society of the Philippines (WSSP)

PROCEEDINGS II

19th ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE

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Weed Utilization

Production of Vermi-compost from Weed Biomass Vis-A-Vis their Utilization in Rice (*Oryza sativa*) Nutrition.

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Abstract: The study was conducted in 2000 and 2001 to evaluate the biomass of two weed species viz., *Ipomoea carnea* and *Eichhornia crassipes* for the production of vermicompost utilizing native earthworm species. Results clearly indicated the possibility of utilizing such biomass with farm yard manure at 60:40 ratio to production vermicompost containing 2.0-2.5% N, 1.3-1.8% P, 1.8-2.5% K, 1.0-1.2% Ca, 0.3-0.5% Mg, 0.4-0.5% S within 60-70 days utilizing the earthworm species *Eisenia foetida* or *Amynthas diffringens*. Results also indicated the superiority of vermicompost from *I. carnea* than *E. crassipes*. The effect of vermicompost thus prepared and the incorporation of fresh biomass of the above weed species were also evaluated on rice in a field experiment conducted for two seasons in an acid Inceptisol. Significant improvement in rice productivity, nutrient uptake as well as improvement in soil nutrient status was recorded due to the incorporation of vermicompost and weed biomass. Results clearly indicated the possibility of saving 25-50% fertilizer nitrogen in transplanted rice.

Key words: weed biomass, earthworm,. rice productivity, soil nutrient status, soil microbial population

INTRODUCTION

Productivity of soils can not be sustained only through the use of chemical fertilizer. Of late, much emphasis is being put globally on increased use of organic sources of nutrients for sustaining soil fertility and crop productivity. Escalating fertilizer prices, growing environmental concern and global energy crisis has necessitates the search for alternative and cheap sources of plant nutrients to supplement chemical fertilizer. Weeds such as *I. carnea* and *E. crassipes* are very much available in and around farm environment and contain a quite high percentage of plant nutrients. Tender and succulent biomass of such weeds may be utilized for direct incorporation in puddled rice soils as well as for the production of vermicompost having low C:N ratio utilizing earthworms. Available literature revealed the positive influence of plant biomass and vermicompost incorporation on crop growth and yield (Raju *et al.* 1987; Minhas and Sigh 1998; Rajkhowa and Gogoi 2001; Victor *et. al.* 2001). Earthworms are reported to be instrumental in the decomposition of different bio-wastes and improving soil health (Fletcher 1991); Kale *et al.* 1992). Since the information relating to utilization of weed biomass for production of vermicompost and their effect on crop nutrition is very much scarce, the present study was proposed.

MATERIALS AND METHODS

Pot studies

The studies on vermicomposting of weed biomass viz., *I. carnea* and *E. crassipes* were conducted during summer and winter seasons of 2000 and 2001 in concrete pots of size 1m x 0.75 m x 0.5 m. The biomass of the respective weeds were collected, chopped (4-6 cm) and heaped under the sun for 7-10 days. A layer of ground soil (4 -5 cm) was placed at the bottom of the each concrete pot above which a layer of partially decomposed cowdung (5-10 cm) was placed. The weed biomass and partially decomposed cowdung at 60:40 ratio (weight basis) was placed in alternate layers up to a height of 2 ft. The earthworm species i.e., *E. foetida and A. diffringens* at 3 kg t^{-1} biomass were introduced to the concrete pots separately. Sprinkling of water was done to keep the compost mixture moist (70-80 %). The pots were then covered with hessian cloth. Necessary measures were taken to protect the pots from direct sunshine and rain. The pot studies were replicated in Completely Randomized Block Design. The maturity of compost was judged visually. The observations on earthworm population, cocoon number and other parameters were taken at harvest. The vermicompost produced were analysed for their nutrient content following standard procedures. The microbial load in the vermicompost was also determined.

Field Experiment

The field experiment was conducted at Assam Agricultural University, Jorhat in dry (DS) and wet seasons (WS) of 2001. The soil in the test field was acidic (pH 5.2) with organic carbon 8.2 g kg^{-1}, available N 240 kg ha^{-1}, P 6.2 kg ha^{-1} and K 74 kg ha^{-1}. The treatments comprised of five sources of organic manures viz., fresh biomass of *I. crnea* (S1); fresh biomass of *E. crassipes* (S2); vermicompost prepared from *I. crnea* (S3); vermicompost prepared from *E. crassipes* (S4) and FYM (S5); and two N-substitution levels *viz.* 25 per cent substitution (N1) and 50 per cent substitution (N2) along with two control -No nitrogen (C1) and recommended dose of nitrogen (C2). The experiment was laid out in factorial randomized block design with three replications. The rice variety "Lachit" and "Ranjit" were grown during DS and WS, respectively. The weed biomass were collected, chopped and incorporated in soil 15 days before final puddling, while vermicompost and Farm Yard Manure (FYM) were applied at final puddling of the soil. The different growth parameters in rice were recorded at the active growth stage of the crop and the yield components and yield were recorded at harvest. The soil and plant samples were analyzed for their nutrient content following standard procedures. The build up of microbial population in soil was studied at harvest of the crop.

RESULTS AND DISCUSSION

The biomass of *I. carnea* and *E. crassipes* could successfully be utilized for production of vermicompost round the year. The time required for composting such biomass varied from 59-65 days during summer and 72-78 days during winter seasons. The vermicompost recovery ranged from 54-67 per cent and was more or less same during both summer and winter seasons.

Table 1. Vermicomposting of weed biomass during summer and winter seasons.

Treatment	Days required for vermicomposting	Per cent recovery
Summer		
E.crassipes +A. diffringens	65	59.02
E.crassipes+ E. foetida	59	67.53
I. carnea + A. diffringens	69	54.30
I. carnea+ E. foetida	61	58.0
CD (P=0.05)	NS	NS
Winter		
E.crassipes +A. diffringens	72	57.45
E.crassipes+ E. foetida	72	57.45
I. carnea + A. diffringens	78	54.84
I. carnea+ E. foetida	72	58.04
CD (P=0.05)	NS	NS

Results also revealed that both species of earthworm viz., *E. foetida and A. diffringens* were similar in their composting ability. The multiplication rate of *E. foetida* was comparatively higher over *A. diffringens* (Table 2) and the multiplication rate was slightly lower during winter season.

Table 2. Population dynamics of *E. foetida* and *A. diffringens* during summer and winter seasons during composting of weed biomass.

Treatment	Initial population	Final population (adult+young)
Summer		
E.crassipes +A. diffringens	85	1206
E.crassipes+ E. foetida	92	1824
I. carnea + A. diffringens	85	1194
I. carnea+ E. foetida	92	1424
CD (P=0.05)	-	125
Winter		
E.crassipes +A. diffringens	85	1184
E.crassipes + E. foetida	92	1545
I. carnea + A. diffringens	85	1129
I. carnea+ E. foetida	92	1224
CD (P=0.05)	-	76

Chemical analysis of vermicompost showed that vermicompost prepared from the weed biomass was rich in major and micro nutrients and had narrow C:N ratio showing its superiority over many other conventional sourced of organic manures.

Results also revealed that vermicompost from *I. carnea* contained relatively higher amount of plant nutrients than from *E. crassipes*. The vermicompost prepared from weed biomass was also rich in fungal and bacterial population. The fungal population was relatively higher in the vermicompost prepared from *I. carnea* while, the bacterial population was higher in the vermicompost from *E. crassipes* (Table 4).

Table 3. Nutrient content of vermicompost prepared from *I. carnea* and *E.crassipes.*

Nutrient Content	Vermicompost	
	I. carnea	*E.crassipes*
N (%)	2.5	2.2
P (%)	1.8	1.3
K (%)	2.9	1.8
Ca (%)	1.2	1.0
Mg (%)	0.5	0.3
S (%)	0.5	0.4
Fe (%)	1.03	1.4
Mn (ppm)	760	1730
Cu (ppm)	1313	1175
Zn (ppm)	21	29.5

Table 4. Microbial population in vermicompost prepared from *I. carnea* and *E.crassipes.*

Microbial population	Vermicompost	
	I. carnea	*E.crassipes*
Fungi	1.3×10^6	2.4×10^5
Bacteria	7.0×10^7	1.6×10^8

Data on changes in C:N ratio of weed biomass during composting showed that C:N ratio of the weed biomass narrowed down to about 11 to 19. Results showed that irrespective of weed species tried, the C:N ratio was comparatively lower with the use of *E. foetida* than *A. diffringens* showing slightly higher efficiency of *E. foetida* over *A. diffringens*

Table 5. Changes in C:N ratio of weed biomass during vermicomposting

Treatment	Days				
	O	15	30	45	60
I. carnea+ E. foetida	37.9	34.0	30.5	22.6	11.27
I. carnea + A. diffringens	37.9	36.5	32.0	29.5	16.33
E.crassipes+ E. foetida	50.6	37.3	27.3	19.8	13.7
E.crassipes +A. diffringens	50.6	47.1	37.4	27.9	19.2

Effect on crops

The chlorophyll content and leaf nitrate reductase activity in rice increased significantly due to different sources of organic manure applied. Use of vermicompost prepared either from *I. carnea* or *E. crassipes* was at par and resulted in higher chlorophyll content over the fresh biomass incorporation of the weeds. The rapid and increased availability of nutrients particularly N from vermicompost with narrow C:N ratio might have resulted in increased chlorophyll content. The increase in chlorophyll content was significantly higher over no nitrogen but was at par with the treatment receiving recommended nitrogen.

Table 6. Chlorophyll content and nitrate reductase activity in rice as influenced by organic manures and N-substitution.

Treatments	Chlorophyll Content (mg g^{-1} tissue)	NRA (ήmoleNO_2 g^{-1} fr.wt.30 min^{-1}
Sources of organic manure		
Fresh biomass of *I. carnea*	1.83	1410
Fresh biomass of *E. crassipes*	1.81	1620
Vermicompost prepared from *I. carnea*	2.30	2118
Vermicompost prepared from *E. crassipes*	2.18	2052
FYM	2.06	1710
CD(P=0.05)	0.21	86.3
Levels of N substitution		
25 % N-substitution	2.11	1774
50 % N-substitution	1.96	1790
CD(P=0.05)	NS	NS
Treatment mean V Control		
Treatment mean	2.03	1782
No nitrogen	1.31	890
CD (P=0.05)	0.13	38.6
Recommended dose of Nitrogen	2.20	2040
CD (P=0.05)	NS	NS

Nitrate reductase acivity (NRA) also followed the similar trend as that of chlorophyll content. All sources of organic manure tested significantly increased the NRA over no nitrogen. Among the sources of organic manure, vermicompost prepared from either *I. carnea* or *E. crassipes* resulted in the highest NRA. Increase in NRA following addition of vermicompost was also earlier reported by Rajkhowa and Gogoi (2001).

Effect on rice yield

Grain yield of rice significantly varied due to different sources of organic manure. Vermicompost prepared from *I. carnea* or *E. crassipes* produced the highest yield of both dDS and WS rice. The increased and prolonged availability of nitrogen from vermicompost might have resulted in increased yield attributes of rice which ultimately resulted in higher yield. Similar results were also earlier reported by Vasanthi and Kumaraswamy (1999). The lowest yield of rice on the other hand, obtained from the treatment that received incorporation of fresh biomass of *E. crassipes*. Contrary to the fresh biomass incorporation of *E. crassipes*, rice yield was higher with the incorporation of fresh biomass of *I. carnea* and was also at par with the treatment that received farm yard manure. This might be due to relatively higher nutrient content of *I. carnea* particularly nitrogen over *E. crassipes*. This confirms the earlier findings of Shamim and Rajkhowa (1999). The overall increase in yield of DS and WS rice due to different sources of organic manure was significantly higher over no nitrogen (control) but was at par with the treatment that received recommended dose of nitrogen. Different nitrogen substitution levels failed to bring any significant change in rice yield.

Table 7. Yield of rice as influenced by organic manures and N-substitution

Treatments	Grain Yield (t ha^{-1})	
	Dry season rice	Wet season rice
Sources of organic manure		
Fresh biomass of *I. carnea*	3.2	4.1
Fresh biomass of *E. crassipes*	2.8	3.9
Vermicompost prepared from *I. carnea*	3.8	4.4
Vermicompost prepared from *E. crassipes*	3.9	4.4
FYM	3.2	4.0
CD(P=0.05)	0.3	0.2
Levels of N substitution		
25 % N-substitution	3.4	4.1
50 % N-substitution	3.4	4.3
CD(P=0.05)	NS	NS
Treatment mean V Control		
Treatment mean	3.4	4.2
No nitrogen	2.1	3.1
CD (P=0.05)	1.2	0.5
Recommended dose of Nitrogen	3.8	4.0
CD (P=0.05)	NS	NS

Effect on nitrogen uptake

Significant variation in N uptake by crop was recorded due to the different sources of organic manure. Use of vermicompost resulted in significantly higher nitrogen uptake over the treatments receiving incorporation of fresh weed biomass. Among the different sources of organic manure, the lowest nitrogen uptake was recorded in the treatment that received fresh biomass incorporation of *E. crassipes*. The higher mineralization of nitrogen from vermicompost compared to fresh biomass incorporation might have supplied more amount of nitrogen which consequently led to increased N uptake by crop.

As regard to nitrogen substitution level, 50% substitution through organic manure resulted in significantly higher nitrogen uptake over 25% substitution. Reduced leaching loss of N with higher substitution rate through manure might have resulted in increased N uptake. The results are in accordance with the findings of Minhas and Singh (1998).

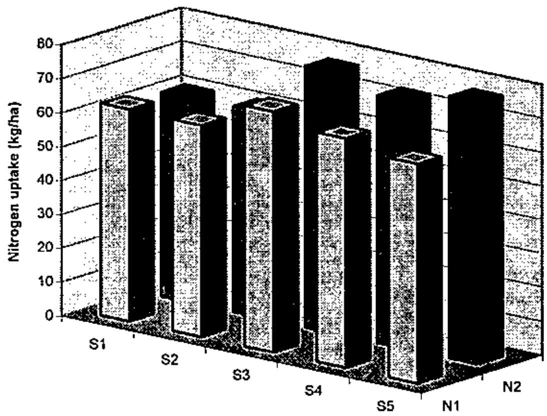

Figure 1. N uptake by dry season rice as influenced by interaction effect of sources of organic manure and N substitution rate.

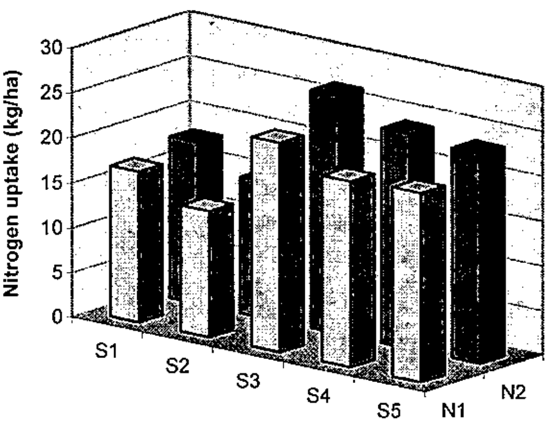

Figure 2. N uptake by wet season rice as influenced by interaction effect of sources of organic manure and N substitution rate

Effect on soil health

Addition of different organic manures significantly increased the soil organic carbon and available N over no N and recommended dose of N after two rice crops. This was in conformity with the findings of Bhandari *et al.* (1992). Vermicompost prepared from *I. carnea* or *E.crassipes* resulted in higher build up of organic carbon in soil over the incorporation of fresh biomass of the weeds or farm yard manure.The increase in available nitrogen was highest with the use of vermicompost from *I. carnea* and was at par with the treatment that received fresh biomass incorporation of *E. crassipes*. As regards to nitrogen substitution levels, 50 % substitution with organic manure led to significantly higher build up of available N in soil over 25% substitution. Reddy and Reddy (1998) also found significantly higher build up of available N in soil over 25% substitution. Such increase in available N due to addition of organic manures might be due to mineralization of nitrogen from organic sources and solubilization of native N.

Similar results were also reported by Bhandari *et al.*(1992) and Minhas and Singh (1998).

Table 8. Effect of organic manures and N-substitution on soil organic carbon and N-status at harvest (after two rice crops)./

Treatments	Organic carbon ($g\ kg^{-1}$)	Available N ($kg\ ha^{-1}$)
Sources of organic manure		
Fresh biomass of *I. carnea*	11.5	258
Fresh biomass of *E. crassipes*	11.4	292
Vermicompost prepared from *I. carnea*	13.3	292
Vermicompost prepared from *E. crassipes*	13.4	278
FYM	12.5	259
CD(P=0.05)	01.0	11.0
Levels of N substitution		
25 % N-substitution	12.3	262
50 % N-substitution	12.5	272
CD(P=0.05)	0.6	8.0
Treatment mean V Control		
Treatment mean	12.4	267
No nitrogen	7.6	162
CD (P=0.05)	3.3	10.0
Recommended dose of Nitrogen	9.8	247
CD (P=0.05)	2.4	9.0

Effect on soil microorganisms

Bacterial and fungal population in soil increased significantly due to addition of organic sources over no N and recommended dose of N applied as fertilizer. Vermicompost prepared either from *I. carnea* or *E.crassipes* resulted in the highest build up of bacterial and fungal population in soil followed by the treatment that received farm yard manure. This might be due to higher microbial load in the vermicompost applied to the soil. Higher microbial load in the vermicompost was also reported by Indira *et al,* (1996).

Among the nitrogen substitution level, 50 per cent substitution through organic manures resulted in significantly higher bacterial and fungal population in soil over 25 per cent substitution.

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Table 9. Bacterial (x10^6 per g soil) and fungal (x10^4 per g soil) population after harvest of wet season rice.

Treatments	Bacteria	Fungi
Sources of organic manure		
Fresh biomass of *I. carnea*	3.13	3.9
Fresh biomass of *E. crassipes*	3.20	3.38
Vermicompost prepared from *I. carnea*	4.73	6.73
Vermicompost prepared from *E. crassipes*	4.90	6.62
FYM	3.68	4.88
CD(P=0.05)	0.22	0.67
Levels of N substitution		
25 % N-substitution	3.83	4.82
50 % N-substitution	4.03	5.38
CD(P=0.05)	0.14	0.43
Treatment mean V Control		
Treatment mean	3.93	5.10
No nitrogen	2.50	3.30
CD (P=0.05)	0.26	0.59
Recommended dose of Nitrogen	3.06	4.43
CD (P=0.05)	0.27	0.62

LITERATURE CITED

Bhandari, A. L *et al.*,1992. Integrated nutrient management in a rice wheat system. *J. Indian Soc. Soil Sci.* 40: pp:742-747.

Fletcher, K. E. (1991). The utilization of organic waste by earthworms. *Agric. Zool. Rev.* 4: 241-253.

Indira, B. N. *et al.*, 1996. Microflora of vermicompost. Pages 51-52 in G. K. Veeresh and K. Shivashankar, eds. (In) Abs. Of papers National Seminar on Farming and Sustainable Agriculture.

Kale R. D. *et al.*, (1992). Influence of vermicompost application on the available micronutrients and selected microbial population in a paddy field. *Siol Bio. Bichem.* 24 (12): 1317-1320. Agriculture .

Minhas, R. S. and Singh, Golab 1998. Utilization of weed plants alone or in conjunction with fertilizer in a wheat maize cropping sequence on a Hapludalf in hilly region of Himachal Pradesh. *J. Indian Soc. Soil Sci.* 46 :50-54.

Raju, R. A. *et al.*,1987. Green leaf and paddy straw incorporation as supplemental source of nitrogen to rice. *Indian J. Agron.* 32 (2): pp: 170-171.

Rajkhowa, D. J. and Gogoi, A. K. 2001. Organic Farming-key sustainable Agriculture. Pages 120-124 in A. K. Yadav and S. Roychoudhuri, eds. Three Decades in Biofertilizers and Organic Farming in North East India. levels on soil available nutrient status in maize-soybean cropping system. *J. Indian Soc. Soil Scii.* 46:474.

Shamim, S. A. and Rajkhowa, D. J. 1999. Recycling of weed biomass for nitrogen economy in rice. M. Sc. (Agri) Thesis submitted to Assam Agricultural University.

Vashanthi, D. and Kumaraswami, K. 1996. Efficacy of vermicompost on the yield of rice and on soil fertility. Abstract *National Seminar on Organic Farming and Sustainable Agriculture.* Bangalore. Pp:40

Victor, R. *et al.,* 2001. Studies on utilization of prominent weeds for vermicomposting. *Indian J. Weed Sci.* 33:229-230.

The Anti-Bacterial Property of Selected Weeds

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Abstract: This study was conducted to screen the anti-bacterial property of twenty weed species against three species of bacteria namely: *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* It also aimed to evaluate the ability of the weed extracts to be a protectant and an eradicant against the pathogens. The methods used were divided into two parts, the screening study and the evaluation study through *in vitro* bioassay. These were done using paper disc technique. Results of the screening study revealed that 17 out of 20 weed crude extracts possess anti-bacterial property. The weed crude extracts of *Ruellia tuberosa* L., *Althernanthera sessilis* R. Br. ex. Roem. and Schult., *Celosia argentea* L., *Gomphrena celosioides* Mart., *Chromolaena odorata* R.M.King and H. Robinson, *Ipomoea obscura* (L.) Ker-Gawl., *Sida rhombifolia* L., *Mimosa invisa* Mart. ex Colla, *Pennisetum polystachyon* (L.) Schult, *Physalis angulata* L. and *Stachytarpheta jamaicensis* (L.) Vahl. were found effective in inhibiting the growth of *E. Coli, P. aeruginosa* and *S. aureus.* While the weed crude extracts of *Emilia sonchifolia* (L.) D.C. ex Wight, *Tridax procumbens* L. and *Solanum ferox* L. were effective only against *E. coli* and *S. aureus.* On the other hand, *Boerhavia erecta* L. and *Macroptilium lathyroides* (L.) Urb. were effective only against *P. aeruginosa,* whereas, *Vernonia cinerea* (L.) Less. was effective only against *S. aureus.* The weeds that gave the biggest zone of inhibition in the screening study against *E. coli, P. aeruginosa* and *S. aureus* were evaluated as to their ability to be protectant and as eradicant. Evaluation study showed that all the weed crude extracts of *R. tuberosa, A. sessilis, C. argentea, G. celosioides, C. odorata, I. obscura, S. rhombifolia, M. invisa* and *P. polystachyon* can all be used as protectant against the pathogens but failed to show that they can be used as eradicant.

Key words: Anti-bacterial property, crude extracts, eradicant, in vitro bioassay, protectant, zone of inhibition

INTRODUCTION

About 8,000 species of plants behave as weeds, and these are those plants that grow where people do not want them to grow (FAO1994). They are considered out of place, undesirable and with negative value. Plants are called weeds wherever they grow because they are useless and they interfere with the successful growth of crops, for they shelter insects and diseases that damage the economic crops. They compete with crops for light, water, and nutrients in the soil that reduce both the quantity and quality of crops.

On the other hand, weeds also have some importance in our ecosystem. They can be enjoyed as ornamentals for they are pleasing to the eyes because of their flowers and seedpods. They appear and grow quickly to cover unsightly scars of the landscape caused by man and nature. Weeds prevent or reduce soil erosion, and they provide in some instances, excellent forage for livestock during certain periods of the year. They are helpful to birds, rabbits and other wildlife for they provide them with shelter and foods (Anderson 1983). Some of these weed plants contain compounds that maybe used to control the effect of microorganisms like bacteria.

Bacteria are widely distributed in nature and they have adapted to every conceivable habitat. They are found within and upon our bodies and in the food we eat, the water we drink and in the air we breathe (Smith 1980). The abundance of bacteria makes the people prone to them, especially the body part, which is usually exposed, and that is the skin.

Bacteria and other microorganisms invade the body through areas where the skin has been broken. Bacteria cause infections such as boils, impetigo and others. The microorganisms such as *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* are some of those invaders causing diseases to animals especially man. Bacteria's great adaptation to every conceivable habitat makes them abundant. Failure to control them can cause diseases and even death.

This study on weeds once proven to inhibit growth of micro-organisms, especially opportunistic pathogens maybe used as effective anti-bacterial agents. These weeds are readily available and their cost is far less than buying anti-bacterial agents that are usually available in tubes, which are really expensive. In addition to this, weeds will be allowed to grow and mechanical as well as chemical control of weeds will be avoided that truly cost much and bring harm to crops, human and wildlife.

The main objective of the study is to determine the anti-bacterial property of some weeds against three different species of bacteria namely: *E. coli, S. aureus* and *P. aeruginosa*. Specifically, it aimed to determine which of the three bacterial isolates is more sensitive to the different crude extracts of the weed plants.

MATERIALS AND METHODS

I. Screening Study

Twenty weeds were collected on a bright sunny morning to be sure that photosynthesis was taking place. Only undamaged and actively growing stem and leaves were cut and placed in plastic bags. After the collection crude extracts, paper discs and bacterial pure culture were prepared.The sterile cotton swab was dipped into the prepared bacterial culture. The entire Mueller – Hinton II agar (MH agar) surface was swabbed evenly then it was rotated and swabbed again. Then the extract impregnated oven dried discs were seeded equidistantly into the agar plate using sterile forceps.

In this study, three bacteria were used. For each bacterium five swabbed plates were used to accommodate the 20 extract impregnated discs plus two control discs. In each plate, four different discs soaked in different plant extracts were seeded plus the alcohol and distilled water soaked discs (control) placed at the middle. This was done in three replications. The disc-seeded plates were incubated for 24 hours at 37 °C before microbial effect was determined by means of zone of inhibition.

The diameter of zone of inhibition was measured to the nearest mm using a foot ruler. Appearance of zone of inhibition means effectivity of weeds against test pathogens and absence means resistance of organism to the weed extract. The weed plants that gave the biggest zone of inhibition in the first part of the study, the screening study, were used in the second part, the evaluation of the weed extract as protectant from the pathogens and as eradicant.

II. Evaluation through *In Vitro* Bioassays

As Eradicant Against the Pathogens

Filter paper disc was prepared. It was sterilized in an autoclave, ovendried, and then soaked into the weed extract with anti-bacterial property as proven in the first part, the screening study. Paper discs for two controls were prepared, soaked in alcohol and sterile distilled water. Bacterial culture was prepared and it was adjusted using 0.5 McFarland Standard. After that, the adjusted bacterial culture was swabbed into the Mueller-Hinton agar plates. This swabbed plate was incubated for 24 hours and after 24 hours of incubation disc extract was seeded onto it. The disc-seeded plates were incubated for 24 hours and after the incubation period if no zone of inhibition was visible the experiment was terminated. But if zone of inhibition appeared, the extract in impregnated disc was removed from its position. A prick was made on the area where the extract has been removed, and then the pricked piece was swabbed into the nutrient agar plate. These plates were incubated for 24 hours and if after 24 hours of incubation growth of bacteria was visible, it only meant that the extract inhibited the growth of the bacteria but did not kill the bacteria.

As Protectant Against the Pathogens

Filter paper disc was prepared. It was sterilized in an autoclave, ovendried, then soaked into the bacterial culture. Paper discs for two controls were soaked in distilled water and the other one in alcohol. The impregnated discs were seeded into the Mueller Hinton II agar (MH agar) containing 5 ml of the plant extracts with antibacterial property as proven in the first part, the screening study. The plant extract was only added to the media after sterilization and when it is already cool. The disc-seeded plates were incubated for 24 hours at 37 °C before determining the microbial effect by means of the inhibition of pathogen's growth. Three replications for each treatment were prepared.

The degree of colonization of the pathogen that developed in the media with the plant extracts was determined using the scale as follows:

1 – Pathogen colonized 100% of the media with the plant extract
2 – Pathogen colonized 75% of the media with the plant extract
3 – Pathogen colonized 50% of the media with the plant extract
4 – Pathogen colonized 25% of the media with the plant extract
5 – No growth of the pathogen in the media with the plant extract

RESULTS AND DISCUSSION

I. Screening for Anti-Bacterial Property

Twenty weeds were screened for antibacterial property using three bacterial species namely: *E. coli, P. aeruginosa* and S. *aureus.* The crude extracts of *R. tuberosa, A. sessilis,* C. *argentea,* G. *celosioides,* C. *odorata, E. sonchifolia, T. procumbens, I. obscura,* S. *rhombifolia, M. invisa, P. polystachyon, P. angulata, S. ferox and S. jamaicensis* showed inhibitory effects on *E. coli,* clear zones were formed around the crude extract discs (Table 1). However, *P. aeruginosa* was inhibited by a great number of the plant weed crude extracts. The weeds that inhibited the growth of this bacterium were *R. tuberosa, A. sessilis,* C. *argentea,* G. *celosioides,* C. *odorata, I. obscura, S. rhombifolia, M. invisa, B. erecta, M. lathyroides, P. polystachyon, P. angulata, and* S. *jamaicensis* (Table 1) S. *aureus,* on the other hand, was inhibited by *R. tuberosa, A. sessilis,* C. *argentea,* G. *celosioides,* C. *odorata, E. sonchifolia, T. procumbens, V. cinerea, I. obscura,* S. *rhombifolia, M. invisa, P. polystachyon, P. angulata, S.ferox* and *S.jamaicensis* (Table 1).

All weeds listed above formed a clear zone around the crude extract impregnated disc. This indicates that the crude extracts inhibited the growth of the bacteria used. This may further indicate the presence of antibacterial compounds in those weed crude extracts against *E. coli, P. aeruginosa* and S. *aureus.* This study is an attempt to look into valueless plants that may possess antibacterial property. Anderson (1983) defined weeds as plants for which man has not yet found use but the result of this study is a very real possibility that a plant considered valueless may be the sources of some chemical compounds that can be used to check the growth of pathogenic microorganisms.

The fact that some of these weed plants were reported to be medicinal by Quisumbing (1978), hence their ability to inhibit the growth of the test organisms. The test plants that were reported as medicinal were *S. rhombifolia* (use for boils, cuts, itches, chicken fox, broken bones, fever, menses, herpes, headache, infantile diarrhea and boils); *A. sessilis* (used for eye wash, snake bite and boils); *C. argentea* (used for bruises, sores, wounds, skin eruption, buboes, abscesses, eczema, gonorrhea, diarrhea and menses); *S. ferox* (used for cuts, wounds, swelling, severe bruises, syphilis and fever); *S. jamaicensis* (used for fever, cough, itchiness and bruises); *V. cinerea* (used for wounds, humid herpes, eczema, febrile infection, wounds, sores, malarial fever, conjunctivitis, asthma and bronchitis); *E. sonchifolia* (used for cuts, wounds, eye inflammation, astringent, sore, swelling, sore ears, ulcers, cough, fever and blindness). Hence, there is the possibility of the presence of active ingredient that is inhibitory to *E. coli, P. aeruginosa* and *S. aureus.*

II. Evaluation of Weed Extract as Eradicant

The weeds that showed the biggest zone of inhibition against the bacterial isolate in the screening study were further evaluated as eradicant. In the screening study, *R. tuberosa, G. celosioides, C. odorata, I. obscura, S. rhombifolia* and *M. invisa*

Table 1. Zone of inhibition (mm) effected by crude extracts in the screening study.

Weed Plant	*E. coli*	*P. aeruginosa*	*S. aureus*
Ruellia tuberosa	8.8	7.6	8.0
Althernanthera sessilis	6.6	7.0	6.3
Celosia argentea	6.6	7.0	6.6
Gomphrena celosioides	8.5	7.3	7.2
Chromolaena odorata	8.6	8.3	8.7
Emilia sonchifolia	6.6	6.0	6.6
Tridax procumbens	6.3	6.0	6.6
Vernonia cinerea	6.0	6.0	6.6
Ipomoea aquatica	6.0	6.0	6.0
Ipomoea obscura	9.6	7.5	7.6
Ipomoea triloba	6.0	6.0	6.0
Euphorbia heterophylla	6.0	6.0	6.0
Sida rhombifolia	9.0	7.8	9.0
Mimosa invisa	8.6	7.3	8.3
Boerhavia erecta	6.0	6.6	6.0
Macroptilium lathyroides	6.0	6.3	6.0
Pennisetum polystachyon	6.6	6.6	7.5
Physalis angulata	6.6	6.6	6.6
Solanum ferox	6.6	6.0	6.6
Stachytarpheta jamaicensis	6.3	6.3	6.6

Zone of inhibition – average of three replications
Paper disc is 6mm in diameter

gave the biggest zone of inhibition against *E. coli.* These weeds were used for the evaluation study if these plants can be used as eradicant. These weeds showed no zone of inhibition in the evaluation study. These weeds therefore could not be used as eradicant the moment the microbes that initiated the disease had already multiplied in number. *R. tuberosa, A. sessilis,* C. *odorata, I. obscura, S. rhombifolia* and *M. invisa* gave the biggest zone of inhibition in the screening study for *P. aeruginosa* but gave negative results on the evaluation study as eradicant. On the other hand, *R. tuberosa,* G. *celosioides,* C. *odorata, I. obscura,* S. *rhombifolia, M. invisa* and *P. polystachyon* gave the biggest zone of inhibition against S. *aureus* but the evaluation study as eradicant showed negative results.

The results indicate that the test pathogens were not affected by the used crude extracts. According to Smith (1980), if the bacteria are susceptible to the antibiotic a zone of inhibition encircles the disc and if the bacteria are unaffected or resistant to the drug, growth will cover the area around the disc. Since the bacteria were already established in the media, the extracts were not able to inhibit the already growing bacteria. Hence, the efficacy of the extract could not be measured using the zone of inhibition. Probably, amount of the extracted active component is not enough to control the growth of the established pathogen. Hence, another method of evaluating weed as eradicant is recommended to test the efficacy of weed crude extracts.

III. Evaluation of Weed Extract as Protectant

The weed that showed zone of inhibition against the three bacterial isolates in the screening study were also evaluated as protectant against the microbes. The degree of pathogen colonization that developed in the media with the weed extract was determined using the scale of 100%, 75%, 50%, 25% and no growth. The degree of *E. coli* colonization in the media with weed extracts showed that *R. tuberosa, G. celosioides, C. odorata, I. obscura, S. rhombifolia* and *M. invisa* can be very good protectant, no growth of pathogen was observed in the media after 24 hours of incubation (Table 2). For *P. aeruginosa,* the weed crude extracts of *R. tuberosa, A. sessilis, C. argentea, G. celosioides, C. odorata, I. obscura, S. rhombifolia, M. invisa* and *P. polystachyon* effected no growth of pathogen (Table 2). *S. aureus* did not grow in the media with crude extracts of *R. tuberosa, G. celosioides, C. odorata, I. obscura, S. rhombifolia, M. invisa* and *P. polystachyon* (Table 2).

Table 2. Degree of colonization of *E. coli, P. aeruginosa* and *S. aureus* in the media with the weed extracts.

Weed Plant	*E. coli*	*P. aeruginosa*	*S. aureus*
R. tuberosa	5	5	5
A. sessilis	NE	5	NE
C. argentea	NE	5	NE
G. celosioides	5	5	5
C. odorata	5	5	5
I. obscura	5	5	5
S. rhombifolia	5	5	5
M. invisa	5	5	5
P. polystachyon	NE	5	5

- 5- No growth of pathogen in the media with the plant extract
- NE – Not evaluated

Results showed that the test pathogens did not grow in the media after 24 hours of incubation and even if the incubation period was prolonged. This means that the bacteria cannot grow in the media with the weed crude extracts, because the weed crude extracts might have some important ingredient that inhibit the growth of the test pathogens. Due to rapid multiplication of the bacteria within a few hours, there are many microorganisms in the inoculated culture (Smith 1980). That is, if the test pathogens are unaffected by the crude extract mixed with the media, growth within 24 to 48 hours of incubation could have developed, but it did not. Therefore, all the weed crude extracts that were evaluated can be used as protectant against *E. coli, P. aeruginosa* and *S. aureus*. This shows that these weeds contain chemical compounds that can check the growth of pathogenic bacteria. It means that weeds are not undesirable after all. They could also be important in some other ways like source of important compounds that may be inhibitory especially to pathogenic microbes.

Based on the results, further study must be carried out to identify the active principles from these weeds that are responsible for the inhibitory effects of the weed crude extracts against the test bacteria. It is also recommended that other methods of evaluating the effects of weeds as eradicant be conducted.

LITERATURE CITED

Anderson, P.W. 1983. Weed Science Principles. USA. West Publishing Company. 2nd edition.

Breed, R.S, E.G.D. Murray and N.R.Smith. 1957. Bergey's Manual of Determinative Bacteriology. Baltimore: The Williams and Wilkins Company. 7th edition.

Castillo, M.B and A.Q. Torres. 1996. *In Vitro* Study on the Anti- Bacterial Activity of Caimito (*Chrysophyllum cainito*), Duhat *(Syzigium cuminii)* and Eucalyptus *(Eucalyptus tereticornis)* leaves against *Escherichia coli.* CLSU Scientific Journal.

Damaso, A.F. 1997. Screening of some plant extracts for growth inhibition of *Aeromonas hydrophila* (Chester) Stainer using *in vitro* bioassay. Unpublished. Undergraduate Thesis. Central Luzon State University. Munoz Nueva Ecija.

Franje, N.S. 1990. Bioassay of plant extracts for their fungicidal/ bactericidal property. CMU Journal of Science. 3(1): 2-24.

Gwynne, D.C. and R.B. Murray. 1985. Weed Science Biology and Control in Agriculture and Hortic\llture. Typeset by Deltatype. Ellesnue Port. UK.

Mantaring- Chua, N.M. 1991. Quantitative Microbial Assay, Clinical Testing and Stability Studies of the Crude leaf extract of *Bidens pilosa* Linn. Acta Manilana 39.31-37.

Moody, K, C.E. Munroe, R.T. Lubigan and E.C. Paller Jr. 1984. Major Weeds of the Philippines. UPLB. College, Laguna.

Narne, A.B. 1995. *In Vitro* Bioassay of Plant Extracts Against *Staphylococcus aureus* Rosenbach. Unpublished Undergraduate Thesis. Central Luzon State University. Munoz, Nueva Ecijia.

Nickell, L.S. 1959. Anti- Microbial Activity of Vascular Plants. Econ. Bot.

Ongtengco, D.C, M. Talaue, L.J. Cruz, T.V. Capal and L.A. Dayap. 1999. The MICs of Betel Oil against common clinical pathogens. ACTA Manilana 47. 61-66.

Osborne, E.M. 1993. Anti- bacterial activity shown by plant extracts.Brits.J. Exp. Pathol.

Pancho, J.V. and S.R. Obien. 1995. Manual of Ricefield Weeds in the Philippines. Philippine Rice Research Institute. Munoz, Nueva Ecija.

Pederson, C.S. and P. Fisher. 1994. The bacterial action of cabbage and other vegetable juices. N .V State Exper. Stat.

Quisumbing, E. 1978. Medicinal Plants of the Philippines. Katha Publishing Co., Inc.

Reyes, B.S. 1992. *In Vitro* Study on the Anti-bacterial Effects of Garlic and Guava. Unpublished Undergraduate Thesis. CLSU. Munoz, Nueva Ecija.

Sasidharan, V .K, T. Krishnakumar and C.B. Manjula. 1998. Antimicrobial activity of nine common plants in Kerala, India. Phil. Journal of Science. Vol. 127 No.1. 65-71.

Modeling

Weed Database: Significance In Indian Agriculture

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Abstract: In India, information on weeds with respect to their floral distribution in different crops and cropping systems, soil types, climatic conditions, growing season, phenology and effective control measures is either not available or insufficient. Such information will be useful for importing or exporting agricultural commodities as per WTO agreement. Hence, an attempt is being made to develop a national database on weeds at NRC Weed Science, Jabalpur, to address these problems. This database will help in retrieving the information on weeds in different agro-climatic zones of the country in various crops and cropping systems, soil types and climatic conditions. The collection of data from all the agro-ecological regions (AER) during last 20 years revealed that the number of most frequently encountered weed species in Indian agriculture varied from 60-70 in humid, per humid, sub humid, coastal and island ecosystems, 30-40 in semiarid and 15-20 in arid ecosystems. The rainfall distribution directly influences the weed growth. The heavy growth of invasive species was confined in the high rainfall humid and per humid region of the country. In the present modules of weed database the desired information is recorded in a text form in an elaborative way and as a consequence, searching for the answer to a specific query is often difficult. Considering the need for easy retrieval of data, the codel nomenclature may be of great use. The coding of the different data field/ data type in the database system may help in minimizing the text and reduce the required space and also help the users for rapid retrieving of the information. In this nomenclature, the weeds may be grouped into various modules and combined together. A single window by putting the coded nomenclature symbols would help to retrieve the desired information within a short period of time.

Key words: agro-climatic zones, agro-ecological regions, coding, database, invasive weeds

INTRODUCTION

Database of weeds in different ecosystems can supply necessary information for prevention, prediction and the pooling of resources for developing the effective control measures against the problematic weeds of the varied agro-ecological regions. A database would also function as a baseline for understanding patterns and change and is a priority (Cronk 1995). Very often basic autecological facts are unknown for different weed species and this is particularly true of ecological behavior in the native range. There is sufficient knowledge on the current status of weeds and other plant species nationally but this information are placed in a very unorganized way in present database. A need was felt to collect all these sporadic information and represent them in a systematic manner and in easily retrievable format so that it could fulfill the requirements of various end users. Considering the existing scenario of software and hardware availability in the country, the guidelines chosen for the selected database were (a) to have collapsible data fields, (b) to be IBM compatible, (c) to run under

Windows, and (d) to be user friendly. Similar guidelines were proposed by Frost *et al.* (1995).

METHODOLGY

Data collected on weeds of different crops and cropping systems and non-cropped areas during the last 22 years (1978-2000) by the All India Coordinated Research Project on Weed Control centers of different agro-ecological regions (19 Agro-ecological regions) of the country were used in the database. The weed survey works done in the different locations of the country were ranked according to the intensity and importance value index (IVI) and also its importance in the production system. These processed information are utilized for the database program and the preparation of weed distribution maps of the country. Information was also gathered from the different weed scientist working in the plant sciences departments of different organizations of the country. This information was compiled district wise. A number of weed species were recorded in the cropped and non-cropped situations.

The information are classified into different groups viz. crops, cropping system, degree of infestation. Out of the major weed species five most dominant weed species has been considered for the database preparation. Database searches could be made as follows:

1. State: The state search form provides a list of twenty states for the user to choose form.
2. District: This form provides a list of total number of districts of the state.
3. Crop: This form provides major crops of the district.
4. Season: This form provides the growing season of the crop in the district and state.
5. Weed: This form provides the list of major five weeds of the crop of the district
6. Infestation: This form provides the degree of infestation of five dominant weed species in the major crops grown in the district.
7. Distribution: This forms provides the distribution of each weed species in the different districts of the state as well as in the country based on their occurrence and degree of infestation. This form also provides the distribution of major crops of the state and in the country.
8. Ranking: The user can select a weed species in different crops of the district, state and country. The ranking includes five different categories each one identified with a number as follows:
   - 5- Very high infestation (> 80 percent)
   - 4- High infestation (60-80 Percent)
   - 3- Moderate infestation (40-60 percent)
   - 2- Low infestation (20-40 percent)
   - 1- Very low infestation (< 20 percent)

Along with the database program, distribution maps of the different dominating weeds are also being prepared by using the software Arcview. Maps will be prepared accordingly for five dominant weeds of major crops of the state. It is also proposed to prepare the maps of major invasive weeds of the country. These maps will show the distribution and its pattern of dominance.

Considering the need for easy retrieval of data, the codel nomenclature (Table 1) has been introduced to minimize the descriptive nature of the different data fields. This will not only maximize the input entry into the program but also help the researcher for reviewing the retrieved information effectively.

Table 1. Key for decoding weed nomenclature.

Ontogeny	**Season**	**Crop**		**Emergence**	**Ranking**	**Control Measure**
A - Annual B - Biennial C - Perennial	1 - Kharif 2 - Rabi 3 - Summer	C - Cereals	C1- Rice C2- Wheat C3- Maize	E-Early merged L - Late emerged	5 - Serious Weed 3 - Principal Weed 1 - Common Weed	1 - Chemical 2 - Cultural 3 - Mechanical 4 - Biological
		P - Pulses	P1- Pea P2-Lentil P3-Greengram P4-Blackgram			
		O - Oil seeds	O1-Rapeseed & Mustard O2-Sunflower O3-Groundnut O4-Castor O5-Niger O6-Linseed			

Decoding

Weed : *Phalaris minor* Retz. Life form: Annual (A) Control: Chemical (1)
Season : *Rabi* (R) Ranking: Serious weed (5) Phalaris minor Retz.: R C_2 A 5 E 1
Associated crop : Cereal Wheat (C_2) Emergence: Early (E)

SIGNIFICANCE

Weeds are an integral component of all ecosystems. However, information on weeds with respect to floral distribution in different crops and cropping systems, soil types, climatic conditions are either not available or insufficient. Such a database will be of tremendous use, as it would aim at effective and economical weed management practices. Besides enhancing crop production, identification of major weeds in different crops/ cropping systems and agro-climatic regions will also help in world trade. In the WTO agreement, it has become essential or mandatory to make all possible information on pest status (including weeds) available before exporting or importing agricultural commodities (Gogoi *et al.* 2002). Information gathered in the database program will help the researchers and planners for easier retrieving of information. The infestation and weed species distribution in different crops and cropping systems will help in identifying the recommended weed control practices for adoption in the crop field. This database will also indicate the status of weed flora distribution in an agro-climatic zone and act as benchmark survey report. The weed flora shift or changes could be monitored in future crop production systems. It will also help into studying the impact of various crop management practices on weed crop interference and their effects on crop growth and yield. Pests, including weeds, play an important role in sustainability of any production system under a specific set of environment. The interactions of different variables on weeds could be monitored in advance by using the information available in a database system. The documentation of weed distribution maps could be of great use for the planners and extension personnel in suggesting the plan of activities related to the constraints in different production systems under varied agro-ecological situations of the country. The information available to the district level even helps in planning up to microlevel projects. The information on weeds and their risk (weed risk) are important production indices in the present era of globalization of agriculture, where the movement of agricultural products becoming more easier and increased by many folds. Keeping record of pests (including weeds) will help the farming community in preparing the budget and crop production balance sheet in advance.

LITERATURE CITED

Cronk, Q.C.B. 1995. Changing Worlds and changing weeds, Symp. Proc. No. 64, Weeds in a Changing World, BCPC, Brighton, November 20, 1995, P. 3-15.

Frost, H.M., P.J. Terry and P. Bacon 1995. A feasibility study into the creation of a database on weeds and invasive plant species. Symp. Proc. No. 64, Weeds in a Changing World, BCPC, Brighton, November 20, 1995, P. 35-49.

Gogoi, A.K., A. Dixit and N.T. Yaduraju 2001 Database on Weeds : A tool for information technology. Abst. ISWS Conf., May, 23-24, Bangalore, India, P.185.

Agro-ecological and Modeling Approach to Emergences of *Echinochloa* Species and Rice

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Abstract: Pot and laboratory experiments were conducted to understand the nature of seedling emergence of *Echinochloa* spp. and rice (*Oryza sativa*) and develop a prediction model of their seedling emergences as affected by soil depth and soil temperature. Initial data analyses indicated that *Echinochloa* spp. could also emerge from deeper soil up to 10 cm as compared with rice. *Echinochloa* spp. required lower temperature for seedling emergence, and emerged faster than rice. When the Gompertz curve was fitted at each soil depth and soil temperature, the parameter *B* did not vary with soil depth and temperature. The parameter *C* decreased with soil depth, but it increased with temperature, and the changes were logistic. The parameter *M* increased with soil depth, while it decreased with temperature, and the changes were exponential. Therefore, incorporating the logistic curve for C and the exponential curve for M combined the Gompertz curve, and the combined model well described the seedling emergence of rice and *Echinochloa* spp. as affected by soil depth or soil temperature. Thus, the combined model can be used to simulate the seedling emergence of weeds buried at different soil profiles and exposed to a certain temperature condition.

Key words: *Echinochloa* spp., rice, emergence, Gompertz curve, modelling, temperature, soil depth

INTRODUCTION

In rice cropping, *Echinochloa* spp. has long been considered as one of the most troublesome weeds, causing significant yield losses, and has a wide regional adaptability ranging from north to south (Holm et al. 1977). *Echinochloa* spp. can emerge more quickly than rice and its low-temperature adaptability is also greater than rice. *Echinochloa* spp. can emerge even at 11.0°C, while rice can emerge at higher than 12.3°C (Kwon et al. 1996). Moreover, it can also emerge from deeper soil profile than rice. Kim (1993) reported that *Echinochloa* spp. can emerge from soil profile deeper than 10 cm in a pot test, and this ability was due to its mesocotyl growth. These characteristics thus render *Echinochloa* spp. as much more competitive than rice at lower temperatures.

The accurate prediction of weed seedling emergence is a key element in crop-weed competition and population dynamics studies, and decision-making for weed management. As seedling emergence is influenced by climatic and soil conditions such as temperature, soil water content, soil depth, and so on, many mathematical modeling approaches have been made to develop a model to simulate seedling emergences of crops and weeds under various conditions (e.g., Cussans et al. 1996; Vleeshouwers 1997). However, little information is available for the model development for seedling emergences of *Echinochloa* spp in combination with rice.

In this study, we applied a general emergence model to simulate *Echinochloa* spp. and rice seedling emergence and further tried to develop a new combined model by incorporating the influences of temperature or soil depth into the general emergence model.

MATERIALS AND METHODS

Model development

The emergence model describes mathematically the emergence pattern of seedlings, which is expressed as the time course of cumulative emergence. Several models were developed but the simplest model is Gompertz curve (1837), which has widely been used by many weed scientists (e.g., Cussans et al. 1996),

$$y = C \Big/ e^{e^{-B(T-M)}} \qquad [1]$$

where y is the cumulative emergence at days (T) after sowing, C is the maximum emergence, B is the rate of increase of emergence once it is initiated, M is a time lag before emergence commences.

As seedling emergence is influenced by environmental factors (*i*), each parameter in Eqn 1 will also be affected. Therefore, Eqn 1 can be rewritten as follows,

$$y = C_i \Big/ e^{e^{-B_i(T-M_i)}} \qquad [2]$$

Eqn 2 is the most complex model. To predict the emergence of a plant under a given condition (*i*), each parameter must be estimated for a wide range of levels. However, if the parameter changes in a continuous simple form with increase or decrease, the change of the parameter can be modeled and the model can be incorporated in to Eqn 2. In this study, wide ranges of soil depth and soil temperature were examined, and modeled changes of parameters were incorporated into the Eqn 2.

Data generation

Effect of soil depth

Pot experiments were carried out at the Experimental Field Station of Seoul National University in 1993. A rice cultivar (*Oryza sativa* L. cv. Dongjin) and three *Echinochloa crus-galli* varities *crus-galli*, *oryzicola*, and *praticola* were sown at different soil depths in pots. Seeds were covered with different amounts of sandy clay loam soil to adjust the soil depths to 0, 1, 2, 3, 4, 6, 8, 10, and 12 cm on 4 April and 6 May 1993. The pots were then placed in a side-opened glasshouse to ensure the temperature to follow closely outside conditions and to prevent the pots from rain. The soil water content was maintained to 30% (w/w) by top irrigation using a hand-sprayer. The emerged seedlings were recorded daily until 25 days after sowing. The experiment consisted of three replicates.

Effect of soil temperature

A laboratory experiment was conducted in an aluminum block apparatus designed to confer a linear continuous temperature gradient to the seeds from cooling to heating ends of the apparatus (Kwon et al. 1996). The apparatus consisted of 8 holes in the same temperature row and 16 rows with different temperatures (8 x 16) ranging from 10 to 30^{O}C. A rice cultivar (*O. sativa* L. cv. Ilpoom) and *E. crus-galli* var. *crus-galli* were sown and covered with sandy clay loam soil to adjust soil depth to 1 cm. The soil moisture content was maintained at 30% (w/w) by top irrigation using hand-sprayer. The emerged seedlings were recorded every 8 hours after the first emergence. The experiment consisted of four replicates repeated twice.

Statistical analysis

All measurements were initially subjected to analysis of variance (ANOVA). Non-linear regression was used to fit the Gompertz curve (1837) and other models. There was no evidence of lack of fit of the most complex model (eqn 2), so each model in the sequence was compared with its predecessor by calculating the F-value as follows

$$F = \left(\frac{RSS_{j+1} - RSS_j}{df_{j+1} - df_j} \right) \Big/ \left(\frac{RSS_a}{df_a} \right) \qquad [3]$$

where *RSS* and *df* represent the residual sum of square and the degree of freedom, respectively, j+1 represents the reduced model from its predecessor (j) and a represents ANOVA. If the F-value was lower than the tabulated F-value (5% level) with (df_{j+1}-df_j, df_a) degrees of freedom, the reduced model could be accepted.

RESULTS AND DISCUSSION

Modeling seedling emergence as affected by soil depth

For each soil depth, non-linear regression analyses were conducted to fit the Gompertz curve (Eqn 1) to the emergence data for each plant species. When parameters (C, B, and M) were plotted against soil depth to explore the relationships between soil depth and parameters, the plots clearly showed that the soil depth affected parameters C and M but not B (Fig. 1). As B did not change with soil depth, it was assumed that B would be constant. When eqn 4 was fitted, there was no evidence that this fitted less well than Eqn 2, so there was no evidence that B varied with soil depth.

$$y = C_i \Big/ e^{e^{-B(T - M_i)}} \qquad [4]$$

As shown in Figure 1, M appeared to increase exponentially with soil depth, so M_i in Eqn 4 was replaced by exponential curve ar^i. F-test, after fitting Eqn 5, also revealed that there was no evidence that Eqn 5 fitted less well than Eqn 4, confirming that the exponential increase of M in Eqn 5 can describe the delayed seedling emergence with increasing soil depth.

$$y = C_i \Big/ e^{e^{-B(T - ar^i)}} \qquad [5]$$

(A) (B)

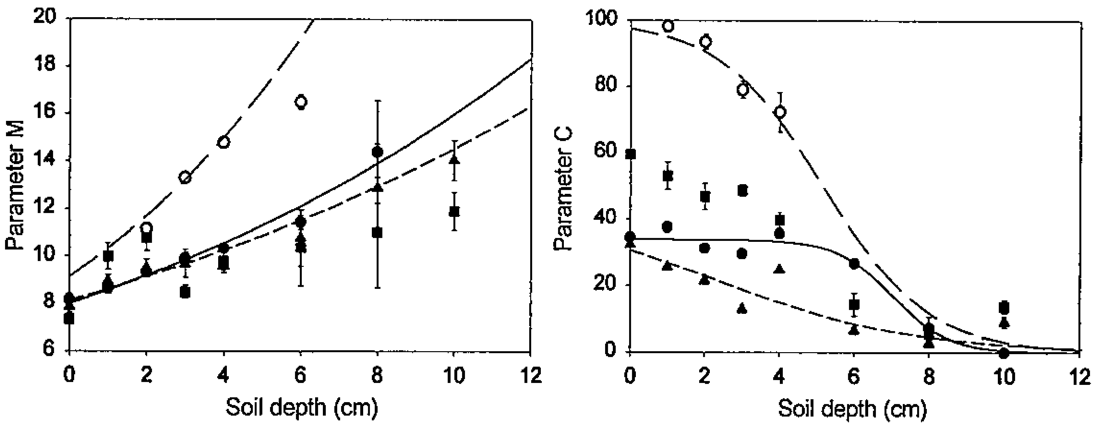

Figure 1. The relationships between soil depth and parameters *M* (A) and *C* (B). The parameters were estimated using Eqn 2 for rice (*O. sativa* cv. Dongjin; ○) and *Echinochloa crus-galli* vars. *crus-galli* (■), *oryzicola* (●), and *praticola* (▲).

Parameter *C* obtained after fitting Eqn 5 showed that *C* still decreases with soil depth in a logistic form. Thus, C_i in Eqn 5 was replaced by the logistic curve to give Eqn 6. When Eqn 6 was fitted, there was no evidence that this fitted less well than Eqn 5, indicating that *C* decreases logistically with increasing soil depth.

$$y = \left(C_{\max} \Big/ \left(1 + \left(i/e^{n} \right)^{d} \right) \left(e^{e^{-B(T - ar^{i})}} \right) \right) \qquad [6]$$

When Eqn 6 was fitted, parameters were estimated for each rice and *Echinochloa* spp. (Table 1). Although *C* was greater in rice, parameters *n* and *d* showed that the emergence (%) of rice decreases most significantly with soil depth than *Echinochloa* spp. implying that *Echinochloa* spp. can emerge in soil deeper than rice. *B* for rice was greater than that for *E. crusgalli* var. *crusgalli*, indicating that rice can emerge more quickly than *E. crusgalli* var. crusgalli after its first emergences. Like this, we often observe delayed emergence of weeds in field conditions. Although the first emergence of weeds is faster than crops, the spread of emergence in time is greater in weeds. Unlike crop seeds, each weed seed is not uniform, having different physiological abilities in dormancy, germination and seedling growth, thus resulting in smaller *B*. Parameters *a* and *r* were also greatest in rice. However, the greater *a* indicates that the lag time of rice to get to a certain emergence takes longer than *Echinochloa* spp. at each soil depth. Moreover, the greater *r* also indicates that the lag time of rice increases more significantly than *Echinochloa* spp. with increasing soil depth. In conclusion, Eqn 6 and estimated parameters clearly describe eco-physiological differences in seedling emergence between rice and *Echinochloa* spp. The simulated emergences of these species are illustrated in Figure 2.

Table 1. Parameter estimates for the seedling emergences of rice and *Echinochloa* spp. as affected by soil depth. The numbers in parentheses are standard errors.

Plant species	Parameter estimates					
	C_{max}	n	d	B	a	r
Rice (*O. sativa*)	100.000 (-)	1.623 (0.068)	2.4340 (0.3270)	0.9332 (0.0750)	9.977 (0.118)	1.1365 (0.0051)
E. crusgalli var. *crusgalli*	52.038 (1.578)	1.6596 (0.0371)	4.067 (0.400)	0.3098 (0.0299)	7.934 (0.287)	1.0600 (0.0135)
var. *oryzicola*	33.822 (0.506)	1.9326 (0.0151)	10.201 (0.970)	0.5128 (0.0371)	8.186 (0.152)	1.0603 (0.0055)
var. *praticola*	32.046 (1.058)	1.2383 (0.0756)	1.279 (0.119)	0.5401 (0.0713)	7.992 (0.206)	1.0646 (0.0069)

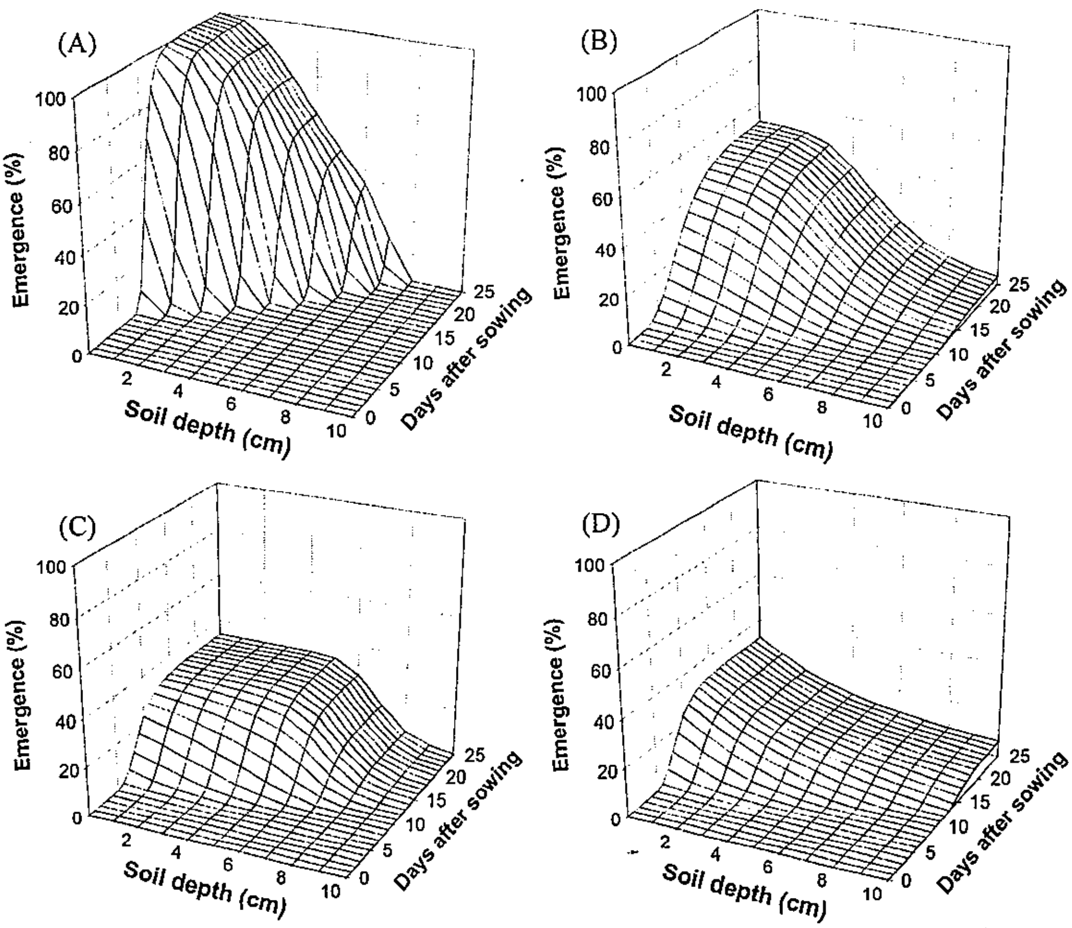

Figure 2. Simulated seedling emergences of rice (*O. sativa* cv. Dongjin) (A), *Echinochloa crus-galli* vars. *crus-galli* (B), *oryzicola* (C), and *praticola* (D) as affected by soil depth, using eqn 6 and parameter estimates given in Table 1.

Modelling seedling emergence as affected by soil temperature

With the same sequences as described, Eqn 2 was remodelled by incorporating the change of each parameter with increasing soil temperature (Fig. 3). Like the cases of soil depth, B appeared to be constant regardless of soil temperature. In cases of the other parameters, M decreased exponentially with increasing soil temperature, and C increased in a logistic form with increasing soil temperature. Thus, the final model was the same as Eqn 6.

$$y = C_i \Big/ e^{e^{-B_i (T - M_i)}} \qquad [2]$$

↓ B_i = constant

$$y = C_i \Big/ e^{e^{-B (T - M_i)}} \qquad [4]$$

↓ M_i = exponential increase or decrease

$$y = C_i \Big/ e^{e^{-B (T - ar^i)}} \qquad [5]$$

↓ C_i = logistic decrease or increase

$$y = \left(C_{max} \Big/ \left(1 + \left(i/e^{n} \right)^{d} \right) \left(e^{e^{-B (T - ar^i)}} \right) \right) \qquad [6]$$

Figure 3. Model tree for seedling emergences of rice (*O. sativa* cv. Dongjin) and *Echinchloa* spp. as affected by soil depth or soil temperature.

In conclusion, Eqn 6 can be used to describe the seedling emergence of rice and *Echinochloa* spp. as affected by either soil depth or soil temperature. This approach can also be applied to incorporate the effects of the other environmental factors, such as soil strength and water content on seedling emergences of crops and weeds. As this model was developed using the data generated from pot studies, further works are required based on field data for validation and parameter adjustment.

LITERATURE CITED

Cussans, G. W., Raudonius S., Brain P., Cumberworth S. 1996. Effects of depth of seed burial and soil aggregate size on seedling emergence of *Alopecurus myosuroides*, *Galium aparine*, *Stellaria media* and wheat (*Triticum aestivum* L.). Weed Research 36: 133-142

Gompertz, B. 1832. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Phil. Trans. Roy. Soc. London, 123: 513-585.

Kim, D. S. 1993. Characterization of emergence and early growth of *Echinochloa* spp. - and rice under the condition of direct-seeding rice culture. MSc thesis, Seoul National University.

Kwon, Y. W., Kim D. S., Park S. W. 1996. Effect of soil temperature on the emergence-speed of rice and barnyardgrasses under dry direct-seeding condition. Kor. J. Weed Sci. 16: 81-87.

Vleeshouwers, L. M. 1997. Modelling the effect of temperature, soil penetration resistance, burial depth and seed weight on pre-emergence growth of weeds. Annals of Botany, 79: 553-563.

Plant-level Responses of Cotton and Bladder Ketmia

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Abstract: This study was undertaken to understand the intra-specific responses of plant-plant interactions of crop and weed plants. The experiment results revealed that cotton (*Gossypium hirsutum*) plant height at maturity decreased linearly with increasing plant densities (0.8,5,10,20 plants m^{-2}). However, when plant height was considered for young plants (looking at main stem first reproductive node), crowded plants grew taller than the isolated plants. The average length of main-stem vegetative internodes and main-stem first internodes were significantly longer in dense populations than in sparse populations. Isolated plants produced greater main stem leaf areas than crowded plants. Individually grown bladder ketmia (*Hibiscus trionum*) plants developed the longest first internode as compared to higher plant densities (5,10,20 cm spacings). Increasing plant density did not affect main-stem height for the first reproductive node whereas crowded plants were significantly shorter than plants at other spacings (10,20,120 cm). The main-stem leaf area and biomass of the weed exhibited a linear increase with increasing spacing.

Key words: L-system, 3D digitizer, morphogenesis, plant architecture, *Gossypium hirsutum, Hibiscus trionum*

INTRODUCTION

In preparation for detailed studies of plant-plant interactions of cotton (*Gossypium hirsutum*) with bladder ketmia (*Hibiscus trionum*), this study investigated the underlying intra-specific responses of these crop and weed plants.

Cotton is an important cash crop in Australia. It occupied approximately half a million ha during 2000-01 and produced 2.4 million bales with 260% of world average yield (Anon. 2001). Excerpts of a cotton growers survey in New South Wales revealed that annual weed control in cotton costs \$A187 ha^{-1}, the major components included \$A76 ha^{-1} for herbicides and \$A67 ha^{-1} for hand chipping where the predominant weeds were Noogoora burr (*Xanthium occidentale*, *X. spinosum*), various sedges (*Cyperus* spp.), (*Physalis* spp.), bladder ketmia and morning glory (*Ipomea lonchophylla* (Charles 1991). Cotton lint yield reductions due to Noogoora burr and fierce thornapple (*Datura ferox*) averaged 36% and 12%, with maximum distances of influence of 1.71 m and 1.65 m, respectively (Charles et al. 1998).

Bladder ketmia is an annual weed found around the world in different crops. It occurs throughout Queensland, except in the north and the dry northwest. Its occurrence in the western region of New South Wales is restricted mainly to grey clay flood plains, less commonly on red earths (Cunningham et al. 1981). Early slow growth of cotton provides a niche for bladder ketmia to establish and complete its life cycle before cotton covers the inter-row space. This characteristic of bladder ketmia has made it an important weed in cotton-based cropping systems.

Plants grow in communities and forage for common resources like photo-synthetically active radiation, soil nutrients, soil moisture and carbon dioxide. The below- and above-ground plant parts play an important role in acquiring different resources and sensing proximity to other plants well before they are shaded and serve as an early warning signal future competition. The process of obtaining resources and further utilization of these resources for the acquisition of more resources is a preemptive exercise to competitive interactions (Bazzaz 1990).

Little emphasis has been given to understanding the adaptive architecture and morphology of individual plant in a plant community scenario. In preparation for studies on inter-specific interactions, this study investigated how the cotton with cotton and bladder ketmia with bladder ketmia plants interacts at the plant level under varying plant densities. Further these interactions will be simulated using L-systems for visualization and better understanding of interactions.

MATERIALS AND METHODS

Glasshouse experiments were conducted during May to December 2001 at the University of Queensland, St. Lucia, Brisbane, Australia. The experiments were conducted using a complete randomized design. Cotton (Sicala-40) and bladder ketmia seeds were pre-germinated and planted at different spacings (5,10,20, and 120 cm) in galvanized sheet troughs (120 cm x 40 cm x 30 cm) painted off-white with washable-finish on the inside. Holes at the bottom and in the corners of the troughs drained excess water. A 2 cm silica sand layer with a nylon mesh (105 μm) on top at the bottom of the troughs was laid out to avoid the mixing up of roots. Acrylic sheets were used to create sections for different plant spacing in troughs. Steamed nursery pot mixture fertilized with Osmocote (6 kg m^{-3}) was used as soil media. Cotton and bladder ketmia plants were watered regularly using water cane.

L-system models for the simulation and visualisation of the morphogenesis of cotton plants (Hanan and Room 1997) were used. In the formulation letters are used to represent specific plant components. The basic model of development of the main stem is captured in the following growth rule

A → I[L][B]A

where apex **A** produces (→) an internode **I**, a leaf **L**, and a bud **B** in the axil of the leaf and the apex continues to grow. The square brackets [and] mark the beginning and end of branches.

Development is expressed as a function of thermal time (Hanan, 1997) so each component has an associated parameter for degree-days. For the apex, degree-days are accumulated until the plastochron is reached, then production rule (A → I[L][B]A) is triggered. The apex also keeps track of the number of nodes produced, and assigns that value to each component, so that component lengths can be related to their position in the plant (Prusinkiewicz et al. 2001). The final form of the production rule is then

A(n,dd) : dd> PLC→ I(n,0)[L(n,0)][B(n,0)]A(n+1,dd-PLC+Ddd)

where **n** is the node number, **dd** is the accumulated degree-days for each component, **PLC** is the plastochron, **Ddd** is the current day's degree-days, and the condition **dd>PLC** controls the application of the rule with the addition of rules for cotyledon production and growth of leaves and internodes, the simulation produces images as the in (Figure 1).

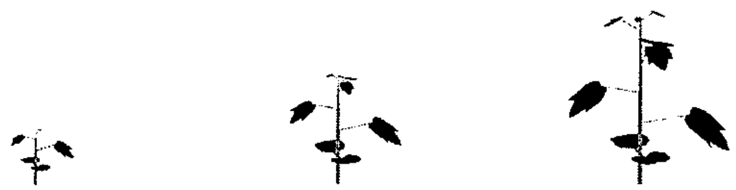


Figure 1. Three stages in the development of a virtual cotton plant.

Morphology of cotton and bladder ketmia plants was measured and recorded with Floradig (software; Hanan and Room 1997) compatible Sonic digitiser Model GP12-XL (Science Accessories Corp. 1994) at 3 day intervals and discontinued one month prior to harvest to have final digitization at harvest. Floradig software and its user manual can be downloaded from http://www.cpai.uq.edu.au. Digitization was initiated when cotton and bladder ketmia plants attained their first true leaf of thumbnail size and continued till the finish of the experiment. Multiple *.txt data files were imported into an Access database. Data were sorted by Access queries. Data was analysed employing Mini-tab software with Tukey's pairwise comparisons.

RESULTS AND DISCUSSION

Effect of plant spacing on growth and development

Cotton – Plants grown in dense population produced significantly longer first internode and average internode length of main-stem vegetative internodes as compared to the sparse population (Figs. 2 and 3). However, 10 cm spacing cotton plants spaced at 10 cm recorded significantly smaller average vegetative internodes than 5 cm spacing. Isolated cotton plants grew tallest amongst the four spacings and proved to be statistically taller than all other spacings at harvest whereas plants spaced at 5 cm recorded minimum final plant height. This trend in plant height was almost reversed for first reproductive node of main shoot resulting in statistically taller cotton plants in 5 cm spacing than the isolated ones. Isolated cotton plants amassed maximum main-stem total leaf area that was statistically higher than all other spacings, while leaf area exhibited linear increase with spacing. Main-stem biomass accumulation also followed a similar trend to that of the main-stem leaves. Sparsely grown cotton plants accumulated maximum main-stem leaf biomass, which was significantly higher than other spacings (5, 10, 20 cm).

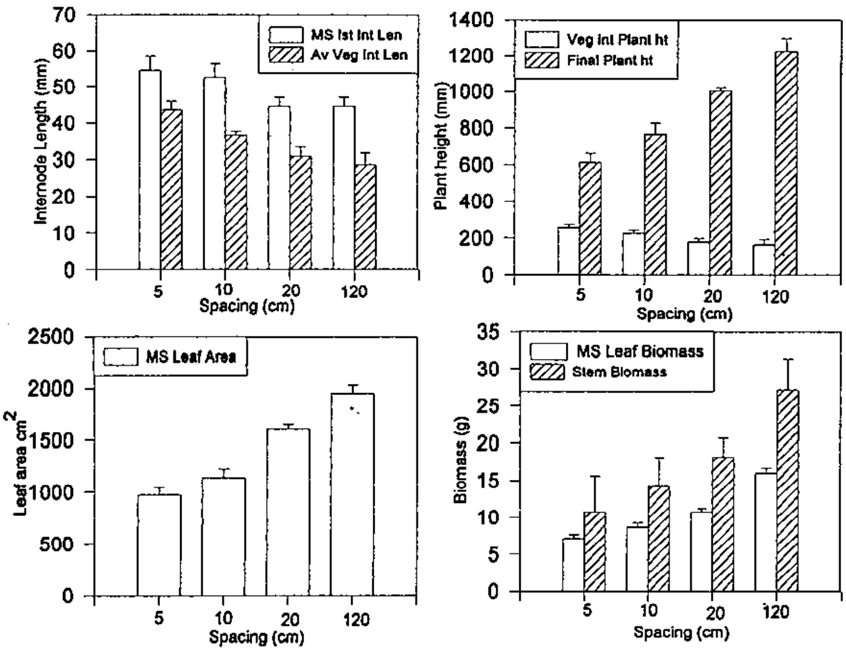
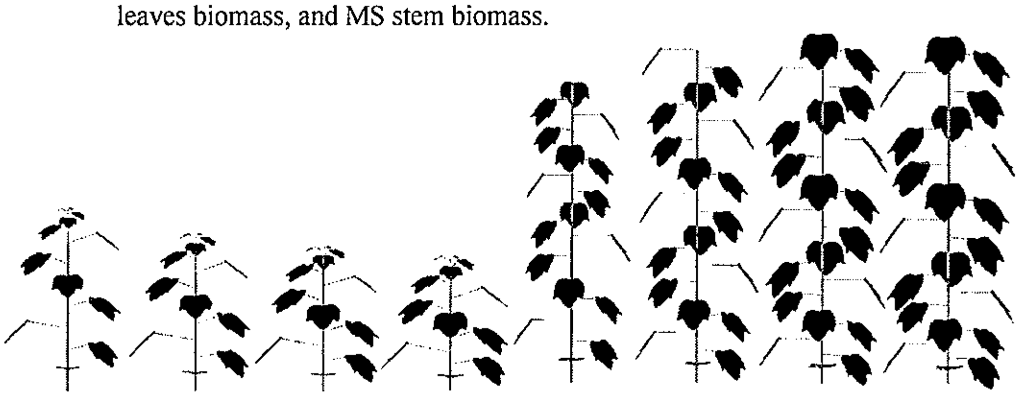


Figure 2. Effect of cotton spacing on MS internode length, plant height, MS leaf area, MS leaves biomass, and MS stem biomass.



First Reproductive Node Stage **At Plant Harvest Stage**

Figure 3. Virtual cotton models showing plant height at first reproductive node stage (left hand four plants) and at harvest (right hand four plants). The spacings from left to right in each half of the figure are 5, 10, 20 and 120 cm.

The internode, petiole, and laminae lengths in this virtual cotton model are expressed in visualisation rules using the following fitted equations based on plant density and nodal position.

Main stem vegetative nodes and leaves

Internode length=0.9007*density+27.968; R^2=0.8932

Petiole length =-.4381*density+102.24; R^2=0.8731
Lamina length=-0.4536*density+85.132; R^2=0.4059
Main stem reproductive nodes and leaves
Internode length=-0.3816*density+43.437; R^2=0.7364
Petiole length=-1.7526*density+101.76; R^2=0.946
Laminae length=-1.1099*density+99.354; R^2=0.9708

Bladder ketmia –The different spacings used for the bladder ketmia plants did not influence the average internode length of the main shoot (Fig. 4). However, the first internode produced by isolated plants was statistically longer than that seen at the other spacings (5, 10, 20 cm). Plant height, when measured from the vegetative internodes, was also not affected by spacing. However, the crowded plants at harvest were statistically shorter than all other spacings (10, 20, 120 cm). Main-stem leaf area was minimum and maximum for crowded and uncrowded plants, respectively. The intermediate spacings (10, 20 cm) did not manifest any statistical difference among them but were significantly inferior to isolated plants and were superior to crowded ones. The biomass of main-stem leaves was also observed to be a minimum in 5 cm spacing but the sparse spacings (20, 120 cm) did not record any significant difference between them. However, main-stem biomass showed a linear increase with increased spacing (5-120 cm).

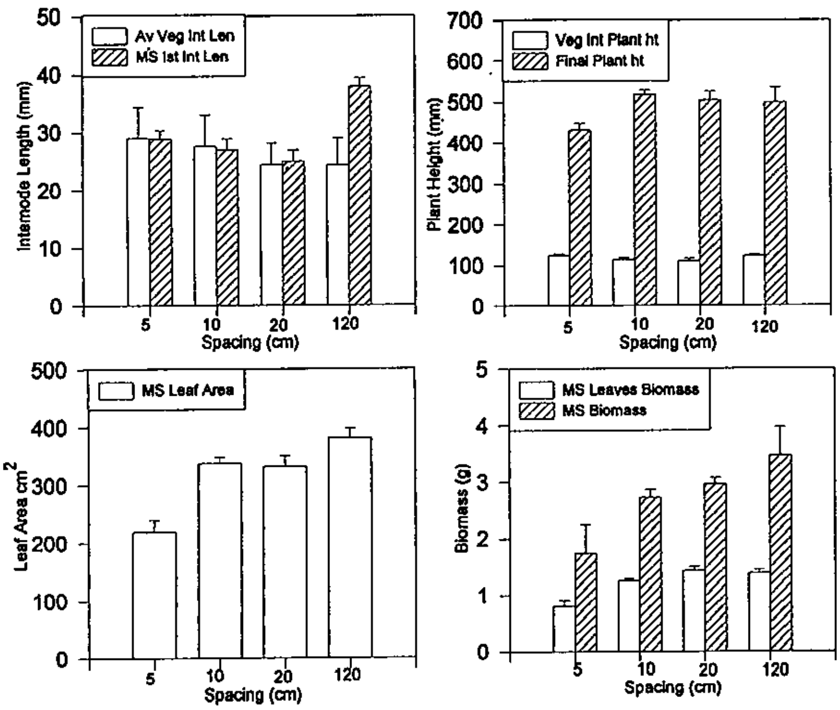


Figure 4. Effect of bladder ketmia spacing on MS internode length, plant height, MS leaf area, MS leaves biomass, and stem biomass.

These results are similar to the findings that reduction in R:FR in plant canopy promotes stem elongation and grew taller stems relative to dry matter accumulation, stem diameter and leaf area and produce few branches ((Ballaré et al. 1987; Child and Smith 1987; Collins and Wein 2000; Schmitt et al. 1987; Jurik 1991). Response to neighbour shade has been examined in *Polygonum* spp. and observed that relative branch length and branching were negatively related to density; plants at high density were smaller and less branched (Geber 1989). In the present study, crowded cotton plants grew taller earlier in their life. However, at harvest uncrowded plants were the tallest, presumably because they had produced a greater number of internodes than the crowded plants. This is because height is the product of internode length and internode number.

Biomass differences in cotton can be mainly attributed to branching proliferation. On the contrary, bladder ketmia did not exhibit similar responses in main shoot growth to cotton. Presumably, bladder ketmia has a shorter life cycle and poor apical dominance. This triggered simultaneous growth of vegetative branches and main stem that might have not left enough time to respond to differences in plant spacing.

This project will now collate these intra-specific responses of cotton and bladder ketmia with new findings on inter-specific interactions of these two species under different soil moisture regimes.

LITERATURE CITED

Anon. (2001). The industry in figures: Cotton Yearbook, 2001:50-62

Ballaré, C.L. et al. 1987. Early detection of neighbour plants by phytochromes perception of spectral changes in reflected sunlight. Plant Cell Environment, 10:551-557.

Bazzaz, F.A. 1990. Plant-plant interactions in successional environments. Pages 239-263 In: J.B. Grace and D. Tilman, editors. Perspectives on plant competition. Academic Press, San Diego, California, U.S.A.

Charles, G.W. 1991. A grower survey of weeds and herbicide use in the New South Wales cotton industry. Australian Journal of Experimental Agriculture, 13(3):387-392

Charles, G.W. et al. (1998). Competition of noogoora burr (*Xanthium occidentale*) and fierce thornapple (*Datura ferox*) with cotton (*Gossypium hirsutum*). Weed Science, 46:442-446.

Child, R. and H. Smith. 1987. Phytochrome action in light-grown mustard: Kinetics, fluence-rate compensation and ecological significance. Planta, 172:219-229.

Cunningham, G.M. et al. 1981. Plants of Western New South Wales. Soil Conservation Services of N.S.W.

Collins, Beverly and Gary Wein. 2000. Stem elongation response to neighbour shade in sprawling and upright Polygonum species. Annals of Botany, 86:739-744.

Geber, M. 1989. Interplay of morphology and development on size inequality: a *Polygonum* greenhouse study. Ecological Monographs 59:267-288.

Hanan, Jim. 1997. Virtual plants-integrating architectural and physiological models. Environmental Modelling & Software, 12(1):35-42.

Hanan, Jim S. and Room, Peter M., 1997. Practical aspects of virtual plant research. In: Michalewicz, M.T.(Ed.), Plants to ecosystems: Advances in Computational Life Sciences. CSIRO Publishing, Melbourne, pp. 28-44.

Jurik, T.W. 1991. Population distributions of plant size and light environment of giant ragweed (*Ambrosia trifida* L.) at three densities. Oecologia, 87:539-550.

Prusinkiewicz, P. et al. 2001. The use of positional information in the modeling of plants. Proceedings of ACM SIGGRAPH Conference in Los Angles 2001, 289-300.

Science Accessories Corp. 1994. SACs GP-12 digitizing system. Science Accessories Corp. Columbia, Maryland.

Schmitt, J.J. et al. 1987. Dominance and suppression, size-dependent growth, and self-thinning in a natural *Impatiens capensis* population. Journal of Ecology, 75:651-665.

Problem Weeds

Sagittaria aginashi Makino, a Perennial Paddy Weed in Korea

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Abstract: *Sagittaria aginashi* Makino is a paddy perennial weed. It lives on volcanic soil areas in Tohoku region. However, the species is very difficult to find in southern Japan now. The Red Data Book of Vascular Plant, Threatened Wildlife of Japan (2000) shows that the species is grouped NT (next threatened, rare species). The Red Data Book of Kinki, Threatened Wildlife of Japan (2001) shows that the species group A (highly endangered plant). The species looks like *S. trifolia* before corm development in September. We observed the species in paddy ecosystems in Korea from 1998 to 2002. We found 2 specimens in Kyoto Univ. from Kyonggi-do, Korea in 1930's. *S. aginashi* was not found in paddy areas in Seoul, Suwon, Choron, Andon, Tegue, Miliyan and Pusan areas. At most, specimen and pictures of *S. aginashi* showed that the species was narrow leaf type of *S. trifolia*. *S. aginashi* was present in Jeju Island.

Key words: perennial paddy weed, *Sagittaria aginashi*

INTRODUCTION

The benefits of paddy fields in environment preservation so far consist of several functions such as preventing flood, fostering water resources, preventing soil erosion and landslide, soil purification, preserving the rural landscape and recreational amenities, and so forth. In addition to these functions, this investigation shows how the preservation function of bio-diversity occurs in paddy field ecosystem and examines the methods that promote the development of sustainable agriculture, which makes bio-diversity an indicator.

In contrast, Japan's agriculture is facing an increasingly difficult situation due to internationalization and the imbalance between supply and demand of agricultural products. Regional vitality is being lost as a result of the aging of residents and depopulation, and the increase of non-farmer households and part-time farmers. Attitudes in rural communities are thus changing. In addition to higher agricultural productivity, the nation has come to expect much of rural communities as maintaining and improving the living environment and diverse functions of rural community areas. Such communities are expected to conserve the land, water and natural resources (Itoh 2000).

To meet these expectations, it is essential to consider various aspects of rural communities, including reorganization of the agricultural area, and the development of management technology to maintain the activity of the rural area and improve agricultural infrastructure and facilities for sustainable agricultural production to harmoniously maintain the rural ecosystem.

An integrated report entitled "Rice Agro-ecosystem of the Muda Irrigation Scheme, Malaysia" was published by MADA and MINT in 1998. Preliminary work related to the bio-diversity of tropical paddy fields is included in this book.

Bio-diversity in rural ecosystems has been threatened recently by the loss of habitats and water pollution caused through the runoff of pesticides and fertilizer from rice fields. Minimum pesticides are required for production in rice fields.

Observation site

Sagittaria aginashi, an endangered plant, was targeted and compared with *S. trifolia*, a serious paddy weed in Tsuchikawa, Nishiki village, Akita Prefecture, Japan as regards the *in-situ* condition.

S. aginashi, *S. trifolia* forma *suitensis*, and several endangered plants were tested as indicator plants of herbicide runoff, especially sulfonylurea (SU) herbicides from paddy fields. We estimated the LD_{50}, GR_{50} and deformity of leaf in the species for bensulfulonmethyl (BSM) and pyrazosulfulonethyl (PSE).

Vegetation surveys were done in poorly drained paddy fields in Seoul, Suwon, Choron, Andon, Tegue, Miliyan and Pusan areas with agriculture extension workers. These fields consisted of about 50 abandoned muddy fields, 10 water plantain, watercress, *Oenanthe javanica* and water vegetable fields, 7 irrigation and natural ponds and 1000 rice fields from 1998 to 2002. We got information about *S. aginashi* in Korea from universities, institutes and museums. Especially, we met Dr. Yong No Lee, the president of the Korea Plant Research Institute and he introduced paddy areas in Seoul and Choron.

RESULTS

A 1/100 recommended dose of BSM in rice fields caused deformity of *S. trifolia* forma *suitensis* leaf 2 weeks after treatment. I_{50} for the species toward BSM and PSE, were 1/10 (7.5 g a.i. ha^{-1}) and 1/3 (7 g), respectively, in pot experimental conditions. GR_{50} for plant height of *S. aginashi* toward BSM were 1/18 (4.18 g a.i. ha^{-1}) in the pot experiment (Fig.1 & Table 1). GR_{50} for dry weight of *Azolla japonica* toward BSM, was 1/50 (1.5 g a.i. ha^{-1}) in the pot experiment. *S. aginashi, S. trifolia* forma *suitensis, Azolla japonica* and *Brasenia shreberi*, were among the good indicator plants for herbicide runoff from paddy fields.

We found 2 specimen of *S. aginashi* in the herbarium of Kyoto University from Kyonggi-do, Korea in 1930's. They have corms in the specimen.

We would like to find the species using companion plants, such as *Epilobium angustifolium* (yanagiran), *Lysimachia vulgaris* (kusaredama), *Scirpus fuirenoides* (hime-matsukasa-susuki), *Eriocaulon robustius* (hiroha-inunohige). We found in the paddy areas of Tohoku District in Japan with *S. aginashi*. We could not find *S. aginashi* in paddy areas in Seoul, Suwon, Choron, Andon, Tegue, Miliyan and Pusan areas in Korea, whereas, these companion plants were present in most places. We found a lot of *S. trifolia* in every observed fields from narrow leaves to wide leaves (Lee, 1997).

BSM (g a.i./ha)	Plant height (cm ±S.D.)	D.W. (g S.D.)
0	13.36 (±0.70)	0.12 (±0.02)
0.25	13.07 (±0.54)	0.12 (±0.06)
0.75	12.62 (±0.88)	0.10 (±0.01)
2.5	8.44 (±1.88)	0.03 (±0.01)
7.5	2.92 (±0.50)	0.02 (±0.01)
25	2.12 (±0.65)	0.01 (±0.005)
75	0.80 (±0.03)	0.01 (±0.002)

Table 1. Effect of BSM on *S. aginashi* at 40 days after application.

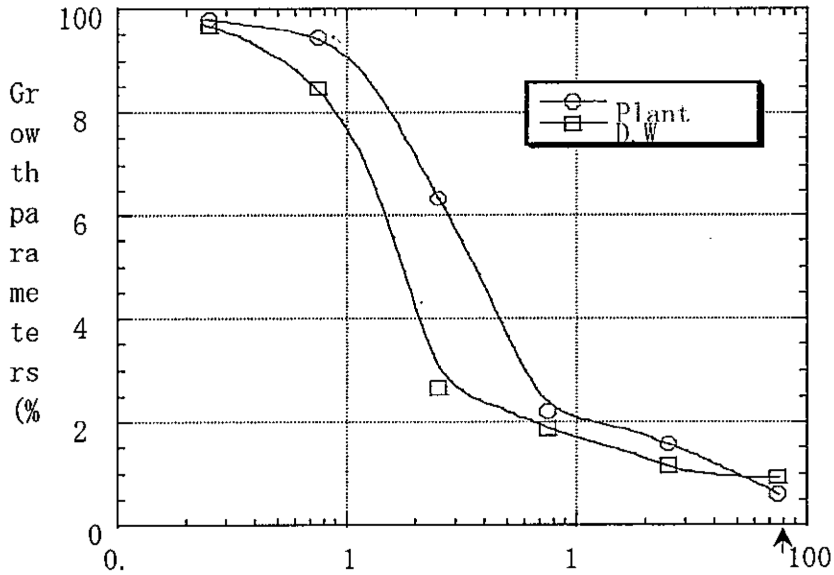

Figure 1. Inhibition rate in growth parameters of. *S. aginashi.*

At most herbarium specimens and pictures of illustrated books published in Korea are known as *S. aginashi* was a narrow leaf type of *S. trifolia S. aginashi* in Jeju Island in a Korean volcanic island was found by Lee, Yong No

DISCUSSIONS

Sagittaria aginashi, an endangered plant, was targeted and compared with *S. trifolia*, a serious paddy weed in Tsuchikawa, Nishiki village, Akita Prefecture, Japan as regards the *in-situ* condition Table 2 shows the niche differentiation between *S. aginashi* and *S. trifolia*. The paddy was situated on a narrow hillside with poor drainage and no consolidated land for rice cultivation and a water shield (*Brasenia schreberi*). *S. trifolia* is found only inside paddy fields, whereas *S. aginashi* is found in and around water-shield ponds, and outside or around paddy fields (Itoh 1996). The species adapt around paddy fields and exists only in a non-herbicide condition. The Red Data Book of Vascular Plant, Threatened Wildlife of Japan (2000) shows the species is grouped NT (next threatened, rare species). The Red Data Book of Kinki, Threatened Wildlife of Japan (2001) shows the species is in grouped A (highly endangered plant). The species looks like *S. trifolia* before corm development in September.

Table 2. Niche differentiation between *S. aginashi*, an endangered plant and *S. trifolia*, a paddy weed in Tsuchikawa, Sishi-senboku Village. Akita Prefecture, Japan (Itoh,1996).

Place	S. Aginashi	(% Total	S. triflolia	Total
Around water shield pond	6	(100)	0	6
Bank of stream	14	(82)	3	17
Ditch around paddy field	8	(82)	2	11
Paddy levee	5	(83)	1	6
Inside paddy field	1	(2)	43	44
Tillaged, submerged paddy field	1	(13)	7	4
Non-tillaged, submerged fallow field	2	(100)	0	2

As mentioned in the preface, the use of the pesticides occupies a big factor in the development of modern agriculture in Asia. Since rice cultivation depends on the use of pesticides not only in Japan but also in Korea, a discussion on the rights or wrongs of their use from the perspective of bio-diversity conservation cannot be avoided. When considering the relationship between bio-diversity and pesticides, most important is how the pesticides affect the non-target organisms inhabiting the same agro-ecosystems. It is certain that not only the concentration but also the mode of action of pesticides appreciably affects non-target organisms. The side effects of pesticides occur also in soil by degradation products. Some observation indicates that the degradation product is not always safe to non-target organisms.

The quantity of pesticides is very important, because the lower the input of pesticides to the environment, the lesser the impact on the environment. To harmonize agro-productivity with environmental protection, it is necessary to (1) monitor pesticides in the environment, (2) predict the fate of pesticides in the environment, (3) examine the influence on non-target organisms to confirm both the safety concentration and safety-releasing pattern and (4) regulate the amount of pesticides released outside the agro-environment. The qualitative properties of pesticides are also important. Pesticide development is changing from the production of biocidal agents to bioregulators such as pheromones. And the formulation of pesticides is also changing from rapid release formulation to slow release formulation such as coating or micro capsule pesticides (Takagi & Ueji 1997; Ueji and Inao 2001).

Even though pesticides have to be used in order to secure yield through the pesticide development approach, the adverse impact of pesticide use on the ecosystems may be reduced and sustainable agriculture with rich bio-diversity may be achieved.

In this project, we could not find *S. aginashi* in Korean paddy ecosystem. If you have any information about the species, please contact following e-mail: kitoh@affrc.go.jp.

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LITERATURE CITED

Environment Agency of Japan 2000. Threatened Wildlife of Japan, Red Data Book 2nd ed. volume 8, Vascular Plants pp.660.

Itoh, K. 1996. Differentiation of habitat similarity of natural vegetation between *Sagittaria aginashi* and *S. trifolia.* Akita Shizenshi Kenkyu, **33:** 1-4. (in Japanese)

Itoh, K. 2000. Bio-diversity in weeds in agro-ecosystem for rice production. *In* Proc. International Workshop on Biology and Management of Noxious Weeds for Sustainable and Labor Saving Rice Production. National Agriculture Research Center, 222-236. Tsukuba.

Lee, Y.N. 1997. Flora of Korea

MINT & MADA, 1998. Rice Agroecosystem of the Muda Irrigation Scheme, Malaysia. Eds. Nashriyah. M. et al. pp.256. Malaysian Institute for Nuclear Technology Research.

Red Data Book Kinki Kenkyukai 2001. Conservative plants in Kinki region, Red Data Book Kinki 2001, 2nd ed., pp.164.

Takagi, K. and M. Ueji, 1997. Use, research and development of pesticides in relation to sustainable agriculture in Japan. JARQ **31:**13-20.

Ueji, M. and K. Inao 2001. Rice paddy field herbicides and their effects on the environment and ecosystem. Weed Bio. & Manag. **1:** 71-79.

Transgenic Crops

Management Strategies for Transgenic Creeping Bentgrass (*Agrostis palustris*) Resistant to Glyphosate

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Abstract. Creeping bentgrass (*Agrostis palustris* Huds.) is a self-sterile, wind pollinated perennial grass. This species is native to Eurasia but has become naturalized in North America. Creeping bentgrass is one of the most tolerant cool-season turfgrasseswhich ca n tolerate continuous, close mowing at heights as low as 0.5 cm. This species is widely used for putting greens and fairways on the golf courses of temperate climates around the world. The development of transgenic *Agrostis palustris* resistant to glyphosate [N-(phosphonomethyl) glycine] has led to our research to identify alternative strategies for control of these and related *Agrostis* species. Field studies were conducted in 2002 with several modes of action of herbicides. Glyphosate at 1.68 kg ha^{-1} alone and sequential applications of 1.68 kg ha^{-1} of glyphosate followed by an application of glyphosate (1.68 kg ha^{-1}) 4 weeks later completely controlled one-year old susceptible creeping bentgrass sod. Clethodim at 0.28 kg ha^{-1}, fluazifop at 0.42 kg ha^{-1} and isoxaflutole at 0.22 kg ha^{-1} provided excellent (>95%) control of one-year old creeping bentgrass. Fluazifop and sethoxydim were not effective in controlling four-year old creeping bentgrass. Atrazine at 2.24 kg ha^{-1} completely killed creeping bentgrass sod up to 12 WAT. Tanxit GTA at 0.7 kg ha^{-1} followed by a sequential application of 0.7 kg ha^{-1} controlled 94% of creeping bentgrass. Similar creeping bentgrass control was also obtained with sequential applications of Mon 44951 at 0.14 followed by an application of 0.14 kg ha^{-1} 4 weeks later. Results indicate alternative management strategies to control the glyphosate resistant creeping bentgrass under various environments.

Key words: *Agrostis palustris* Huds, clethodim, fluazifop, glyphosate, isoxaflutole, mesotrione, resistant turfgrass, sethoxydim

INTRODUCTION

Creeping bentgrass (*A. palustris* Huds.) is a self-sterile, wind pollinated perennial grass. This species is native to Eurasia but has become naturalized in North America. Creeping bentgrass is one of the most tolerant cool-season turfgrasses, which can tolerate continuous, close mowing at heights as low as 0.5 cm. This species is widely used for putting greens and fairways on the golf courses of temperate climates around the world.

With the advent of biotechnology, glyphosate [N-(phosphonomethyl)glycine] resistant crops such as maize (*Zea mays* L.) and soybeans [*Glycine max* (L.) Merr.] have been developed. Creeping bentgrass plants are currently being developed (Scotts and Monsanto Company). Glyphosate resistant creeping bentgrass plants are being developed utilizing the co4 epsps gene, which codes for an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme (Lee et al. 1996; Lee et al.

2002). The development of glyphosate resistant turfgrass species was reported as a beneficial technology by Johnson et al. (1989). However, creeping bentgrass is a self-sterile, wind-pollinated species and has the potential to transfer the glyphosate resistance trait via pollen to non-glyphosate resistant creeping bentgrass and related species (Belanger et al. 2001; Davies 1953; Johnson and Riordan,1999; Wipff and Freeker 2001). Gene transfer has also been repeatedly demonstrated between a crop plant and a related species growing in close proximity (Arias and Rieseberg,1994; Hancock et al. 1996; Rodgers and Parkes 1995).

Glyphosate controls plants by inhibiting the enzyme EPSP synthase involved in the synthesis of aromatic amino acids (Amrhein et al. 1980). Cyclohexanedione and aryloxyphenoxy-propionate class herbicides control grass species by inhibiting the enzyme acetyl-CoA carboxylase (ACCase), which is involved in fatty acid biosynthesis (Burton et al. 1989; Focke and Lichtenthaler 1987). Isoxaflutole, a HPPD inhibitor herbicide, controls broadleaf and grass species by inhibiting p-hydroxyphenylpyruvate dioxygenase, an enzyme involved in the conversion of p-hydroxyphenylpyruvate to homogentisate in plastoquinone biosynthesis (Luscombe et al. 1995; Kushwaha and Bhowmik 1999). Mesotrione, another HPPD inhibitor, controls susceptible species in a similar manner. The molecular target for mesotrione is the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme is involved in the pathway that converts the amino acid tyrosine to plastoquinone. Mesotrione is structurally similar to the substrate p-hydroxyphenylpyruvate and acts by competitive inhibition, which results in the blockage of carotenoid synthesis (Lackey et al. 2000).

Grass control herbicides belonging to cyclohexanedione and aryloxyphenoxy-propionate classes are commonly used to control both annual and perennial grass weed species in a wide variety of broadleaf agronomic crops. Several of these herbicides such as clethodim, fenoxaprop, fluazifop and sethoxydim are also labeled for grass weed control in ornamental crops or in several turfgrass species. Isoxaflutole can be used to selectively control creeping bentgrass in other established turfgrass species (Bhowmik and Drohen 2001). Glufosinate and glyphosate provided complete control of colonial bentgrass (*Agrostis capillaris* L.) 42 days after treatment in Oregon (Cook et al. 1996).

Currently, limited information is available on the response of creeping bentgrass and related bentgrass species to the above-mentioned herbicides. The development and potential commercialization of glyphosate resistant *Agrostis* species poses a management challenge to our current system. The potential for unintended transfer of the glyphosate resistant gene from creeping bentgrass to related *Agrostis* species necessitates further studies for alternative control strategies. The objective of this research was to examine the potential for selective control of creeping bentgrass stands.

MATERIALS AND METHODS

Experiments were conducted at the University of Massachusetts Turfgrass Research Facility in South Deerfield, MA. The soil was a Hadley fine sandy loam (Typic Udifluvents) with 3.5% organic matter and a pH of 6.5. The area was fertilized once a month with N fertilizer at 24.39 kg ha^{-1}. This trial area was moderately infested with

Poa annua. Mowing was done weekly and the clippings were not removed. Mowing was maintained at a 1.27 cm height. In all experiments, POST treatments were applied to the established creeping bentgrass. An untreated control was included in each experiment for the comparison. All treatments were applied using a CO_2-backpack sprayer with Teejet XR 11004 VS nozzles at 152 kPa using a spray volume of 467 L ha^{-1}. Treated turfgrass area was weekly mowed after the treatment application. Turfgrass injury was visually estimated on a scale of 0 to 100%, where 0 = green healthy turf and 100 = dead brown turf. Turfgrass injury was determined every two weeks up to 16 weeks after treatment (WAT).

Experiments were conducted in a randomized block design and all treatments were replicated three or four times. Analysis of variance (ANOVA) was used to determine differences in treatment effects. Error terms were obtained by calculating the expected mean squares (Damon and Harvey 1987). The appropriate error terms were used in the hypotheses tests using the ANOVA program of SAS (SAS Institute, 1995). Means were separated by Student-Newman-Keuls test at p=0.05.

RESULTS AND DISCUSSION

Effect of age on creeping bentgrass control

Glyphosate at 1.68 kg ha^{-1} alone and sequential applications of 1.68 kg ha^{-1} of glyphosate followed by an application of glyphosate (1.68 kg ha^{-1}) 4 weeks later controlled creeping bentgrass over 95% 1 WAT and provided complete control up to 16 WAT (Table 1). Clethodim at 0.28 kg ha^{-1}, fluazifop at 0.42 kg ha^{-1} and sethoxydim at 0.42 kg ha^{-1} controlled only 27 to 38% 1WAT. However, at 4 WAT, these treatments provided over 95% control, except the sethoxydim treatments, which provided only 87% creeping bentgrass control. Similar results with these herbicides have been reported by Hart et al. 2002; Bhowmik and Riego 2003; and Sutton 2002. Isoxaflutole at 0.28 kg ha^{-1} controlled creeping bentgrass by 50, 88 and 95% 1, 2, 4 WAT, respectively. The sequential treatments of clethodim, fluazifop or isoxaflutole 4 weeks after the initial application improved creeping bentgrass control up to 16 WAT. Glyphosate either alone or sequential treatments provided complete control of one-year old creeping bentgrass 16 WAT. Sethoxydim was not an effective treatment. In general, glyphosate, clethodim and isoxaflutole completely controlled *Poa annua* 4 WAT.

Effect of timing of application on creeping bentgrass control

Glyphosate at 1.68 kg ha^{-1} alone and sequential applications of 1.68 kg ha^{-1} of glyphosate followed by an application of glyphosate at 1.68 kg ha^{-1} 4 weeks after the initial application controlled 4-year old creeping bentgrass (>95%) throughout the 16-week period (Table 2). Clethodim at 0.28 kg ha^{-1}, fluazifop at 0.42 kg ha^{-1} and sethoxydim at 0.42 kg ha^{-1} controlled only 17 to 35% 1WAT. However, at 4 WAT, these reatments provided over 90% control except the fluazifop treatments which

Table 1 Response of one-year old creeping bentgrass sod to postemergence applications of various sequential treatments.

Treatment	Rate	Control, WAT[1]						
		Agrostis palustris						*Poa annua*
		1	2	4	8	12	16	4
	Kg ha^{-1}	%						
Glyphosate[2]	1.68	98 a[5]	100 a	100 a	99 a	98 a	98 a	100 a
Glyphosate + Glyphosate[4]	1.68 + 1.68	98 a	100 a	97 a	98 a	98 a	98 a	100 a
Clethodim[3]	0.28	27 c	87 bcd	98 a	91 a	75 a	67 ab	99 a
Clethodim + Clethodim[4]	0.28 + 0.28	28 c	90 b	99 a	97 a	98 a	98 a	99 a
Fluazifop[3]	0.42	38 c	83 d	95 a	82 a	63 a	53 b	37 b
Fluazifop + Fluazifop[4]	0.42 + 0.42	37 c	85 cd	96 a	92 a	92 a	92 a	35 b
Sethoydim[3]	0.42	27 c	83 d	87 b	70 a	23 b	18 c	28 b
Sethoxydim + Sethoxydim[4]	0.42 + 0.42	28 c	85 cd	87 b	85 a	68 a	60 ab	32 b
Isoxaflutole	0.28	50 b	88 bc	95 a	93 a	90 a	92 a	95 a
Isoxaflutole + Isoxaflutole[4]	0.28 + 0.28	50 b	88 bc	95 a	95 a	96 a	96 a	93 a
Untreated	0.0	0 d	0 e	0 c	0 c	0 c	0 c	0 c

[1]WAT = weeks after initial treatment application prior to planting
[2]Glyphosate applied as Roundup Pro formulation
[3]Fluazifop, clethodim, sethoxydim and isoxaflutole applied with crop oil concentrate at 1% (v/v).
[4]Sequential application 4 weeks after the initial application.
[5]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.

treatments of fluazifop and sethoxydim 4 weeks after the initial application did not improve creeping bentgrass control 16 WAT. Glyphosate either alone or with sequential treatments provided complete control of 4-year old creeping bentgrass. The sequential applications of clethodim and isoxaflutole provided excellent control 16 WAT. Fluazifop and sethoxydim were not effective in controlling 4-year old creeping bentgrass. In general, glyphosate, clethodim and isoxaflutole completely controlled *Poa annua* 4 WAT.

Effect of timing and frequency of application on bentgrass control

This trial was conducted on a 4-year old creeping bentgrass stand mixed with Kentucky bluegrass patches. Sequential applications of glyphosate at 1.68 kg ha^{-1}, clethodim at 0.28 kg ha^{-1}, fluazifop at 0.42 kg ha^{-1} or sethoxydim at 0.42 kg ha^{-1} 4 and 8 weeks after the initial application resulted in excellent control (>99%) of creeping bentgrass stand 12 WAT. Creeping bentgrass control with HPPD inhibitors (isoxaflutole and mesotrione) was slightly different. Isoxaflutole at 0.22 kg ha^{-1} resulted in 93% control 2 WAT, while it controlled only 79% 8 WAT. Sequential applications of 0.11 kg ha^{-1} of isoxaflutole followed by an application of 0.11 kg ha^{-1} of isoxaflutole 4 weeks later resulted in 87 and 53% control of creeping bentgrass 2 and 8 WAT, respectively. Mesotrione at 0.22 kg ha^{-1} resulted in 87% control 2 WAT, while it controlled only 45% 8 WAT. Sequential applications of mesotrione at 0.11 kg ha^{-1} followed by 0.11 kg ha^{-1} of mesotrione application 4 weeks later resulted in 91 and 57% control of creeping bentgrass 2 and 8 WAT, respectively. Similar results have been reported earlier (Hart et al. 2002; Bhowmik and Domingo 2003; Bhowmik and Drohen 2001). Atrazine at 2.24 kg ha^{-1} completely killed creeping bentgrass sod up to 12 WAT. Tanxit GTA at 0.7 kg ha^{-1} followed by a sequential application of 0.7 kg ha^{-1} controlled creeping bentgrass by 94%. Similar creeping bentgrass control was also obtained with sequential applications of Mon 44951 at 0.14 followed by an application of 0.14 kg ha^{-1} 4 weeks after the initial application. Results indicate alternative management strategies to control the glyphosate resistant creeping bentgrass under various environments.

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Table 2 Response of 4-year old creeping bentgrass sod to postemergence applications of various sequential treatments.

Treatment	Rate	Control, WAT[1]						
		Agrostis palustris						*Poa annua*
		1	2	4	8	12	16	4
	Kg ha^{-1}	%						
Glyphosate[2]	1.68	97 a	100 a	100 a	98 a	97 a	95 a	100 a
Glyphosate + Glyphosate[4]	1.68 + 1.68	100 a	100 a	100 a	100 a	98 a	97 a	100 a
Clethodim[3]	0.28	35 d	92 b	99 a	99 a	96 a	93 a	99 a
Clethodim + Clethodim[4]	0.28 + 0.28	30 d	92 b	98 ab	94 ab	99 a	97 a	98 a
Fluazifop[3]	0.42	23 d	83 c	82 c	67 c	42 b	15 cd	0 c
Fluazifop + Fluazifop[4]	0.42 + 0.42	27 d	83 c	83 c	73 bc	65 b	53 b	0 c
Sethoydim[3]	0.42	17 d	87 bc	91 abc	77 abc	52 b	15 cd	5 c
Sethoxydim + Sethoxydim[4]	0.42 + 0.42	20 d	87 bc	92 abc	78 abc	62 b	25 c	0 c
Isoxaflutole	0.28	58 c	90 b	90 abc	78 abc	60 b	22 c	92 a
Isoxaflutole + Isoxaflutole[4]	0.28 + 0.28	78 b	87 bc	87 bc	83 abc	96 a	91 a	85 b
Untreated	0.0	0 c	0 d	0 d	0 d	0 c	0 d	0 c

[1]WAT = weeks after initial treatment application prior to planting
[2]Glyphosate applied as Roundup Pro formulation
[3]Fluazifop, clethodim, sethoxydim and isoxaflutole applied with crop oil concentrate at 1% (v/v).
[4]Sequential application 4 weeks after the initial application.
[5]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.

Table 3 Response of creeping bentgrass as a sod to timing and frequency of postemergence applications of various treatments.

Treatment	Rate	*Agrostis palustris* Control, WAT[1]					
		1	2	4	6	8	12
	Kg ha^{-1}	%					
Glyphosate[2]+ Glyphosate[3]	1.68 + 1.68	100 a[7]	100 a	100 a	100 a	99 a	87 ab
Glyphosate + Glyphosate[5] + Glyphosate[6]	1.68 + 1.68 + 1.68 100 a	100 a	100 a	100 a	100 a	99 a	
(Glyphosate + Fluazifop)	1.68 + 0.42						
+ (Glyphosate + Fluazifop)[5]	1.68 + 0.42	100 a	100 a	100 a	100 a	98 a	53 cd
Clethodim[3] + Clethodim[5]	0.28 + 0.28	82 ab	93 a	99 a	100 a	100 a	99 a
Clethodim[3] + Clethodim[5]+ Clethodim[6]	0.28 + 0.28 + 0.28 75 ab	96 a	97 a	99 a	100 a	100 a	
Fluazifop[3]+ Fluazifop[5]	0.42 + 0.42	75 ab	95 a	99 a	99 a	99a	88 ab
Fluazifop + Fluazifop[5] + Fluazifop[6]	0.42 + 0.42 + 0.42 80 ab	97 a	95 a	100 a	100 a	98a	
Sethoxydim[3] + Sethoxydim[5]	0.42 + 0.42	82 ab	94 a	82 ab	97 a	99 a	58 bcd
Sethoxydim[3] + Sethoxydim[5] + Sethoxydim[6]	0.42 + 0.42 + 0.42 73 ab	92 a	91 a	98 a	99 a	97 a	
Quizalafop-p[4]+ Quizalafop-p[5]	0.092 + 0.092	70 ab	92 a	92 a	99 a	95 a	77 abc
Sulfometuron[3]	0.070	28 c	92 a	99 a	100 a	100 a	99 a
Imazaquin[4]	0.56	63 b	90 a	93 a	97 a	97 a	85 ab
Imazaquin[4] + MSMA	0.56 + 2.0	42 c	87 a	88 a	89 a	90 a	65 a-d
Atrazine[3]	2.24 100 a	100 a	100 a	100 a	99 a	99 a	
Metribuzin + MSMA	0.28 + 2.24	96 a	97 a	93 a	98 a	98 a	57 bcd
Tranxit GTA[4] + Tanxit GTA[5]	0.070 + 0.070	82 ab	93 a	95 a	100 a	99 a	94 a
MON 44951[4] + MON 44951[5]	0.140 + 0.140	78 ab	90 a	82 ab	98 a	100 a	95 a
Isoxaflutole	0.11	92 ab	93 a	72 ab	81 ab	79 a	42 de
Isoxaflutole + Isoxaflutole[5]	0.11 + 0.11 80 ab	87 a	37 c	62 c	53 b	15 ef	
Mesotrione	0.22	78 ab	87 a	62 abc	70 bc	45 b	13 ef
Mesotrione + Mesotrione[5]	0.11 + 0.11	83 ab	91 a	53 bc	77 abc	57 b	27 ef
Untreated check	0.0	0 d	0 b	0 d	0 d	0 c	0 f

[1]WAT = weeks after initial treatment application prior to planting; [2]Glyphosate applied as Roundup Pro formulation
[3]Fluazifop, clethodim, sethoxydim, sulfumeturon, atrazine applied with crop oil concentrate at 1% (v/v).
[4]Imazaquin, Tanxit GTA, MON 44951, quizalafop applied with a non-ionic surfactant, X-77 at 0.25% (v/v)
[5]Sequential application 4 weeks after the initial application; [6]Sequential application 8 weeks after the initial application.
[7]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.

LITERATURE CITED

Amrhein, N., B. Deus, P. Gehrke and H.C. Steinrucken. 1980. The site of the inhibition of the shikimate pathway by glyphosate. Plant Physiology, 66:830-834.

Arias, D.M. and L.H. Rieseberg. 1994. Gene flow between cultivated and wild sunflowers. Theoritical Applied Genetics, 89:655-660.

Belanger F.C., T.R. Meagher and P.R. Day. 2001. Herbicide resistant creeping bentgrass:risks from outcrossing with related species. Weed Science Society of America Abstracts, 41:141-142.

Bhowmik, P.C. and A. Drohen. 2001. Differential response of cool-season Turfgrass species to isoxaflutole. International Turfgrass Society Research Journal, 9:995-1000.

Bhowmik, P.C. and D.C. Riego. 2003. Management alternatives for glyphosate resistant creeping bentgrass. Proceedings of the Northeastern Weed Science Society, 57:(in press).

Burton, J.D., J.W. Gronwald, D.A. Somers, B.G. Gengenbach and D.L. Wyse. 1989. Inhibition of corn acetyl-CoA carboxylase by clyclohexanedione and aryoxyphenoxypropionate herbicides. Pesticide Biochemistry Physiology, 34:76-85.

Cook, T., K. Stollero and L. Wohler. 1996. 1996 UHS non-selective trial. OSU Turfgrass field Day Report. Oregon State University, Lewis Brown Farm, pp. 17-25.

Damon, R.A. Jr. and W.R. Harvey. 1987. Experimental Design, Anova and Regression. Harper and Row, Publishers, Inc. NY.

Davies, W.E. 1953. The breeding affinities of some Btitish species of *Agrostis*. British Agricultural journal, 5:513.

Focke, M., H.K. Lichtenthaler. 1987. Inhibition of the acetyl-CoA carboxylase of barley chloroplsts by cyloxydim and sethoxydim. Z. Naturforsch, 42c:1361-1363.

Hancock, J.F., R. Grumet and S.C. Hokanson. 1996. The opportunity for escape of engineered genes from transgenic crops. HortScience, 31:1080-1085.

Hart, S.E., D.W. Lycan, M. Fletti, E.K. Nelson and G. Marquez. 2002. Response of glyphosate resistant and susceptible bentgrass (*Agrostis spp.*) to postemergence herbicides. Weed Science Society of America Abstracts, 42:79-80.

Johnson, P.G. and T.P. Riordan. 1999. A review of issues pertaining to transgenic turfgrasses. HortScience, 34(4):594-598.

Johnston, D.T., A.J.P. Van Wijk and D. Kilpatrick. 1989. Selection for tolerance to glyphosate in fine-leaved *Festuca* species. Proceedings of the 6th International Turfgrass Conference. Japan Society of Turfgrass Science, Tokyo, pp. 103-105.

Kushwaha, S. and P.C. Bhowmik. 1999. Inhibition of pigment biosynthesis in cucumber cotyledons by isoxaflutole. Photosynthetica, 37:553-558.

Lackey, B.A., R.A. Wichert, D.W. Bartlett, and J.K. Townson. 2000. Mode of action, absorption, translocation and metabolism of mesotrione in weeds and corn. Proceedings of the Northeastern Weed Science Society, 54:55.

Lee, L., S. Guo, W. Horman, E. Nelson and R. Harriman. 2002. Development of Roundup Ready creeping bentgrass. Proceedings of the SIVB (IAPTC & B Congress), Abstracts (in press).

Lee, L., C.L. Laramore, P.R. Day and N.E. Turner. 1996. Transformation and regeneration of creeping bentgrass (*Agrostis palustris* Huds) protoplasts. Crop Science, 36:401-406.

Luscome, B.M., K.E. Pallet, P. Loubiere, J.C. Melgarejo and T.E. Vrabel. 1995. RPA-201772:a novel herbicide for broadleaf and grass weed control in maize and sugarcane. Proceedings Brighton Crop Protection Conference-Weeds, 2:35-42.

Rodgers, H.J. and H.C. Parkes. 1995. Transgenic plants and the environment. Journal of Experimental Botany, 46:467-488.

[SAS] Statistical Analysis Systems. 1995. SAS/STAT User's Guide. Version 6, 4th Ed., Volume 2, Cary, NC 27513. Statistical Analysis Systems Institute, 846 p.

Sutton, D. 2002. Monsanto Company, St. Louis, MO. (Personal communication).

Wipff, J.K. and C. Frecker. 2001. Gene flow from transgenic creeping bentgrass (*Agrostis stolonifera* L.) in the Willamette Valley, Oregon. International Turfgrass Society Research Journal, 9:224-24

Assessment on Gene Flow Through Detection of Sexual Compatibility Between Transgenic Rice with *Bar* and *Echinochloa crusgalli* var. *mitis*

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Abstract: The possibility of gene flow between two varieties of transgenic rice with *bar* gene (Y0003 and 99-t) (male) and barnyard grass (*Echinochloa crusgalli* var. *mitis*) (female) was studied by means of reproductive biology. The germination and growth of rice pollen grains on barnyard grass stigmas at different periods, 30 minutes up to 4 hr, after crossing by hand were observed with optical-microscope. The cross results were compared with the germination and growth of barnyard grass pollen grains at the corresponding time after self-pollination. The results showed that the pollen grain germination and growth of the two varieties were similar on barnyard grass stigmas and differed from self-pollination of barnyard grass significantly. Pollen grains germinated and pollen tubes penetrated stigmas normally, and the number of pollen grains condensing or releasing their inclusions or having released them increased with time after self-pollination. Pollen grains of transgenic rice on the stigmas of barnyard grass failed to germinate or grow normally after cross, nor could penetrate the stigmas of barnyard grass. Therefore, it could be concluded that the sexual incompatibility between transgenic rice with bar gene and barnyard grass lay in that the rice pollen is not able to penetrate the stigma of barnyard grass. The fact that the emasculated barnyard grass pollinated with the rice pollen grains did not seed further proved the incompatibility between them.

Keyword: *Echinochloa crusgalli* var. *mitis*, gene flow, transgenic rice with *bar* gene, sexual compatibility.

INTRODUCTION

While benefiting human significantly, herbicide-resistant crops could induce potential harm to environment since herbicide-resistant gene could transfer to weedy relatives. In a specific environment, the relatives of some crops are malignant weeds or wild relatives. If the fitness of weedy relatives are increased by herbicide-resistant gene, these weedy relatives will become hardly-controlling weeds, which will produce new weed-control problems in the arable field, and affect ecological environment (Kling 1996; Qian et al. 1999; Wei et al. 1999; Gressel 2000). From the above reasons, it is necessary to assess the potential gene flow accurately. Barnyard grass and rice belong to *Echinochloa* and *Oryza* respectively. As barnyard grass exists in rice field widely, and can propagate largely to harm the crops severely. It can even mimic rice strongly (Barrett 1983), the issue whether herbicide-resistant gene of transgenic rice could transfer barnyard grass has raised wide concerns. However there have been no reports about this research. In general, there are three methods to assess the gene flow of herbicide-resistant transgenic crops. First, by spontaneous hybridization (Thomas et al. 1996; Chevre et al. 1997; Darmency and Fluery 2000). The results of this method have high correlation with experimental design and environment, and the period of this method is long and covers large scale analysis. Second, by hand pollination (Lefol et al.

1996; Metz et al. 1997). Manual pollination was done before anthesis so that the possibility of pollen cross would be increased artificially. The hybrid F_1 must be tested in following season, so the period of this method is also long. Third, by ovary culture in vitro or by embryo rescue techniques (Kerlan et al. 1992; Lefol et al. 1997). This method is resorted to when the relatedness with parent is far and sexual compatibility is poor leading to embryo abortion. By this means it is possible to obtain hybrids between female and male, but gene transfer in nature is unlikely. The weighty matter is these methods overlook the principal condition namely the sexual compatibility so that the reason and the stage of sexual incompatibility couldn't be known. Thus the gene flow between herbicide-resistant crops and weedy relatives cannot be assessed objectively and rapidly.

In this experiment, the gene flow between two varieties of transgenic rice with *bar* gene (Y0003 and 99-t) (male) and barnyard grass (*Echinochloa crusgalli* var. *mitis*) (female) was studied by means of reproductive biology. The purpose was to explore the method how to test the gene flow at earlier stage. Furthermore, the firsthand evidence of whether the herbicide-resistant gene could transfer from transgenic herbicide-resistant rice to barnyard grass could be provided by this experiment.

MATERIALS AND METHODS

1. Materials: The two experimental transgenic rice with bar gene Y0003 and 99-t were provided by International Rice Institute. Barn yard grass was collected from rice field in Nanjing, China. The experimental soil was clay which was collected from vegetable field in Nanjing Agricultural University.

2. Methods: The experiment was conducted in the greenhouse and weed research laboratory of Nanjing Agricultural University. The germination and growth of pollen grains were observed with an optical-microscope. More than 30 florets were treated for each treatment.

2.1 The emasculation technique for barnyard grass

The blooming spikes of barnyard grass were selected as experimental material. The anthers were drawn out with vacuum pump when glumes just opened. The emasculated florets were marked and others were pulled. Twenty spikes were treated. Ten spikes were self-pollinated, the other 10 spikes were not pollinated. After 10d of treatment, the seed-setting rates were examined in order to test the emasculating rate and the vigor of stigmas.

2.2 Barnyard grass pollen germination and growth at different times after anthesis

The stigmas of barnyard grass were taken out carefully at 0.5 1, 2, 3, 4 hr after anthesis. These stigmas were put on clean microscope slides, and dropped with a little distilled water, then covered with cover slides. The prepared stigmas were observed using an optical-microscope.

2.3 Hybridization in greenhouse

The emasculated florets of blooming barnyard grass spikes were pollinated with experimental rice pollen grains. At 0.5, 1, 2, 3, 4 hr after pollination, the stigmas were prepared with the method of 1.2.2.The germination and growth of the rice pollen grains on the stigmas of barnyard grass were observed with optical-microscope and pictures were taken. More than 550 pollinated florets were bagged in order to test the seed-setting rate after pollination.

RESULTS AND DISCUSSION

Research on the emasculating technique for barnyard grass

The emasculating rate was 100% because no seed was produced in the 398 emasculated florets. This showed that the emasculation with vacuum pump was perfect. Two hundred ninety eight emasculated florets pollinated with the rice pollen and produced 295 seeds with a setting rate of 98.9%. This indicated that stigma's vigor of barnyard grass was not influenced by emasculation with vacuum pump. From the above reason, the emasculation with vacuum pump was used so that the germination and growth of the rice pollen grains on the stigma of barnyard grass could be estimated correctly.

Barnyard grass pollen germination and growth at different time after anthesis

At 0.5h after anthesis, almost 85% of pollen grains germinated and pollen tubes had penetrated the stigmas of barnyard grass, and 16.79% of germinated pollen grains had released or were releasing their inclusions. At1-4h after anthesis, the number of pollen penetrating the stigma had no significant change, reaching 90%. The percent of pollen condensing or releasing inclusions or having released inclusions doubled at 1h compared with that at 0.5h after anthesis. The percent of those pollen grains at 2h after anthesis was close to that at 3h, rising by 10% than that at 1h. At 4h after anthesis, the percent of pollen grains releasing inclusions increased significantly compared with that before 4h, reaching 57%, and the percent of pollen grains condensing or releasing inclusions decreased slightly (Fig. 1).

Hybridization in greenhouse

The pollen grain germination and growth of the two varieties were similar on barnyard grass stigmas and differed from self-pollination of barnyard grass significantly. At 0.5h after pollination, 95% pollen grains did not germinate and formed global pollen tube from germinal pore, which cannot penetrate the barnyard grass's stigma. The remaining 5% germinated successfully. At 1-4 hr after pollination, 55% of the pollen grains germinated, but pollen tubes did not grow normally and penetrate the stigmas of barnyard grass. Some pollen tubes were twisted or hooked, changing the growth route after encountering the barnyard grass's stigmas. Some of them enlaced the self pollen grains or the stigmas of barnyard grass. Others had swelled at the tube terminal. The pollen was distorted and formed various shapes so that pollen grains cannot penetrate the stigmas of barnyard grass. Forty five percent of pollen grains did not germinate. Meantime, no pollen grains which were condensing and releasing or had released inclusions were observed. The fact that rice pollen grains cannot normally germinate on the stigmas of barnyard grass demonstrated that the sexual compatibility between barnyard grass and rice was very poor. The 620 emasculated florets pollinated with Y0003 and 570 florets pollinated with 99-t did not produce seeds. The seed setting rate was zero. This result indicates that failure of emasculated barnyard grass florets to seed also showed the sexual incompatibility between barnyard grass and experimental transgenic rice.

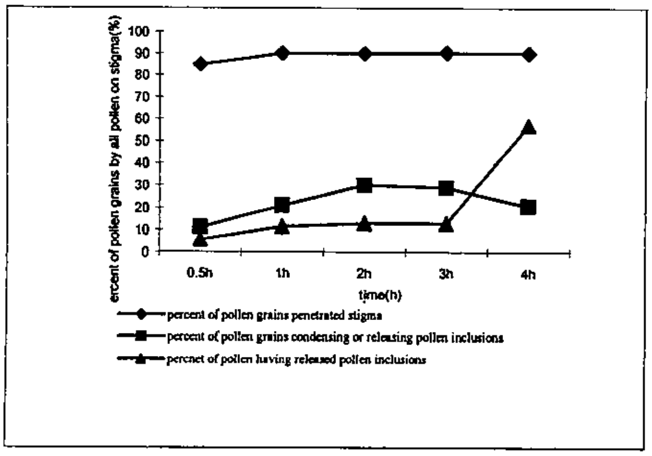
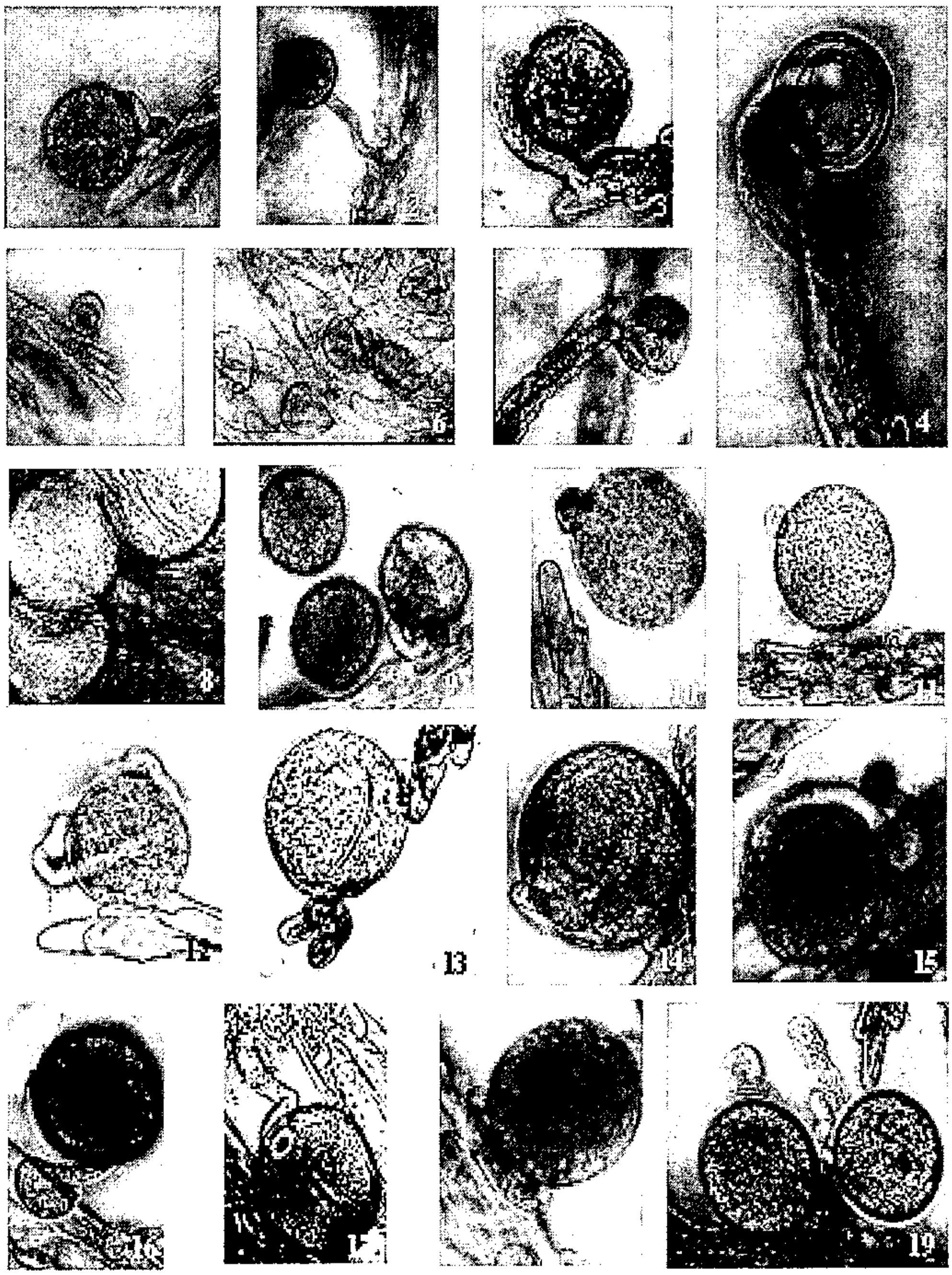


Figure 1. Pollen grain germination and tube growth of barnyard grass after self-pollination.

DISCUSSION

It maybe possible that the herbicide-resistant gene of rice can transfer to barnyard grass, considering that barnyard grass is a concomitant of rice. The probability of occurrence, however is very low considering that the relatedness of the two species is very far. There have been no reports about whether they are sexually incompatible or at what stage and what degree of sexual incompatibility occur. This experiment could offer the direct evidence for the sexual incompatibility of the plants at early stage. The germination of pollen grains on the stigma need signal transmission among cells. The stigmas are not only important barriers against exterior pollen grains, but also can supply germination environment for pollen grains as well as the signal of pollen recognition (Chen and Yang 2000). In this experiment, although some pollen grains of herbicide-resistant transgenic rice could germinate on the stigma of barnyard grass, the pollen tube grew so abnormally that they failed to penetrate the stigma of barnyard grass. This result demonstrates that pollen grains of transgenic rice are unable to transmit signal with the stigma of barnyard grass normally.

Many researchers (Dale 1992; Schefler and Philip 1994; Snow et al. 1999) hold the view that successful hybrid formation depends not only on the sexual compatibility between the crop plants and recipient species.The two species must also flower at the same time, share the same insect pollinator (if insect-pollinated) and be at a sufficiently close distance for the transfer of viable pollen. If the recipient species is sexually compatible with the crop, the consequences of transfer of the transgenes will depend on the sexual fertility of the hybrid progeny, their vigor and sexual fertility in subsequent generations, or their ability to propagate vegetatively. Among these important conditions for gene transfer, the sexual compatibility was the most important. Without this condition, the possibility of gene transfer was very poor. In our experiment,



1-7 Pollen germination and growth of *E. crusgalli* var. *Mitis* after self-pollination: 1-3 Pollen tube had penetrated the stigma, 400×, 4-5 The contents of pollen were contracting and releasing, 4, 200×, 5, 400×, 6-7 The contents of pollen had released, 4, 200×, 5, 400×; 8-19 Pollen germination and growth of transgenic rice with *bar* on the stigma of *E. crusgalli* var. *Mtis*: 8-9 No-germinating pollen grain (8- Y0003, 9-99-t), 10-11 Just germinating pollen grain without penetrating the stigma of *E. crusgalli* var. *Mtis* (10- Y0003, 11-99-t), 400×, 12-19 Abnormal pollen tube couldn't penetrate the stigma of *E. crusgalli* var. *Mtis* herbicide-resistant transgenic rices were sexually incompatible with barnyard grass. This showed that the possibility of herbicide-resistant gene transferring to barnyard grass is very low. In addition, the emasculated barnyard grass pollinated with pollen grains of transgenic rice but unable to seed demonstrated further that gene transfer cannot occur by pollen crossing.

The cross between transgenic rice and barnyard grass was inter-specific. Meng et al. (1995) reported that expression positions of hybridization incompatibility included stigma, style, and embryo sac. The incompatibility between transgenic rice and barnyard grass occurred at the first step of incompatibility. That decreased the possibility of horizontal gene transfer significantly. The transgenic herbicide-resistant gene could escape not only by cross-pollination but also by horizontal gene transfer (Lorenz and Wackernagel 1994; Gebhard and Smalla 1999). The same cellular mechanisms that enable the artificial vector carrying the foreign genes to insert into the genome can also mobilize the vector to jump out again to reinsert at another site or to infect other cells. If incompatibility between transgenic rice and barnyard grass wouldn't occur the first step, the herbicide-resistant gene could release in embryo of barnyard grass to increase the risk of horizontal gene transfer. The low possibility of horizontal gene transfer was also proven by this experiment. The fast test method for those species whose incompatibility occurs at early stage of pollen germination was provided by this experiment.

LITERATURE CITED

Barrett, S C H. 1983. Crop mimcry in weeds. Economic Botany. 37:25-282.

Chen S R. and Yang H Y. 2000. The mechanism of pollen-pistil interaction. Plant Physiology Communications. 36:356-360.[in Chinese]

Chevre A M. et al. 1997. Gene flow from transgenic crops. Nature. 389:924.

Dale P J. 1992. Spread of engineered genes to wild relatives. Plant Physiol. 100:13-15.

Darmency H. and Fluery A. 2000. Mating system in *Hirschfeldia incana* and hybridization to oilseed rape.Weed Research. 40:231-238.

Kling J. 1996. Could transgenic super crops one day breed super weeds?, Scinece, 274:180-181.

Qian Y Q. et al. 1999. Application and potential problems of transgenic crops. Chin. J. Appl. Environ. Biol.. 5:427-423. (in Chinese)

Gebhard F and Smalla K. 1999. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiology Ecology.28:261-272

Gressel J. 2000. Molecular biology of weed control. Transgenic Research. 9: 355-382.

Kerlan M C. et al. 1992. Risk assessment of out crossing of transgenic rapeseed related species: inter-specific hybrid production under optimal condition with emphasis on pollination and fertilization. Euphytica. 62:145-153.

Lefol E. et al. 1996. Predicting hybridization between transgenic oilseed rape and wild mustard.Field Crops Research. 45:153-161.

Lefol E. et al. 1997. Sexual hybridization in crosses of cultivated Brassica species with the crucifers *Erucastrum gallicum* and *Raphanus raphanistrum* potential for gene introgression. Euphytica. 95:127-139.

Lorenz M G. and Wackernagel W. 1994. Bacterial gene transfer by natural genetic transformation in the environment. Microbiol. Rev..58:563-602.

Meng J L. et al. 1995. Plant Reproductive Genetics. Beijing: Science Press. 296-308. (in Chinese)

Metz P L J. et al. 1997. The impact on biosafety of the phosphinothricin tolerant transgene in inter-specific *B. rapa*× *B. napus* hybrids and their successive backcrosses.Theor Appl Genet. 95:442-450.

Schefler J A. and Philip J D.1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Research. 3: 263-278.

Snow A A. et al. 1999. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. Molecular Ecol..8:605-615.

Thomas R. M. et al. 1996. The risks of crop transgene spread. Nature. 380: 31.

Wei W. et al. 1999..Gene flow between transgenic crop and their wild related species. Acta Botanica Sinica. 41:343-348. (in Chinese)

Weeds, Mycorhizae, and Economics of Roundup-Ready Hybrid Corn (*Zea mays*) Grown Under Conservation Versus Full Tillage System in Lampung (Indonesia)

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Abstract: This field study that was conducted in 2000 (first) and 2001 (second) growing seasons to document (1) weeds, (2) mycorhizae, and (3) economics of farming system of RR-Corn compared with other corn cultivars (hybrid C-7 and open pollination Bisma) in conservation (CT) and full tillage (FT). Weeds were sprayed twice with Roundup in the CT plots (1st season: RR-Corn and C-7; 2nd season: RR-Corn, C-7 and Bisma). Manual weeding was applied in the FT plots (1st season: C-7 and Bisma; 2nd season: RR-Corn, C-7 and Bisma). Major constraints for producing corn under CT were planting difficulty and low growth success (1st season), coverage of the ialang grass (*Imperata*), drought, and downy mildew disease (2nd season). Planting difficulty in CT was due to remnants of (thorny, woody and stubby) weeds. In any cultivar and tillage system, broadleaved weeds dominated over grasses. The number of mycorhiza was 5 to 172 chlamydospores and averaged 51.8 chlamydospores per 5 g soil. Mycorhizal density in RR-CT was lower than that in BISMA-FT. There were indications that root infection by mycorhizae was supressed in CT system (i.e., RR and C7). Growing RR-Corn in a CT system in the 1st season could have been beneficial if its growth success was adequately good. In terms of labor, however, RR-Corn CT was more efficient than the other farming systems. In contrast, in the second season there was no major difference in the overall efficiency between CT and FT. The overwhelming farming inefficiency was due to the drought and cost for eradicating the diseased plants.

Key words: conservation tillage, Roundup-ready corn

INTRODUCTION

One of the serious problems that cause low corn production in Lampung is weeds. Uncontrolled weeds hinder corn plant growth, affect soil characteristics, and worsen pest problems in the agroecosystem. Therefore, weed management is a must in agriculture (Kishore *et al.* 1992).

Conservation tillage system has been considered promising to control weeds and conserve soil biodiversity (Utomo 1995; Utomo 1997). However, its effectiveness has been quite dependent on herbicide application. Corn plants themselves are frequently affected by herbicide application (leaf injury or severe growth). Consequently, growing an herbicide-tolerant corn variety becomes a viable alternative to cope with such condition.

Recent progress in gene technology has facilitated the introduction and expression of genes to the development of crop plant genotypes that are resistant to a specific herbicide (Kishore et al. 1992). Herbicide, like Roundup has been developed by combining two powerful tools, chemical synthesis and screening of representative plants species, both of weeds and crop. Since 1987, the gene of glyphosate (active ingredient of Roundup) tolerance has been transferred into tomato. Recently, Roundup-resistant corn has been engineered by the name of RR-Corn.

Thus, this study was done to (1) examine weed problems, (2) document effects on mycorhizae, and (3) assess the economic feasibility of farming system of RR-Corn as compared with other corn cultivars (hybrid C-7 and open pollination Bisma) grown under conservation tillage (CT) versus full tillage (FT) system.

MATERIALS AND METHODS

The study was conducted in an experimental land of the Assessment Station for Agricultural Technology, Natar, South Lampung (ca. 20 km north of Bandar Lampung) during the dry season of 2001 (June to November 2001).

Three corn varieties were planted in six plots (size 80 m x 50 m; planting distance: 25 cm x 75 cm) differing in tillage systems (C7-CT = C7 grown under conservation-tillage; RR-CT = RR grown under conservation-tillage; Bisma-CT = Bisma grown under conservation tillage; RR-FT = RR grown under full tillage; C7-FT = C7 grown under full-tillage; Bisma-FT = Bisma grown under full-tillage). In the FT system, seeds were sown after the land was plowed completely. No plowing and mechanical weeding were done in the CT system. In the CT system, Roundup 75 WSG (74.7% monoammonium gliphosate) was sprayed twice during the growing season (Roundup rate 1.5 kg ha^{-1}, and 1.0 kg ha^{-1}, spray volume 450 L ha^{-1}).

Weeds

The indicators of weed infestation, i.e., total and dominant weed species were observed using the quadrat (50 cm x 50 cm) method. Additionally, herbicide phytotoxicity to the corn plants was evaluated using visual scoring valuation (Komisi Pestisida Departemen Pertanian 1989).

Mycorhizae

The densities of mycorhizae were observed from 0.5 kg soil samples using decantation and centrifugation method and their population density was determined (Daniel and Skipper 1982). Earthworms were hand-sorted from 3 quadrats (25 cm x 25 cm of 30 cm deep) per plot. The earthworms were collected, tallied and weighed. The population density and the biomass density (extrapolated to 1 m^2) were observed twice, i.e. before planting and at the vegetative phase of the plants.

Economics

The economic information was tabulated and descriptively analyzed. Efficiency problems recognized in the field were inventoried and confronted with their respective agronomic relevance. Efficiency of growing each of the three corn varieties (RR, C7 and Bisma) under CT or FT system was calculated using analysis of Revenue Cost Ratio (R/C) as follows.

$$R/C = \frac{\text{Gross Revenue}}{\text{Cost}}$$

Meanwhile, the break-even point (BEP), i.e., the minimal plant yield (kg ha^1) above to that is considered profitable, was calculated using the following formula.

$$BEP = TFC/(P\text{-}VCU)$$

where TFC = total fixed cost, P = output price per kg yield and VCU = variable cost per kg. Yield used for the economic analysis was of the dry seeds (13-15% water content; see Agronomic Performance). Price was Rp950,00 kg^{-1} seeds.

RESULTS AND DISCUSSION

Weeds

The first Roundup application to RR-CT plot was done at 3 weeks after planting (WAP) when weeds covered 22.4% of the plot. Table 1 shows that the herbicide could suppress the weed growth so that at 6 WAP weed coverage in the RR-CT plot was lower than that in the rest of the plots. The pattern of weed growth suppression was in accordance with the suppression of weed dry weight collected from the corresponding plots. The second Roundup application also effectively suppressed the weed growth.

At the time of the first herbicide application, both plots were 10 % covered with weeds. The weeds seemed to be quite sensitive to the herbicide so that at the time of planting most of the weeds looked toxicated. At 3 WAP (5 weeks from the start), weed coverage was more than 50% with *Ipomoea triloba, Mimosa invisa, Euphorbia geniculata,* and LCC dominating.

The second Roundup application to C7-CT or Bisma-CT was done 7 weeks following the first application, i.e., when the weed coverage was more than 85% over either plot. In terms of the existing weed coverage, the second herbicide application seemed to be a little late. However, that was safer for the crop although 15% of the corn plants in either plot were still toxicated by the herbicide. Toxicated plants were characterized by yellowing and drying of their under leaves.

Overall, weed coverage and dry weight of the C7-CT plot were similar to that of the Bisma-CT plot. However, weed coverage in either C7-CT or Bisma-CT plot was much higher than that in the RR-CT plot. That was also true for the weed dry weight at 6 WAP (Table 1).

Weed conditions in all FT plots (RR, C7 and Bisma) were relatively similar, especially in terms of the weed dry weight. Weed coverage at both observations (3 WAP and 6 WAP) was above 41%. The weeds were new which originated from their seed deposits (except *Imperata cylindrica*). Weed growth was faster due to looser (tilled) soil and more open surface (no-mulch) than those in the CT systems. In addition, mechanical weeding that has been done could not seem to suppress the weeds effectively, especially against *I. cylindrica* and other weeds that grew along the plant rows (*M. invisa* and *I. triloba*).

Table 1. Weed coverage (%) and weed dry weight (g 0.25 m^2).

	Weed Coverage		Weed Dry Weight	
	3 WAP	6 WAP	3 WAP	6 WAP
Conservation Tillage				
RR	22.4 c	16.8 d	1.10 b	4.88 c
C7	55.0 ab	85.6 a	4.78 ab	20.02 a
Bisma	56.0 ab	93.0 a	2.46 ab	19.52 ab
Full Tillage				
RR	66.6 a	62.6 b	6.56 a	14.00 abc
C7	41.4 bc	48.4 c	5.70 a	10.00 bc
Bisma	41.6 bc	62.0 b	3.96 ab	14.00 abc
LSD 0.05	*21.6*	*11.3*	*4.47*	*9.88*

Mycorhizae

The number of mycorhiza fluctuated in a range of 5 to 172 chlamydospores and averaged 51.8 chlamydospores per 5 g soil (Fig. 1, Table 2). Mycorhizal density in RR-CT was lower than that in BISMA-FT but it was rather unclear whether in general mycorhizal population was supressed in the CT system. The presumed supression phenomenon, however, was more clearly shown by the mycorhizal infection. Mycorhizal infection to RR or to C7 plant roots was significantly lower under CT system than that under FT system. That indicated that the pattern of mycorhizal population density (the potential) in soil was not necessarily parallel with the pattern of their interactions with the plat roots (the actual). The actual interaction (i.e., infection) should be affected by the plant characters. In other words, if supression of mycorhizal infection was real under CT system (due to herbicide effect, perhaps) then that might be the case for the hybrid corn varieties (RR and C7) but that was not necessarily true for the open pollinated variety (BISMA).

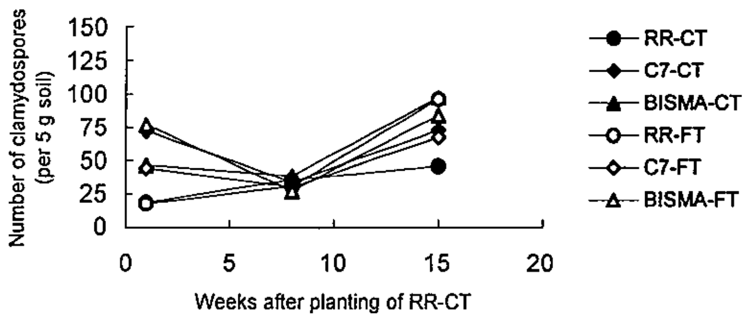

Figure 1. Population density of mycorhizae in soil where three varieties of corn plants were managed under conservation tillage (CT) or full tillage (FT). (RR = Roundup Ready, hybrid variety), C7 = hybrid variety and Bisma = open pollination variety

Economic performance

Farming analysis for 2001 growing season (Table 3) showed that growing RR in CT system was the only farming practice that provided net profit, i.e. Rp.742.700,00 ha^{-1} or R/C = 1.13. The others were unprofitable (R/C < 1). The overwhelming farming failures were due to the unsuitable growing season where in general rainfed water was lacking. But the efficiency of all tested plots (RR-CT included) was actually sub-normal due to excessive use of "unecessary" man-days for artificial watering (surface irrigation) and manual control for the downy mildew disease which contributed 20—25% of the total labor cost. The superiority of RR-CT over others occurred only in the plot area where *Imperata* grass did not exist.

Table 2. Average mycorhizal density and mycorhizal infection in plots where three corn varieties were grown under conservation versus full tilllage systems in Natar South Lampung, 2001 growing season.

Tillage systems and corn varieties	Mycorhizal density (number $5g^{-1}$ soil)	Mycorhizal infection (%)
Conservation Tillage		
RR	33.1 b	47.7 b
C7	59.5 a	44.7 b
Bisma	60.6 a	63.8 a
Full Tillage		
RR	48.0 ab	69.7 a
C7	47.2 ab	71.2 a
Bisma	62.6 a	57.5 ab
LSD 0.05	15.5	15.3

Values in the same column followed by the same letter indicated no siginificant difference by LSD 0.05(Lentner and Bishop, 1986; Little and Hills, 1978); infection = number of chopped corn roots with mycorhizal mycelium multiplied by 100 and divided by total number of chopped corn roots in a five-meter row.

The break-even point (BEP) analysis provided information on the minimum level above which the yield of any variety-tillage combination was considered profitable (Table 4). The BEPs of the second season were higher than that of the first season (> 5000 kg ha^{-1} versus < 4,000 kg ha^{-1}) meaning that (1) to reach a minimum profit, the 2001 growing season had to produce more seeds and (2) farming inefficiency in this season was higher. The source of inefficiency was quite apparent as aforementioned, i.e., uses of extra man-days for surface irrigation (as a consequence of the harsh dry season) and for manual control of the downy mildew disease. In addition, it was evident that the BEP was the highest for the highest performers (RR-CT for the second season and C7-FT for the first season). Curiously, the most profitable corn farming was the most inefficient farming.

Table 3. Financial analysis for farming three corn varieties under two tillage systems ha^{-1}.

Description	No *Imperata*			With *Imperata*		
	RR	C7	BISMA	RR	C7	BISMA
I. Conservation Tillage (CT)						
a. Grain yield (kg ha^{-1})	6,830	4,620	2,720	4,850	-	2,050
b. Gross revenue (Rp) [c)]	6,488,500.00	4,389,000.00	2,584,000.00	4,607,500.00	-	1,947,500.00
c. Farming cost						
- Purchased input (Rp)	1,363,000.00	1,363,000.00	1,053,000.00	1,363,000.00	-	1,053,000.00
- Pre-harvest labor (Rp)	3,290,000.00	2,957,500.00	2,905,000.00	3,290,000.00	-	2,905,000.00
- Harvest & post-harvest (Rp)	1,092,800.00	739,200.00	435,200.00	776,000.00	-	328,000.00
Total Farming Cost	5,745,800.00	5,059,700.00	4,393,200.00	5,429,000.00	-	4,286,000.00
Net Revenue (Rp)	742,700.00	- 670,700.00	- 1,809,200.00	- 821,500.00	-	- 2,338,500.00
R/C	1.13	0.87	0.59	0.85	-	0.45
II. Full Tillage (FT)						
a. Grain yield (kg ha^{-1})	2,050	2,710	2,280	-	-	-
b. Gross revenue (Rp) [c)]	1,947,500.00	2,574,500.00	2,166,000.00	-	-	-
c. Farming cost						
- Purchased input (Rp)	1,133,000.00	1,133,000.00	823,000.00	-	-	-
- Pre-harvest labor (Rp)	3,372,500.00	3,372,500.00	3,285,000.00	-	-	-
- Harvest & post-harvest (Rp)	328,000.00	433,600.00	364,800.00	-	-	-
Total Farming Cost	4,833,500.00	4,939,100.00	4,472,800.00	-	-	-
Net Revenue (Rp)	- 2,886,000.00	- 2,364,600.00	- 2,306,800.00	-	-	-
R/C	0.40	0.52	0.48	-	-	-
Exchange rate: Rp1000,00 = US $ 0.095						

Table 4. Break-even-point analysis of the 2000 versus 2001 corn growing season (kg/hectare)

Tillage systems	2001 season	2000 season
Conservation Tillage (CT)		
RR	5,890	3,018
C7	5,469	3,018
Bisma	5,010	-
Full Tillage (FT)		
RR	5,703	-
C7	5,703	3,788
Bisma	5,200	3,369

LITERATURE CITED

Daniels, B.A. and H.D. Skipper. 1982. Method for recovery and quantitative estimation of propagules from soil. Pages: 29-36, in Methods and principles of mycorrhizal research. N.C. Schenck (ed.). APS, St. Poul, Minnesota.

Kishore, G. M., S. R. Padgette, and R. T. Fraley. 1992. History of herbicide-tolerant crops, methods of development and current state of the art-emphasis on glyphosate tolerance. Weed Tech. 6:626-634.

Komisi Pestisida Departemen Pertanian. 1989. Pedoman pengujian efikasi untuk pendaftaran pestisida. Koperasi Daya Guna Direktorat Perlindungan Tanaman Pangan, Departemen Pertanian. Jakarta.

Lentner, M. and T. Bishop. 1986. Experimental design and analysis. Blacksburg: Valley Book. 565 pp.

Little, T.M. and F.J. Hills. 1978. Agricultural experimentation, design and analysis. New York etc.: John Wiley & Sons. 350 pp.

Utomo, M. 1995. Reorientasi kebijakan pengolahan tanah. P. 1—7. In: M. Utomo, F.X.

Susilo, D.R.J. Sembodo, Sugiatno, H. Susanto, A. Setiawan (ed.) Prosiding Seminar nasional V Budidaya Pertanian Olah Tanah Konservasi. Universitas Lampung. Bandar Lampung, 8—9 Mei 1995.

Utomo, M. 1997. Olah tanah konservasi: Teknologi pengolahan tanah lahan kering berkelanjutan. Pidato Pengukuhan Guru Besar Universitas Lampung. Bandar Lampung. 33 pp.

Environmental Benefits From the Use of Roundup Ready® Cotton

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Abstract: Roundup Ready® Cotton has been successfully introduced into the Australian cotton industry on the basis of its technical fit and the benefits the weed control system offers. With the use of the Roundup Ready technology, however, comes the potential increase in the amount of the herbicide glyphosate (N-(phosphonomethyl) glycine isopropylammonium, Roundup) applied. Although glyphosate has been used in Australia for many years with favourable results for weed control, no other Australian studies have examined the potential environmental impact of this increased glyphosate use. The current study aims to assess the relative environmental risk of glyphosate when used as the principal herbicide on glyphosate-resistant genetically enhanced cotton varieties compared with other industry-typical conventional herbicide programs. The project has comprised desktop risk-ranking modelling studies and a field study involving water, suspended sediment and soil sampling.

Two approaches to modelling, the Pesticide Impact Ranking Index (PIRI) (Kookana *et al.* 1998) and the use of fugacity modelling (Mackay 2001 and Sanchez-Bayo *et al.* 2002), have been used to compare the probable fate of glyphosate and other herbicides in runoff and groundwater. The relative risk of each herbicide to the aquatic environment was calculated based upon the toxicity of each chemical and their concentrations in the relative environmental phases, determined by the respective modelling approaches.

The field study undertaken at Auscott Midkin in the Gwydir Valley (northern New South Wales, Australia) consisted of four trial fields. All four fields were sown with Roundup Ready® Cotton (*Sicot 189RR*). Two fields were subjected to typical conventional herbicide programs over the 2001/02 growing season whilst the other two were subjected to typical glyphosate-dominated herbicide programs. Other herbicides used in the study, for comparative purposes, included 2,4-D, diquat dibromide, diuron, fluometuron, paraquat dichloride, pendimethalin, prometryn and trifluralin. The field data has also enabled a level of validation of the desktop risk-ranking assessments.

These assessments show that the use of Roundup Ready® Cotton with Roundup Ready® herbicide provides a lower environmental risk than using conventional applications of herbicides.

Key words: Australian cotton industry, ecological risk assessment, glyphosate, Roundup Ready cotton

INTRODUCTION

Roundup Ready® Cotton has been introduced into the Australian cotton industry on the basis of its technical fit and the benefits the weed control system offers. With the use of the Roundup Ready technology however, comes the potential increase in the amount of the herbicide glyphosate (N-(phosphonomethyl)glycine isopropylammonium, Roundup®) applied. Although glyphosate has been used in Australia for many years with favourable results for weed control, no other Australian studies have examined the potential environmental impact of this increased glyphosate use. Glyphosate has low affinity for the organic phase ($logK_{ow}$ reported in the negative range –4.59 to –1.7) and high water solubility, ranging from 10 to 15.7 g L^{-1} (Tomlin 1999-2000; Giesy *et al.* 2000). At first sight, this data suggests that glyphosate could be prone to leaching or appearing in run-off water. However, a number of studies have shown that glyphosate is relatively immobile in the environment as it binds tightly to soil, with sorption coefficients (K_{oc}), ranging from 9 to 60,000 L kg^{-1}, with a geometric mean of 2,072 L kg^{-1} (Giesy *et al.* 2000).

The objective of this study was to assess the environmental risk of glyphosate use for weed control on glyphosate-resistant genetically enhanced cotton varieties relative to other commonly used herbicides, including diuron, 2,4-D, fluometuron, pendimethalin, prometryn and trifluralin. This objective was achieved by comparing results of risk assessments for two groups of herbicide application scenarios, genetically engineered (GE) and conventional. The GE scenarios involved the use of glyphosate as a main feature, because of the tolerance to glyphosate of genetically engineered Roundup Ready® cotton varieties such as *Sicot 189RR*. Irrigation runoff data obtained from the field studies were used for the risk analysis in relation to water quality guidelines and toxicological data from glyphosate and other herbicides.

MATERIALS AND METHODS

This project consisted of a field study involving actual herbicide treatment programs and desktop risk assessment, for several theoretical herbicide application programs.

Modelling

Two desktop risk assessment approaches, the Pesticide Impact Ranking Index (PIRI) (Kookana *et al.* 1998) and the use of fugacity modelling (Mackay 2001 and Sanchez-Bayo *et al.* 2001) with ecological risk assessment, were used. The relative risk of each herbicide to the aquatic environment can be calculated based upon the toxicity of each chemical and their concentrations in runoff and groundwater.

Field studies

The field study undertaken at Auscott Midkin in the Gwydir Valley (northern New South Wales, Australia) consisted of four trial fields. All four fields were sown with Roundup Ready® Cotton (*Sicot 189RR*). Two fields were subjected to typical conventional herbicide programs over the 2001/02 growing season whilst the other two were subjected to typical glyphosate-dominated herbicide programs. Other herbicides used in the study, for comparative purposes, included 2,4-D, diquat dibromide, diuron, fluometuron, paraquat dichloride, pendimethalin, prometryn and trifluralin. The herbicide application scenarios assessed were based upon industry-typical application programs over an entire growing season.

The four fields, with slope gradients of 1:1400, were uniform light to medium grey cracking soils of 50-60% clay content. Soil organic carbon levels were measured as 0.8-1.2%, and pH levels were within the range of 7.5 to 8.6.

Herbicide applications

Actual applications of herbicides for the four fields of the field experiment are shown in Table 1. The use of glyphosate in a post emergence 'knockdown' application is not possible on a conventional cotton variety but was made possible here through the planting of the glyphosate tolerant *Sicot 189RR* variety on all four fields. The ability to rate the relative risk of individual herbicides was not affected by the application of glyphosate for knockdown in the conventional program fields. The applications were chosen without the benefit of prior relative risk assessment.

Chemical Analysis

Pesticide residues other than glyphosate

Composite water and suspended sediment samples were collected from the tail drains of the fields following irrigations. The water samples were extracted three times with dichloromethane and the extracts were combined and then reduced in volume by evaporation and made to volume in hexane. All herbicides except for the phenylurea chemicals were analysed by GC/MS. For analysis of phenylurea herbicides, solvent exchange between hexane and methanol was conducted on the hexane extract obtained from this method. Residues were determined by HPLC–UV.

Table 1: Herbicide applications on the four fields used in the field experiment. Figures in brackets indicate application rates of active ingredient in kg ha^{-1}.

Fields (Herbicide Program)	Herbicide Applications – 2001/02 Cotton Season[a]			
	Knockdown	Pre-Emergence	Knockdown	Layby
24 (Roundup)	-	Diuron (1)/Trifluralin (2.3) (early Sept)	Glyphosate (1.5) (end Oct)	Glyphosate (1) (mid Dec)
29 (Conventional)	-	Diuron (1)/Trifluralin (2.3) (early Sept) Fluometuron/ Prometryn (early Oct)	Glyphosate (1.5) (late Oct) Glyphosate (1) (early Nov)	Fluometuron (1.5)/ Prometryn (1.5)/ Diuron (1) (early Dec)
83 (Roundup)	2/4-D (0.9)/ Glyphosate (1) (mid Aug)	Diuron (1)/Trifluralin (2.3) (mid Sept)	Glyphosate (1.5) (mid Oct)	Glyphosate (1) (mid Dec)
84 (Conventional)	2/4-D (0.9)/ Glyphosate (1) (mid Aug)	Diuron (1)/ Pendamethalin (3) (mid Sept) Fluometuron (1.7)/ Prometryn (1.7) (late Sept)	Glyphosate (1.5) (mid Oct)	Fluometron (1.2)/ Diuron (0.8) (mid Dec)

[a] All herbicide applications occurred in 2001

Sediment and soil samples were extracted by shaking with an acetone/dichloromethane (50/50 v/v) mixture. Residues were then partitioned three times with dichloromethane and the solvent then exchanged with hexane. Determination and confirmation of pesticides other than the phenylurea herbicides was conducted by GC-MS. For analysis of phenylurea herbicides, solvent exchange between hexane and methanol was conducted on the hexane extract obtained from this method. Analysis was conducted by HPLC–UV and/or HPLC-MS. Confirmation was by HPLC-MS.

Glyphosate residues in water and filtered sediment

Water samples were acidified, dried and the residue taken up in the mobile phase for analysis by HPLC using a post-column reaction specific for primary amines. Glyphosate was oxidised in a post-column reactor coil at 55° C with hypochlorite solution to form glycine, then reacted with o-phthalaldehyde (OPA) in the presence of mercaptoethanol in a second coil to form a fluorophor, detected fluorometrically. AMPA, a major breakdown product of glyphosate, also forms a fluorophor under these conditions and was also detected.

Sediment from water passed through a 0.7 µm filter paper was extracted with 0.5N ammonium hydroxide, reduced in volume, acidified with hydrochloric acid and taken to dryness. The residue was then taken up in mobile phase and filtered before analysis by HPLC.

RESULTS AND DISCUSSION

Application of Pesticide Impact Rating Index (PIRI) to assess relative risk rating for potential off-site migration and toxicity to rainbow trout (*Oncorhynchus mykiss*) and water flea (*Daphnia sp.*)

PIRI is a simple index that integrates different factors influencing the off-site migration potential of pesticides (Kookana *et al.* 1998 and 1999). PIRI is based on three components, namely: the value of the asset (water resources threatened) (*V*), the source (s) of threat (pesticide use) to the asset (*L*), and the transport pathways through which the threat is released to the asset (*T*). The detriment to water quality is assumed to be the product of *V*, *L* and *T*, summed over all the pesticides used on a catchment.

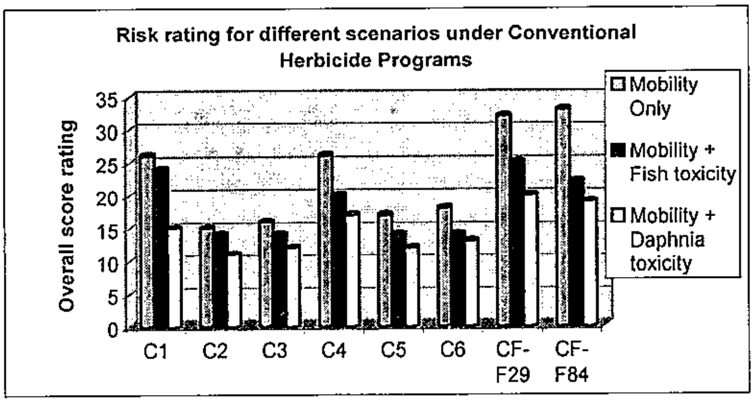

Figure 1: The overall scores for the 8 different scenarios under the conventional herbicide programs.

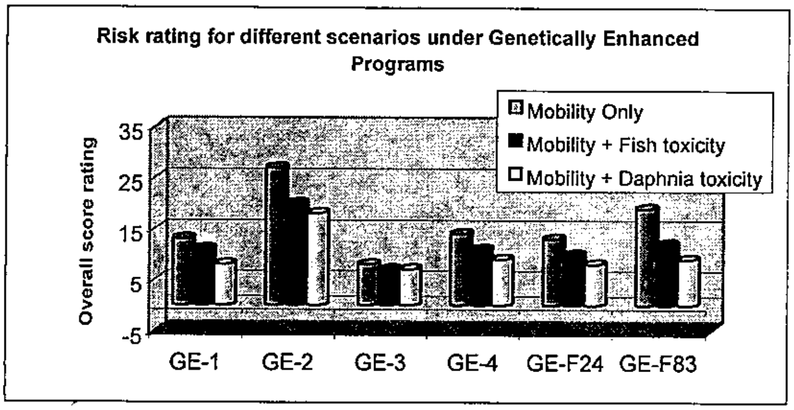

Figure 2: The overall scores for the 6 different scenarios under the genetically enhanced herbicide programs

Under conventional herbicide programs (Fig. 1), the highest PIRI risk rating was associated with the two actual field scenarios (CF-F29 and CF-F84) and the conventional herbicide scenarios C2, C3 had the lowest risk rating.
Under GE programs, the PIRI scores (Fig. 2) were lower than those in the conventional programs. Within GE programs, the highest risk rating was associated with GE-2. In contrast the GE herbicide scenario GE-3 had the lowest risk rating.

Fugacity

Fugacity modelling (Mackay, 2001) allows estimation of the concentrations of herbicides in environmental phases for theoretical herbicide applications scenarios. The data obtained from fugacity modelling were then used to develop an environmental risk assessment for these scenarios.

Comparisons between data from actual field experiments and modelled fugacity data were made. Fugacity assumes that partitioning occurs instantaneously after application. Irrigations and irrigation run-off sampling are usually conducted some time after the initial pesticide application. Thus, the values obtained by modelling using fugacity are usually overestimates because environmental degradation is not included. To compare such fugacity data with field data, half-lives must be taken into account to obtain more realistic values.

Agreement between fugacity calculations and actual concentrations was acceptable. In the case of glyphosate, fugacity models concentrations that fall below the limit of detection. No glyphosate was detected above the LOD in the field data validating this model. On the other hand, the results for sediment in runoff from field F24 showed good agreement for trifluralin and diuron for modelled and field data.

The herbicide program chosen for field F29 employed multiple applications of some herbicides (Table 1). However, there is good agreement between the modelled and the field data.

In summary, the results showed that good agreement was observed between fugacity modelled data and real field data. The largest disagreement, in the case of prometryn, was less than an order of magnitude greater. It follows that it was appropriate to use fugacity to model 'worst case' concentrations of herbicides for the risk assessment of the theoretical herbicide scenarios.

Field trial data

The results obtained from the field data, summaries shown in Table 2, show that glyphosate was detected above the limit of detection in a small number of runoff samples within suspended sediments. Diuron and trifluralin were detected more often above their limits of detection (LOD) in both the suspended sediment and the aqueous phase.

The other herbicides, pendimethalin, fluometuron, prometryn and metolachlor were also detected in some samples. It can be noted that some herbicides, e.,g. fluometuron and prometryn, were detected in runoff samples though not applied to all fields. This is likely a result of the reuse of contaminated irrigation runoff to irrigate successive fields. The study assessed the data presented in Table 2 with respect to water quality guidelines, however, the guidelines are incomplete and do not prescribe values for fluometuron, prometryn or pendimthalin. Glyphosate was found to be well below the 370 μg L^{-1} limit (ANZECC/ARMCANZ, 2000). Diuron was found to be above its limit (0.2 μg L^{-1}), which is purposely low due to lack of available toxicity data. Therefore, the herbicide concentrations in runoff were used to formulate the relative risk between the herbicides based upon toxicological data.

Table 2. Summary of results of the runoff studies showing averages and ranges of herbicide concentrations (μg L^{-1}) in water for each field.

Field	Chemical	Glyphosate	Diuron	Pendimethalin	Fluometuron	Prometryn	Trifluralin	Metolachlor
24	Ave	-	16.1	0.4	1.3	1.3	0.6	0.1
	Low High	LOD	1.6 45.8	LOD 1.01	0.3 2.2	LOD 1.43	0.07 1.35	LOD 0.09
29	Ave	-	26.6	1.0	10.9	3.2	1.8	0.2
	Low High	LOD	1.4 73.8	LOD 2.5	LOD 32.9	LOD 6.75	LOD -6.7	LOD 0.18
83	Ave	-	16.9	0.1	1.1	0.8	1.0	0.1
	Low High	LOD	0.51 63.3	LOD 0.27	LOD 1.5	LOD 1.44	LOD 3.82	LOD 0.18
84	Ave	-	37.3	3.7	38.8	4.4	0.1	0.1
	Low High	LOD	0.59 57.7	LOD 6.47	LOD 93.2	0.36 13.68	LOD -0.12	LOD 0.09

LOD=limit of detection; 0.05 μg L^{-1} for pendimethalin, prometryn, trifluralin and metolachlor, 10 μg L^{-1} for glyphosate, and 0.3 μg L^{-1} for diuron and fluometuron.

Risk to aquatic ecosystems for field experiment scenarios

Relative risk (RR) was expressed by comparing the toxic exposure of the herbicides of two common wetland species, rainbow trout (*Oncorhynchus mykiss*) and water flea (*Daphnia sp.*) following recognised risk assessment framework (Norton *et al.*, 1992). The field results were used to determine the probability of exposure and the likely level of exposure is given by the median value of all data collected (Table 3).

Exposure (X)

Estimation of exposure is a key aspect of determining risk and knowledge of the concentrations (C) of pesticides in the ecosystem is essential to determine exposure. The following equation (1) was used to determine exposure (X):

$$X = C\ P\ t_{1/2}\,(BCF) \qquad (1)$$

The probability of exposure (P) is determined by the frequency of positive values 'n (+)' for each chemical compared to the number of samples in the data set "N". The half-life ($t_{1/2}$) is included to represent persistence and "BCF" refers to the bioconcentration factor in organisms (Chiou *et al.* 1977).

A summary of herbicide concentrations used for the ecosystem risk assessment is shown in Table 2. The median data (Table 3) were used to determine the exposure as they are considered to give a more realistic representation of the runoff data for risk assessment purposes. Averaged data are often skewed too low and are therefore not useful for sensible ecosystem management. As shown in Table 3, glyphosate had the lowest probability of detection. No significant difference was observed in the runoff data between scenario types (conventional vs. GE) for trifluralin. In the case of glyphosate a negative BCF factor is given, due to its extremely low affinity for organic matter. This value is obtained from a negative $\log K_{OW}$ and was disregarded for the exposure calculations contained in Table 3.

Table 3: Median herbicide concentration in runoff water calculated from the field trials and determination of exposure of each chemical to the ecosystem.

Chemical	Median ($\mu g\ L^{-1}$)		Probability (P) = n(+)/N		Half-life (t1/2)	BCF	X = C * P * t1/2*BCF	
	Conv	GE	Conv (P)	GE (P)	days	log	Conv.	GE
Glyphosate	<10	<10	0.33	0.46	22	-2.6	72.6[a]	101.2[a]
Diuron	43.68	7	1.00	1.00	60	3.2	8255.5	1323.0
Fluometuron	11.9	0.9	1.00	-	85	2.7	2761.4	-
Prometryn	2.09	1.2	1.00	-	60	3.4	423.9	-
Pendimethalin	1.91	0.18	1.00	-	21	5.2	209.8	-
Trifluralin[b]	0.42		0.89		25	4.9	45.6	

[a] using a concentration of 10 $\mu g\ L^{-1}$ and disregarding negative BCF

[b] Combined runoff data, conventional and GE as no significant difference was found between program residue detection

Risk (R)

Applying the information generated on exposure (X) and toxicity (Tox), Tox=LC_{50} (Tomlin, 1999-2000) to the following equation (2) allows computation of the risk to the ecosystem:

$$R = X/Tox \quad (2)$$

The results of the risk assessment are presented in Table 4. The risk categories were subjective, chosen as high (R>10), medium (10>R>1), low (1>R>0.01) and negligible (R<0.01) and were applied to obtain relative distribution within the results of the risk assessment.

Table 4 Relative risk scores and categories of the herbicides used in the field trial scenarios.

Chemical	Conventional				GE			
	Trout	**Category**	*Daphnia*	Category	**Trout**	**Category**	*Daphnia*	Category
Glyphosate	0.0009	Negligible	0.006	Negligible	0.001	Negligible	0.008	Negligible
Diuron	7.5	Medium	0.2	Low	1.2	Medium	0.03	Low
Fluometuron	0.05	Low	0.3	Low	-	-	-	-
Prometryn	0.08	Low	0.01	Low	-	-	-	-
Pendimethalin	4.2	Medium	ID	-	-	-	-	-
Trifluralin[a]	1.1	Medium	0.08	Low	1.1	Medium	0.08	Low

[a]Combined Conventional and GE
ID Insufficient data

The results in Table 4 indicate that glyphosate has negligible risk compared to use of the other herbicides to the two-wetland species, by many orders of magnitude. The chemicals yielding lowest risk after glyphosate were prometryn and fluometuron, which are categorised as low. From Table 4 diuron and pendimethalin pose medium risk to trout. Diuron, pendimethalin and trifluralin posed the greatest risk to trout, however, the risk to *Daphnia* from the use of these chemicals was categorised as low.

In assessing the conventional versus GE herbicide programs selected, with hindsight it can be concluded that the use of prometryn and fluometuron in a GE program instead of trifluralin and diuron would further reduce risk associated with the overall GE program. Further improvement of the GE application scenario depends upon the interchangeability of efficacy in the herbicide treatments from physical properties. Any increase in application rates must increase the exposure and associated risk to ecosystems proportionally. A principle of ecosystem protection is that the lower the mass of a chemical applied to the environment the lower the risk of ecological contamination by that chemical.

Risk to aquatic ecosystems for the theoretical herbicide application scenarios

The main purpose of the fugacity model was to produce an estimation of the concentration of herbicides in runoff to enable risk assessment of the theoretical herbicide scenarios. The total runoff data obtained from the fugacity modelling was used to assess the risk of the theoretical herbicide treatments based upon the toxicity of the two-wetland species as detailed previously.

The results from the theoretical scenarios presented in Figure 3 showed good agreement with the risk categories determined for the actual field experiment scenarios. Diuron and trifluralin consistently gave high risk to trout and low and medium risk respectively to *Daphnia*. The fluometuron assessment indicated high and medium risk existed for *daphnia* and trout respectively. The theoretical scenarios allowed metolachlor, pyrithiobac-sodium and clethodim to be assessed and these results showed that the risk of using these chemicals at the indicated rates was usually very low. The risk associated with the use of glyphosate was consistently found to be negligible.

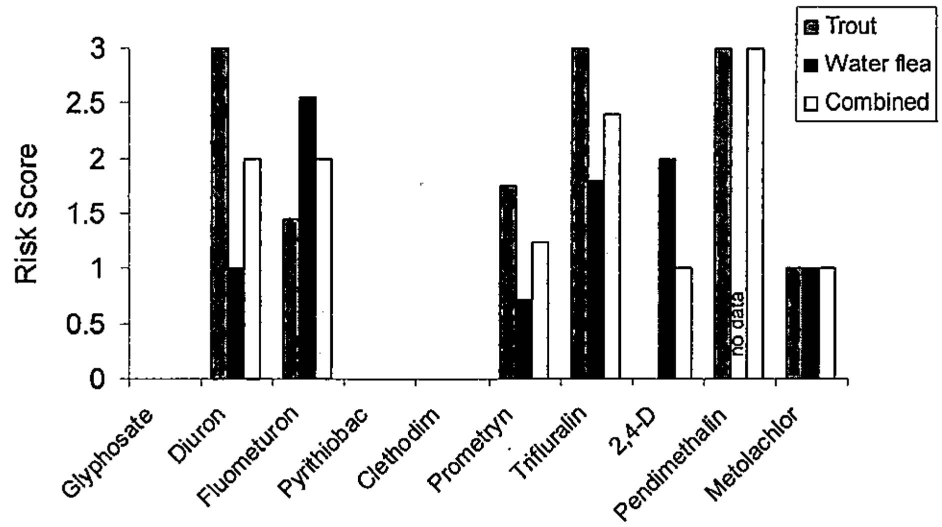

Figure 3. Risk score for each herbicide; presented as averages of all scores from all scenarios.

CONCLUSIONS

The two approaches to risk assessment illustrated here are complementary. Obviously, they can both be used to optimise environmental risk reduction if employed as decision tools for choosing application programs. Whilst desktop approaches can indicate the likely risks, risk assessment using real data provides validation for such management decisions. Better information on the toxicity of herbicides to local ecosystems, including plant species, would be desirable for this purpose.

These assessments indicate that the use of prometryn and fluometuron in a GE program with glyphosate instead of trifluralin and diuron would result in a significant reduction of risk associated with the overall GE program. Although there was no significant difference in the illustrative conventional and GE herbicide programs chosen in this study, this can easily be rectified. The interchangeability of the herbicide treatments as allowed by efficacy should allow further reduction of risk.

The risk assessment presented indicates that the introduction of Roundup Ready® Cotton for weed management in cotton production lowers the risk of ecosystem contamination, consistent with similar studies carried out on transgenic corn varieties (Esters *et al.* 2001; Wauchope *et al.* 2001). The current study has not assessed the risk of leaching to ground water. The unique interaction of glyphosate with soil, a result of its zwitterionic structure, suggests that environmental fate studies involving a number of Australian soils, especially with respect to leaching, would be appropriate. Some studies have shown that glyphosate behaviour in soil depends upon localised soil characteristics (Piccolo *et al.* 1994, 1996; de Jonge *et al.* 2000), but detailed computer modeling of glyphosate leaching behaviour in a highly vulnerable US setting showed no movement below 2 m (Estes et al. 2001).

Therefore, the use of Roundup Ready® Cotton with Roundup Ready® herbicide potentially provides a lower environmental risk than other approaches using conventional applications of herbicides.

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LITERATURE CITED

ANZECC/ARMCANZ (2000). *Guidelines for Fresh and Marine Water Quality.* Australian and New Zealand Environment and Conservation Council; Agricultural and Resource Management Council of Australia and New Zealand.

Chiou, G.T., Freed, B.H., Schmedding, D.W. and Kohnert, K.L. (1977) Partition coefficients and bioaccumulation of selected organic chemicals. *Environ. Sci. Technol.*, 11: 475.

de Jonge, H., L. W. de Jonge, and O. H. Jacobsen. (2000) [C-14]glyphosate transport in undisturbed topsoil columns. *Pest Management Science* 56:909-915.

Estes, T. L., Allen, R., Jones, R. L., Buckler, D. R., Carr, K. H., Gustafson, D. I., Gustin, C., McKee, M. J., Hornsby, A. G., & Richards, R. P. (2001). Predicted impact of transgenic crops on water quality and related ecosystems in vulnerable watersheds in the United States. Paper presented at the Soil and Water Mini-Symposium, British Crop Protection Council (BCPC) Conference, Weeds 2001. Brighton, UK.

Giesy, J. P., S. Dobson, and K. R. Solomon. (2000) Ecotoxicological Risk Assessment for Roundup Herbicide. *Rev Environ Contam Toxicol* 167:35-120.

Kookana RS, Correll RL and Simpson, B. (1998). Assessing risk at catchment or regional level through a Pesticide Impact Rating Index (PIRI). Land and Water Research and Development Corporation (LWRRDC) Occasional Paper 23/98, page no. 81-88, Canberra.

Kookana RS, Correll RL and Miller R. (1999). Pesticide Impact Rating Index (PIRI). In: *Rivers for Future*, Spring 1999 issue, Land and Water Research and Development Corporation (LWRRDC), Canberra, Australia.

Mackay, D. (2001) Multimedia Environmental Models: The Fugacity Approach. Lewis Publishers, Michigan.

Norton, S.B., Rodier, D.J., Gentile, J.H., van der Schalie, W.H., Wood, W.P. and Slimak, M.W. (1992) A framework for ecological risk assessment at the EPA. *Environ. Toxicol. Chem.*, 11: 1663-1672.

Piccolo, A., G. Celano, M. Arienzo, and A. Mirabella. (1994) Adsorption and Desorption of Glyphosate in Some European Soils. *Journal of Environmental Science & Health - Part B: Pesticides, Food Contaminants, & Agricultural Wastes* 29:1105-1115.

Piccolo, A., G. Celano, and P. Conte. (1996) Adsorption of Glyphosate by Humic Substances. *Journal of Agricultural & Food Chemistry* 44:2442-2446.

Sanchez-Bayo, F., S. Baskaran, and I. R. Kennedy. (2002) Ecological relative risk (EcoRR): another approach for risk assessment of pesticides in agriculture. *Agriculture, Ecosystems and Environment* 91: 31-57.

Tomlin, C. D. S. (1999-2000) The e-Pesticide Manual, Eleventh edition, Version 1.1. British Crop Protection Council, Surrey.

Wauchope, R. D., T. L. Estes, R. Allen, J. L. Baker, A. G. Hornsby, R. L. Jones, R. P. Richards, and D. I. Gustafson. (2001) Predicted impact of transgenic, herbicide tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Management Science* 58:146-160.

The Use of Carfentrazone-ethyl For The Control Of Roundup Ready Cotton And To Aid In The Knockdown Of Difficult To Control Broadleaved Weeds

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Abstract: Carfentrazone-ethyl has been evaluated for use as a mixing partner for the knockdown herbicide glyphosate with the objectives of increasing weed spectrum, the reliability of broadleaf weed control and the speed of activity.

Carfentrazone has shown a high degree of activity on some of the harder-to-control broadleaf weeds such as marshmallow *(Malva parviflora* L.*)*, Paterson's curse *(Echium plantagineum* L.*)*, and sub-clover *(Trifolium subterraneum* L.*)*. As well as double gee *(Emex australis* Steinh.*)* and *Brassica* species, particularly wild radish *(Raphanus raphanistrum* L.*)*, both of which are showing increasing levels of Group B herbicide resistance. After the limited release of glyphosate tolerant cotton (Roundup Ready®(RR) Cotton) in Australia during the 2001/2002 season and its rapid adoption in the 2002/2003 season. The control of volunteer seedling RR cotton plants in a cropping system that is dominated by the use of glyphosate for fallow and crop establishment weed control is a true challenge.

Initial herbicide screening work by Roberts (2001) demonstrated that various commercially available products commonly added to glyphosate for broadleaved weed control had limited effect on RR cotton seedlings. However carfentrazone was identified as one active that did provide commercially acceptable levels of control of RR cotton.

The 2002 winter cropping season saw the launch of carfentrazone in a 240 g ai L^{-1} EC formulation branded as Hammer EC by FMC (Chemicals) Pty Ltd. Hammer is distributed in Australia by Crop Care Australasia Pty Ltd specifically for use as a mixing partner with the major knockdown herbicides for use in the southern, temperate, cropping regions of Australia. Further field trials were undertaken in the cotton growing regions to determine the suitability of carfentrazone for use in herbicide programs for the control of RR cotton and other common broadleaved weeds. It was established that use rates of 18 to 24 g ai ha^{-1} provided the best and most robust control of RR Cotton (97% – 100%).

Carfentrazone has many unique benefits. However one of significant interest to the farming community is that it has no soil carryover and therefore no plant back restrictions - a particular benefit when used prior to sowing legumes or oilseeds and in opportunistic cropping systems utilizing unreliable rainfall.

Keywords: broad-leaf weeds, carfentrazone-ethyl, glyphosate, knockdown herbicides, Roundup Ready® Cotton

INTRODUCTION

Herbicides control plants by disrupting different functions of the plant cell. Carfentrazone-ethyl is a light-dependent herbicide that inhibits the enzyme protoporphyrinogen oxidase (commonly abbreviated as protox) and in doing so, disrupts lipid and cell membranes. The lethal effect is caused by natural plant compounds that accumulate in the cells of weeds treated with carfentrazone. Protox is the site of action, but induction of lipid peroxidation, which results in membrane disruption, is the mechanism of action. Membrane disruption is the overall mode of action.

The following points summarize the protox mechanism of, and course of action for, carfentrazone in plants;

- Sunlight and photosynthesis are required. Light is required for activity since photosynthesis must be in progress. Newly developing leaves, actively making plant pigments, are most susceptible.
- Protox inhibition leads to a build-up of protoporphyrin IX, an intermediate in chlorophyll synthesis.
- In light, proptoporphyrin IX is very efficient at transferring energy to oxygen-yielding, unstable forms including singlet oxygen.
- The cell membrane is damaged, releasing cell contents into intercellular spaces, causing the cell to collapse and die.
- Cellular destruction results in plant tissue necrosis (death).

The process and onset of action is very rapid. Plants treated with carfentrazone become necrotic and die shortly after treatment. Initial symptoms are observed within 2 to 4 days and mortality occurs within 7 to 14 days dependent upon environmental conditions.

Leaf penetration is the key to control with carfentrazone and weeds should be in an active growth stage – producing chlorophyll – to maximize effects.

Roundup Ready® (RR) Cotton

In many broadacre crops, weeds are the most economically damaging pests. The improved ability to control weeds is usually rapidly adopted by farmers and as such herbicide tolerant crops are likely to dominate farming fields in the future. The limited commercial release of RR Cotton in the 2000/2001 growing season saw the beginning of a new era in weed management for cotton production in Australia. An additional management difficulty with herbicide tolerant crops is the control of volunteers in the following year.

This is particularly important if the volunteer plants being controlled have the Roundup Ready gene and the following crop is cotton, as the use of glyphosate will not control these seedlings. Initial herbicide screening work by Roberts (2001) demonstrated that the products commonly added to glyphosate for broadleaved weed control had limited effect on RR cotton seedlings.

Carfentrazone-ethyl was identified as one product that provided commercially acceptable levels of control of RR cotton. Consequently a series of trials were designed to further evaluate the potential of carfentrazone as a viable tool for the management of volunteer cotton plants and other broadleaf weeds within a weed management system principally bases on glyphosate.

MATERIALS AND METHODS

A. Cotton Trial

An initial screening trial was conducted by FMC (Chemicals) Pty Ltd mid season 2001/2002 to evaluate the use of carfentrazone for the control of both conventional and RR cotton seedling volunteers. An additional series of 9 trials were conducted at the beginning of the 2002/2003 cotton seasons in Australia to confirm the results of the initial trial, as well as to evaluate the effectiveness of carfentrazone on other common broadleaved weeds that occur during the cotton-seeding period.

All trials were of a complete randomized block design with 3 replications. Treatments were applied in 75 L ha^{-1} total spray volume using Flat Fan 110-01 Teejets at 200 kPa. The rate of glyphosate (450 g ai L^{-1}) used ranged from 1000 to 1500 mL ha^{-1} dependent on weed size and represented commercial use rates. All treatments had the spray adjuvant "Supercharge" added at 0.5% volume/volume (ie: 0.5 L $100L^{-1}$) unless otherwise stated.

Assessments of Visual Percent Control were made at 7 & 14, Days After Application (DAA), results not shown. Final weed counts were performed at 28 DAA to ensure sufficient time for any re-growth to occur.

Trial sites were chosen in fields that had been pre-watered 10 to 20 days prior to seeding of this seasons cotton crop with application being just prior to seeding or post seeding pre-emergent of the cotton crop. At the time of application targeted broadleaf weeds ranged in size from cotyledon to 6 leaves.

B. Wheat Trial

A series of 8 trials were also conducted in the Western Australia wheat belt during the winter of 2001 to evaluate the efficacy of carfentrazone when mixed with glyphosate and applied prior to crop establishment or as fallow sprays. The weed species targeted were marshmallow, sub-clover, wild radish and doublegee.

Trial methods were the same as outlined above with the exception that treatments were applied in 60 L ha^{-1} total spray volume and the rate of glyphosate (450 g ai L^{-1}) used ranged from 600 mL to 1000 mL ha^{-1} dependent on weed size and represented commercial use rates.

The results presented in Table 5 are the average of these data sets and thus statistical analysis has not been included.

At the time of application the broadleaf weeds ranged in size from cotyledon to 6 leaf. This was considered to be typical of weed sizes at crop establishment.

RESULTS

The use of carfentrazone either alone or in mixtures with glyphosate provided excellent levels of control of both Roundup Ready and conventional cotton seedlings up to the 6 Leaf growth stage. The level of control provided was superior to glyphosate alone or the standard mixtures of glyphosate plus oxyfluorfen or glyphosate plus fluroxypyr when applied to conventional cotton.

These mixtures were not applied to the first trial #1, because Roberts (2001) had already clearly showed that both oxyfluorfen and fluroxypyr had no commercial acceptable effect on RR cotton.

Additional trials were conducted to evaluate the use of glyphosate plus carfentrazone mixtures on a range of commonly found broadleaved weeds at the time of planting cotton.

Carfentrazone when used alone has provided excellent control of both RR and conventional cotton. There is a clear rate response, with 6 g ai ha^{-1} failing to provide commercially acceptable levels of control (64% to 92%). The use of 12 g ai ha^{-1} was acceptable (94% to 100%) when applied in 100 L ha^{-1} total volume. However, the final result was very dependent on the quality of application as can be seen by the drop in efficacy (86% & 78%) when applied in 50 L ha^{-1} total volume. Both of these rates are considered to be too unreliable for commercial recommendation.

Use rates of 18 to 24 g ai ha^{-1} have provided the greatest and most robust levels of control (97% to 100%). Additional field demonstrations on a commercial scale (not reported), have confirmed that use rates of 18 to 24 g ai ha^{-1} applied in a minimum total volume of 75 L ha^{-1} constantly provide commercially acceptable results of 95% control or greater.

Traditionally glyphosate is relied upon to control conventional cotton volunteer seedlings. Often that level of control is not total (79%), the addition of carfentrazone at 18 g ai ha^{-1} increased the level of control to 97%. The addition of either oxyfluorfen (71%) or fluroxypyr (82%) to glyphosate, whilst broadening the spectrum of weeds controlled did not increase the level of conventional cotton control.

When using carfentrazone alone the rate of spray adjuvant (Supercharge) used was 1.0% v/v, this was reduced to 0.5% v/v due to the extra wetter loading that is present in the glyphosate formulation.

Table 2: Represents a summary of all broadleaved weeds tested to date, where effective control is defined as commercially acceptable 95% or greater. Many broadleaved weed species were effectively controlled by glyphosate alone. The addition of carfentrazone did not cause any antagonism to glyphosate and was a benefit in terms of speed of control with full plant desiccation being observed by 7 (DAA).

The addition of carfentrazone significantly increased the level of control achieved compared to glyphosate alone on three weed species as well as RR and conventional cotton. Glyphosate is commonly mixed with fluroxypyr when targeting these weeds, particularly cow/peach vine *(Ipomea lonchophylla)*. Carfentrazone had limited effect on bellvine *(Ipomea plebeia)* and the use of fluroxypyr remains the best choice for this weed.

Table 1. Percentage control of Roundup Ready and conventional cotton seedling volunteers *(Gossypium hirsutum)*.

Treatment	Rate g ai ha^{-1}	#1 RR Cotton	#1 Con. Cotton	#2 Con. Cotton.
Carfentrazone	6	92	64	
Carfentrazone	12	96	100	
Carfentrazone	18	100	100	
Carfentrazone	24	100	100	
Carfentrazone **	12	86	78	
Glyphosate	450 / 562	0	0	79
G + Carfentrazone	6			81
G + Carfentrazone	12	100	100	94
G + Carfentrazone	18	100	100	97
G + Carfentrazone	24			97
G + Oxyfluorfen	19			71
G + Fluroxypyr	90			82
LSD (P=0.05)		8.3	20	
Av: Plant Size		4 Leaf 70% 6 Leaf 30%	4 Leaf 35% 6 Leaf 65%	Cotyledon to 2 Leaf

** A single treatment applied in 50 L ha $^{-1}$ Total Volume.

#1: Trial Number AU12-02-H501, Conducted mid season 2000/2001 by seeding both Roundup Ready and Conventional cotton. All treatments applied in 100 L ha $^{-1}$ total volume with "Supercharge" at 1.0 % v/v.

#2: Is the averaged results from three trials, AU12-03-H525, H526, H527, conducted at the beginning of the 2002/2003 season. All treatments applied in 75 L ha $^{-1}$ total volume with "Supercharge" at 0.5% v/v.

Carfentrazone at use rates of 18 to 24 gai ha $^{-1}$, has significantly increased the level of control for both native rosella *(Abelmoschus ficulneus)* and cow/peach vine *(I. lonchophylla)* compared to glyphosate alone (Table 3). Carfentrazone has provided equivalent and at times numerically, although not significantly, greater levels of control of both native rosella and cow/peach vine compared to the standard fluroxypyr. Oxyfluorfen, another Group G active, had no useful effect on cow/peach vine and only limited effect on the native rosella.

Table 4 shows that carfentrazone when mixed with glyphosate has not responded to the addition of extra wetter (47%) compared to nil adjuvant (48%), which is not surprising considering the wetter loading that most glyphosate formulation already have. There has also been minimal response to the addition of a wetter/oil combination such as DC Trate (55%).

Table 2. Common broadleaved weeds tested with glyphosate plus carfentrazone mixtures.

Common Name	Scientific Name	Plant Size
Species effectively controlled by glyphosate alone.		
Boggabri	*Amaranthus mitchellii*	Cot. – 4 Leaf
Bladder ketmia	*Hibiscus trionum*	Cot. – 2 Leaf
Mint Weed	*Salvia reflexa*	
Pigweed Black	*Trianthema portulacastrum*	Cot. – 2 Leaf
Sesbania Pea	*Sesbania cannabina*	Cot. – 2 Leaf
Shepherds Purse	*Capsella bursa-pastoris*	4 – 12 Leaf
Spiny Headed Sida	*Sida acuta*	Cot. – 2 Leaf
Species effectively controlled by glyphosate plus carfentrazone.		
Cow/Peach Vine	*Ipomea lonchophylla*	Cot. – 4 Leaf
Native Rosella	*Abelmoschus ficulneus*	Cot. – 2 Leaf
Pigweed Red	*Portulaca oleracea*	10 – 30 cm diameter
Vol. Cotton	*Gossypium hirsutum*	Cot. - 6 Leaf
Vol. RR Cotton	*Gossypium hirsutum*	Cot. – 6 Leaf
Species NOT effectively controlled by glyphosate plus carfentrazone		
Bellvine	*Ipomea plebeia*	Cot. – 6 Leaf

Table 3. Percentage control of native rosella *(A. ficulneus)* and cow/peach vine *(I. lonchophylla)*.

Treatment	Rate G ai ha^{-1}	Native Rosella (28 DAA)	Cow / Peach Vine (28 DAA)
Glyphosate	450 / 562	88	44
G + Carfentrazone	6	98	34
G + Carfentrazone	12	96	70
G + Carfentrazone	18	98	88
G + Carfentrazone	24	100	93
G + Oxyfluorfen	19	94	41
G + Fluroxypyr	90	100	85
LSD (P=0.05)		8	20
Av: Plant Size		4 Leaf 70% 6 Leaf 30%	4 Leaf 35% 6 Leaf 65%

The last three products are considered to be premium adjuvants and are commonly used with grass selective herbicides for their ability to increase activity. All have resulted in significantly higher levels of control than either BS1000 or DC Trate. Uptake (72%) was numerically inferior to Hasten (81%) and Supercharge (83%), although not significantly. Hasten and Supercharge have clearly resulted in the highest levels of activity.

Table 4: Effect of different spray Adjuvants on the control of cow/peach vine.

Treatment*	Rate % v/v	Cow/Peach Vine (28 DAA)
Nil		48
BS1000	0.25	47
DC Trate	1.0	55
Uptake	0.5	72
Hasten	0.5	81
Supercharge	0.5	83
LSD (P=0.05)		13
Av: Plant Size		6 to 12 Leaf

*All treatments consisted of glyphosate (675 g ai ha^{-1}) plus carfentrazone (18 g ai ha^{-1}) with the various
adjuvants added, and were applied in 75 L ha^{-1} total volume.
BS1000; (wetter) 1000 g L^{-1} alcohol alkoxylate
DC Trate; (oil/wetter) 763 g L^{-1} petroleum oil
Uptake; (Adjuvant) 582 g L^{-1} paraffinic oil + 208 g L^{-1} non-ionic surfactant.
Hasten; (Adjuvant) 704 g L^{-1} esterifield canola oil and non-ionic surfactant.
Supercharge; (Adjuvant) 432 Mineral oil
Adjuvants often consist of other ingredients including penetrant's.

Whilst no grass species results are presented, most sites had either annual ryegrass *(Lolium rigidum* Gaudin*)* or barley grass *(Hordeum leporinum* Link *)* present ranging in size from Zadok Z12 to Z21. There was no evidence of carfentrazone having any activity on the grass species present. These grasses were controlled 100% by all treatments with symptoms typical of glyphosate use (Table 5).

The use of both carfentrazone and oxyfluorfen resulted in rapid leaf desiccation and increased speed of death of the target broadleaved weeds. Both carfentrazone and oxyfluorfen significantly increased the speed of control at 7 DAA, compared to glyphosate alone. Carfentrazone was noticeably quicker acting than oxyfluorfen on all specials tested.

Carfentrazone at 12 to 18 g ai ha^{-1} when mixed with glyphosate, has resulted in commercially acceptable levels of weed control (>90%) for all target weeds and is significantly better than glyphosate alone, and was numerically superior to oxyfluorfen on all weed species targeted. The addition of oxyfluorfen to glyphosate significantly improved the level of control (94%) of Marshmallow compared to glyphosate alone (15%), but had limited effect on the other weed species. Whilst the higher rate (30 g ai ha $^{-1}$) of oxyfluorfen has clearly been antagonistic compared to the lower rate (18 g ai ha $^{-1}$).

DISCUSSION

Carfentrazone has provided excellent levels of control of both conventional and Roundup Ready cotton seedling volunteers when applied alone at rates of 18 to 24 g ai ha $^{-1}$

Table 5. Percentage control of marshmallow *(Malva parviflora)* subterranean clover *(Trifolium subterranean)*, wild radish *(Raphanus raphanistrum)*, and spiny emex *(Emex australis)*.

Treatment	Rate g ai ha $^{-1}$	Marsh-mallow	Sub-clover	Wild Radish	Spiny Emex
Glyphosate	270	15	70	90	66
G + carfentrazone	6	88	87	95	78
G + carfentrazone	12	97	93	98	89
G + carfentrazone	18	99	97	99	94
G + oxyfluorfen	18	94	75	92	72
G + oxyfluorfen	30	82	69	90	66
Av: Plant Size		Cotyl. to 8 Leaf	Cotyl. to 4 Leaf	3 to 4 Leaf	1 to 4 Leaf

Results are the average of 3 to 4 trials for each weed species.

The key to control is leaf penetration and full coverage of all growing points from the plant apex down to below the cotyledons. Thus, the use of an appropriate spray adjuvant, specifically either Hasten or Supercharge, and sufficient total spray volume (> 75 L ha $^{-1}$) is important.

A mixture of carfentrazone and glyphosate can be used to control Roundup Ready cotton seedling volunteers, as well as the usual weeds glyphosate is targeted against. In addition to this carfentrazone has activity on most broadleaved weeds and has shown additive effects when mixed with glyphosate ranging from increased speed of control to greater levels of control of some of the more difficult to control species.

The difference in modes of action from glyphosate, which inhibits EPSP Synthase (group M) and carfentrazone which, inhibits protoporphyrinogen oxidase (group G) is important, as this allows carfentrazone to control glyphosate tolerant cotton and also control other weeds using a different herbicide group. Rotating herbicide chemistry is an important component of any preventative herbicide resistance strategy.

All broadleaved weeds tested in the Western Australian series of trials (Table 5) proved to be susceptible to carfentrazone. There was no evidence of any antagonism between glyphosate and increasing rates of carfentrazone in these trials.

However, there was a common trend for a numerical inverse rate response to the use of oxyfluorfen. This is a commonly observed field experience where rates of oxyfluorfen (24 g ai ha^{-1} or higher) are used with glyphosate rates of 450 g ai ha^{-1} or less. It is the opinion of the author that if a high enough rate of oxyfluorfen is combined with a low rate of glyphosate then the movement of the glyphosate to its sites of action can be reduced, resulting in initial foliage desiccation from the oxyfluorfen followed by new growth emerging from the lower growing points. Further work may be warranted to fully understand this issue.

The demonstrated effectiveness of carfentrazone on species such as wild radish and spiny emex provides another useful tool in the resistance management of these and other species, whilst at the same time providing excellent control levels for some of the more difficult broadleaved weed species.

REFERENCE

Roberts, M. (2001). Herbicide Tolerant Cotton–Its Role in Sustainable Farming Systems. In Proceedings of the 11th Australian Cotton Conference.

Herbicides

Effect of Different Formulations of Glyphosate-Based Herbicides on Liquid Fastburn Symptom Development

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Abstract: This study was initiated to evaluate the short-term efficacy of several liquid formulations of glyphosate-based herbicides on the development of fast burn symptoms. Two dry herbicide formulations (Roundup ProDry® and QuikPRO® powered by Roundup® from Monsanto Co.) were included in the experiments. QuikPRO® powered by Roundup® has been developed as a fastburn glyphosate product. All glyphosate-based herbicide treatments were applied on a kg ae^{-1} (acid equivalent) glyphosate per acre basis and were compared to glufosinate (Finale® 2SC) treatment applied at 1.7 kg ae ha^{-1}. Six different formulations of glyphosate-based herbicides were applied at three different rates (5, 5.88 and 6.8 kg ae ha^{-1}). The percent "brown-out" symptomology of individual weed species was observed at 1, 2, 4, 7 and 14 days after treatment (DAT). The effect of the treatments was observed on creeping bentgrass, common lambsquarters, prostate knotweed, velvetleaf, and wild mustard. Two days after application the QuikPRO® powered by Roundup® formulation applied at 6.8 kg ae ha^{-1} rate resulted in 25% brownout of the common lambsquarters, while the Finale® treatment led to 6% brownout of the lambsquarters population. The higher rate of application (6.8 kg ae ha^{-1}) of QuikPRO® powered by Roundup® resulted in 64% more brownout of common lambsquarters, compared to the lower application rate (5 kg ae ha^{-1}) at 4 DAT. The fastburn response on prostate knotweed was much slower than the effect on lambsquarters, wild mustard and velevet leaf. MON 79141, MON 78997, MON 78567, and QuickPRO® powered by Roundup® treatments applied at 6.8 kg ae ha^{-1} resulted in greater brown-out symptoms on creeping bentgrass compared to the Roundup ProDry® and Finale® treatments at 1 DAT. There was no significant difference in level of brownout symptomology development of creeping bentgrass between the 5.88 and 6.8 kg ae ha^{-1} rate of MON 79141 and MON 78567 at 2,4, 7, 8 and 14 DAT.

Key words: brownout, formulation, Roundup®, glufosinate, surfactants

INTRODUCTION

Different formulations of glyphosate-based herbicides have been very effectively used for non-selective weed control. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid biosynthetic pathway (Amrhein et al. 1980). EPSP inhibition leads to the depletion of the aromatic amino acids phenylalanine, tyrosine and tryptophan, all needed for protein synthesis in plants (Ahrens 1994). Roundup ProDry® and QuikPRO® powered by Roundup® are dry formulations of glyphosate. Roundup ProDry® contains an ammonium salt of glyphosate, sodium sulphite and some surfactants, while QuikPRO® powered by Roundup® contains an ammonium salt of glyphosate and diquat dibromide. Different liquid formulations of glyphosate-based herbicides are being developed with improved surfactant systems in order to optimize their fastburn properties.

Objectives

The objectives of the research were;

1. To evaluate the short term efficacy of the different liquid formulations of glyphosate for fastburn symptomology
2. Compare the liquid formulations to the dry formulations of glyphosate based on brown-out symptoms development

MATERIALS AND METHODS

Two sets of experiments were conducted in the spring and fall of 2002 at the California State Polytechnic University, Pomona. The first experiment was conducted in a fallow land with a very high weed population. The predominant weed species were common lambsquarters (*Chenopodium album* L.), prostate knotweed (*Polygonum aviculare* L.), velvet leaf (*Abutilon theophrasti* Medicus), and wild mustard (*Brassica kaber* Wheeler). Common lambsquarters were in the 7-leaf stage, prostate knotweeds were in the 10-leaf stage, velvet leafs were in the 12-leaf stage and the wild mustards were in the 10-12-leaf stage when the treatments were applied. In this experiment MON 78995, MON 78996, MON 78997, QuikPRO® powered by Roundup®, MON 78567, and Roundup ProDry® was applied at two rates, 5.0, and 6.8 kg ae ha^{-1}. All the treatments were compared to an untreated check plot and a Finale® 2SC (glufosinate) treatment applied at 1.7 kg ae ha^{-1}. All the treatments were laid out in a randomized block design with three replicates. The treatments were applied over the top with a carrier volume of 100 gal $acre^{-1}$.

The second set of experiments was conducted on a creeping bentgrass (*Agrostis palustris*) putting green built according to the USGA specification (USGA 1993). In this experiment MON 79141, MON 78997, MON 78567, QuikPRO® powered by Roundup®, and Roundup ProDry® was applied at three rates (5, 5.88, and 6.8 kg ae ha^{-1}). All the treatments were compared to an untreated check and a Finale® 2SC (glufosinate) treatment applied at 1.7 kg ae ha^{-1}. The treatments were laid out in a randomized block design with three replicates. Percent brown-out symptomology was observed for all individual species at 1, 2, 4, 7 and 14 days after treatment (DAT).

Statistics

Data was summarized with Agriculture Research Manager (ARM) software. Analysis of variance (ANOVA) was conducted and Duncans New Multiple Range Test at $P = 0.05$ was used to separate the means of the discontinuous variables, while regression was used for all continuous variables like rate of application.

RESULTS AND DISCUSSIONS

In the first experiment common lambsquarters developed brown-out symptoms 1 day after treatment (DAT) with the QuikPRO® powered by Roundup® application applied at 6.8 kg ae ha^{-1}. There was no statistically significant difference between any of the other treatments at 1 DAT. None of the treatments affected the prostate knotweed population at 1 DAT. During the second DAT the common lambsquarters showed a 23% higher brownout in the QuikPRO® powered by Roundup® treatment (6.8 kg ae ha^{-1}) than the untreated check and the Finale® treatment. There was no significant difference in common lambsquarters brownout at 2 DAT between any of the other treatments. QuikPRO® powered by Roundup® (6.8 kg ae ha^{-1}) resulted in 7% brownout symptom development of the prostate knotweed population 2 DAT, while there was no significant difference between the other treatments. After 4 DAT the Finale® treatment resulted in 70% brownout of common lambsquarters and 63% brown out of prostate knotweed, which was significantly higher than the other treatments (Fig. 1).

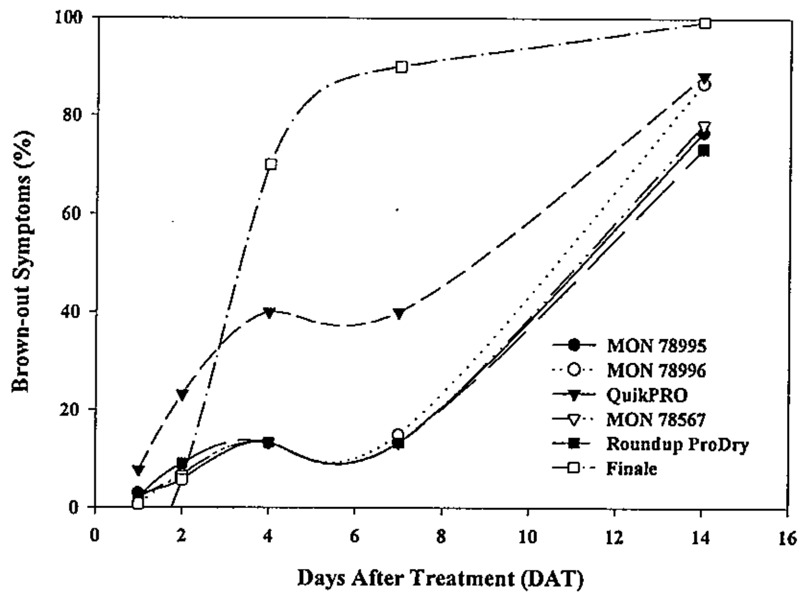

Figure 1. Effect of different glyphosate-based herbicide formulations applied at 6.8 kg ae ha^{-1} and glufosinate applied at 1.7 kg ae ha^{-1} on common lambsquarters brown-out as observed 1, 2, 4, 7 and 14 days after treatment (DAT). The LSD (P = 0.05 level) was 2.87, 5.70, 3.16, 8.07, and 16.46 for the data set obtained at 1, 2, 4, 7 and 14 DAT, respectively.

After 4 DAT and 7 DAT the Finale® treatment resulted in optimum brown-out of lambsquarters and prostate knotweed followed by the QuikPRO® powered by Roundup® treatment (6.8 kg ae ha^{-1}), while the other treatments did not differ significantly. At 14 DAT all the treatments resulted in over 70% brownout of both lambsquarters and prostate knotweed except the Roundup ProDry® (5 kg ae ha^{-1}) and there was no significant difference among the treatments.

In the second experiment Roundup ProDry® did not have any effect on creeping bentgrass untill 4 DAT. MON 79141, MON 78997, MON 78567, and QuikPRO® powered by Roundup® treatments applied at 6.8 kg ae ha^{-1} resulted in greater brown-out symptoms on creeping bentgrass compared to the Roundup ProDry® and Finale® treatments at 1 DAT. There was no significant difference in brownout symptomology of creeping bentgrass between the 5.88 and 6.8 kg ae ha^{-1} rate of MON 79141 (Fig. 2) and MON 78567 at 2,4, 7, 8 and 14 DAT.

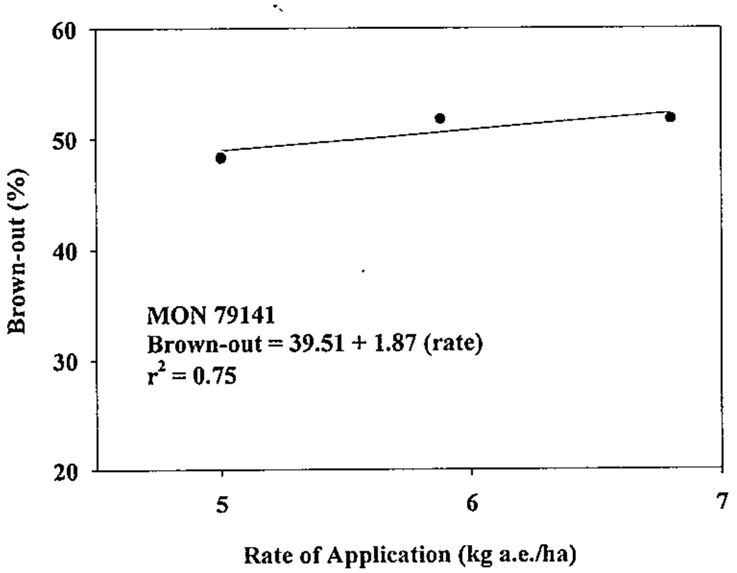

Figure 2. Development of brownout symptoms on creeping bentgrass due to application of MON 79141 as observed at 7 DAT. MON 79141 was applied at 5, 5.88, and 6.8 kg ae ha^{-1} rates. The points are mean brownout percentage and the line was constructed with a linear regression model.

At 7 DAT the Finale® treatment resulted in over 71% brownout of creeping bentgrass, which was significantly higher than all the other treatments. MON 79141, MON 78997 and QuikPRO® powered by Roundup® treatments applied at 6.8 kg ae ha^{-1} resulted in significantly higher brown-out symptoms on creeping bentgrass compared to MON 78567 and Roundup ProDry® at 1, 2, 4, and 7 DAT (Fig. 3).

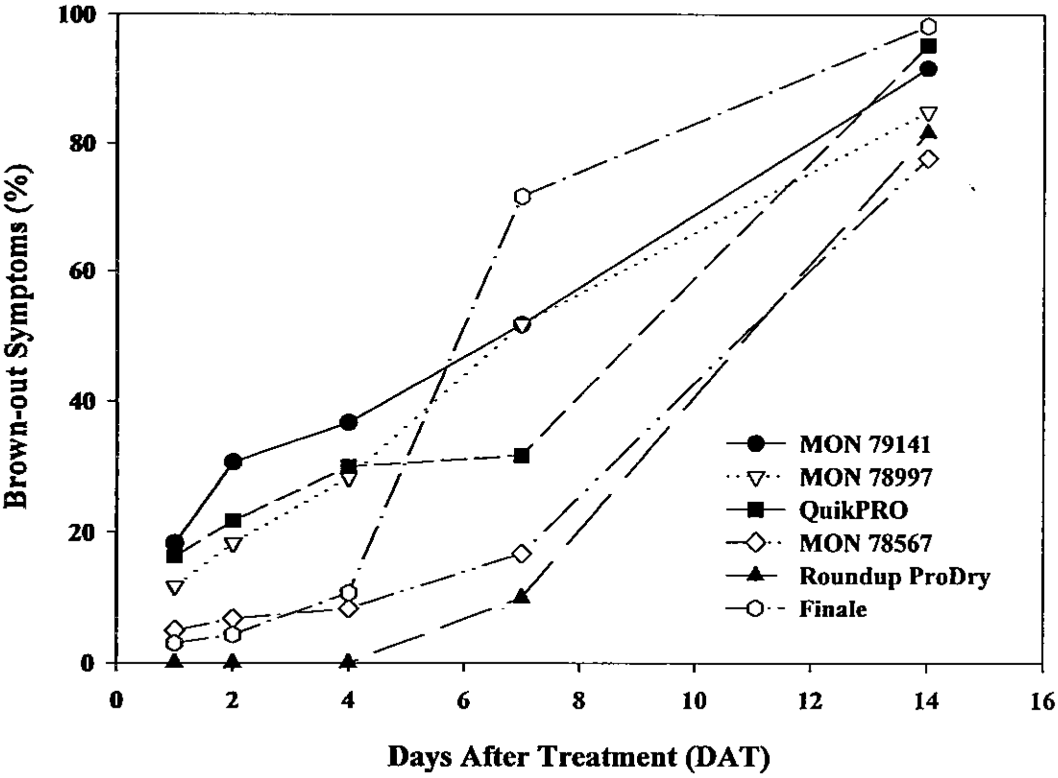

Figure 3. Effect of different formulations of glyphosate-based herbicides applied at 6.8 kg ae ha^{-1} and glufosinate applied at 1.7 kg ae ha^{-1} on creeping bentgrass brown-out symptoms development as observed 1, 2, 4, 7 and 14 days after treatment (DAT). The LSD (P = 0.05 level) was 6.31, 9.54, 11.32, 16.28, and 12.96 for the data set obtained at 1, 2, 4, 7 and 14 DAT, respectively.

Two days after application the QuikPRO® powered by Roundup® formulation applied at 6.8 kg ae ha^{-1} rate resulted in 25% brownout of the common lambsquarters, compared to only 6% due to the Finale® treatment. The higher rate of application (6.8 kg ae ha^{-1}) of QuikPRO® powered by Roundup® resulted in a 64% more brownout symptomology of common lambsquarters, compared to the lower application rate (5 kg ae ha^{-1}) at 4 DAT. The fast burn response on prostate knotweed was much slower than the effect on lambsquarters, wild mustard and velvetleaf. After 4 DAT the Finale® treatment resulted in 70% brownout of the common lambsquarters compared to only 40% by the QuikPRO® powered by Roundup® treatment. There was no statistically significant difference in fast burn symptom development on prostate knotweed between all the glyphosate based herbicide treatments 7 DAT. There was no significant difference in lambsquarter brown-out between the Finale® treatment and all the other glyphosate-based herbicide treatments except the Roundup ProDry® treatment applied at 5 kg ae ha^{-1} rate at 14 DAT.

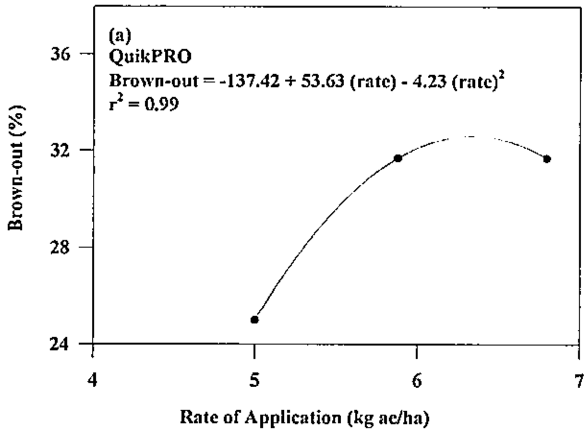

Figure 4. Development of brownout symptoms with the application of MON 78997 on creeping bentgrass as observed at 7 DAT. MON 78997 was applied at 5, 5.88, and 6.8 kg ae ha rates. The points are mean brownout percentage and the line was constructed with a linear regression model.

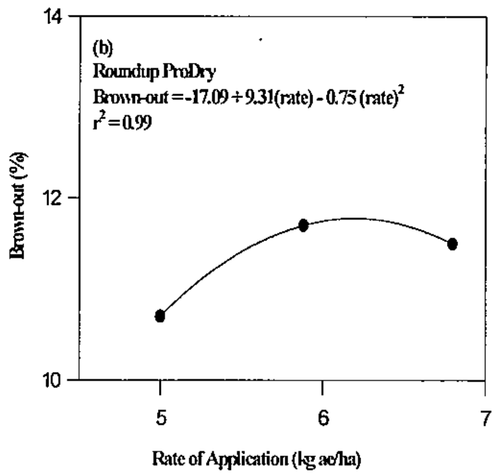

Figure 5. Regression curves indicating the brown-out symptoms on creeping bentgrass observed 7 DAT due to QuikPRO® powered by Roundup® (a) and Roundup ProDry®(b) applications.

The brownout symptoms development on creeping bentgrass at different application rates with the liquid formulations of glyphosate-based herbicides could be explained with a linear regression model, while a quadratic regression model was used to explain the effects due to the dry formulation treatments (QuikPRO® powered by Roundup® and Roundup ProDry®) (Fig. 5). There was no significant difference in creeping bentgrass brown-out between the 5.88 and 6.8 kg ae ha^{-1} rates of MON 79141, MON 78997, QuikPRO® powered by Roundup®, MON 78567, and Roundup ProDry® formulations 14 DAT.

LITERATURE CITED

Ahrens, W. H. 1994. Herbicide Handbook, Seventh Edition. Pages 149-152. Weed Science Society of America, Champaign, Illinois.

Amrhein, N., B. Deus, P. Gehrke, H. C. Steinrucken. 1980. The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. Plant Physiology. 66: 830-834.

USGA. 1993. USGA Recommendations for putting green construction. United States Golf Association, Far Hills, New Jersey.

Herbicidal Scheduling in Maize (*Zea mays* L.)-Potato (*Solanum tuberosum* L.)-Sunflower (*Helianthus annus* L.) Cropping System Under Irrigated Ecosystem of Northern India

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Abstract: Field investigation was carried out for two consecutive years (2000-2002) to develop herbicidal schedule in maize-potato-sunflower cropping system grown under an irrigated ecosystem of alluvial tract of Gangetic plain at Kanpur. Six sets of the herbicidal treatments were compared in complete randomized block design with four replications. Results revealed that allowing weed growth throughout the cropping span caused 34.91%, 26.84% and 30% yield reductions in maize, potato and sunflower crops, respectively. Atrazine (0.75 kg ha^{-1}) applied in maize prevented weed competition but did not show any residual effect on weed emergence in the succeeding potato and sunflower crop of the sequence. Similarly, atrazine (0.75 kg ha^{-1}) in maize and in potato (0.25 kg ha^{-1}) brought about significant reductions in associated weed flora in these crops but failed to reduce weed growth in the succeeding sunflower crop. Application of atrazine (0.75 kg ha^{-1}) in maize and pendimethalin (1 kg ha^{-1}) in sunflower proved to be an effective herbicidal schedule of weed control and optimum yield of component crops under maize-potato-sunflower cropping system grown under the irrigated ecosystem of northern India.

Keywords: Herbicides, maize, potato, sunflower, weeds, cropping system

INTRODUCTION

Maize-potato-sunflower is a newly introduced cropping system in the alluvial tract of northern India. Weeds pose serious problem in growing the component crops of this system. Maize is heavily infested with rainy season weeds mainly carpet weed (*Trianthema monogyna* L.), amaranthus (*Digera arvensis* Forsk), nutgrass (*Cyperus rotundus* L.), dayflower (*Commelina benghalensis* L.), wild rice (*Echinochloa colona* (L.) Link) etc. Immediately after harvesting of maize during second fortnight of September, potato planting is done in succession. Rainy season annuals during the early stage and winter annuals during later stage often emerge and offer severe competition in potato crop. The succeeding sunflower crop is sown during first fortnight of March. Owing to frequent irrigations and congenial temperature, this crop is adversely affected through winter and summer annuals. Thus, different types of flora compete with all the component crops of this cropping system. Keeping these facts in view, the present investigation was undertaken to develop an herbicidal schedule in maize-potato-sunflower cropping system.

MATERIALS AND METHODS

Field investigation was carried out for two consecutive years (2000-2002) to develop herbicidal schedule in maize-potato-sunflower cropping system grown under irrigated ecosystem of alluvial tract of Gangetic plain at Kanpur. Six sets of the herbicidal treatments *viz.*, T_1 maize (atrazine 0.75 kg ha^{-1}) - potato (untreated) - sunflower (untreated) T_2 maize (atrazine 0.75 kg ha^{-1}) - potato (atrazine 0.25 kg ha^{-1}) – sunflower (untreated) T_3 maize (atrazine 0.75 kg ha^{-1}) - potato (atrazine 0.25 kg ha^{-1}) – sunflower (pendimethalin 1 kg ha^{-1}) T_4 maize (atrazine 0.75 kg ha^{-1}) – potato (manual weeding) – sunflower (pendimethalin 1 kg ha^{-1}) T_5 maize (weedy) – potato (weedy) – sunflower (weedy) T_6 maize (manual weeding) – potato (manual weeding) – sunflower (manual weedng) were compared in randomized complete block design with four replications. The experimental fields were sandy loam in texture having medium organic carbon (0.54%), phosphorous (17/1 kg ha^{-1}) and potassium (160 kg ha^{-1}). Soil pH was slightly alkaline (7.6). Maize (cv. *Azad uttam* 75-80 days duration), potato (cv. *Kufri chandramukhi* 85-90 days duration) and sunflower (cv. Morden 80-85 days duration) were raised during rainy, winter and spring season, respectively, under sequencial cropping in *situ.* The component crops were raised under recommended package of practices under irrigated condition. As per treatment, atrazine and pendimethalin were applied second day after sowing through *knapsack* sprayers fitted with flood jet noozle maintaining spray volume of 800 l ha^{-1}. The populations of weeds were recorded using a 50 cm x 50 cm quadrat at two places from each plot at 60 days after sowing. Dry matter accumulation of weeds was estimated after sun drying and then in electric oven at 60°C till constant weight. Manual weeding was done twice at 20 and 40 days after sowing in each crop as per treatment through manual labors using *khurpi* – a hand tool.

RESULTS AND DISCUSSIONS

Weed Studies

Maize crop was mainly invaded with carpet weed (*T. monogyna* L.), wild rice (*E. colona* (L.) Link), day flower (*C. benghalensis* L.) and nutgrass (*Cyperus rotundus* L.). Infestations of lambsquarter (*Chenopodium album* L.), pimpeerneel (*Anagallis arvensis* L.), sweet clover (*Melilotus alba* Desr.) and swinecress were the major weed flora in sunflower. *Antrazine* (0.75 kg ha^{-1}) reduced the population of *T. monogyna, C. benghalensis* and *E. colona* significantly during both years of experimentation. Balyan *et al.* (1994) also reported effective control of *T. monogyna* and *D. arvensis* due to application of atrazine in maize crop. The crop over effect of atrazine was found ineffective in reducing the associated weed flora in succeeding potato crop. Low dose of atrazine (0.25 kg ha^{-1}) in potato was found advantageous in diminishing the emergence and growth of *C. album, A. arvensis, M. alba* and *C. didymus.* Further, the residual effect of atrazine (0.75 kg ha^{-1}) applied in maize and low dose of atrazine (0.25 kg ha^{-1}) applied could not be reflected in reducing the population of *T. monogyna* a major weed in sunflower crop (Table 1). The application of pendimethalin (1 kg ha^{-1}) registered discernible effects on the mortality of *T. monogyna* in sunflower crop. Similar results have been reported by Itnal *et al.* (1992) in groundnut+sunflower intercropping. Pooled data on dry matter of weeds behaved in similar fashion (Table 2). Significant reductions in weed dry weight were recorded due to effect of herbicides in all crops under study. Application of atrazine (0.75 kg ha^{-1}) in maize, atrazine (0.25 kg ha^{-1}) in potato and pendimethalin (1.0 kg ha^{-1}) in sunflower depressed the dry matter accumulation by 46.42%, 40.00% and 43.87%, respectively. However, no residual effects of atrazine could be manifested on the mortality of weeds in the succeeding potato and sunflower crop. Owing to adequate rainfall received in maize crop, there was faster degradation of applied atrazine in maize. Consequently no carry over effects in succeeding potato could be seen either on weed flora or on crop growth. Potato plot also enjoyed congenial conditions for microbial degradation of atrazine due to frequent irrigations and high applications of nitrogen. As a result, no residual effect could be visualized in the succeeding sunflower crop. In a field experiment conducted under similar agroecosystem at Lucknow, Srivastava *et al.* (1999) observed the persistence of atrazine in soil varying from 45-60 days and its content declined well with increasing nitrogen and moisture level.

Table 1: Weed population 50 cm^2 at 60 days after sowing under maize-potato-sunflower cropping system.

Treatments	Maize							
	T. monogyna		*E. colona*		*C. benghalensis*		*C. rotundus*	
	2000	2001	2000	2001	2000	2001	2000	2001
T_1	2.50 (1.73)	3.75 (2.06)	3.25 (1.93)	3.25 (1.93)	4.75 (2.29)	4.25 (2.18)	16.00 (4.06)	35.50 (6.00)
T_2	3.25 (1.93)	3.25 (1.93)	4.25 (2.18)	4.00 (2.10)	3.25 (1.93)	3.25 (1.93)	17.50 (4.24)	34.50 (5.91)
T_3	3.50 (2.00)	3.75 (2.06)	3.00 (1.87)	3.25 (1.93)	4.75 (2.29)	4.00 (2.10)	16.50 (4.12)	32.25 (5.72)
T_4	2.25 (1.65)	3.75 (2.06)	3.75 (2.06)	4.00 (2.10)	3.75 (2.06)	4.00 (2.10)	19.50 (4.47)	33.00 (5.78)
T_5	7.00 (2.73)	9.50 (3.16)	5.00 (2.34)	5.50 (2.44)	5.25 (2.39)	6.50 (2.70)	23.75 (4.92)	35.25 (6.02)
T_6	1.50 (1.40)	2.00 (1.57)	1.00 (1.20)	1.50 (1.40)	2.25 (1.65)	2.00 (1.57)	16.25 (4.09)	27.25 (5.26)
C. D. 5%	0.31	0.30	0.37	0.26	0.46	0.39	NS	NS

Treatments	Potato							
	C. album		*A. arvensis*		*M. alba*		*C. didymus*	
	2000	2001	2000	2001	2000	2001	2000	2001
T_1	7.25 2.78	10.75 3.35	12.25 3.57	8.25 2.95	8.00 2.91	7.25 2.78	6.25 2.59	7.50 2.82
T_2	2.75 1.80	10.75 3.35	12.25 3.57	4.00 2.10	3.75 2.06	3.25 1.93	2.00 1.57	4.50 2.23
T_3	2.75 1.80	4.00 2.10	3.00 1.87	4.50 2.23	4.25 2.18	3.50 2.00	2.00 1.57	3.25 1.93
T_4	3.75 2.06	5.25 2.39	5.00 2.34	4.50 2.23	4.50 2.23	5.50 2.44	1.50 1.40	3.50 2.00
T_5	8.50 3.00	8.50 3.00	10.75 3.35	8.50 3.00	8.50 3.00	5.00 2.34	4.25 2.18	8.75 3.04
T_6	2.00 1.57	2.00 1.57	4.50 2.23	1.50 1.40	3.75 2.06	2.00 1.57	2.00 1.57	2.00 1.57
C. D. 5%	0.28	0.32	0.26	0.36	0.44	0.42	0.29	0.27

Treatments	Sunflower			
	T. monogyna		*C. rotundus*	
	2001	2002	2001	2002
T_1	14.75 (3.90)	9.25 (3.12)	14.75 (3.90)	26.75 (5.22)
T_2	14.75 (3.90)	9.50 (3.16)	15.50 (4.00)	28.75 (5.36)
T_3	6.00 (2.50)	3.75 (2.06)	12.00 (3.53)	26.50 (5.22)
T_4	6.50 (2.70)	4.00 (2.00)	11.75 (3.50)	26.75 (5.22)
T_5	13.25 (3.70)	9.00 (3.08)	14.50 (3.87)	27.75 (5.31)
T_6	3.75 (2.06)	2.00 (1.57)	8.25 (2.95)	24.25 (4.97)
C. D. 5%	0.31	0.22	NS	NS

Table 2: Dry weight of weeds and yield of component cops (Pooled of two years)

Treatments	Dry weight of weeds (kg ha^{-1})			Yield of component crops		
	Maize	Potato	Sunflower	Maize	Potato	Sunflower
T_1	1185	890	299	2430	14.99	1067
T_2	1195	640	287	2473	18.17	1100
T_3	1235	540	174	2489	18.15	1362
T_4	1200	635	181	2510	16.49	1363
T_5	2305	900	310	1961	14.49	1078
T_6	735	520	104	3013	19.81	1550
C. D. 5%	213	91	67	201	2.02	133

Crop Studies

Pooled data revealed that the yields of component crops were significantly influenced by the weed control treatments (Table 2). Result showed that allowing weed growth throughout the cropping period caused on an average, 34.91%, 26.84% and 30% yield reductions in maize, potato and sunflower, respectively. Application of atrazine (0.75 kg ha^{-1}) in maize, atrazine (0.25 kg ha^{-1}) in potato and pendimethalin (1.0 kg ha^{-1}) in sunflower recorded significant to be increase in the yield over the untreated. The extent of increase in the yield was found 26.92% inmaiza, 25.25% in potato and 26.34% in sunflower. The residual effects of these herbicides were not visible on the growth and yield of component crops. In an earlier field investigation carried out at Kanpur, application of simazine (1 kg ha^{-1}) in maize field did not show the residual effect in the succeeding potato and wheat crop (Rathi and Tewari 1979). It was thus concluded that pendimethalin (1 kg ha^{-1}) in sunflower proved to be an effective herbicidal schedule for intended weed control and optimum yield of component crops grown under irrigated ecosystem of north India.

LITERATURE CITED

Srivastava, T. K.et al. 1999. Efficacy of weed control measures and soil persistence of atrazine in sugarcane (*Saccharum officinsrum*) as influenced by irrigation and nitrogen. Indian Journal of Agronomy 44 (3):833-840.

Blyan, R.S. *et. al.* 1994. Atrazine as post emergence herbicide for weed control inmaize (*Zea mays* L.). Indian Journal of Weed Science 26 (3 & 4) 35-39.

Itnal, C. J. *et. al.* 1992 Weed Management in groundnut and sunflower intercropping. Indian Journal of Weed Science 24 (1 and 2):75-77.

Rathi, K.S. and tewari, A.N. (1979) Chemical weed control in maize through simazine and its residual effect on succeeding crops. Indian Journal of Agriculture Research 13 (1): 43-46.

Impact of Metsulfuron-methyl on the Yield, Oil Quality and Growth of Oil Palm

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Abstract: A field study was conducted to evaluate the impact of continued application of metsulfuron-methyl (Ally®) at 15 g ha^{-1} and 30 g ha^{-1} and tank-mix of Ally® + glyphosate at 15 + 540 g ha^{-1} on the yield, oil quality and growth of oil palm. Initial results indicated that after the 12th consecutive round of normal circle spray over a 3-year period, all treatments produced normal fresh fruit bunch (FFB) yields, oil extraction rate (OER) and vegetative growth of oil palm. The half-lives of Ally®at 15 and 30 g ha^{-1} in the field were 6.3 and 7.9 days, respectively, from the first-order kinetics. The results demonstrated that Ally® has short half-life in the soil under an oil palm ecosystem.

Key words: First-order kinetics, fresh fruit bunch, kernel extraction rate, metsulfuron-methyl, vegetative growth

INTRODUCTION

Metsulfuron-methyl is a low-use-rate sulfonylurea herbicide widely used for post-emergence broadleaf weed control in oil palm plantations. It inhibits the enzyme acetolactate synthase (ALS), also known as acetohydroxy acid synthase (AHAS), which participates in the biosynthesis of the branched-chain amino acid valine, leucine and isoleucine (Ray 1985; Brown 1990). The deprivation of these essential amino acids results in the rapid cessation of plant cell division and growth. Metsulfuron-methyl has good selectivity against oil palm and is very effective against a wide range of broadleaf weeds at application rates of 15 to 30 g ha^{-1} (Khairuddin and Teoh 1992; Chung 1997).

The increasing use of metsulfuron-methyl has led to concerns about its fate in soils and its bioavailability, which depends on its degradation and its retention on soil constituents. In pursuing these aims, extensive research has been carried out on the mobility and degradation of metsulfuron-methyl in laboratory and field studies (Nordh-Christerson and Bergstrom 1989; Wadd and Drennan 1989; Walker et al. 1989; Bergstrom 1990).

In general, sulfonylurea herbicides degrade in soils primarily by chemical hydrolysis and microbial metabolism. There have been several publications, which elucidate the significance of microbial degradation (Beyer et al. 1988; Vega et al. 1992; Ismail and Lee 1995; Brown and Kearney 1991). Chemical hydrolysis of metsulfuron-methyl has been shown to be very rapid at low pH. The hydrolysis at 45°C increased from 2.1 days at pH 5 to 33 days at pH 7 (Brown and Kearney 1991). The degradation rate of metsulfuron-methyl is affected by soil temperature, moisture, pH, and soil microbial viability. The half-life of metsulfuron-methyl ranges from 2.5 days [soil conditions: pH 3.1, 35 °C, 80% field water holding capacity (FC)] to 36 days (soil conditions: pH 5.7, 10 °C, 60% FC) depending on these factors (Ismail and Lee 1995; James et al. 1995). The degradation of metsulfuron-methyl in the soil under temperate conditions is well documented. However, there is a lack of knowledge about the behavior of metsulfuron-methyl in the tropical soils especially in oil palm agro-ecosystem.

This study evaluated the long-term impact of metsulfuron-methyl on the yield, oil quality and growth of oil palm under field conditions.

MATERIALS AND METHODS

This study was established in a 1988 planting of D X P oil palms planted 9 m (triangular) to give 136 palms ha^{-1}. Oil palm yield in the selected site from the 3rd to 10th year of harvesting ranged from 24 to 26 t $ha^{-1}year^{-1}$. The selected field was sprayed with metsulfuron-methyl (Ally® 20DF) at the label recommended rate of 15 g ha^{-1}, twice the recommended rate of 30 g ha^{-1} and tank-mix of Ally® and glyphosate at 15 + 540 g ha^{-1}. Spray applications were carried out using PB Crossmark knapsack sprayer fitted with the red multi-cone nozzle to deliver a volume rate of 450 L per blanket hectare. Treatments were applied over a 1.2 m wide swath in the harvesting paths and 1.8 m radius palm circles. All treatments were sequentially applied at 3 monthly intervals. Manual weeding was carried out in the untreated plots every 6-8 weeks to maintain the trial site.

Plot size consisted of 16 rows with 12 palms per row. Twenty palms in the middle 4 rows were marked for recording, which means each recording plot was separated with 12 rows on the left and right from each other. This is to avoid contamination of treatments through lateral movement. The randomized complete block design (RCBD) with 4 replications was used for this study. The number and weight of fresh fruit bunches were recorded for individual palms at each harvesting round of 10 days interval. After the bunch was harvested from the palm, one bunch from each plot was sampled for bunch analysis following the method developed by Rao et al. (1983). The vegetative measurement described by Corley et al. (1971) was carried out at 6 months interval for all the 20 palms in each plot. Data were subjected to analysis of variance and treatment means were compared using Tukey's Test.

Soil samples were collected at 0-10, 10-20, 20-30, 30-40 and 40-50 cm depth using a soil auger at 1, 3, 7, 14 and 21 days after 2nd application of treatment. Five soil samples at approximately 0.5 kg each were randomly collected from each replicate across the study area. After collection from the field, these samples were air dried at room temperature and passed through a 2 mm sieve. The sieved samples were well mixed and homogenized. The soil samples were analyzed according to the method described in by Walker et al. (1989).

RESULT[illegible]

[illegible] bunch (FFB) yields a[illegible] month of [illegible] years of harvesting as wel[illegible] ment. No significant difference was ob[illegible] Ally® at both tested rates and tank-mi[illegible] rate were found to produce the same [illegible] after 12th consecutive rounds of treatm[illegible] every month throughout the whole expe[illegible] the 18th to 20th month. The total FF[illegible] significant different among treatmen[illegible]

The same results we[illegible] impact of treatments was not [illegible] uniform, ranging from 20.3[illegible]

Vegetative gro[illegible] leaflet, total frond an[illegible] first 6 months befo[illegible] (Tables 3a to 3d).

The al[illegible] that 12 co[illegible] up to twi[illegible] any d[illegible] ported [illegible] palm

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RESULTS AND DISCUSSION

Fresh fruit bunch (FFB) yields are summarized in Tables 1a to 1d for every month of the 3 years of harvesting as well as the first 6 months before the first treatment. No significant difference was observed between treatments. Plots treated with Ally® at both tested rates and tank-mix of Ally® + glyphosate at field recommended rate were found to produce the same FFB yield compared to the untreated check after 12th consecutive rounds of treatment. The fresh fruits bunch weight fluctuated every month throughout the whole experiment with the highest bunch weight obtained in the 18th to 20th month. The total FFB yield over the period of 36 months was not significant different among treatments (Table 4).

The same results were obtained on the oil extraction rate (OER), where the impact of treatments was not significantly different. The OER data obtained were quite uniform, ranging from 20.30% to 28.88% throughout the experiment (Tables 2a to 2d).

Vegetative growth data collected on rachis length, frond production, number of leaflet, total frond and leaf area index at 6 month interval for 3 years as well as the first 6 months before treatment were not significantly different between the treatments (Tables 3a to 3d).

The above results on FFB, OER and vegetative growth obtained clearly indicated that 12 consecutive rounds of normal circle sprays over a 3 year period with Ally® at up to twice the recommended rate and tank-mix of Ally® + glyphosate does not cause any deleterious effects on FFB, OER and vegetative growth of oil palm. This was supported by many studies showing that Ally® has excellent crop selectivity against oil palm (Khairuddin and Teoh 1992; Chung 1997).

The results on degradation of Ally® in the soil at 15 and 30 g ha^{-1} were shown in Figure 1. The data suggest that the degradation of Ally® at both application rates followed first-order kinetics as both correlation coefficients, at $R^2 > 0.9$. To calculate the half-life, residue values were logarithmically transformed and a linear fit of the values against time. Half-lives of 6.3 and 7.9 days were estimated from the first-order kinetics of the degradation process for both application rate (Table 5). As expected from known behavior of Ally®, rapid degradation occurs in acidic soil with high organic content and temperature. The high microbial activity in the oil palm ecosystem also enhanced the degradation of Ally® in the soil. The results concurred with previous studies by Reed and Chang (1992). Thus, it can be concluded that the repeated use of Ally® does not give rise to residues in the environmental compartment of soil.

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Table 1a. Impact of Ally® and tank-mix of Ally® + glyphosate on fresh fruit bunch (FFB)- in the 1st year.

Treatment Application		Pre-treatment			1st Application			2nd Application			3rd Application			Cumulati FFB/ha (MT)
Treatment	Rate (g ha^{-1})	FFB/ha (MT)												
		Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99	Sep 99	Oct 99	Nov 99	Dec 99	
1. Ally®	15	1.13 a	0.99 a	1.80 a	1.87 a	2.26 a	2.19 a	2.49 a	3.08 a	1.30 a	2.36 a	1.90 a	1.56 a	22.93 a
2. Ally®	30	1.90 a	1.26 a	1.94 a	2.88 a	3.02 a	2.42 a	2.77 a	3.05 a	1.44 a	2.38 a	1.80 a	1.39 a	26.25 a
3. Ally® + glyphosate	15 +540	1.45 a	1.03 a	1.34 a	2.46 a	2.65 a	1.84 a	2.31 a	3.27 a	1.66 a	2.57 a	2.33 a	1.13 a	24.04 a
4. Check	-	1.11 a	1.05 a	1.50 a	2.77 a	2.63 a	2.06 a	2.85 a	3.32 a	1.49 a	2.31 a	2.22 a	1.37 a	24.68 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 1b. Impact of Ally® and tank-mix of Ally® + glyphosate on fresh fruit bunch (FFB)- in the 2nd year.

Treatment Application		4th Application			5th Application			6th Application			7th Application			Cumulati FFB/ha (MT)
Treatment	Rate (g ha^{-1})	FFB/ha (MT)												
		Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00	Sep 00	Oct 00	Nov 00	Dec 00	
1. Ally®	15	1.13 a	1.24 a	1.29 a	1.64 a	1.91 a	2.69 a	3.43 a	4.06 a	2.31 a	1.56 a	1.00 a	0.60 a	22.86 a
2. Ally®	30	1.90 a	0.75 a	1.04 a	1.71 a	1.94 a	1.90 a	2.71 a	3.70 a	2.98 a	2.03 a	1.04 a	0.68 a	22.38 a
3. Ally® + glyphosate	15 +540	1.38 a	0.92 a	1.06 a	1.54 a	1.64 a	2.30 a	3.03 a	3.32 a	2.61 a	2.00 a	1.07 a	0.73 a	21.60 a
4. Check	-	1.34 a	1.18 a	1.19 a	1.38 a	2.56 a	2.13 a	2.42 a	3.60 a	2.86 a	1.94 a	0.95 a	0.65 a	21.20 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 1c. Impact of Ally® and tank-mix of Ally® + glyphosate on fresh fruit bunch (FFB)- in the 3rd year.

Treatment Application		8th Application			9th Application			10th Application			11th Application			Cumulati FFB/ha (MT)
Treatment	Rate (g ha^{-1})	FFB/ha (MT)												
		Jan 01	Feb 01	Mar 01	Apr 01	May 01	Jun 01	Jul 01	Aug 01	Sep 01	Oct 01	Nov 01	Dec 01	
1. Ally®	15	1.44 a	1.84 a	1.26 a	1.70 a	1.48 a	2.13 a	1.35 a	2.10 a	2.08 a	2.53 a	0.89 a	1.21 a	20.01 a
2. Ally®	30	1.46 a	1.75 a	1.46 a	1.79 a	1.70 a	1.82 a	1.15 a	2.06 a	2.41 a	2.51 a	1.21 a	1.74 a	21.06 a
3. Ally® + glyphosate	15 +540	1.79 a	1.70 a	1.34 a	1.41 a	1.19 a	1.82 a	1.12 a	1.91 a	2.09 a	2.38 a	1.07 a	1.84 a	19.60 a
4. Check	-	1.53 a	2.09 a	1.31 a	1.70 a	1.43 a	1.91 a	1.27 a	1.94 a	1.86 a	2.26 a	0.83 a	1.51 a	19.64 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 1d. Impact of Ally® and tank-mix of Ally® + glyphosate on fresh fruit bunch (FFB)- in the 4th year.

Treatment Application		12th Application			13th Application			Cumulative FFB/ha (MT)
Treatment	Rate (g ha^{-1})	FFB/ha (MT)						
		Jan 02	Feb 02	Mar 02	Apr 02	May 02	Jun 02	
1. Ally®	15	1.78 a	0.68 a	1.92 a	2.19 a	1.93 a	0.52 a	9.02 a
2. Ally®	30	1.80 a	0.94 a	2.09 a	2.73 a	1.99 a	0.66 a	10.21 a
3. Ally® + glyphosate	15 +540	1.67 a	0.75 a	1.99 a	2.91 a	1.45 a	0.81 a	9.58 a
4. Check	-	1.76 a	0.85 a	2.11 a	2.33 a	1.81 a	0.59 a	9.45 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 2a. Impact of Ally® and tank-mix of Ally® + glyphosate on oil extraction rate (OER)- in the 1st year.

Treatment Application		Pre-treatment			1st Application			2nd Application			3rd Application		
Treatment	Rate (g ha^{-1})	OER (%)											
		Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99	Sep 99	Oct 99	Nov 99	Dec 99
1. Ally®	15	23.91 a	26.42 a	23.81 a	25.69 a	26.23 a	24.91 a	24.22 a	24.59 a	26.48 a	23.35 a	27.30 a	24.10 a
2. Ally®	30	22.50 a	25.01 a	23.46 a	25.69 a	23.09 a	22.03 a	23.79 a	23.61 a	22.47 a	24.78 a	25.36 a	28.14 a
3. Ally® + glyphosate	15 +540	21.45 a	25.03 a	23.67 a	25.30 a	24.05 a	26.33 a	24.56 a	21.29 a	24.47 a	22.86 a	25.93 a	25.94 a
4. Check	-	20.30 a	27.78 a	26.30 a	21.80 a	28.88 a	24.24 a	25.41 a	22.82 a	24.63 a	21.16 a	25.67 a	24.62 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 2b. Impact of Ally® and tank-mix of Ally® + glyphosate on oil extraction rate (OER)- in the 2nd year.

Treatment Application		4th Application			5th Application			6th Application			7th Application		
Treatment	Rate (g ha^{-1})	OER (%)											
		Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00	Sep 00	Oct 00	Nov 00	Dec 00
1. Ally®	15	23.91 a	24.15 a	23.60 a	24.59 a	20.11 a	21.52 a	23.34 a	20.86 a	22.04 a	25.22 a	24.35 a	25.76 a
2. Ally®	30	22.50 a	25.48 a	23.33 a	22.02 a	21.72 a	22.27 a	24.98 a	22.68 a	21.52 a	22.10 a	26.26 a	23.74 a
3. Ally® + glyphosate	15 +540	21.45 a	22.83 a	23.76 a	23.85 a	23.22 a	21.27 a	23.34 a	21.45 a	23.93 a	19.44 a	26.50 a	25.08 a
4. Check	-	20.30 a	25.83 a	20.78 a	25.07 a	23.25 a	21.26 a	22.55 a	24.06 a	19.69 a	24.12 a	26.55 a	25.68 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 2a. Impact of Ally® and tank-mix of Ally® + glyphosate on oil extraction rate (OER)- in the 3rd year.

Treatment Application		8th Application			9th Application			10th Application			11th Application		
Treatment	Rate (g ha^{-1})	OER (%)											
		Jan 01	Feb 01	Mar 01	Apr 01	May 01	Jun 01	Jul 01	Aug 01	Sep 01	Oct 01	Nov 01	Dec 01
1. Ally®	15	21.45 a	22.46 a	22.37 a	22.48 a	21.29 a	24.02 a	23.36 a	23.58 a	23.56 a	24.62 a	21.88 a	23.87 a
2. Ally®	30	21.53 a	22.41 a	23.78 a	24.63 a	23.80 a	25.11 a	26.08 a	24.75 a	23.17 a	26.88 a	24.50 a	24.02 a
3. Ally® + glyphosate	15 +540	24.28 a	22.23 a	22.44 a	24.96 a	23.96 a	25.95 a	25.80 a	24.88 a	21.39 a	25.42 a	24.50 a	24.38 a
4. Check	-	24.45 a	25.95 a	24.17 a	24.05 a	23.32 a	25.19 a	22.08 a	24.12 a	23.15 a	24.87 a	23.30 a	23.33 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 2b. Impact of Ally® and tank-mix of Ally® + glyphosate on oil extraction rate (OER)- 4th year.

Treatment Application		12th Application			13th Application		
Treatment	Rate (g ha^{-1})	OER (%)					
		Jan 02	Feb 02	Mar 02	Apr 02	May 02	Jun 02
1. Ally®	15	25.30 a	22.22 a	24.80 a	25.22 a	25.86 a	23.26 a
2. Ally®	30	26.66 a	25.48 a	26.13 a	23.92 a	24.73 a	24.08 a
3. Ally® + glyphosate	15 + 540	25.02 a	22.15 a	27.06 a	23.38 a	25.93 a	25.68 a
4. Check	-	26.78 a	21.92 a	23.32 a	26.93 a	26.02 a	23.81 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 3a. Impact of Ally® and tank –mix of Ally® + glyphosate on vegetative growth of oil palm – in the 1st year.

Treatment Application		Pre-treatment					6th months after first application				
Treatment	Rate (g/Ha)	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index
1. Ally®	15	544.84 a	0	170.56 a	40.97 a	4.66 a	559.94 a	19.01 a	173.71 a	38.15 a	4.98 a
2. Ally®	30	558.71 a	0	173.05 a	41.58 a	4.90 a	571.08 a	19.05 a	175.96 a	41.47 a	5.40 a
3. Ally® + glyphosate	15 + 540	547.70 a	0	171.72 a	41.10 a	4.75 a	561.03 a	19.18 a	174.83 a	34.95 a	4.67 a
4. Check	-	545.26 a	0	173.14 a	43.36 a	5.09 a	570.46 a	18.77 a	175.84 a	42.58 a	5.88 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 3b. Impact of Ally® and tank –mix of Ally® + glyphosate on vegetative growth of oil palm – in the 2nd year.

Treatment Application		12th months after first application					18th months after first application				
Treatment	Rate (g/Ha)	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index
1. Ally®	15	571.65 a	20.13 a	176.92 a	39.65 a	5.02 a	563.50 a	10.70 a	180.20 a	33.10 a	7.97 a
2. Ally®	30	586.32 a	19.25 a	177.09 a	42.05 a	5.53 a	618.21 a	10.74 a	179.53 a	32.79 a	8.75 a
3. Ally® + glyphosate	15 + 540	567.35 a	21.23 a	176.88 a	36.43 a	4.98 a	536.27 a	11.60 a	173.20 a	34.13 a	8.30 a
4. Check	-	584.43 a	19.77 a	176.91a	43.15 a	5.78 a	580.68 a	11.84 a	183.26 a	35.89 a	8.36 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 3c. Impact of Ally® and tank –mix of Ally® + glyphosate on vegetative growth of oil palm – in the 3rd year.

Treatment Application		24th months after first application					30th months after first application				
Treatment	Rate ($g\ ha^{-1}$)	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index
1. Ally®	15	601.15 a	12.25 a	179.48 a	35.21 a	5.53 a	597.71 a	20.61 a	183.69 a	34.34 a	5.65 a
2. Ally®	30	639.00 a	11.00 a	179.00 a	34.00 a	5.84 a	620.81 a	20.60 a	189.08 a	34.47 a	5.88 a
3. Ally® + glyphosate	15 + 540	605.72 a	11.89 a	181.83 a	34.76 a	5.77 a	621.30 a	20.81 a	183.48 a	33.72 a	5.69 a
4. Check	-	610.16 a	11.91 a	181.49 a	35.67 a	6.09 a	608.63 a	21.16 a	183.91 a	34.48 a	5.76 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 3d. Impact of Ally® and tank –mix of Ally® + glyphosate on vegetative growth of oil palm – in the 4th year.

Treatment Application		36 months after first application				
Treatment	Rate (g ha^{-1})	Rachis Length (cm)	Frond Prod.	No. of Leaflet	Total Frond	Leaf Area Index
1. Ally®	15	603.17 a	12.04 a	177.51 a	37.23 a	5.61 a
2. Ally®	30	622.24 a	12.00 a	180.57 a	38.81 a	5.44 a
3. Ally® + glyphosate	15 + 540	600.91 a	11.32 a	178.64 a	38.00 a	5.39 a
4. Check	-	608.90 a	11.72 a	178.67 a	38.49 a	5.49 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 4. Impact of Ally® and tank –mix of Ally® + glyphosate on the total FFB of oil palm .

Treatment	Rate (g ha^{-1})	Total FFB/ha (MT)
1. Ally®	15	74.84 a
2. Ally®	30	79.90 a
3. Ally® + glyphosate	15 + 540	74.88 a
4. Check	-	74.97 a

Values in same columns having the same letter are not significantly different at $p \leq 0.01$

Table 5: First order rate constants (K), half-lives ($T_{1/2}$) and correlation coefficient of metsulfsufon-methyl at two application rates.

Concentration (g ha^{-1})	R^2	K (days^{-1})	$T_{1/2}$ (Days)
1. 15	0.9159	0.1093	6.3
2. 30	0.9202	0.0877	7.9

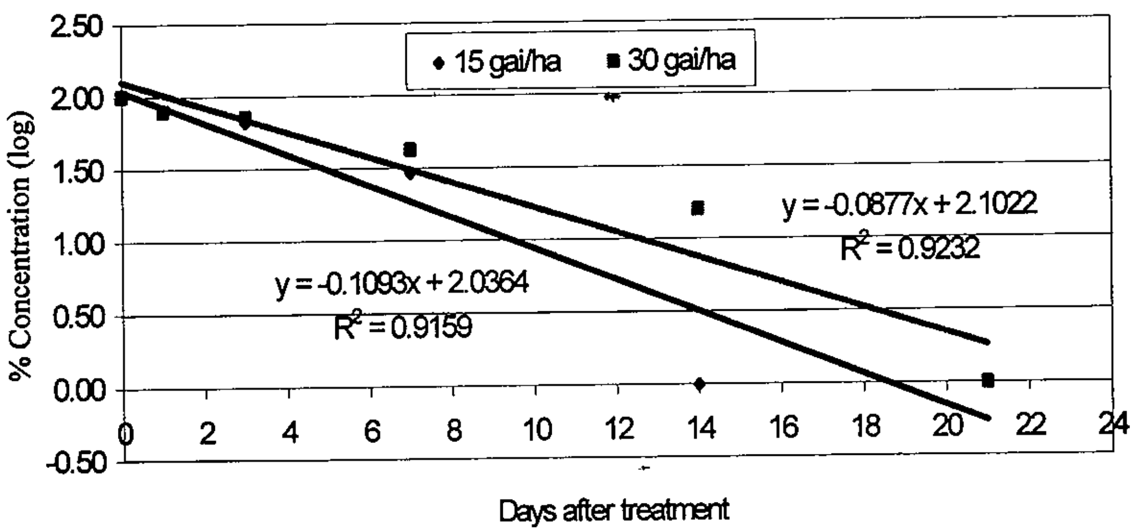

Figure 1: Degradation of metsulfuron-methyl in the field from two application rates.

LITERATURE CITED

Bergstrom, L. 1990. Leaching of chlorsulfuron and metsulfuron-methyl in three Swedish soils in field lysimeters. J. Environ. Qual., 9:701-706.

Beyer, E.M. Jr. et al. 1988. Sulfonylurea. Pages 117-190 in P.C. Kearney and D. Kaufman, eds. Herbicides: Chemistry, Degradation and Mode of Action Vol. 3. Marcel Dekker, New York.

Brown, H.M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci., 29: 263-281.

Brown, H.M. and P.C. Kearney. 1991. Plant biochemistry, environmental properties, and global impact of the sulfonylurea herbicides. Pages 32-49 in D.R. Baker, J.G. Fenyes and W. K. Moberg, eds. Synthesis and Chemistry of Agrochemicals I, ACS Symposium Series 443.

Chung, G. F. 1997. Efficient weed management. The Planter, 73 (861): 645-670.

Corley, R.H.V et al. 1971. Analysis of growth of the oil palm (*Elaeis guineensis* Jacq.). I. Estimation of growth parameters and application in breeding. Euphytica, 20: 307-315.

Ismail, B.S. and H. J. Lee. 1995. Persistence of metsulfuron-methyl in two soils. J. Environ. Sci. Health, B30: 485-497.

James, T.K. et al. 1995. Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. Weed Research, 35: 113-120.

Khairuddin, H, and C. H. Teoh. 1992. Evaluation of new herbicides for general weed control in oil palm. The Planter, 68 (794): 257-269.

Nordh-Christerson, M. and L. Bergstrom. 1989. Field observation of soil movement and residues of sulfonylureas in Sweden. Proc. British Crop Prot. Conf. – Weeds. p. 1127-1132.

Reed, W.T. and S.H. Chang. 1992. Resistance in weeds to sulfonylurea herbicides and its relevance to tropical weed control. In: Proc. 3rd International Conf. on Plant Protection in the Tropics.

Ray, T.B. 1985. The site of action of the sulfonylurea herbicides. Proc. British Crop Prot. Conf. – Weeds. p. 131-138.

Rao, V. et al. 1983. A critical re-examination of the method of bunch quality analysis in oil palm breeding. PORIM Accasional Paper, Palm Oil Research Institute of Malaysia., Kuala Lumpur, p. 1-28.

Vega, D. et al. 1992. Degradation chimique ou microbilogique des sulfonylurea dans le sol II. Cas de metsulfuron-methyle. (Chemical or microbial degradation of sulfonylurea in the soil. II. Metsulfuron-methyl. Weed Res. 31:149-155.

Wadd, D.J. and D.S.H. Drennan. 1989. A field study of the persistence and leaching of chlorsulfuron and metsulfuron methyl. Proc. British Crop Prot. Conf. – Weeds. p. 1133-1138.

Walker, A. et al. 1989. Adsorption and degradation of chlorsulfuron and metsulfuron-methyl in soils from different depths. Weed Res., 29: 281-187.

Bromacil Residues in Soil and Groundwater Following its Use in Asparagus Crops

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Abstract: Bromacil is a persistent, soil active herbicide with high water solubility. It is widely used for controlling weeds in citrus, pineapples, sugar cane and forestry. In New Zealand, bromacil is also registered for controlling weeds in asparagus. In the study reported here, two asparagus fields were sampled over a 1–3 year period to determine the fate of the applied bromacil. Wells were drilled on the field margins and groundwater samples were collected at regular intervals throughout the study. In addition, soil samples were taken to 1 m depth on nine occasions. Bromacil residues were extracted from the water by solid phase extraction (SPE) and eluted with methanol. Soil samples were extracted with aqueous sodium hydroxide and partitioned into dichloromethane. All samples were then evaporated and redissolved in methanol/water for analysis by HPLC. At Site 1, which had a low organic matter sandy soil, bromacil residues were routinely traced in soil samples to 1 m depth. Concentrations in the top 10 cm ranged from 2160 $\mu g\ kg^{-1}$ immediately after application to 166 $\mu g\ kg^{-1}$ after 1 year. At 1 m depth concentrations fluctuated up to 18 $\mu g\ kg^{-1}$ throughout the year. In the groundwater sampling wells associated with Site 1, bromacil was found in concentrations ranging from 0.30 – 84 $\mu g\ L^{-1}$. At Site 2, which contained more organic matter and less sand, soil residues were not found deeper than 50 cm. The groundwater was generally free of bromacil at this site, but occasionally contained concentrations up to 0.34 $\mu g\ L^{-1}$.

Key words: Bromacil, degradation, groundwater, leaching, persistence, soil residues

INTRODUCTION

Since its introduction in the early 1960s, bromacil has been used in a variety of agricultural and non-agricultural situations for control of annual and perennial weeds. However, its principal use is for weed control in citrus orchards and industrial sites including railway lines. Bromacil is very persistent in the soil with a half life of 4 - 6 months (Gardiner et al 1969) and is also very soluble and, thus, prone to leaching (Smith et al 1975). Bromacil is more strongly adsorbed by organic matter than by clay particles and is thus more persistent and less mobile in soils with a high organic matter content (Rhodes et al 1970; Gerstl and Yaron 1983a, b). Also, Weber and Best (1972) reported that bromacil was more persistent in an acid soil than a neutral soil.

In Europe, bromacil has been found in wells within 100 m of railway tracks where it had been used for weed control (Leistra and Boesten, 1989). In his review of groundwater contamination in the USA, Ritter (1990) reported that bromacil was found in the groundwater of two states and attributed its presence to agricultural use. In areas where bromacil has been widely used in avocadoes and citrus crops, such as California and Florida, USA, it is now included in the "Pesticides on Groundwater Protection List".

Latest pesticide use trends in these areas show that bromacil use is declining steadily (CDPR 2002).

In New Zealand, bromacil is registered for controlling weeds in asparagus (*Asparagus officinalis*) and is applied annually to the majority of the crop. The type of soil usually chosen for asparagus cropping is sandy in nature. Generally, this type of soil is highly prone to leaching of bromacil, resulting in groundwater contamination (Hebb and Wheeler 1978; Gómez de Barreda et al 1991). However, most New Zealand soils are acidic and have high organic matter content. Also, the clay fraction of many soils is of volcanic origin, especially in the North Island where clay content may consist of 100% allophane (Burney et al, 1975). Because of these features, it is difficult to predict the behaviour and fate of bromacil in New Zealand soils and groundwater without local data.

MATERIALS AND METHODS

Two study sites with a history of bromacil use were set up in established asparagus crops in the Waikato region of New Zealand. Both sites were treated with bromacil (Hyvar, 800 g ai kg^{-1}) at 2.0 kg ai ha^{-1} in August 1990, 1991 and 1992 as part of their normal agricultural practices. For collection of groundwater samples, several wells were drilled with a Gillings rig and lined with 75 mm slotted PVC pipe fitted at the top with a screw cap and sealed around the soil surface with bentonite clay. Site 1 had five wells located on low lying land on either side of the asparagus crop. Wells 1A and 1B were drilled in 1990 and Wells 1C, 1D and 1E in 1991. At this site water samples were also collected from the owners' domestic well (1F) sited about 70 m from the asparagus crop. Site 2 had four wells located at the ends of shelter belts in the middle of an asparagus block. Wells 2A and 2B were drilled in 1990 and Wells 2C and 2D in 1991. Water samples (0.5 – 2.0 l) were collected from each well at regular intervals with a stainless steel dipper fitted with a one-way valve at the bottom. Stratified soil samples were collected at selected time intervals from both sites during the growing season of 1991/92 with a modified motorised soil sampler fitted with a 50 mm diameter split tube inside the auger. Three soil cores were collected from the treated area adjacent to the wells at each sampling time and after stratification the corresponding layers were bulked together, sieved (2 mm), and subsampled (2 x 20 g) for dry matter determination. Some soil properties for the stratified layers at both sites are presented in Table 1.

Full details of the methods for extraction, cleanup and determination of bromacil have been provided elsewhere (James and Lauren 1995). Briefly, bromacil was extracted from the groundwater by SPE (C18) followed by elution with methanol. Extraction from the soil was by aqueous sodium hydroxide followed by partitioning into dichloromethane. Both the methane and dichloromethane fractions were then evaporated and redissolved in methanol/water for analysis. Bromacil concentration was determined by HPLC on a reversed phase C18 column with a methanol/water mobile phase and UV detection. The detection limits were 0.05 μg L^{-1} for a 100 ml groundwater sample and 2.0 mg kg^{-1} for a 50 g soil sample.

Table 1: Some soil characteristics of the two field study sites.

Soil depth (cm)	Soil property				
	pH	Organic C (%)	Sand (%)	Silt (%)	Clay (%)
Site 1					
0-10	6.1	2.3	88	10	2
10-20	5.9	0.9	97	2	1
20-30	5.9	0.5	95	5	0
30-50	5.8	0.9	94	6	1
50-70	7.1	0.4	97	2	1
70-100	6.8	0.2	97	2	1
Site 2					
0-10	6.7	6.6	66	23	11
10-20	6.0	4.9	76	19	5
20-30	5.7	6.0	79	20	1
30-50	6.0	1.6	91	9	0
50-70	6.3	0.6	95	5	0
70-100	6.2	0.4	96	4	0

RESULTS AND DISCUSSION

Groundwater

At Site 1 bromacil was found in all wells adjacent to the asparagus field (Wells 1A, 1B, 1C and 1D) as well as in the domestic well (1F) at all sampling dates (Table 2). The level of contamination fluctuated during the year but there was always a large increase of bromacil found within the first few months of it being applied to the soil surface. With time, the levels of bromacil usually decreased over the year but never completely disappeared. No bromacil was used after the 1992 application and its residues diminished over time, particularly in the most contaminated well (1C), and reached low levels 2 years after the final application. No bromacil was ever found in the well (1E) situated about 20 m away from the treated area but in the same low lying topography as the other wells.

Bromacil was only occasionally found in the wells at Site 2 and then only at concentrations up to 100 fold lower than at Site 1. This was despite the water tables being at similar depths at the two sites (Table 2). Gerstl and Yaron (1983a) found bromacil to be only weakly adsorbed by clay but strongly adsorbed by soil organic matter (OM). It is likely that the higher levels of organic carbon (OC) at Site 2 adsorbed the bromacil more strongly, effectively stopping it from leaching downwards in large quantities.

The situation here, which has resulted in bromacil leaching to the groundwater, appears to be similar to some overseas cases. Ritter (1990) and Hebb and Wheeler (1978) both reported bromacil leaching to the groundwater under sandy soils in the USA. In the latter case, the soil involved (91% sand, 1.9% OM, pH 4.9) was very similar to our Site 1. However, bromacil in this case was applied at at 10 fold greater rate (22 kg ai ha^{-1}) to control weeds in pines and the highest amount found in the groundwater was 1250 $\mu g\ l^{-1}$ 135 days after application. In citrus orchards in Spain, Gómez de Barreda et al (1991) found bromacil in well water at 4.4 μg l^{-1}. The soils in this case were similar in texture to our Site 2 except that their soil had considerably less OM (<1%) than at Site 2.

Table 2: Bromacil contamination found in the groundwater and monthly rainfall data. Analysis results are the average of two subsamples.

Sampling date	Bromacil concentration (μg l^{-1}) Well: Site 1[a] 1A	1B	1C[c]	1D[c]	1F	Site 2 2A	2B	2C[c]	2D[c]	Rain-fall[b] (mm)
21.9.90	9.31	0.08			3.10	0.10	0.10			78
	Bromacil applied[d]					Bromacil applied[d]				
2.11.90	37.78	5.20			2.44	0.14	n.d.[e]			152
1.12.90	58.26	5.50			3.99	0.13	dry			108
3.1.91	84.01	7.83			3.95	dry	-			24
9.2.91	67.84	Dry			7.54	dry	-			160
14.3.91	dry	Dry			8.48	dry	-			86
14.4.91	dry	Dry			5.92	dry	-			60
8.5.91	dry	Dry			11.93	dry	-			87
14.6.91	dry	Dry			4.88	dry	-			52
16.7.91	dry	Dry	48.35	dry	8.20	dry	-	n.d.	dry	66
16.8.91	16.12	1.05	55.98	0.23	3.66	n.d.	-	n.d.	0.24	210
19.9.91	6.48	4.38	35.85	0.34	2.94	0.07	-	0.06	0.21	134
	Bromacil applied[d]					Bromacil applied[d]				
16.10.91	4.32	6.32	42.25	0.47	2.69	n.d.	-	n.d.	0.21	88
19.11.91	12.85	3.58	25.16	0.48	1.90	n.d.	-	n.d.	0.34	116
20.12.91	20.06	10.94	40.39	0.43	2.88	n.d.	-	n.d.	0.28	32
31.1.92	10.64	Dry	23.03	0.39	2.05	dry	-	n.d.	dry	136
19.2.92	8.40	Dry	19.21	0.28	1.72	dry	-	n.d.	0.23	68
25.3.92	dry	Dry	18.82	dry	1.33	dry	-	n.d.	dry	50
23.4.92	dry	Dry	32.44	dry	4.46	dry	-	n.d.	dry	60
3.6.92	dry	Dry	43.40	dry	0.77	dry	-	n.d.	dry	101
3.7.92	5.81	2.43	15.03	0.38	1.36	dry	-	n.d.	dry	78
18.8.92	2.11	Dry	13.45	0.38	1.17	n.d.	-	n.d.	dry	279
21.9.92	0.77	-	21.52	0.67	1.22	n.d.	-	dry	0.08	102
	Final bromacil application[d]									
4.12.92	7.10	-	28.09	0.58	1.74	-	-	-	-	273
12.4.92	4.85	-	11.30	0.94	2.68	-	-	-	-	318
5.8.93	3.65	-	8.60	0.91	3.33	-	-	-	-	358
18.11.93	dry	-	7.26	1.53	3.56	-	-	-	-	213
20.2.94	dry	-	4.95	0.90	2.08	-	-	-	-	228
16.5.94	dry	-	4.83	0.70	1.12	-	-	-	-	210
31.8.94	0.46	-	2.82	0.65	0.53	-	-	-	-	464
	Minimum and maximum depth of watertable in well (m) during sampling									
Min.	1.7	2.0	1.7	2.0	6.0	1.8	2.5	1.8	2.5	
Max.	2.1	2.4	3.8	4.3		2.3	3.0	3.4	5.5	

a No bromacil was ever found in Well 1E.
b Rainfall is the total accumulated for the periods between samplings. The first period is for the month prior to the first sampling.
c Well not drilled until 19.6.91.
d Bromacil was applied at 2.0 kg ai ha^{-1}. NB, no bromacil applied at Site 1 in 1993.
e n.d. = not detected at detection limit of 0.05 μg l^{-1}.
\- Well not sampled.

The quantity of bromacil in the groundwater at Site 1 is not considered to pose a health risk. Bromacil has low toxicity and all except one of these analyses showed a concentration below the limit of 80 μg l^{-1} set by the USA Environmental Protection Agency (EPA) Office of Ground Water and Drinking Water (Health Advisory Levels for Selected Pesticides) (Barceló, 1993).

Soil

At both Sites 1 and 2, 66% - 85% of the applied bromacil was recovered from the top 10 cm of the soil (Table 3). Bromacil was also found at lower depths at both sites, both before and after the 1991 application. However, at Site 1, the bromacil concentration in the 10–20 cm was much higher than at Site 2, and increased significantly much sooner after application (within 2 months) than at Site 2 (within 4 months). At Site 1 bromacil was found down to the maximum depth samples (70 - 100 cm), whereas at Site 2 it was not found deeper than 50 cm. At both sites bromacil residues continued to persist in large amounts into the next growing season.

Table 3: Soil residues of bromacil and rainfall data for the 12 month period following herbicide application in September 1991.

Sampling date	Bromacil soil residues (μg kg^{-1}) Sampling depth (cm) 0-10	10-20	20-30	30-50	50-70	70-100	Rainfall[a] (mm)
Site 1							
22.8.91	718	624	995	87	9	n.d.	181
25.9.91	307	339	263	26	n.d.[b]	n.d.	143
Bromacil applied at 2.0 kg ai ha^{-1}							
23.10.91	2160	462	107	86	14	18	71
28.11.91	845	1180	870	619	32	13	95
17.12.91	842	331	214	124	n.d.	n.d.	32
16.1.92	623	663	616	361	15	3	92
25.3.92	403	777	418	113	28	5	162
23.6.92	322	299	1450	156	23	2	232
22.9.92	166	289	377	64	35	10	387
Site 2							
22.8.91	930	160	42	n.d.	n.d.	n.d.	181
Bromacil applied at 2.0 kg ai ha^{-1}							
7.10.91	1510	63	n.d.	n.d.	n.d.	n.d.	173
18.11.91	1680	57	17	n.d.	n.d.	n.d.	136
17.12.91	1230	42	23	35	n.d.	n.d.	32
16.1.92	1400	239	n.d.	n.d.	n.d.	n.d.	92
25.3.92	1260	68	n.d.	n.d.	n.d.	n.d.	162
23.6.92	1240	32	22	n.d.	n.d.	n.d.	232
22.9.92	630	29	20	n.d.	n.d.	n.d.	387

a Rainfall is the total accumulated for the periods between samplings. The first period is for the month prior to the first sampling.

b n.d. = not detected with detection limit of 2 μg kg^{-1}.

These findings complement previous studies in New Zealand by Sanders et al. (1996) who found that residues from single applications of bromacil persisted for about 6 months but repeat applications persisted considerably longer. Their studies showed that repeat applications of bromacil had a detrimental effect on the soil microflora which probably resulted in reduced breakdown and longer persistence of residues. Tucker (1978) investigated the use of bromacil in Florida citrus orchards on sandy soils (95 - 99% sand, 0.38 - 0.42% OM, pH 7.3 - 7.8) and found residues down to 60 cm. This concurs with the results of the present study as bromacil leached very quickly after application on the more sandy soil (Site 1). Several workers have also reported on the rate of degradation of bromacil. Gardiner et al. (1969) and Wolf and Martin (1974) determined the half life ($t_{½}$) of C^{14} labelled bromacil to be 5 - 6 months in silt loam and sandy soils while Zimdahl et al. (1970) determined it to be 7 months at 13.2°C in a loam soil. Zimdahl et al. (1970) also found that the degradation of bromacil followed first order reaction kinetics and the rate was not greatly affected by temperature ($t_{½}$.= 5 months at 31.2°C). In our study the $t_{½}$ for bromacil in the 0 - 10 cm layer differed markedly between sites. At Site 1 rainfall had a significant effect on the dissipation of bromacil residues, rapidly leaching them from the upper soil layers (Table 3). As a result the $t_{½}$ for bromacil in the 0 – 10 cm layer was only 110 days. At Site 2, where there was considerably less leaching, the $t_{½}$ for bromacil was about 330 days.

This study showed that bromacil leached in small concentrations into the groundwater after agricultural use at normal rates of application. The amounts of herbicide involved were generally well within recommended limits set by the World Health Organisation (WHO) and the EPA. The leaching of this herbicide appeared to occur soon after application and probably before it became strongly bound to soil sorptive sites. The worst contamination, both in terms of amount and duration, was from the very sandy site with low soil OC content. Residues in the groundwater decreased markedly once the use of bromacil was discontinued. The second case of contamination (Site 2), which was on soil with higher soil OC, was very minor.

Under the soil and climatic conditions of this study, bromacil was found to be quite persistent, and in some situations mobile. Residues persisted throughout the year and in some instances this repeated use could lead to accumulation of bromacil in the soil over time. Where required, periodic use of alternative herbicides or lower rates of bromacil could effectively be employed to break any cycle of accumulation.

LITERATURE CITED

Barceló, D. 1993. Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water. J. Chromatography, 643:117-143.

Burney, B., Rahman, A., Oomen, G.A.C. and Whitham, J.M. 1975. The organic matter status of some mineral soils in New Zealand. Proc. New Zealand Weed and Pest Control Conference, 28:101-103.

Gardiner, J.A., Rhodes, R.C., Adams, J.B. and Soboczenski, E.J. 1969. Synthesis and studies with 2-C^{14}-labelled bromacil and terbacil. J. Agriculture and Food Chemistry, 17:980-986.

CDPR, 2002. (California Department of Pesticide Regulation). Summary of Pesticide Use Report. http://www.cdpr.ca.gov/docs/pur/pur98rep/98com.htm. 16.10.2002.

Gerstl, Z. and Yaron, B. 1983a. Behavior of bromacil and napropamide in soils:I. adsorption and degradation. Soil Science Society America J., 47:474-478.

Gerstl, Z. and Yaron, B. 1983b. Behavior of Bromacil and Napropamide in Soils:II. Distribution after application from a Point Source. Soil Science Society America. J., 47:478-483.

Gómez de Barreda, D., Lorenzo, E., Gamón, M., Monteagudo, E., Saez, A., De la Cuadra, J.D., Del Busto, A., Ramos, C., and Carbonell, E.A. 1991. Survey of herbicide residues in soil and wells in three citrus orchards in Valencia, Spain. Weed Research, 31:143-151.

Hebb, E.A. and Wheeler, W.B. 1978. Bromacil in Lakeland soil ground water. J. Environmental Quality, 7:598-601.

James, T. K. and Lauren, D. R. 1995. Determination of bromacil in groundwater and in high organic matter soils. J. of Agriculture and Food Chemistry, 43:684-690.

Leistra, M. and Boesten, J.J.T.I. 1989. Pesticide contamination of groundwater in Western Europe. Agriculture, Ecosystems and Environment, 26:369-389.

Rhodes, R.C., Belasco, I.J. and Pease, H.L. 1970. Determination of mobility and adsorption of agrichemicals on soils. J. Agriculture and Food Chemistry, 18:524-528.

Ritter, W.F. 1990. Pesticide contamination of ground water in the United States - a review. J. Environment Science Health, B25:1-29.

Sanders, P., Wardle, D. and Rahman, A. 1996. Persistence of bromacil in soils with different management histories. Proc. New Zealand Plant Protection Conference, 49:207-211.

Smith, A.E., Grover, R., Emmond, G.S. and Korven, H.C. 1975. Persistence and movement of atrazine, bromacil, monuron and simazine in intermittently-filled irrigation ditches. Canadian J. Plant Science, 55:809-816.

Tucker, D.P.H. 1978. Bromacil and diuron residue levels in Florida citrus soils. Pesticides Monitoring J., 12:47-50.

Weber, J.B. and Best, J.A. 1972. Activity and movement of 13 soil-applied herbicides as influenced by soil reaction. Proc. Southern Weed Science Society, 25:403-413.

Wolf, D.C. and Martin, J.P. 1974. Microbial degradation of 2-carbon-14 bromacil and terbacil. Proc. Soil Society America, 38:921-925.

Zimdahl, R.L., Freed, V.H., Montgomery, M.L. and Furtick, W.R. 1970. The degradation of triazine and uracil herbicides in soil. Weed Research, 10:18-26.

Bio-efficacy Of Carfentrazone-ethyl Against Broadleaf Weeds Infesting Wheat (*Triticum aestivum*) In India

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Abstract: Experiments were conducted during the years 1998-1999 and 1999-2000 to evaluate the bio-efficacy of Carfentrazone-ethyl applied as post-emergence foliar spray at the rates of 15, 20, 25 and 30 g ha^{-1}, against broadleaf weeds infesting Wheat. Carfentrazone-ethyl at the rates of 25 and 30 g ha^{-1} gave more than 95 percent control of *Chenopodium album, Rumex dentatus, Medicago denticulata, Melilotus alba, Vicia faba* and *Anagallis arvensis*. Lower rates of application at 15 and 20 g ha^{-1} led to poorer control of these weeds which was on a par with that noticed under 2,4-D (sodium salt) applied @ 500 g ha^{-1}. As a consequence, grain and straw yield under these three treatments were comparable. Carfentrazone-ethyl applied at 25 and 30 g ha^{-1} gave the highest grain and straw yield, which were significantly superior when compared to that obtained under 2,4-D.

Key words: Bio-efficacy, broadleaf weeds, carfentrazone-ethyl

INTRODUCTION

Weeds infesting wheat fields comprise annual grasses such as *Phalaris minor*, *Avena sterilis ssp.ludoviciana* and broadleaf weeds such as *A. arvensis*, *C. album*, *Convolvulus arvensis*, *Lathyrus aphaca*, *M. denticulata*, *M. alba*, *Silene conoidea*, *V. faba* and *R. dentatus* (Bajpai et al. 1992 ; Balyan et al. 1988; Balyan and Malik 1992 ; Singh 2000; Singh et al. 1995 and Suresh Kumar and Singh 1994).

The herbicide, Isoproturon, which is widely used since early 1980's has been found to be very effective against grasses. However, it exhibits poorer bio-efficacy against some broadleaf weeds such as *A. arvensis*, *L. aphaca*, *M. denticulata*, *M. alba* and *R. dentatus* (Bajpai et al. 1992 ; Balyan et al. 1990 and Balyan and Malik 1988). Besides tank mixing 2,4-D with isoproturon, stand alone application of 2,4-D has also been recommended for controlling weed flora predominated by broad leaf weeds (Phogat et al. 1991; Sharma et al. 1987; Singh and Sharma 1984; Singh et al. 1997). Inappropriate timing of application of 2,4-D has been found to lead to malformation of spikes and reduction in grain yield in wheat (Balyan et al. 1990; Balyan and Panwar 1997; Bhagwati et al. 1989; Bhan et al. 1976 ; Pinthus and Natowitze 1967 and Sharma et al. 1987).

Hence, there is need for alternative effective herbicides to control broadleaf weeds in wheat without causing attendant malformation of spikes. Metsulfuron-methyl which is effective against broadleaf weeds has been evaluated and recommended (Rajvir and

Pahuja 2001; Sharma and Sharma 1997; Tewari et al. 1998 and Walia et al. 1998). In this paper we report the usefulness of carfentrazone-ethyl as a post-emergence, contact herbicide for the effective control of broadleaf weeds.

MATERIALS AND METHODS

Field experiments were carried out during the Rabi season (November to April) of 1998-1999 and 1999-2000 in the State of Punjab. Soil of the experimental field was sandy loam and slightly alkaline in reaction (pH 8.0). Wheat variety PBW 343 was sown on November15,1998 and November 22,1999 at a row to row spacing of 20cm with a tractor mounted seed-cum-fertilizer drill using a seed rate of 100 kg ha^{-1}. Recommended levels of NPK (125 kg N, 62.5 kg P_2O_5 and 30 kg K_2O ha^{-1}) were applied. Half of N and full P_2O_5 and K_2O were applied at the time of sowing and remaining N was given with first irrigation. All other agronomic and plant protection practices were followed as per the Package of Practices, PAU, Ludhiana. Four irrigations were given. Treatments of carfentrazone-ethyl @15,20,25 and 30 g ha^{-1} were compared with the standard treatment of 2,4-D sodium salt applied @ 500 g ha^{-1}, weed free and weedy check plots. Plot size was 6 m X 6 m and all the treatments were arranged in randomized block design with four replications. Herbicide treatments were imposed as post-emergence blanket foliar spray at 35 days after sowing using a knapsack sprayer fitted with WFN 040 nozzle and spray volume of 450 litres of water per hectare.

Visual assessment of phytotoxicity of herbicide treatments on crop was recorded on a scale of 1 to 10 (1= adverse effect of the herbicide on the crop and 10= 100% adverse effect of the herbicide on crop). Species-wise count of weeds was recorded at 60 days after sowing within 1.0m x 1.0m quadrat at three places at random in each plot. Dry weight of weeds was recorded after drying the weed samples in an oven at 70^0 C until constant weight was achieved. Grain and straw yield was recorded at harvest.

RESULTS AND DISCUSSION

Phytotoxicity

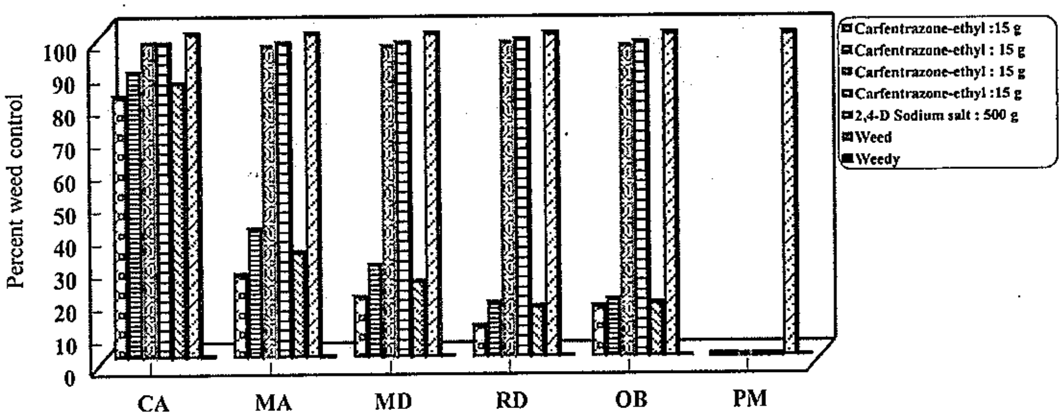
Wheat plants in the experimental plots treated with carfentrazone-ethyl exhibited mild speckling of leaves three days after imposing treatments. The plants became normal ten days after treatment. Treatment with carfentrazone-ethyl did not lead to leaf injury, vein clearing, wilting, necrosis, epinasty and hyponasty symptoms in plants.

Weed flora

At the time of imposing treatments, plots were predominantly infested with the broadleaf weeds (65%), *C. album, M. alba, M. denticulata,* and *R. dentatus.* Other broad leaf weeds were *A. arvensis, C. arvensis, L. aphaca, Silene conoidea, Cornopus didymus* and *V. faba. Phalaris minor* was the grassy weed observed during both the years.

Weed control

Treatment with carfentrazone-ethyl @ 15 and 20 g ha^{-1} gave 78% to 91% weed control of *C. album* and less than 30 per cent control of *M. alba, M. denticulata* and *R. dentatus* and other broadleaf weeds (Fig. 1). These treatments were comparable to 2,4-D applied @ 500 g ha^{-1}. Poor bio-efficacy of 2,4-D Sodium salt against *Rumex* sp., *M. denticulata, A. arvensis, C. didymus, Silene conoidea, Fumaria parviflora, C. arvensis, L. aphaca and V. sativa* has earlier been reported (Balyan et al., 1992). Carfentrazone-ethyl @ 25 and 30 g ha^{-1} gave 95 to 98% control of *C. album, M. alba, M. denticulata, R. dentatus* and other broadleaf weeds.



CA :*Chenopodium* ; MA :*Melilotus* ; MD:*Medicago* ;
RD:*Rumex dentatus* OB : Other broadleaf weeds (see text) *Phalaris*

Figure 1. Effect of treatments on weed control (Mean of 1998-1999 & 1999-2000)

Dry weight of weeds

Weedy check treatment recorded the highest dry weight of weeds (Table 1). There was a significant reduction in the dry weight of weeds under the treatments of carfentrazone-ethyl @ 15 and 20 g ha^{-1} and 2,4-D applied @ 500 g ha^{-1}. A further reduction in the dry weight of weeds was noticed in the treatments of carfentrazone-ethyl @ 25 and 30 g ha^{-1}. Dry weight of weeds was the lowest in weed free plots because besides broadleaf weeds, the grass weed *P. minor* was also removed.

Table 1: Influence of treatments on dry weight of weeds at 60 days after sowing

Treatments (g a.i. ha^{-1})	Dry weight of weeds ($g\ m^{-2}$) *					
	Chenopodium album	*Melilotus alba*	*Medicago denticulata*	*Rumex dentatus*	*Other broad leaf weeds* **	*Phalaris minor*
Carfentrazone ethyl : 15	4.6	38.3	77.4	58.7	17.1	196.2
Carfentrazone ethyl : 20	2.9	30.7	72.3	54.4	15.4	201.2
Carfentrazone ethyl : 25	0.2	1.2	1.1	0.1	1.2	201.5
Carfentrazone ethyl : 30	0.1	0.4	0.4	0.3	0.8	199.7
2,4-D Sodium salt : 500	3.2	33.7	73.5	55.5	19.0	198.6
Weed free	0.1	0.2	0.4	0.2	0.2	0.9
Weedy check	60.5	74.0	99.3	71.6	24.7	205.2
CD at 5%	7.2	9.2	15.7	11.3	6.2	23.7

* Mean of 1998-1999 & 1999-200

** See text

Effective tillers and yield

Number of effective tillers, grain and straw yield were the lowest in weedy check plot, demonstrating the adverse effects of weed infestation throughout the duration of the crop (Table 2). Highest number of tillers, grain and straw yield were recorded in the weed free plots. Values of these parameters in plots treated with 2,4-D @ 500 g ha^{-1} were significantly higher when compared to those of weedy check plots. Similar findings have been reported in earlier studies (Dilraj Sidhu et al. 2000; Kurchania et al. 2000; Sahadeva Singh et al. 1997 and Singh and Sharma 1984). Carfentrazone-ethyl @ 25 and 30 g ha^{-1} was significantly superior to its lower rates of 15 and 20 g ha^{-1} and to that of 2,4-D in respect of number of effective tillers, grain and straw yield but was inferior to hand weeding. This is due to the fact that carfentrazone-ethyl controlled broadleaf weeds but not grasses where as under hand weeded treatment both grasses and broadleaf weeds was effectively removed. Visual assessment of the crop during the reproductive phase did not reveal any adverse effect of carfentrazone-ethyl on spike formation.

Table 2: Effect of treatments on effective tillers, grain and straw yield.

Treatments (g a.i. ha^{-1})	**Effective tillers* (No $sq.m^{-1}$)**	**Grain yield* (kg ha^{-1})**	**Straw yield* (kg ha^{-1})**
Carfentrazone ethyl : 15	117.6	3138	4092
Carfentrazone ethyl : 20	118.7	3214	4229
Carfentrazone ethyl : 25	122.5	3868	5175
Carfentrazone ethyl : 30	122.6	3957	5260
2,4-D Sodium salt : 500	117.2	3063	4067
Weed free	129.7	5014	6478
Weedy check	68.4	2492	3374
CD at 5%	2.3	302	320

* Mean of 1998-1999 & 1999- 2000

Higher grain and straw yield obtained under treatments with carfentrazone-ethyl at 25 and 30 g ha^{-1} provided further proof that the herbicide has no adverse effects on spike formation. Results of the present study, thus, indicate that application of carfentrazone-ethyl @ 25 and 30 g ha^{-1} at 35 days after sowing leads to successful control of broadleaf weeds with favorable implications on grain yield.

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LITERATURE CITED

Balyan, R.S. et al. 1988. Effect of herbicides and crop rotation on weed complex. Haryana agric. Univ. J. Res. 18: 100-107.

Balyan, R.S. et al. 1992. Effect of rates, time and methods of application of tralkoxydim on wild oat in wheat. Trop. Pest Manage. 38: 411-415.

Balyan, R.S. and Malik, R.K. 1988. Effect of time of application of Isoproturon + 2,4-D on the control of weeds in wheat. Haryana agric. Univ. J. Res. 18: 47-49.

Balyan, R.S. and Malik, R.K. 1992. Chemical control of wild oat and other weeds in wheat. J. Trop. Agric. And vety. Sci..27:427-433.

Balyan,R.S. et al. 1990. Sensitivity of wheat cultivars to fluroxypyr and 2,4-D combination. Indian J.Agron.35:408-409.

Balyan, R.S. and Panwar, R.S. 1997. Herbicidal control of broadleaf weeds in wheat. Haryana J. Agron. 13: 33-38.

Bhagwati, P.C. et al. 1989. Competition between wheat and associated weeds at different nitrogen rates. J. Trop. Agric. And vety. Sci. 27:427-433.

Bhan,V.M. et al.1976.Spike formation by 2,4-D in dwarf wheats. Indian J. Weed Sci. 8(1):53-59.

Bajpai, R.P. et al. 1992. Evaluation of weed control method in wheat fields in northern hill zone of Madhya Pradesh. Abstr. Ann. Weed Sci. Conf. 1992, Hissar, p.56.

Dilraj, S. et al. 2000. Herbicidal control of *Rumex spinosus* (Kandiali palak) in wheat (*Triticum aestivum*). Indian J. Weed Sci. 32(3&4): 156-159.

Kurchania, S.P. et al. 2000. Bio-effciacy of metsulfuron-methyl and 2,4-D combinations for broadleaf weed control in wheat. Indian J. Weed Sci. 32(1&2): 67-69.

Pinthus, M.J. and Natowitze, 1967. Response of spring wheat to the application of 2,4-D at various growth stages. Wed Res. 7(2): 95-101.

Phogat, B.S. et al. 1991. Bio-efficacy of herbicides mixture in wheat. Indian J. Agron. 36: 102-103.

Rajijvir, S. and Pahuja, S.S. 2001. Bio-efficacy of chlorsulfuron, metsulfuron methyl and metribuzin alone and in combinations for weed control in wheat (*Triticum aestivum*). Indian J. Weed Sci. 33(3&4): 112-115.

Sahadeva, S. et al. 1997. Efficacy of sulfonyl urea herbicides for weed control in wheat. Indian J. Weed Sci. 29 (3&4): 163-166.

Sharma, J.J. and Sharma, V. 1997. Management of complex weed flora with herbicide mixture in wheat (*Triticum aestivum*). Indian J. Weed Sci. 29 (3&4): 125-128.

Sharma, A.K. et al. 1987. Studies on weed control in wheat with herbicide mixtures. Haryana agric. Univ. J. Res. 17: 341-345.

Singh, Samunder, 2000. Unpublished data of weed survey of March-April 2000 of farmers in Haryana State.

Samunder, S. et al. 1995. Distribution of weed flora of wheat in Haryana. Indian J. Weed Sci. 27: 114-121.

Singh,S.P. and Sharma, H.C.1984. Effect of 2,4-D and hand weeding on weed population, growth and yield of wheat. Haryana Agric. Univ. Res.17:341-345.

Singh, S. et al. 1997. Studies on the chemical weed control in wheat. Indian J. Weed Sci. 29 (3&4): 100-102.

Suresh, K. and Singh, C.M. 1994. Weed management studies in wheat. Indian J. Weed Sci. 26(3&4): 125-128.

Tewari,A.N. et al. 1998. Efficacy of metsulfuron methyl on associated weeds in wheat (*Triticum aestivum*). Indian J.of Agri.Sci. 68 (2):121-122

Walia, U.S. et al. 1997. Control of *Rumex spinosus* with sulfonyl urea herbicides in wheat. Indian J. Weed Sci. 29:103-105.

Usefulness Of Pre-Mix Formulation Of Clomazone + 2,4-D EE For Weed Management In Transplanted Paddy (*Oryza sativa*) In India

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Abstract: The study was conducted during the years 1999-2000 and 2000-2001 to evaluate the usefulness of pre-mix formulation of clomazone and 2,4-D EE for weed management in transplanted paddy. EC formulation containing clomazone @ 175 and 200 g litre^{-1} with each of the two levels of 2,4-D EE @ 180 and 270 g litre^{-1} were evaluated for bio-efficacy against weeds and safety to crop. Standards for comparison were butachlor 50EC @ 1250 g ha^{-1}, clomazone @ 175 and 200 g ha^{-1} and 2,4-D EE @ 180 and 270 g ha^{-1}. Weed flora in the experimental plots comprised the grasses, *Echinochloa crus-galli* and *E. colona,* the sedges, *Cyperus difformis, C. iria, Fimbristylis miliacea* and the broadleaf weeds, *Eclipta alba, Monochoria vaginalis, Commelina benghalensis, Sphenoclea zeylanica* and *Ludwigia parviflora.* Pre-emergence soil surface spray of clomazone + 2,4-D EE @ 175 + 270 and 200 + 270 g ha^{-1} within three days after transplanting paddy led to higher percent control of weeds and lowest dry weight of weeds, in observations recorded at 30 and 45 days after transplanting. Grain and straw yield under these treatments were the highest and were significantly superior to that of all the treatments included as standards for comparison.

Key words: broadspectrum weed control, pre-mix formulation of clomazone+2,4-DEE, transplanted paddy

INTRODUCTION

India is one of the major rice growing countries in the world. As per the agricultural statistics, rice was cultivated in 44.6 million hectares with a production of 85.9 million tons and an average productivity of 1928 kg per hectare in the year 2000. Severe infestation of weeds is one of the main factors responsible for the low productivity of rice. Weed flora in transplanted paddy fields in different rice growing regions of India comprises grasses such as *E. crus-galli, E. glaberescens*; *E. colona* and broad leaf weeds such as *Ammania baccifera, Marsilea quadrifolia, L. parviflora, M. vaginalis, S. zeylanica* and sedges such as *C. difformis, C. iria, C. rotundus,* and *F. miliacea.*(Bali et al. 1994; Ghosh and Ganguly 1993; Kurmi and Das 1993; Mandal et al. 1986; Mukhopadhyay 1968, 1969 and 1971; Ray 1973; Sahu and Bhattacharya 1964 and Singh and Rao 1970).

Uncontrolled infestation of weeds has been reported to lead to crop loss of 30 to 70 per cent (Anantha Kumari and Rao 1993; Chaudhary et al. 1995; Gautam and Mishra 1995; Jena and Mishra 1992 and Raju and Reddy 1995). Herbicides such as anilofos, benthiocarb, butachlor, oxadiargyl, oxadiazon, pendimethalin, 2,4-D and pretilachlor have been found to be useful for weed management. Butachlor is the most widely used herbicide mainly because of its broadspectrum activity. Continual use of the same herbicide having the same mode of action leads to shift in weed flora and evolution of resistance in weeds (Ahn et al. 1975; De Datta 1977, Ho and Zuki 1988; Kandasamy and Sankaran 1995 and Kim 1983). In order to prevent such a problem and to present farmers with a wider choice of affordable and effective herbicides, there is a need to develop molecules of newer chemistries and combination products having herbicide partners with different mode of action. The present study was conducted with this objective.

MATERIALS AND METHODS

An experiment each was conducted in the State of Karnataka during Kharif season (June to November) of 1999-2000 and 2000-2001. The trials were laid out in randomized block design with three replications. Thirty days old seedlings of rice cv. IR-64 were transplanted at a distance of 20 cm x 15 cm during both the years in sandy clay loam soils with pH of 7.8 on July 05 and July 15 of the respective years. Fifty kg P_2O_5 and 50 kg K_2O per hectare were applied at planting and 100 kg nitrogen was given in three equal splits at the time of transplanting, 20 and 40 days after transplanting. The treatments consisted of pre-mix formulation of clomazone + 2,4-DEE applied at 175+180, 175+270, 200+180 and 200+270 g $ha.^{-1}$, besides weed free and weedy check treatments. Standards for purposes of comparison were stand-alone application of butachlor @ 1250 g ha^{-1}, clomazone @ 175 and 200 g ha^{-1}, 2,4-DEE @ 180 and 270 g ha^{-1}. Plot size was 8 m x 8 m. Water in the experimental plots was drained out before imposing the treatments. Herbicide treatments were applied as pre-emergence soil surface spray three days after transplanting using a knapsack sprayer fitted with flat fan nozzle and a spray volume of 600 litres of water per hectare. Water was impounded in the experimental plots one day after treatment.

Phytotoxicity scoring was done at five and ten days after herbicide application based on 1 to 10 scale (1= no adverse effect of the herbicide on the crop and 10= 100% adverse effect of the herbicide on the crop). Species-wise number of weeds occurring within $1.0m^2$ quadrat in each plot was recorded at random at 30 days after transplanting. Dry weight of weeds under each treatment was recorded after drying the weeds in an oven at 70^0 C until constant weight was achieved. Grain and straw yield were recorded at harvest.

RESULTS AND DISCUSSION

Phytotoxicity

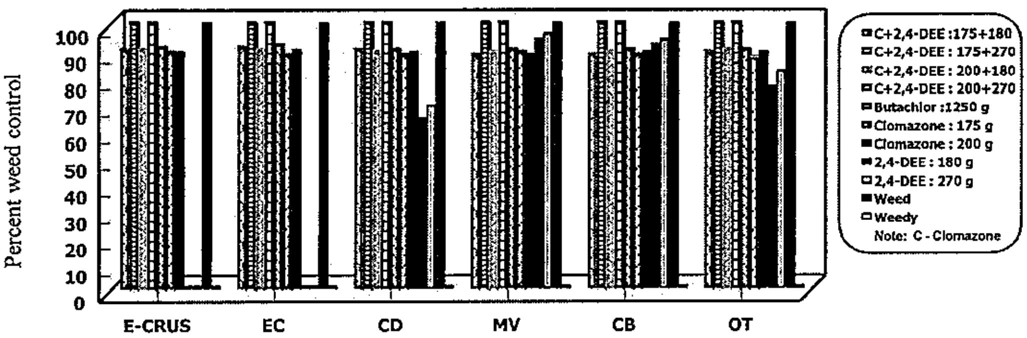
Plants in the plots treated with clomazone alone and the combination of clomazone + 2,4-DEE at all the dosages exhibited slight bleaching of leaves of paddy at five days after imposing treatments. Plants regained greenness of leaves at ten days after treatment. Similar observations were reported by Caseley et al. (1997) and Andrade (1988). Treatment with stand alone application of clomazone and pre-mix formulation of clomazone + 2,4-DEE did not lead to leaf injury, vein clearing, wilting, necrosis, epinasty and hyponasty symptoms in plants.

Weed flora

E. crus-galli, E. colona, M. vaginalis, C. benghalensis, L. parviflora, S. zeylanica, E. alba, C. difformis, C. iria and *F. miliacea* were the weeds noticed in the weedy check plots at 30 and 45 days after transplanting.

Weed control

Treatment with the combination of clomazone + 2,4-DEE @ 175+180 and 200+180 g ha^{-1} gave 80 to 88% control of the weeds (Fig. 1). These treatments were comparable to stand alone application of clomazone at both the rates and butachlor. 2,4-DEE @ 180 and 270 g ha^{-1} gave control of broadleaf weeds and sedges to the extent of 70 to 90% and 57 to 73%, respectively. Treatment with clomazone + 2,4-DEE at 175+270 and 200+270 g ha^{-1} gave 88 to 100% control of the weeds, *E. crus-galli, E. colonum, M. vaginalis, C. benghalensis, C. difformis* and others comprising *L. parviflora, S. zeylanica, E. alba, C. iria* and *F. miliacea*..



E-CRUS : *Echinochloa crus-galli* ; EC: *Echinochloa colonum* ; CD: *Cyperus difformis* ;
MV: *Monochoria vaginalis* ; CB : *Commelina benghalensi* ; OT: Others (see text)

Figure 1. Effect of treatments on weed control (Mean of 1999-2000 and 2000-2001

Dry weight of weeds

Dry weight of weeds was the highest in weedy check plots (Table 1). There was a significant reduction in the dry weight of weeds of all the species in the treatments of clomazone + 2,4-DEE @ 175 +180 and 200+180 g ha^{-1}. These treatments were comparable to butachlor @ 1250 g ha^{-1} and stand-alone application of clomazone @ 175 and 200 g ha^{-1}. Lowest dry weight of weeds of all the species was recôrded under clomazone + 2,4-DEE @ 175+270 and 200+270 g ha^{-1} which was comparable to weed free plots. In earlier investigations, stand alone application of clomazone has been found to be useful for weed management in Rice (Scherder et al. 2000; Spiridonov et al. 1995; Webster et al. 1999; Webster and Baldwin 1998; and Talbert et al. 1999). Jordan and Kendig 1998 and Carvalho et al. 1990 hypothesized that combined application of clomazone and 2,4-DEE might enhance the spectrum of weeds controlled. Results of the present investigation provide evidence to the hypothesis.

Table 1. Influence of treatments on dry weight of weeds and yield.

Treatments (g a.i. ha^{-1})	Dry weight of weeds (g m^{-2}) *						Grain yield*	Straw yield*
	Echinochloa crusgalli	*Echinochloa colona*	*Cyperus difformis*	*Monochoria. vaginalis*	*Commelina benghalensis*	Others**	(kg ha^{-1})	
Clomazone + 2,4-DEE : 175 + 180	2.2	2.1	2.8	4.1	4.8	4.7	4563	7863
Clomazone + 2,4-DEE : 175 + 270	0.1	0.1	0.1	0.1	0.1	0.1	5080	8726
Clomazone + 2,4-DEE : 200 + 180	2.2	2.3	2.7	4.1	4.8	4.2	4600	7925
Clomazone + 2,4-DEE : 200 + 270	0.1	0.1	0.1	0.1	0.1	0.1	5087	8844
Butachlor : 1250	2.0	2.0	2.6	5.2	5.4	4.4	4653	8107
Clomazone : 175	2.6	2.9	3.6	6.0	5.5	5.2	4500	7902
Clomazone : 200	2.1	2.3	3.5	4.7	5.4	4.8	4520	8036
2,4-DEE : 180	100.8	39.8	5.5	5.1	5.7	8.7	3603	6152
2,4-DEE : 270	100.1	39.8	6.1	4.8	5.5	8.2	3714	6961
Weed free	0.1	0.1	0.1	0.1	8.7	0.1	5051	9106
Weedy check	99.9	38.9	42.5	3.7	35.8	36.4	2546	5178
CD at 5%	2.2	1.6	1.9	2.8	1.6	2.2	196	406

* Mean of 1999 –2000 & 2000-2001 ; ** See text

Yield

Weedy check plot recorded significantly lower grain and straw yield during both the years (Table 1). This is due to the presence of weeds throughout the duration of the crop resulting in severe crop-weed competition. Among the herbicide treatments, treatment with 2,4-DEE @ 180 and 270 recorded the lowest grain and straw yield. This is attributed to the control of broadleaf weeds and sedges by 2,4-DEE but not grasses. Similar results obtained in other studies were attributed to the bio-efficacy of 2,4-D against broadleaf weeds and sedges but not grasses (Ampong-Nayarko 1999 and Bernasor and De Datta 1986). Treatment with clomazone + 2,4-DEE @ 175+180, 200+180 g ha^{-1} and stand alone application of clomazone @ 175 and 200 g ha^{-1} recorded grain and straw yields which were on par with that of butachlor @ 1250. Treatment with clomazone + 2,4-DEE @ 175+270 and 200+270 g ha^{-1} recorded the highest grain and straw yields which were significantly superior to that of butachlor @ 1250 g ha^{-1}.

Thus, the results of present study has clearly established that treatment with pre-mix formulation of clomazone + 2,4-DEE @ 175+270 g ha^{-1} and 200+270 g ha^{-1} was superior to stand alone application of clomazone and butachlor for weed management of broad spectrum of weeds in transplanted paddy with favourable implication on grain and straw yields.

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LITERATURE CITED

Ahn, S.B. et al. 1975. Effect of repeated annual application of pre-emergence herbicide on paddy field weed populations. In: Proc. 5th Asian-Pac. Weed Sci. Soc.Conf. Tokyo, Japan. pp. 287-292.

Ampong - Nyarke, K. and De Datta, S.K. 1991. A handbook for weed control in rice. IRRI, Manila, Philippines, pp. 113.

Andrade,VA.de.1988. Chemical control of *Echinochloa* spp., in irrigated rice Analis-17a. Reuniao-da-cultura-do-Arroz-Irrigado-Pelotas-RS-de-26-a-30-de-Setembro de.356-358.

Anantha Kumari, P. and Rao,V.N.1993. Effects of herbicides on weed control in low land rice. Indian J. Weed Sci. 25: 105-107.

Bali, A.S. et al. 1994. Weed control in transplanted rice (*Oryza sativa*). Indian J.Agron. 39(1):16-18.

Bernsaor, P.C. and De Datta, S.K.1986. Chemical and cultural control of bulrush (*Scirpus maritimus*L.) and annual weeds in lowland rice. Weed Res. 26(4): 233-244.

Carvalho, F.T. et al. 1999. Efficiency of clomazone for pre-emergence control of weeds in rice. EMBRAPA-Agropecuria-Oeste.No.3, 80-83.

Caseley, J.C. et al. 1997. Herbicides with alternative modes of action for the control of propanil and fenoxaprop-p-resistant *Echinochloa colona*. Brighton crop Protection Conference: Weeds. Proc. Off an Int. Conf, Brighton,UK, 17-20, November,1997, Vol.1 : 215-220.

Chaudhury, A.N. et al. 1995. Effect of weed management practices on nutrient loss and yield of rice in Nagaland. Indian J. Agron. 40: 193-197.

De Datta,S.K. 1977. Approaches in the control and management of perennial weeds in Rice. In: Proc. 6th Asian-Pac.Weed Sci.Soc.Conf.Jakarta, Indonesia:204-206.

Gautam, K.C. and Mishra, J.S. 1995. Problems, prospects and new approaches in weed management. Pestic. Information 21: 7-19.

Ghosh, D.C. and Ganguly, S.1993. Cultural factors affecting weed infestation and crop productivity in wetland transplanted rice. Indian Soc.Weed Sci. Vol. 3: 30-33.

Ho, N.K. and Zuki, I.M.1988. Weed population changes from transplanted to direct seeded rice in the Muda area. In:Proc. Of the Nat. Sem. And Workshop on Rice Field Weed Management: 55-67.

Jena, S.N. and Mishra, S.S. 1992. Chemical weed control in transplanted rice. Ann. Conf. Indian Soc. Weed Sci. Hisar on 3-4, March. P.35.

Jordan, D.L. and Kendig, J.A.1998. Barnyard grass (*Echinochloa crus-galli*) control with post-emergence applications of propanil and clomazone in dry seeded *rice (Oryza sativa)*.Weed Tech. 12(3):537-541.

Kandasamy, O.S. and Sankaran, S.1995. Weed succession of long-term application of herbicides in lowland rice system. In.Proc.15th Asian-Pac.Weed Sci. Soc.Conf.Vol.II, Tsukuba, Japan, July 25-28.1995:129-131.

Kim, K.U.1983. Control of perennial weeds in rice in temperate zones In:Int.Rice Res.Inst., Weed Control in Rice, Los Banos, Laguna, Philippines 234-255.

Kurmi,K. and Das, G.R.1993. Effect of herbicide on weed control in transplanted rice.Indian Soc. Weed Sci. Vol III:27-29.

Mandal, B.K. et al. 1986. Relative efficiency of selected herbicides for controlling weeds in transplanted rice. Oryza 23:242-248.

Mukhopadhyay, S.K.1968. Major weed flora in the crop fields of West Bengal and recommendations on their control measures. Farm J. 9(8):13-28.

Mukhopadhyay, S.K.1969. Weeds and weed control in field crops. Food Fmg. And Agric. 295:19-20.

Mukhopadhyay, S.K.1971. Nature of weed problems in different rice culture and advances in weed control in rice fields. Paper Presented at the All India Workshop on Crop Protection, Lucknow, 8-11 November.

Raju, R.A. and Reddy, M.N. 1995. Performance of herbicide mixture for weed control in transplanted rice (*Oryza sativa*). Indian J. Weed Sci. 27: 106-107.

Ray,B.R.1973. Weed control in rice : A review .Indian J. Weed Sci.5(1):60-71.

Sahu, B.N. and Bhattacharya, T.K.1964. Effect of 2,4-D and MCPA with and without cultural practices on the control of weeds and the growth and yield of rice. Rice Newsletter. 12:21-29.

Scherder, E.F. et al. 2000. Clomazone for control of propanil resistant and susceptible barnyard grass in rice. Research Series-Arkanas-Agricultural-Experiment-Station.No.476:116-130.

Singh, D.J. and Rao, K.1970. Studies on weed control in rice. Indian J.Weed Sci.2(1):31-36.

Spiridonov, Y.Y. et al. 1995. Herbicidal preparations of the new generations in weed control. Report 2. Effectiveness of Command in weed control in Soybean and rice plantations in far east region of Russia Agrokhimiya.2:86-94.

Talbert, R.E. et al. 1999. Confirmation, population genetics and control of propanil-resistant and susceptible barnyard grass. Research-Series-Arkanas-Agricultural-Experimen-Sation.468:103-118.

Webster, E.O. and Baldwin, F.L.1998. Clomazone use in rice. Research-Series-Arkanas-Agricultural-Experiment - Sation.460: 91-94.

Webster, E.P. et al. 1999. The potential for clomazone use in rice (*Oryza sativa*). Weed Tech.13(2):390-393.

Evaluation of Pre-Mix Formulation of Clomazone + Pendimethalin for Weed Control in Cotton (*Gossypium hirsutum*)

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Abstract: Field experiments were conducted during the years 1999-2000 and 2000-2001 to evaluate the usefulness of pre-mix EC formulation of clomazone + pendimethalin for weed management in irrigated cotton. Dosages of clomazone + pendimethalin applied as pre-emergence soil surface spray within two days after sowing were 175 + 350, 225 + 450, 300 + 600 and 375 + 750 g ha^{-1}. These treatments were compared against weedy check, weed free treatment and stand alone application of clomazone @ 525 g ha^{-1} and pendimethalin @ 1000 g /ha. Per cent weed control was the lowest and dry weight of weeds was the highest in weedy check plot followed by treatments comprising clomazone + pendimethalin @ 175 + 350 g ha^{-1}, 225 + 450 g ha^{-1} and stand alone application of clomazone @ 525 g ha^{-1} and pendimethalin @ 1000 g ha^{-1}. Percent weed control was the highest and the dry weight of weeds was the lowest under the treatments of clomazone + pendimethalin @ 300 + 600 g ha^{-1} and 375 + 750 g ha^{-1} and weed free treatment. The former two treatments gave effective control of *Echinochloa colonum, Commelina benghalensis, Cyanotis axillaris, Digera arvensis, Euphorbia hirta, Trianthema portulacastrum, Paspalum conjugatum* and *Digitaria sanguinalis*. Seed Cotton yield was the highest under the treatments clomazone + pendimethalin @ 300 + 600 and 375 + 750 g ha^{-1} which was at par with that obtained under weed free treatment.

Key words: bio-efficacy, pre-mix formulation and seed cotton

INTRODUCTION

One of the important factors responsible for low yield of cotton in India is competition offered by weeds. Losses in cotton yield range from 40 to 85 per cent depending upon the nature and intensity of weed infestation and duration of their competition (Babiker et al. 1986; Balyan et al. 1983; Bhan and Mishra 1993; Brar and Gill 1983; Jain et al. 1981; Keeley and Thullen 1989; Sandhu et al. 1996; Street et al. 1985 and Thind et al. 1995).

Initial period of 30-35 days is the most critical for crop-weed competition in cotton (Jaya kumar et al. 1990 and Bhan and Mishra 1993). Thus, weed control during early stages is very important to minimize competition and attain high yields. Farmers often resort to conventional methods of weed control like hand weeding and hoeing to contain weeds. These methods besides being expensive and ineffective are not ideal because reinfestation of weeds takes place after each hoeing. Coincidence of rainy season with weed infestation makes hoeing impractical. Evaluation of herbicides to overcome these problems has led to the recommendations on the use of pre-emergence herbicides such as diuron, pendimethalin, fluchloralin, trifluralin, alachlor and metolachlor (Brar et al. 1995; Brar et al. 1999; Kurlekar and Khuspe 1979 and Panwar and Malik 1988). Non-availability of some of these herbicides at the right time and the requirement of pre-plant incorporation in the case of some others have restricted the herbicide options available to farmers. Viewed in this context, there is an imperative need to widen the choice of herbicides available to farmers. The present investigations are undertaken to evaluate the usefulness of pre-mix formulation of clomazone + pendimethalin for weed management in cotton.

MATERIALS AND METHODS

Field investigations were carried out for two years in the State of Karnataka during the Kharif season (June to December) of 1999-2000 and 2000-2001 in a field with medium black soil. One pre-sowing irrigation was done and cotton variety var. LH 1556 was sown in a finely prepared seedbed by dibbling four seeds per hill on May 20, 1999 and June 01, 2000 respectively in the two years. Sowing was done in such a manner that row-to-row spacing of 1.0 m and plant-to-plant distance of 0.75 m was maintained. About one month after sowing, extra plants were removed keeping one plant per hill. Phosphorous @ 30 kg P_2O_5 ha^{-1} and potash @ 30 kg K_2O ha^{-1} given before sowing. Nitrogen @ 100 kg ha^{-1} was applied in two equal splits at thinning and at flowering. First irrigation was given about four weeks after sowing and subsequent ones as and when needed.

Treatments of clomazone + pendimethalin @ 175+350, 225+450, 300+600 and 375+750 g ha^{-1} were compared with stand alone application of clomazone @ 525 g ha^{-1}, pendimethalin @ 1000 g a.i.ha $^{-1}$, weed free treatment and weedy check plots. The plot size was 6 m x 6 m. The treatments were laid out in randomized block design with four replications. All the herbicide treatments were imposed as pre-emergence, blanket soil surface spray two days after sowing using a bakpak sprayer fitted with flat fan nozzle and a spray volume of 750 litres per hectare.

Visual phytotoxicity of herbicide treatments on crop was recorded at five and ten days after sowing on a scale of 1 to 10 (1= no adverse effect of the herbicide on the crop and 10 = 100 per cent adverse effect of the herbicide on the crop). Species-wise count of weeds and dry weight of weeds were recorded at 45 days after sowing within 1.0 m x 1.0 m quadrat at three places at random in each plot. Dry weight of weeds under each treatment was recorded after drying the weeds in an oven at 70^0 C until constant weight was achieved. Seed cotton yield was recorded by harvesting five times

RESULTS AND DISCUSSION

Phytotoxicity

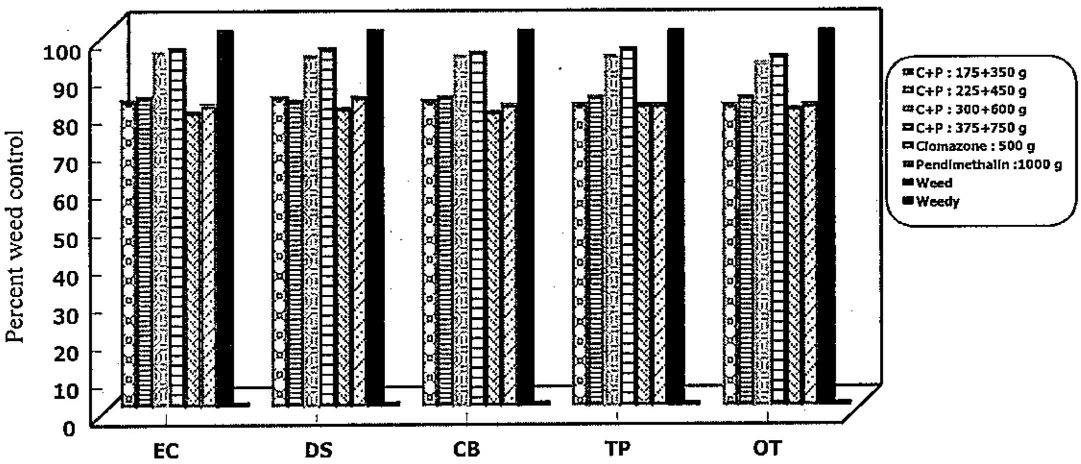
Cotton plants in the experimental plots treated with clomazone alone and combination of clomazone and pendimethalin exhibited light yellowing of cotyledonary leaves of about 20% plants after imposing treatment. The plants became normal at 10 days after sowing. These treatments did not lead to leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty.

Weed flora

E. colonum, D. sanguinalis, C. benghalensis and *T. portulacastrum* were the predominant weeds in the weedy check plots at 45 days after sowing. *Amaranthus viridis, Digera arvensis, Euphorbia hirta, C. axillaris* were the other weeds noticed in the weedy check plot. Infestation of the weeds was highest in the weedy check plots.

Weed control

Treatment with pre-mix formulation of clomazone + pendimethalin at 175+350 and 225+450 g ha^{-1} gave 78-83% control of all weeds (Fig. 1). These treatments were comparable to stand alone application of clomazone @ 525 g ha^{-1} and pendimethalin @ 1000 g ha^{-1}. Similar reduction in population of *T. portulacastrum* and other weed species due to treatment with pendimethalin was noticed in earlier studies (Brar et al. 1995; Karlekar and Khusik 1979; Panwar and Malik 1988). Treatment with the pre-mix formulation of clomazone + pendimethalin @ 300+600 and 375+750 g ha^{-1} gave more than 90% control of *E. colonum, D. sanguinalis, C. benghalensis* and *T. portulacastrum* and the others comprising *D. arvensis, E. hirta, C. axillaris* and *Paspalum conjugatum.*



EC: *Echinochloa colonum* DS: *Digitaria sangunalis* ; CB: *Commelina benghalensis*
TP: *Trianthema portulacastrum* OT: Others (see

Figure 1. Effect of treatments on weed control (Mean of 1999-2000 and 2000-2001

Dry weight of weeds

Weedy check treatment recorded the highest dry weight of weeds (Table 1). Among the herbicide treatments, application of pre-mix formulation of clomazone + pendimethalin @ 175+350, 225+450 g ha^{-1} recorded the highest dry weight of weeds and these treatments were comparable to stand alone application of clomazone @ 525 g ha^{-1} and pendimethalin @ 1000 g ha^{-1}. Treatment with pre-mix formulation of clomazone + pendimethalin @ 300+600 and 375+750 g ha^{-1} recorded the lowest dry weight of weeds which was comparable to that noticed in weed free treatment.

Table 1. Effect of herbicide treatments on dry weight of weeds at 45 days after sowing.

Treatments (g a.i. ha^{-1})	Dry weight of weeds (g m^{-2}) *				
	Echinochloa colona	*Digitaria sanguinalis*	*Commelina benghalensis*	*Trianthema portulacastrum*	Others **
Clomazone + Pendimethalin: 175 + 350	6.8	5.8	5.9	8.9	6.5
Clomazone + Pendimethalin: 225 + 450	6.4	5.1	5.3	7.0	5.7
Clomazone + Pendimethalin: 300 + 600	1.6	1.9	1.3	3.1	2.3
Clomazone + Pendimethalin: 375 + 750	1.1	1.1	0.9	2.2	1.2
Clomazone: 525	7.3	4.5	6.5	9.1	7.3
Pendimethalin: 1000	6.8	5.8	6.1	7.8	6.6
Weed free	0.2	0.4	0.2	0.4	0.5
Weedy check	26.2	28.8	33.7	37.8	21.7
CD at 5%	2.9	2.8	3.1	4.1	3.2

DAS : Days after spraying
* Mean of 1999-2000 & 2000-2001 ; ** see text

Yield

Lowest seed cotton yield was recorded in weedy check plots (Table 2). A significant improvement in yield was noticed under the treatment of clomazone + pendimethalin @ 175+350 and 225+450 g ha^{-1}, clomazone @ 525 g ha^{-1} and pendimethalin @ 1000 g ha^{-1}. Higher yields consequent on superior weed control due to treatment with pendimethalin has earlier been reported (Panwar et al. 1989 and Singh et al. 1988). A further significant improvement of yield which was comparable to that obtained under weed free treatment was evident in plots treated with pre-mix formulation of clomazone + pendimethalin @ 300+600 and 375+750 g ha^{-1}.

It could, thus, be concluded that treatment with pre-mix formulation of clomazone + pendimethalin @ 300 + 600 and 375+750 g ha^{-1} offered broad spectrum weed control in cotton which is superior to that obtained under stand alone application of pendimethalin and clomazone. Bio-efficacy against weeds and seed cotton yield due to treatment with clomazone + pendimethalin @ 300 + 600 g ha^{-1} were comparable to that under treatment with clomazone + pendimethalin @ 375+750 g ha^{-1}. Therefore, application of clomazone + pendimethalin @ 300+600 g ha^{-1} was adequate for effective weed control and enhancing seed cotton yield.

Table 2. Influence of herbicide treatments on seed cotton yield.

Treatments (kg a.i. ha^{-1})	**Seed Cotton yield (kg ha^{-1})**
Clomazone + Pendimethalin: 175 + 350	1082
Clomazone + Pendimethalin: 225 + 450	1136
Clomazone + Pendimethalin: 300 + 600	1406
Clomazone + Pendimethalin: 375 + 750	1425
Clomazone: 525	1041
Pendimethalin: 1000	1021
Weed free	1450
Weedy check	506
CD at 5%	175

* Mean of 1999-2000 & 2000-2001

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LITERATURE CITED

Babiker, A.G.J. et al. 1986. Effect of application time on herbicidal efficacy of oxasiazon on cotton in the Sudan Gazira. Weed Res. 26: 51-57.

Balyan, R.S. et al. 1983. The effect of weed removal at different times on the yield of seed ctton. Cotton Develop. 13: 9-10.

Bhan, V.M. and Mishra, J.S. 1993. Improving crop productivity through weed management. Pesticides Information. XIX (3): 25-36.

Brar, H.S. and Gill, H.S. 1983. Studies on critical period of crop weed competition in cotton. Indian J. Ecol. 10: 148-153.

Brar, A.S. et al. 1999. Integrated weed management in American cotton. J. Res.Punjab aric. Univ. 36(3&4): 194-198.

Brar, A.S. et al. 1995. Integrated weed management in upland cotton (*Gossypium hirsutum*). Indian J. Weed Sci. 27(3&4): 138-143.

Jain, S.C. et al. 1981. Nutrient competition studies in cotton (*Gossypium hirsutum*) under different weed control systems in Madhya Prades. Indian J. Weed Sci. 13: 18-25.

JayaKumar, R. et al. 1990. Interference period of weeds with cotton. Pestology XLV (10): 28-29.

Keeley, P.E. and Thullen, R.J. 1989. Growth and competition of Black Nightshade (*Solanum nigrum*) and Palmer Amaranth (*Amaranthus palmer*) with cotton (*Gossypium hirsutum*). Weed Sci. 37: 326-334.

Kurlekar, V.G. and Khuspe,V.S. 1979. Studies on weed control in cotton. Indian J. Agron. 24: 377-382.

Panwar, R.S. and Malik, R.K. 1988. Integrated weed management in cotton. J. Cotton Res. & Dev. 2: 50-56.

Panwar, R.S. et al. 1989. Evaluation of pre-emergence and post-emergence herbicide in Cotton, Haryana Agric. Univ. J. Res. 19 : 235-239.

Sandhu, K.S. et al.1996. Crop weed competition in American cotton (*Gossypium hirsutum).* Indian J. Weed Sci. 28 : 171-172.

Singh V. et al. 1988. Pattern of dry matter production under weed control systems and nitrogen level in cotton. J. Indian Soc. Cotton Imp. 13(2) : 163-168.

Street, J.E. et al. 1985. Competition of binary weed system with cotton (*Gossypium hirsutum).* Weed Sci. 33: 807-809.

Thind, R.J.S. et al.1995. Weed, interference in American cotton. Indian J. Weed Sci. 27(1&2): 75-79.

Control of Fern, *Stenochlaena palustris*, Using Paraquat Mixed with Triasulfuron

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Abstract: A field experiment was conducted in a mature oil palm plantation to determine the efficacy of paraquat and sulfosate mixed either with triasulfuron or metsulfuron methyl for the control of fern *Stenochlaena palustris*. *S. palustris* could only be controlled by paraquat treatment. The greatest efficacy was found in paraquat 300 g a.i. ha^{-1} mixed with triasulfuron 15 g a.i. ha^{-1} or with metsulfuron methyl 15 g a.i.ha^{-1} and in paraquat 600 g a.i ha^{-1}. The mortality ranged from 91.6% to 93%. The mortality of *S. palustris* treated with paraquat 300 g a.i. ha^{-1} was not different from those treated with paraquat 200 g a.i. ha^{-1} mixed with triasulfuron or metsulfuron methyl at 10 g a.i. ha^{-1}. The mixtures of paraquat with triasulfuron or metsulfuron methyl did not only result in higher mortality of *S. palustris* but also suppressed the weed longer than those treated with paraquat alone. In contrast, sulfosate applied either single or mixed with triasulfuron or metsulfuron showed poor control on *S. palustris*.

Key words: metsulfuron methyl, paraquat, *Stenochlaena palustri*, .triasulfuron

INTRODUCTION

In the last decade, the development of new oil palm plantations has turned from mineral soil to peat soil due to the limitation of mineral soil for new oil palm plantation. The most common weed species associated with oil palm grown on peat soil is fern, especially *Stenochlaena palustris*.

Herbicide application is one of the most common tools for controlling weeds in oil palm plantations nowadays (Chia and Badrulisam, 1997; Sriyani *et a*l. 2001). The herbicide usually offers a more effective and efficient tool in controlling weeds than other tools especially in estates located in a region where labor is expensive. Herbicide application for controlling weeds in a plantation is generally repeated every two to three months because weed growth interferes with crop management. In Indonesia, paraquat has been widely used as a nonselective post emergence herbicide for controlling weeds in oil palm plantations because it acts very quickly to kill the target plants. The study aimed to determine *S. palustris* control using paraquat combined with triasulfuron and metsulfuron methyl.

MATERIALS AND METHODS

A field experiment was carried out in September to December 2001 in an oil palm plantation on peat soil in Labuhan batu Regency, North Sumatra, Indonesia. The oil palm trees were planted in 1994. The field was infested mainly by weed species of *S. palustris*. Herbicides tested were applied in the first week of September 2001. The rates of herbicide treatments used in the study are shown in Table 1. Each herbicide was applied using a lever operated knapsack sprayer with the spray volume of 500 L ha^{-1}. Water used for diluting herbicides was collected from a well in the peat soil area. The plot size was 5 m x 18 m laid down on the inter-rows of oil palm trees.

Table 1. Rates for herbicide treatments used in the study.

Herbicide	Rate (g a.i. ha^{-1})
Paraquat	200
Paraquat + Metsulfuron methyl	200 + 10
Paraquat + Triasulfuron	200 + 10
Paraquat	300
Paraquat + Metsulfuron methyl	300 + 15
Paraquat + Triasulfuron	300 + 15
Paraquat	600
Metsulfuron methyl + Agristick	30 + 2.5% (v/v)
Triasulfuron + Agristick	30 + 2.5% (v/v)
Sulfosate	480
Sulfosate + Metsulfuron methyl	480 + 15
Sulfosate + Triasulfuron	480 + 15

The treatments were arranged in a Randomized Complete Block Design with three replicates. Visual assessment of plant damage (% of foliar control) was made at one, two and four weeks after application (WAA). Coverage of re-growth of the plant was assessed at six to 12 WAA. Data were subjected to ANOVA analysis.

RESULTS AND DISCUSSION

Percent control of *S. palustris* is shown in Table 2. Percent control of *S. palustris* by paraquat at 600 g a.i. ha^{-1} was not different from that of the two combinations, paraquat 300 g a.i. ha^{-1} + 15 g metsulfuron methyl a.i. ha^{-1} and 300 g a.i. ha^{-1} triasulfuron. The three-herbicide treatments controlled *S. palustris* ranging from 91 to 93% at 4 WAA. Similarly, control of *S. palustris* with the application of 300 g a.i. ha^{-1} paraquat was not different from those treated by combinations of paraquat at 200 g a.i. ha^{-1} + metsulfuron methyl 10 g a.i. ha^{-1} and paraquat 200 g a.i. ha^{-1} + triasulfuron 10 g a.i. ha^{-1}. The three treatments controlled of *S. palustris* by 83 to 85%. It is obvious that the application of paraquat + metsulfuron methyl and paraquat + triasulfuron as mixtures increased efficacy on *S. palustris*.

On the other hand, metsulfuron methyl + Agristick, triasulfuron + Agristick, sulfosate, sulfosate + metsulfuron methyl and sulfosate + triasulfuron did not control *S. palustris* satisfactorily. Coverage of *S. palustris* at six to 12 WAA is shown in Table 3. The application of paraquat + metsulfuron methyl and paraquat + triasulfuron as mixtures resulted in lower percent of coverage of *S. palustris*. Both mixtures of 300 g paraquat + 15 g metsulfuron methyl ha^{-1} and 300 g paraquat + 15 g triasulfuron ha^{-1} resulted in 30% coverage of *S. palustris* of which were much lower than that of 300 g paraquat

Table 2. Visual percent control of *S. palustris* by the herbicides used.

Herbicide	Rate ($g\ a.i.\ ha^{-1}$)	% control (WAA)		
		1	2	4
Paraquat	200	63.3 b	70.0 b	73.3 b
Paraquat + Metsulfuron methyl	200 + 10	70.0 bc	76.6 bc	83.3 c
Paraquat + Triasulfuron	200 + 10	73.3 cd	78.3 c	85.0 c
Paraquat	300	75.6 cde	80.0 c	85.0 c
Paraquat + Metsulfuron methyl	300 + 15	80.0 de	81.6 c	93.0 d
Paraquat + Triasulfuron	300 + 15	75.0 cde	81.6 c	91.6 d
Paraquat	600	83.0 e	88.3 d	91.6 d
Metsulfuron methyl + Agristik	30 + 2.5% (v/v)	5.0 a	5.0 a	8.3 a
Triasulfuron + Agristick	30 + 2.5% (v/v)	5.0 a	5.0 a	5.0 a
Sulfosate	480	5.0 a	5.0 a	5.0 a
Sulfosate + Metsulfuron methyl	480 + 15	5.0 a	5.0 a	5.0 a
Sulfosate + Triasulfuron	480 + 15	5.0 a	5.0 a	5.0 a

Means followed by the same letter in the same column are not significantly different at the 0.05 level according to LSD test.

Table 3. Visual coverage of *S. palustris* assessed 6, 8, 10, and 12 WAA.

Herbicide	Rate ($g\ a.i.\ ha^{-1}$)	% coverage (WAA)			
		6	8	10	12
Paraquat	200	35 c	41.6 b	53.3 b	80 b
Paraquat + Metsulfuron methyl	200 + 10	18.3 de	26.6 c	38.3 c	61.6 cd
Paraquat + Triasulfuron	200 + 10	23.3 d	26.6 c	8.3 c	56.6 d
Paraquat	300	16.7 e	25.0 c	36.6 c	71.6 bc
Paraquat + Metsulfuron methyl	300 + 15	8.3 f	16.6 cd	20.0 d	30 e
Paraquat + Triasulfuron	300 + 15	8.3 f	15.0 cd	21.6 d	30 e
Paraquat	600	6.7 f	11.6 d	16.6 d	35 e
Metsulfuron methyl + Agristick	30 + 2.5% (v/v)	93.3 b	100 a	100 a	100 a
Triasulfuron + Agristick	30 + 2.5% (v/v)	100 a	100 a	100 a	100 a
Sulfosate	480	95 ab	100 a	100 a	100 a
Sulfosate + Metsulfuron methyl	480 + 15	96.7 ab	100 a	100 a	100 a
Sulfosate + Triasulfuron	480 + 15	95 ab	100 a	100 a	100 a

Means followed by the same letter in the same column are not significantly different at the 0.05 level according to LSD test.

without mixture (coverage of 70%) at 12 WAA. These results mean that the mixtures of paraquat + metsulfuron methyl and paraquat + triasulfuron suppressed *S. palustris* longer than paraquat without combination. It is not understood how the mixtures of paraquat + metsulfuron methyl, and paraquat + triasulfuron work synergistically in controlling *S. palustris*. In fact, paraquat is a quick acting contact herbicide whereas the sulfonylureas are systemic herbicides (WSSA 1989). In contrast, metsulfuron methyl or triasulfuron (30 g a.i. ha^{-1}) applied singly could not control fern, *S. palustris*. Similarly, sulfosate at 720 g a.i. ha^{-1} applied either singly or mixed with triasulfuron or metsulfuron methyl can not also control *S. palustris*. In conclusion, the study indicates that *S. palustris* could only be controlled by using paraquat. Triasulfuron and metsulfuron methyl at 50 to 75 g. ha^{-1} could increase the efficacy of paraquat in controlling fern, *S. palustris*.

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LITERATURE CITED

Chia, T. H. and Badrulisam, J. 1997. Herbicidal effect in the development of parthenocarpy in oil palm. Proc. 16th APWSS Conf. Kuala Lumpur. p. 303-305.

Sriyani, N., Mawardi, D., Lubis, A. T., Indarto, and Sugianto. 2001. Efficacy of glyphosate 48% herbicide to control weeds in oil palm and rubber plantations. Proc.XV. Weed Sci. Soc. Indonesia, Surakarta. P. 326-334.

WSSA. 1989. *Handbook of Herbicide*. Sixth Edition. WSAA, Champaign, Illinois. 301pp.

Dissipation and Mobility of Butachlor and Pretilachlor in Broadcast-seeded Wetland Rice

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Abstract: A laboratory experiment was conducted to determine the test species suitable for bioassay of herbicide residue and a field experiment was conducted to study the persistence and mobility of butachlor and pretilachlor in broadcast-seeded wetland rice by determining the amount of herbicide residue at various soil depths and at different times after application through plant bioassay.

The laboratory experiment indicated that among the five plant species tested, barnyard grass (*Echinochloa glabrescens* Munroe ex Hook.f.) showed greatest sensitivity to both herbicides in terms of shoot length, hence was used as bioassay test plant for the herbicide dissipation experiment.

The field experiment showed that dissipation in water samples was rapid. Butachlor dissipated faster than pretilachlor at 0 day after treatment (DAT), the amount of butachlor and pretilachlor detected at 0 DAT was 4.25 and 7.5 ppm, respectively. The amount of pretilachlor decreased at a faster rate than butachlor at 3 DAT. Continuous decrease in the amount of herbicide was observed until 14 DAT. Pretilachlor and butachlor were not detected in the water sample from 21 to 84 DAT. Dissipation of butachlor and pretilachlor from the soil samples was faster than dissipation of both herbicides from the water sample. Both herbicides were detected only at 0 DAT at concentrations of 0.25 and 0.3 ppm, respectively. Butachlor and pretilachlor residues were only detected at the 0 to 5 cm depth indicating that the two herbicides were not mobile and ruling out the possible role of leaching in dissipation.

Key Words: bioassay, butachlor, dissipation, herbicide residue, mobility, pretilachlor

INTRODUCTION

Increasing concern on environmental safety has triggered research on the fate and effect of continuous use of pesticides to the environment. Most of these researches involved insecticides, particularly the old ones or those belonging to the organochlorines and organophosphates groups that control a broad spectrum of insects and which are relatively more persistent.

Herbicide use and development have been increasing in the tropics. With the demand for increased yields for an ever-increasing population, there has been a shift in the way rice is produced, from traditional transplanting to direct seeding, due to high cost of production in the former. With this development, herbicide usage has also increased since the weed problem is more serious in direct-seeded than in transplanted rice and consequently making herbicides a necessary input in direct-seeded rice. Herbicides have also been recently pointed out as among the causes of environmental problems. Most of researches on the fate of herbicides in the environment have been confined to temperate soil and climatic conditions which are far different from conditions in the tropics. Herbicide residue studies were also aimed at finding the safe recropping interval and determine the length of time a herbicide will persist and provide effective control of target weed species. Among the very few studies on fate of herbicides is the one conducted by Chen and Chen (1979) in Taiwan that indicated that temperature gave a significant effect on adsorption of butachlor (N-butoxymethyl 2-chloro-2', 6'-diethylacetanilide) by soil. In the Philippines, Mabbayad et al. (1986) reported that dissipation of butachlor in two soil types under laboratory and field conditions was very rapid. There was faster dissipation of the herbicide at higher moistures and higher temperature. Other studies were by Filho (1980) on oxadiazon (2-tert-butyl-4-[2-4-dichloro-5-isopropyloxyphenyl]-1,3,4-oxadiazoline-5-one) and by Berayon and Mercado (1983) on pendimethalin (N-[1-ethylpropyl]-3,4-dimethyl-2,6-dinitrobenzenamine) persistence. There is therefore a great need for tropical countries like the Philippines to generate information on the fate of commonly used herbicides in the soil including their movement or presence at different depths from the soil surface, with time.

Herbicide residues in the soil can be accurately measured and analyzed by chemical and physical procedures. But such quantitative measurements sometimes are not well correlated with plant response because of a number of interacting soil (physical and chemical properties) and environmental factors (rainfall and temperature) (Appleby 1985). Measuring herbicide residue through bioassay offers a more realistic assessment of the amount of the herbicide that is left in the soil over time, since this is the amount, which is available for plant uptake and various degradative processes. The use of both bioassay and chemical analysis to determine the amount of herbicide residues in the soil will surely enrich the data that will be generated.

This study was conducted to: 1) determine the appropriate bioassay test plant for butachlor and pretilachlor; 2) determine the persistence and mobility of butachlor and pretilachlor in a broadcast-seeded wetland rice environment through herbicide residue analysis using plant bioassay; and 3) determine the amount of residues of these herbicides along the soil column at different times after application.

MATERIALS AND METHODS

Determination of appropriate test plant for butachlor and pretilachlor

A laboratory experiment was done to determine the sensitivity of test plants to varying concentrations of butachlor and pretilachlor. Seeds of corn, mungbean, sesame, sorghum and barnyard grass were used to determine response of these plant species as bioassay plants for butachlor and pretilachlor. Twenty seeds of these test species were sown in petri dishes lined with filter paper. Ten mL of different concentrations of the two herbicides ranging from 25 ppm to 0.5 ppm were added. The experiment was laid out in a randomized complete block design with four replications. Germination as well as the general growth (e.g., root and shoot length) of the test species was noted. Shoot and root length of plants were measured after one week.Data were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's Multiple Range Test (DMRT). The test species with the greatest sensitivity to the two herbicides was used as bioassay plant.

Herbicide dissipation

A field experiment was conducted to determine dissipation and mobility of the two herbicides through plant bioassay. Nine concrete plots measuring 1.5 m x 2 m were used. The experiment was laid out using the randomized complete block design with three replications. Three treatments were maintained for this experiment, these are butachlor applied at 0.8 kg ai ha^{-1}, pretilachlor at 0.4 kg a.i. ha^{-1} and untreated check

Land preparation

Soils from two lowland sites were added to the soil in each plot. This was done to ensure the occurrence of weed species associated with lowland rice. The original soil found in the plots (Soil A) and the soils from the lowland areas (Soil B) were mixed separately to homogenize the soils. After mixing, soil was placed in each plot. Soil A was placed at the lower 25 cm while the upper 5 cm was composed of Soil B. The soil in each plot has a depth of 30 cm. It was prepared for lowland culture prior to planting. Plowing and rotavation was done manually in each plot.

Seed preparation, planting and application of treatments

Seeds of PSB Rc-58 were cleaned, soaked in continuously flowing water for 24 h and incubated for another 48 h prior to planting. It was sown in the plots at the rate of 100 kg ha^{-1}. The herbicides were applied at 8 DAS instead of 4 DAS due to unfavorable weather conditions. It was applied. Herbicides were applied using a calibrated sprinkler instead of a knapsack sprayer.

Residue Analysis

Soil and water samples were collected from each plot at 0, 3, 7, 14, 21, 28, 35, 42, 56, 70, 84, 98 days after treatment and at harvest. PVC pipes measuring 6 cm in diameter and 5 cm in length served as soil samplers. Prior to sampling, five samplers were piled one on top of the other and fastened by scotch tape to make to a total length of 25 cm., this served as the soil column. One soil column was implanted at all sampling times for each plot to obtain soil samples. Passing a carpenter's metal spatula through each soil sampler separated soil samplers from the columns. Soils in the sampler were placed in labeled plastic cups and represented soil from each depth. Soil from first (from the top), second, third, fourth and the lowest sampler represented 0-5, 5-10, 10-15, 15-20 and 20-25 cm depths, respectively. The soil at each depth was mixed and water was added to make a paste-like consistency.

Water samples were obtained from the top portion of each plot. Collected water samples were placed in small plastic ice cream cups. Twenty seeds of barnyard grass were sown in each cup containing the soil and water samples. All samples were maintained at the Department of Agronomy screen house.

Shoot length of 10 plants were measured from each treatment and compared to the shoot length of test plants subjected to known concentrations of test herbicides, which served as standard. For all sampling times, a standard was set up and maintained in the same area where the soil and water samples collected were maintained. The shoot length of the test plant that is equivalent to the standard indicated the level of herbicide residue present in the herbicide treated plots at all sampling times. Movement of the herbicides was noted by assessing the amount of herbicide at different depths (0-5, 5-10, 10-15, 15-20 and 20-25).

RESULTS AND DISCUSSION

Development of Bioassay Procedure for Butachlor and Pretilachlor

Effect of Butachlor on Shoot and Root Growth

The effect of butachlor on the shoot and root growth of test plants is shown in Table 1. Plant species used vary in their response to different concentrations of butachlor solution. Corn and mungbean shoot was not significantly affected by varying concentrations of butachlor. Sorghum plants treated with 5 to 25 ppm butachlor showed significantly shorter shoots than those plants treated with 1 and 0.5 ppm of the herbicide and also from the control. Sesame and barnyard grass were the only species whose shoot growth was affected by the varying butachlor concentrations. Sesame's shoot length was greatly reduced at 5 to 25 ppm butachlor concentrations. However, the shoot length of plants subjected to 1 and 0.5 ppm butachlor did not differ from the control.

Among the test species, barnyard grass (*Echinochloa glabrescens* Munroe ex Hook.f.) exhibited the greatest significant reduction of shoot length. Plants treated with 0.5 to 25 ppm butachlor had significantly shorter shoots than the control. Plants applied with 5, 10 and 25 ppm showed comparable shoot lengths. Shoot lengths of barnyard grass was reduced from 81 to 84% when applied with 5 to 25 ppm butachlor; application of 1.0 ppm and 0.5 ppm of the herbicide reduced shoot length by 73% and 47%, respectively.

Table 1. Effect of butachlor on the percentage (%) inhibition of shoot and root of test plant species.

Concentration	Test plant species									
(ppm)	Corn		Mungbean		Sorghum		Sesame		Barnyard grass	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
25	0	0	21	33	72	42	40	72	84	14
10	0	0	28	30	68	26	21	73	84	8
5	0	26	34	21	54	9	12	72	81	0
1	0	0	19	16	14	0	2	25	73	6
0.5	0	0	20	0	0	0	0	17	47	17
0	-	-	-	-	-	-	-	-	-	-

The different test plants had varying response to butachlor concentrations. Corn plants showed minimal sensitivity to the herbicide. Mungbean plants showed sensitivity at 1 to 25 ppm butachlor. Root length of mungbean plants treated with 0.5 ppm butachlor was not significantly different from the control. Sorghum plants showed greater sensitivity than mungbean and corn at 25 ppm. Root length of plants treated with 5 to 25 ppm butachlor was significantly shorter than those treated with 1 ppm and 0.5 ppm and the control.

Among the plants tested, sesame was greatly affected by butachlor. Roots of sesame were greatly reduced from 72 to 73% when treated with 5 to 25 ppm butachlor. At 0.5 to 1.0 ppm, root length was reduced from 17 to 25%.

Barnyard grass showed minimal reduction in root length indicating that the shoot was more sensitive to butachlor than the roots. Root length of the control plants differ significantly from root length of plants treated with 25 ppm and 0.5 ppm. However, plants treated with 1 ppm, 5 ppm and 10 ppm did not differ from the control.

Effect of Pretilachlor on Shoot and Root Growth

The effect of pretilachlor on the shoot and growth of the different test species is shown in Table 2. For corn and mungbean, the response to pretilachlor was the same as their response to butachlor application. Shoot length of corn plants treated with different pretilachlor concentrations did not differ from that of the control, suggesting that corn plants were not sensitive to the herbicide. Shoot length of mungbean varied with pretilachlor concentrations. Significantly shorter roots were observed among treated plants except for those treated with 1 ppm pretilachlor.

Table 2. Inhibition of the shoot and root of test plant species due to varying concentrations of pretilachlor.

Concentration	Corn		Mungbean		Sesame		Barnyard grass	
(ppm)	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
25	0	0	0	63	61	75	84	51
10	0	0	0	56	50	75	79	37
5	0	0	0	54	40	71	77	29
1	0	0	0	48	40	59	58	42
0.5	0	0	28	35	16	34	25	30
0								

Among the plant species tested, sesame and barnyard grass showed the greatest sensitivity to pretilachlor as what was observed with butachlor. However, there was greater reduction of shoot length of barnyard grass than the shoot length of sesame. Sesame plants treated with 0.5 to 25 ppm were significantly shorter than the control. It was observed that as the concentration increased, root length was reduced significantly. Inhibition of the shoot ranged from 16% to 60% when treated with 0.5 to 25 ppm, respectively.

Corn plants did not show any root inhibition. Roof lengths of the other test plants were greatly affected by varying concentrations of pretilachlor. Mungbean plants exhibited significantly shorter roots compared to the control however; plants treated with 1 to 10 ppm did not vary significantly from each other. Root of sesame was greatly reduced at 5 to 25 ppm pretilachlor. Inhibition of the roots was at 59% to 75% at 25 ppm. Inhibition of the roots was even higher than barnyard grass. Greatest inhibition of the root (51%) was observed at 25 ppm.

Results of the laboratory experiments agree with the findings of Elloran (1986). She reported that pretilachlor at 10^{-3} M completely inhibited germination of barnyard grass and rice. At concentrations of 10^{-7} and 10^{-6}, pretilachlor had little or no effect on germination but produced stunted or/malformed seedlings. Based on the results, barnyard grass was chosen as the bioassay plant for the field experiment on dissipation and mobility. Although sesame also exhibited sensitivity of shoot and root growth, some plants rotted at the early stages of the observation period. Its adaptability to lowland conditions has still to be studied and the cost of its use is another consideration. Barnyard grass on the other hand is readily available particularly in broadcast-seeded wetland rice areas where butachlor and pretilachlor are widely used. Barnyard grass showed great sensitivity to the two herbicides in terms of shoot growth and also with root growth. These test plants were not completely killed at the highest concentration of the two herbicides (25 ppm).

Herbicide Dissipation

Dissipation in Water Sample

The concentration of the herbicide that was detected at 0 DAT (3 h after treatment) was estimated, using the standard, to be 4.25 ppm and 7.5 ppm for butachlor and pretilachlor, respectively (Fig. 4). At 3 DAT, concentration of both herbicides decreased with pretilachlor having the greater decrease to 0.9 ppm; corresponding figure for butachlor was 2.6 ppm. Further decrease in the concentration of both herbicides was observed at 7 DAT. Concentration was detected to be 1.35 ppm and 0.47 ppm for butachlor and 0.8 ppm and 0.5 ppm for pretilachlor at 7 and 14 DAT respectively. At 21 DAT no herbicide was detected. Shoot length of test plants were comparable with those of the untreated plots and the 0 ppm in the standard. These results indicate that no herbicide was detectable from 21 to 84 DAT.

Dissipation of butachlor was observed to be very rapid than the dissipation of pretilachlor at O DAT in the water samples indicated by the higher amount of pretilachlor at 7.5 ppm compared with the 4.25 ppm for butachlor. However, the amount of pretilachlor detected decreased at a faster rate than butachlor after 3 days, declining further until 14 DAT. This is due to the differences in properties, particularly solubility of the two herbicides. Pretilachlor is more soluble at 50 mg L^{-1}, while butachlor solubility is 23 mg L^{-1} in water. Chen and Chen (1979) reported that maximum amounts of 2.16 ppm and 0.30 ppm for the 1st and 2nd cropping season was detected in water samples collected at 0 DAT. Furthermore, half-lives of butachlor dissipated from field water were found to be about 0.8 day in the 2nd crop and 5.5 days in the 1st crop. The difference in response of the two cropping season was attributed to the difference in climatic environments. The 2nd cropping was characterized as having a hot and shiny weather, whereas relatively cooler and rather cloudy weather was observed in the 1st cropping.

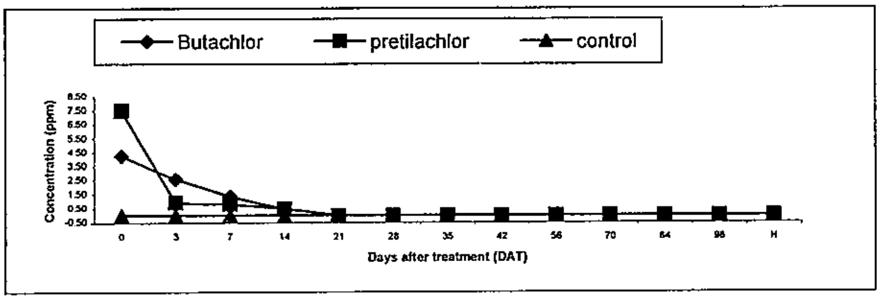

Figure 1. Dissipation of butachlor and pretilachlor in water

Dissipation in soil

Soil samples from varying depth were obtained at different times after herbicide application from the herbicide-treated and control plots. Response of barnyard grass in terms of shoot length indicated that the two herbicides were only detected at 0 DAT in the soil samples and was confined to the 0 to 5 cm depth (Fig. 2). The amount of butachlor and pretilachlor detected at 0 DAT was 0.25 and 0.3 ppm, respectively. At the lower soil depths, no herbicide was detected at 0 DAT. These amounts were reduced to zero at this soil depth at 3 DAT. At 7 DAT no herbicide residue can be detected at all soil depths indicating that both butachlor and pretilachlor were not mobile and that they stayed at the 0-5 cm depth, ruling out the possible contribution of leaching to loss of the herbicides.

Most of the reports on butachlor dissipation indicated higher amounts than what was recorded in this study. The differences in amounts obtained by other authors may be attributed to the method of determination used. Early works on butachlor and pretilachlor involved analysis through chemical means, which may also account for the amount of herbicide adsorbed by the soil particles and that may have been released or made available due to extraction by chemical means. Analysis through bioassay is different from chemical analysis because it does not account for the herbicide that is adsorbed but only the portion that is available for plant uptake and for other degradation processes. Thus, the amount of the herbicide reported in water and soil reflect the portion of what was applied that is available for uptake by weeds and rice.

The acetanilides to which butachlor and pretilachlor belongs, have sufficiently short half-lives under warm, moist field conditions to prelude their build up in the soils (Beestman and Deming 1974). This was also confirmed by succeeding works of several researchers who worked on butachlor under various environmental conditions (Chen and Chen 1979; Kulshresta et al 1981; Mabbayad et al 1986) and for pretilachlor (Suwanketnikom and Kaitboonyarit 1990). Major routes of degradation for butachlor were photodecomposition with several products already identified, adsorption and microbial decomposition.

In the experiments conducted, dissipation through photodecomposition may have contributed greatly to dissipation of both herbicides in the water sample, the effect of which was greater on pretilachlor since decrease in the amounts detected declined rapidly after application until 14 DAT.

Dissipation in soil was faster than in water since no amount of both herbicides were detected at 3 DAT. This may be attributed to absorption of the herbicide by the growing rice plants. Kulshresta (1987) reported that butachlor residues were detected in rice plants 35 days after herbicide treatment. Furthermore, rice grains at harvest contained 0.11 ppm butachlor (Kulshrestha et al 1981) and indicated that the herbicide were absorbed and translocated within the plant.

Another process that may affect dissipation of the two herbicides is adsorption by the soil. The soil used in the experimental plots contained 2.18% organic matter, high enough to hold herbicide particle tightly. Work done by Beestman and Deming revealed that dissipation from Ray silt containing 1% organic matter was two times faster than dissipaton from Wabash silty clay soil containing 3% organic matter. Suwanketnikom and Kaitboonyarit (1990) reported that pretilachlor was the second most adsorbed herbicide of the acetanilide family tested through soil thin layer chromatography. Only a very small fraction of the herbicide was mobile and the main portion remained at the site of application.

The amount of butachlor and pretilachlor that was detected in this study were lower from those obtained by Mabbayad et al. (1986). They reported that at 60 minutes after treatment the detectable amounts of butachlor were 10.2 ppm in loam soil and 8.6 ppm in silty clay loam soil. These figures are much higher than those obtained in this study. Furthermore, butachlor was detected up to 21 days after application in Mabbayad's study while in this study, butachlor in soil was only present at 0 DAT or 3 hours after application.

The results indicate that most of the butachlor and pretilachlor available for plant uptake is in the water. An important implication of these results is that the occurrence of heavy rains close to butachlor and pretilachlor application may reduce the effectiveness of these herbicides and enhance movement of the herbicides to other fields. Another important implication of these results is that in order to enhance the effectiveness of both herbicides in direct-seed rice, they should be applied during the dry season or during the early part of the dry season for wet cropping season. Butachlor and pretilachlor have short residual activity in water. Three weeks after application, no amount of both herbicides available for plant uptake was detectable in the water of treated fields.

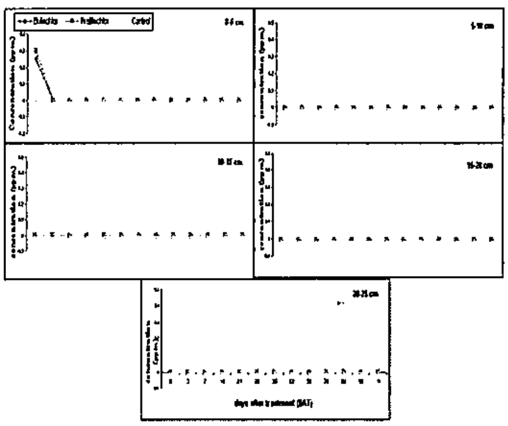

Figure 2. Dissipation of butachlor and pretilachlor in soil at 0-25 cm depth

LITERATURE CITED

Appleby, A.P. 1985. Factors in determining fate of herbicides in soil with bioassays. Weed Sci.33(2):2-6.

Beestman, G.B. and J. M. DEMING. 1974. Dissipation of acetanilide herbicides from soils. Agron. J. 66:308-311.

Berayon, B.E. and B.L. mercado. 1983. Persisitence of pendimethalin in the soil. The Phil Agric. 66(4):367-378.

Chen, Y.L. and J.S. Chen. 1979. Degradation and dissipation of herbicide butachlor in paddy fields. J. Pestic. Sci. 4:431-438.

Filho, A.S. 1980. Persistence in the soil and weed control efficacy of oxidiazon in lowland rice (oryza sativa L.) Unpubl. M.S. Thesis. University of the Philippines Los Banos, College, Laguna.

Kulshresta, G., N. T. yaduraju and V.S. Mani. 1981 Dissipation of butachlor and propanil herbicides in rice crop. Proc. 8th Asian Pacific. Weed Sci. Soc. Conf. Bangalore, India. Pp.469-473.

Kulshresta, G. 1987. Dissipation of herbicide bitachlor from different formulations in direct-seeded and transplanted rice crop. Pesticides 1987:20-24.

Mabbayad, M. O., K. Moody and A.M. Argente. 1986. Effect of soil type, temperature and soil moisture on butachlor dissipation. Philipp. J. Weed Sci. 13:1-10 .

Effect of Simulated Rainfall on Glyphosate Performance Against *Cynodon dactylon*

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Abstract: Two separate glasshouse experiments were conducted to study the effect of simulated rainfall on herbicide performance against *Cynodon dactylon*. Glyphosate formulated as Sting (first experiment) and Touchdown (second experiment) were used at the rates of 0 (control), 1.5, 1.5 + agral and 3.0 kg a.i. ha^{-1}. Paraquat at 1.0 kg a.i. ha^{-1} was also used as a comparison. Ten weeks old *Cynodon dactylon* plants were exposed to a heavy simulated rainfall (10 mm per minute for 3 minutes) at 2, 4, 8 and 24 hours after glyphosate application. The results have showed that injury from glyphosate was decreased when artificial rainfall was applied to *C. dactylon* soon after spraying. At 3.0 kg a.i. ha^{-1}, a 4 h rain free period was sufficient to get good control of *C. dactylon.* Addition of 1% Agral to 1.5 ka a.i. ha^{-1} of both forms of glyphosate increased their efficacy. Rain free periods of only 4 to 8 hours were enough to give good control when 1.5 kg a.i. ha^{-1} were applied with 1% Agral.

Key words: Common bermuda, herbicides, rainfastness, surfactants

INTRODUCTION

As the Cynodon dactylon is a highly competitive perennial grass, which has been ranked second in the world's worst weed (Holm *et al.* 1977). Having rhizomes and stolons makes this species particularly difficult to eradicate especially by mechanical weeding. Stolons and rhizomes cut into fragments can easily regrow and establish if conditions are suitable. The continued use of pre-emergence herbicides that control only annual weeds gives an opportunity for *C. dactylon* to spread. Wakabayashi (1982) suggested that for the effective control of perennial grasses, a toxic amount of foliage applied herbicide must translocate through the entire rhizome system. Hence, systemic post-emergence herbicides, i.e., glyphosate, fluazifop-P-butyl, glufosinate ammonium etc. could be used to control this grass. It has also been reported that certain environmental factors can influence the performance of post-emergence herbicides. Rainfall, soil moisture or drought, temperature, light and relative humidity has been shown to affect the activities of several herbicides (Baird and Begeman 1972; Turner 1981; Bovey *et al.* 1990; Reddy and Sing 1992). Rainfall can effect the efficacy of post-emergence herbicides greatly. Rainfall soon after application of foliar-applied herbicides may drastically reduce their efficacy on herbaceous weeds or woody plants. Numerous studies have reported the effect of rainfall on the efficacy of many foliar applied-herbicides (Caseley *et al.* 1975; Behrens and Elakkad 1976; Coupland *et al.* 1976; Andersen and Arnold 1985). However little is known about the influence of rainfall on the herbicide performance against *C. dactylon.* The objective of the study was to investigate the effect of times of simulated rainfall and the influence of surfactants on herbicide performance against *C. dactylon.*

MATERIALS AND METHODS

Two separate experiments (Sting® experiments) and (Touchdown® experiments) on the effect of simulated rainfall on herbicide performance on *C. dactylon* were conducted in the weed glasshouse. *C. dactylon* plants were established in the glasshouse by planting shoot propagules in 12.5-cm-diameter plastic pots (three shoots per pot) containing John Innes No. 1 compost. Plants were treated at the age of ten weeks after planting. Glyphosate formulated as Sting® and Touchdown® were used at the rates of 0 (control), 1.5, 1.5 + 1 % agral and 3.0 kg ai ha^{-1}. Paraquat at 1.0 kg ai ha^{-1} was also used as a comparison. *C. dactylon* plants were arranged in a 2 m^2 plot outside the glasshouse and herbicides were sprayed onto the plant using a 1 liter size laboratory pot sprayer which delivered about 450 - 500 l ha^{-1}. Herbicides were sprayed to the whole area of the plots, not only onto the plants. All plants were returned to the glasshouse and were separated into 5 groups for simulated rainfall treatments. At 2, 4, 8 or 24 hours after herbicide application the plant were exposed to a heavy simulated rainfall using a regulated hosepipe fitted with a fine spray nozzle to deliver water at the rate of 10 mm per minute for 3 minutes. Other plants were left without a rainfall treatment. Herbicide treated and untreated plants without a simulated rainfall treatment were also included as controls. All plants were kept growing for another eight weeks. The experiment was a factorial design with 4 replications.

RESULTS AND DISCUSSIONS

Table 1 and 2 show the results of the effect of simulated rainfall on glyphosate (Sting® and Touchdown®) efficacy on *C. dactylon.* Plants which received no rainfall treatments developed phytotoxic symptoms about a week after spraying, particularly with the higher doses of herbicides or the use of agral. Visual symptoms of glyphosate developed more slowly on plants subjected to rainfall treatments. Generally, injury from glyphosate was much less when artificial rainfall was applied to *C. dactylon.* This effect of rainfall lasted up to and including 8 hours after glyphosate spray at 1.5 kg ai ha^{-1}. Addition of surfactant (1% Agral) to 1.5 kg ai ha^{-1} of glyphosate significantly ($p<0.05$) increased damage when rainfall was given at 8 hours or earlier. So, rain free periods of only 4 to 8 hours for Touchdown® and Sting® respectively were enough to give good control (>77 % injury) when applied with 1 % Agral. Sting® and Touchdown® at 3.0 kg ai ha^{-1} need only 4 or more hours rain free periods to get good (>85 % injury) control of *C. dactylon.* Rainfall applied 2 hours after glyphosate spray significantly ($p<0.05$) reduced plant injury to the same extent for both formulations. On the average, herbicide damage from both Sting® and Touchdown® were reduced by about half with rainfall after about 1¾ to 2¼ (3.0 kg ai ha^{-1} and 1.5 kg ai ha^{-1} + Agral) or 3¾ hours (1.5 kg ai ha^{-1} alone) (Figure 1 and 2). As a comparison the effect of paraquat at 1.0 kg ai ha^{-1} was not affected by any rainfall treatments.

Table 1. Percentage of injury of C. *dactylon* as affected by the time of simulated rainfall after glyphosate (Sting®) spraying.

Herbicides	% injury					Means
	Simulated rainfall treatments (hours after herbicide spray)					
	2	4	8	24	no rain	
Paraquat 1.0 kg ai ha^{-1}	95	95	100	100	98	97.6
Glyphosate (Sting®) 3.0 kg ai ha^{-1}	52.5	86.3	91.3	93.3	96.3	83.9
1.5 kg ai ha^{-1} + 1 % Agral	38.8	67.5	77.5	77.5	81.3	68.5
1.5 kg ai ha^{-1}	13.8	46.3	57.5	73.8	82.5	54.8
Means	40.0	73.8	81.6	86.2	89.5	

LSD ($P<0.05$) for herbicide = 7.33   LSD ($P<0.05$) for rain = 8.20
LSD ($P<0.05$) herbicide*rain = 16.40

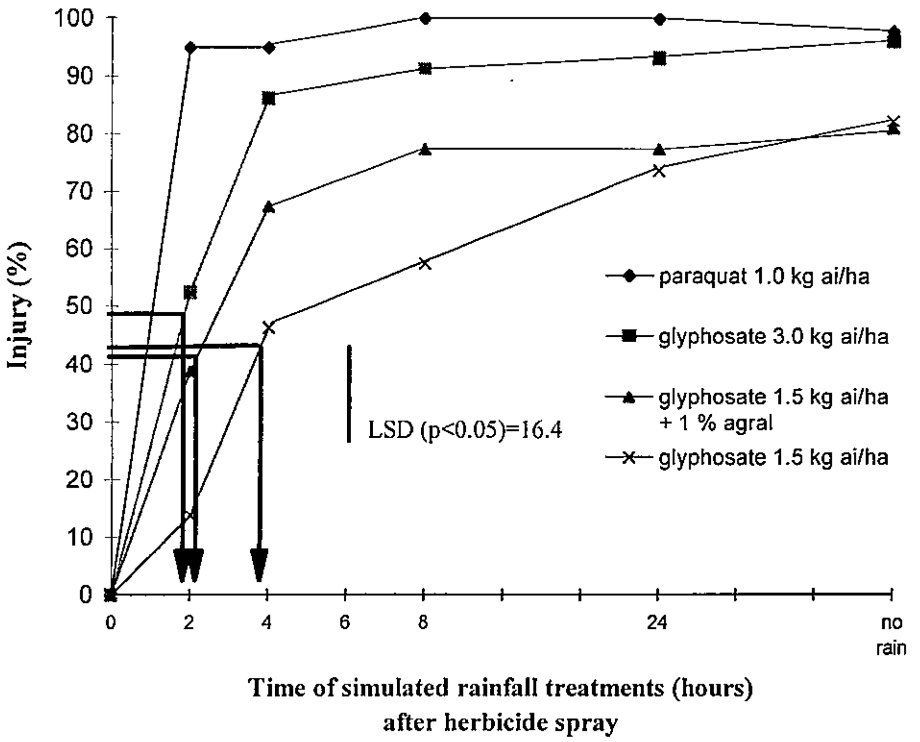

Figure 1. Time for 50 % loss of control by the effect of simulated rainfall on herbicide (Sting®) performance against *C. dactylon.*

Table 2. Percentage of injury of *C. dactylon* as affected by the time of simulated rainfall after glyphosate (Touchdown®) spraying

Herbicides	% injury (after 8 weeks) Simulated rainfall treatments (hours after herbicide spray)					Means
	2	4	8	24	no rain	
Paraquat 1.0 kg ai ha^{-1}	92.5	92.5	95.0	95.0	95.0	94.0
Glyphosate (Touchdown®)						
3.0 kg ai ha^{-1}	53.8	88.8	91.3	93.8	97.0	84.9
1.5 kg ai ha^{-1} + 1 % Agral	40.0	77.5	80.0	82.5	88.8	73.8
1.5 kg ai ha^{-1}	16.3	48.8	60.0	75.0	85.0	57.0
Means	50.7	76.9	81.6	86.6	91.5	

LSD ($P<0.05$) for herbicide =11.4 LSD ($P<0.05$) for rain = 11.3
LSD ($P<0.05$) herbicide*rain =14.5

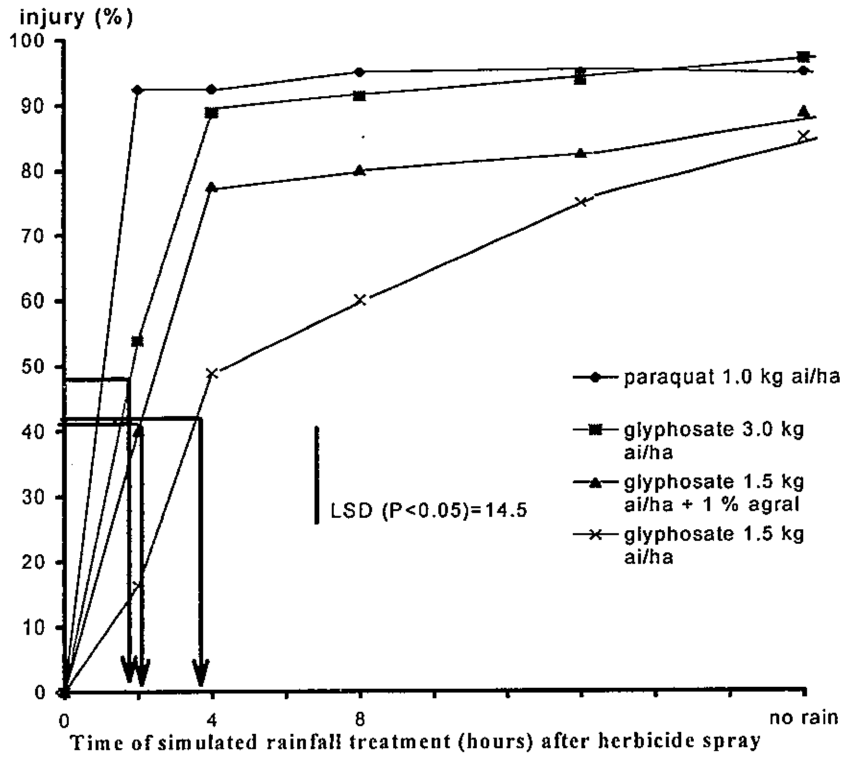

Figure 2. Time for 50 % loss of control by the effect of simulated rainfall on herbicide (Touchdown®) performance against *C. dactylon.*

The studies demonstrated that injury from glyphosate was much less when a heavy artificial rainfall (10 mm min^{-1}. for 3 min) was applied to *C. dactylon* soon after spraying. Behrens and Elakkad (1976) also reported that artificial rainfall at the rate of 1.0 mm $hour^{-1}$ for one hour starting at 5 minute after spraying severely decreased glyphosate activity and rainfall at the rate of 12.5 mm starting at 2 hours after herbicide application resulted in complete loss of its activity in *A. repens*. With both glyphosate formulations applications at the rate of 3.0 kg a.i. ha^{-1} needed only 4 or more hours rain free period to get good control of *C. dactylon*. This agrees with other reports, which indicate that a rain free period of 6-8 hours after spraying glyphosate is needed to give a good level of control (Baird and Begeman 1972 and Behrens and Elakkad 1972). However with 1.5 kg a.i. ha^{-1} alone, early rainfall prevented much herbicide damage. So the effect of rainfall after spraying glyphosate can be overcome by increasing the dose applied and the cost of application. The higher concentration of glyphosate in the spray solution would have increased the rate of foliar absorption. Coupland *et al.* (1976) also observed that increased doses of difenzoquat helped to overcome the effect of adverse environmental conditions on the control of *Avena fatua*.

Manipulation of formulation and/or application methods may reduce the risk of diminished glyphosate activity. The results have showed that by adding surfactant (1 % Agral) to 1.5 kg a.i. ha^{-1} glyphosate, good control could be obtained with rain free periods of only 4 or 8 hr for Touchdown® and Sting® respectively. Turner (1981) reported that adding 0.5 % Ethomeen surfactant to Roundup® increased its performance about fourfold when 5.0 mm of rain was applied to *A. repens* 1 hr after herbicide application. Reddy and Sing (1992) also reported that 'Kinetic', a silicon adjuvant enhanced glyphosate efficacy when *Cyperus esculentus* and *Panicum maximum* were subjected to post-spray rainfall at 60 minutes. The cost of using extra adjuvant would be less than doubling the doses of glyphosate applied. Applying a lower volume of water also has the effect of increasing both herbicide and adjuvant concentrations in the spray drops applied so this should also be investigated for *C. dactylon*.

When other environmental conditions during the critical period after spraying are favorable for uptake then less time is required for penetration of the herbicide, so the herbicide performance and its rain fastness are likely to improve. Caseley *et al.* (1975) demonstrated that there was less reduction in glyphosate activity on *E. repens* due to the wash off effect of heavy rain 2 hours after spraying when sprayed plants were kept at 90 % relative humidity compared to 50 % relative humidity, or when herbicide had been sprayed onto leaves which were already wet. The importance and significance of rain following the application of glyphosate or other foliage-applied herbicides depends on several factors, including the dose applied, rain intensity and the presence of appropriate types and amounts of surfactant. The absence of rain for periods of 8 hours or more increases the opportunity for sufficient amounts of herbicide to penetrate into the plant.

ACKNOWLEDGEMENT

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LITERATURE CITED

Andersen, D. M. and Arnold, W. E. 1985. Rainfall effects on desmedipham and phenmedipham performance. Weed Science. 33 : 391-394.

Baird, D. D. and Begeman, G. F. 1972. Post-emergence characterization of a new quackgrass herbicide. Proceedings of the Northeastern Weed Science Society. 26 : 100-106.

Behrens, R. and Elakkad, M. A. 1976. Influence of rainfall on phytotoxicity of foliar applied herbicides. Proceedings of the North Central Weed Control Conference. 31 : 141

Behrens, R. and Elakkad, M. A. 1972. Quackgrass control with glyphosate. Proceedings of the North Central Weed Control Conference. 27 : 54

Bovey, R. W., Mayer, R. E. and Whisenant, S. G. 1990. Effect of simulated rainfall on herbicide performance in Huisache (*Acacia farnesiana*) and Honey mesquite (*Prosopis glandulosa*). Weed Technology. 4 : 26-30.

Caseley, J. C. , Coupland, B. and Simmonds, R. C. 1975. The effect of precipitation on the control of *Agropyron repens* with glyphosate. Proceedings of the European Weed Research Society. 124-130p.

Coupland, D., Caseley, J. and Simmons, R. C. 1976. The effect of light, temperature and humidity on the control of *Avena fatua* with difenzaquat. Proceeding of Brighton Crop Protection Conference-Weeds. UK 47-53p.

Holm, L., Pancho, J. V., Plucknett, D. L. and Herberger, P. G. 1977. The world's worst weed : Distribution and Biology. University Press. Hawaii.

Reddy, K. N. and Sing, M. 1992. Organosilicone adjuvant effects on glyphosate efficacy and rain fastness. Weed Technology. 6 : 361-365.

Turner, D. J. 1981. The effect of additives on the control of *Agropyron repens* with glyphosate. Proceedings of the AAB Conference. Grass in Cereals in the UK. 167-175p.

Wakabayashi, H. 1982. Translocation of current pesticides developed in Japan. Japan Pesticide Information. Mitsubishi Chemical Industries Limited, Kanagawa, Japan. 41 : 8-13.

Efficacy of Sulfonylurea Herbicides against Perennial Paddy Weeds under Different Planting Depth, Application Time, and Temperature Conditions

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Abstract: Herbicidal responses of sulfonylurea herbicides to *Cyperus serotinus, Eleocharis kuroguwai, Sagittaris trifolia, and Potamogeton distinctus* were evaluated under different planting depths of perennial weeds, application time, and temperature conditions. Herbicidal efficacy of sulfonylurea herbicides was fairly good until 2 to15 cm planting depths, but was poor at more than 15 cm depth, and the control of *E. kuroguwai* decreased remarkably. Azimsulfuron, imazosulfuron, and ethoxysulfuron showed good control of *P. distinctus* regardless of application time, but herbicidal efficacy against *C. serotinus* was reduced with delayed application time. *E. kuroguwai* and *C. serotinus* were regrown by application of azimsulfuron and imazosulfuron at 5 and 10 days after planting, but were killed at 15 days after planting. Weed control efficacy of sulfonylurea herbicides to perennial weeds was highest at 30°C followed by 20 to 25°C, but was poor at 10 to 15°C.

Key words: application time, perennial paddy weed, planting depth, sulfonylurea herbicide, temperature, weed control efficacy

INTRODUCTION

Emergence patterns of perennial weeds are very different in paddy fields by planting depth (Pyon et al. 1990), temperature (Lee and Pyon 2001; Pyon et al. 1990; Pyon 1984). Weed control efficacies of perennial weeds are also different in paddy fields by application time of herbicides (Lim et al. 1991; Lee et al. 1994). Therefore, weed control efficacy of perennial weeds was examined under different planting depths of perennial weeds, application timing of sulfonylurea herbicides and temperatures conditions.

MATERIALS AND METHODS

Planting depth study

Tubers or rhizomes of perennial weeds were planted in 1/5,000 a Wagner pots at 2, 5, 10, 15, 20, 25 cm planting depths in the green house. Fertilizer was applied at 110-45-57 kg ha^{-1} $N-P_2O_5-K_2O$. Azimsulfuron at 15 g ha^{-1}, imazosulfuron at 75g ha^{-1}, and ethoxysulfuron at 21g ha^{-1} were applied at 10 days after planting. Weed control efficacy was examined at 50 days after treatments.

Application time study

Tubers or rhizomes of perennial weeds were planted in 1/5,000 a Wagner pots at 3 cm planting depth in the greenhouse. Azimsulfuron at 15 g ha^{-1}, imazosulfuron at 75 g ha^{-1}, and ethoxysulfuron at 21 g ha^{-1} were applied at 5, 10, 15, 20, 25 days after planting. Weed control efficacy was examined at 10 days interval until 60 days after treatments.

Temperature study

Tubers or rhizomes of perennial weeds were planted in pots (7 cm diameter) at 3 cm planting depth. Growing temperatures were 10, 15, 20, 25, 30°C in the growth chamber. Azimsulfuron at 15 g ha^{-1}, imazosulfuron at 75 g ha^{-1}, and ethoxysulfuron at 21 g ha^{-1} were applied at 10 days after planting. Weed control efficacy was examined at 50 days after treatments by measuring dry weight of weeds.

RESULTS AND DISCUSSION

Effect of planting depth on control of paddy perennial weeds by sulfonylurea herbicides

C. serotinus was controlled by azimsulfuron and imazosulfuron at 2 to 10 cm planting depths regardless of planting depths, but was not emerged at above15 cm depths (Table 1). Ethoxysulfuron controlled *C. serotinus* was controlled by 34% at 2 cm and 5% at 5 cm depths. *Eleocharis kuroguwai* and *Sagittaria trifolia* were controlled well at below 15 cm depths, but control efficacy of the 3 herbicides decreased at 20 and 25 cm depths. *Potamogeton distinctus* was controlled by the 3 herbicides regardless of planting depths.

Table 1. Control of perennial weeds at 50 days after treatments by three sulfonylurea herbicides under different planting depths.

Herbicides	Weed species	% weed control					
		Planting depth (cm)					
		2	5	10	15	20	25
Azimsulfuron	*C. serotinus* Rottb.	99.95	100	100	-	-	-
(15g ai ha^{-1})	*E. kuroguwai* Ohwi.	99.7	100	100	99.8	84.2	58.9
	S. trifolia Rottb.	98.2	98.5	100	94.1	65.9	-
	P. distinctus A. Benn.	97.6	100	99.4	100	99.2	98
Imazosulfuron	*C. serotinus* Rottb.	98.9	98.8	100	-	-	-
(75g ai ha^{-1})	*E. kuroguwai* Ohwi.	99.2	99.8	99.8	97.8	-	46.7
	S. trifolia Rottb.	97.8	93.5	97.0	100	82.5	-
	P. distinctus A. Benn.	100	99.4	99.3	98.8	98.8	98
Ethoxysulfuro	*C. serotinus* Rottb.	34.6	4.9	-	-	-	-
n	*E. kuroguwai* Ohwi.	90.9	97.0	99.6	97.3	74.6	57.0
(21g ai ha^{-1})	*S. trifolia* Rottb.	98.2	95.7	100	100	83.2	-
	P. distinctus A. Benn.	74.7	82.0	97.5	97.4	90.4	91.7

Effect of application time on control of paddy perennial weeds by sulfonylurea herbicides

As herbicide application time was delayed, weed control efficacy of 3 sulfonylurea herbicides against perennial weeds decreased, although control effect was higher in azimsulfuron and imazosulfuron than in ethoxysulfuron (Fig. 1). *C. serotinus* and *E. kuroguwai* were controlled well by azimsulfuron and imazosulfuron at 5 to 15 DAT, but control effects decreased at 20 and 25 DAT application (Table 2). *E. kuroguwai* was controlled by ethoxysulfuron at 5 DAT, but control effects decreased at 15 DAT and late timing. Control effect of *C. serotinus* was generally lower especially at 15 DAT and later timing. *S. trifolia* was controlled well by the 3 sulfonylurea herbicides at 10 and 15 DAT, but control effects decreased at 5, 20, 25 DAT application. *P. distinctus* was controlled completely by azimsulfuron and imazosulfuron regardless of application timing, but control efficacy of ethoxysulfuron was better at 20 and 25 DAT than at 5, 10, and 15 DAT application.

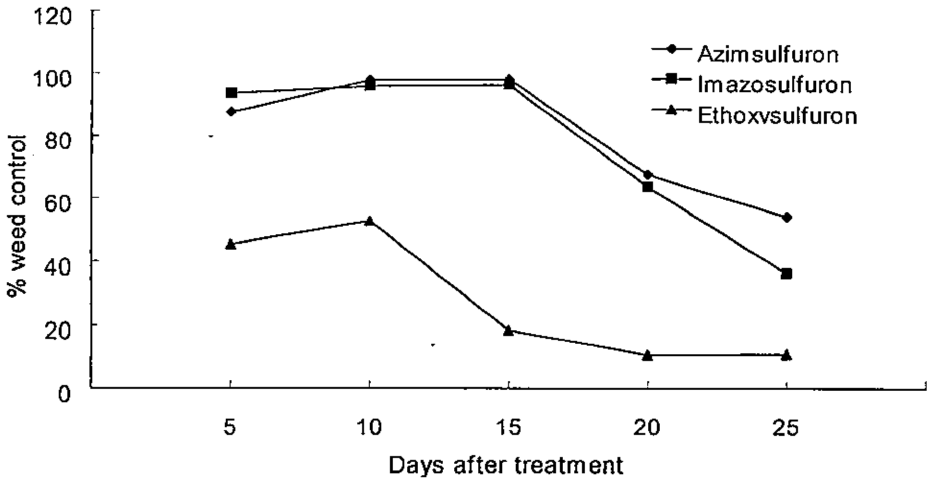

Fig. 1. Control of perennial weeds at 60 days after planting as affected by application times of three sulfonylurea herbicides .

Table 2. Control of perennial weeds at 60 days after planting by species as affected by application times of three sulfonylurea herbicides.

Herbicides	Weed species	% weed control				
		Application time (DAP)※				
		5	10	15	20	25
Azimsulfuron	*C. serotinus* Rottb.	93.7	97.9	99.3	74.6	66.0
(15 g ai ha^{-1})	*E. kuroguwai* Ohwi.	92.5	98.3	95.2	70.6	24.9
	S. trifolia Rottb.	47.4	86.5	88.5	89.7	73.2
	P. distinctus A. Benn.	100	100	100	100	100
Imazosulfuron	*C. serotinus* Rottb.	94.1	95.7	96.4	72.9	56.8
(75 g ai ha^{-1})	*E. kuroguwai* Ohwi.	91.8	96.7	96.8	46.8	23.7
	S. trifolia Rottb.	79.7	86.8	89.6	85.1	84.2
	P. distinctus A. Benn.	100	94.9	100	100	100
Ethoxysulfuro	*C. serotinus* Rottb.	37.0	47.2	8.1	2.0	11.1
n	*E. kuroguwai* Ohwi.	93.5	80.6	62.2	31.5	-
(21 g ai ha^{-1})	*S. trifolia* Rottb.	62.0	84.7	85.0	79.8	77.8
	P. distinctus A. Benn.	2.8	23.8	18.0	60.8	67.3

※DAP: Days after planting

Effect of temperature on control of perennial paddy weeds by sulfonylurea herbicides

Weed control efficacy of the 3 sulfonylurea herbicides against all perennial weeds was highest at 30°C followed by 20-25°C, but control efficacy was poor at 10-15°C. Control of *P. distinctus* was very poor at 10-15°C. *C. serotinus* was controlled completely by azimsulfuron at 30 DAT and imazosulfuron at 40 DAT and efficacy of ethoxysulfuron was lower at 30°C. Control efficacy against *E. kuroguwai* by the 3 herbicides was generally excellent regardless of temperature conditions. Control of *S. trifolia* was also higher at high temperature conditions, but was not completed and its growth was inhibited. Control effects of *P. distinctus* by the 3 sulfonylurea herbicides was poor at 10 and 15°C, but was 100% at 20, 25, and 30°C.

Table 3. Control of perennial weed species at 50 days after treatments by three sulfonylurea herbicides under different temperature conditions.

Herbicides	Weed species	% weed control* Temperature(°C) 10	15	20	25	30
Azimsulfuron (15g ai/ha^{-1})	*C. serotinus* Rottb.	100	94.5	71.7	86.3	100
	E. kuroguwai Ohwi.	88.9	96.7	100	99.8	99.9
	S. trifolia Rottb.	73.3	87.8	81.4	75.6	86.1
	P. distinctus A. Benn.	21.4	60.8	100	100	100
Imazosulfuron (75g ai/ha^{-1})	*C. serotinus* Rottb.	91.7	95.6	81.8	81.0	96.1
	E. kuroguwai Ohwi.	100	90.6	100	99.5	99.5
	S. trifolia Rottb.	73.3	69.4	89.6	93.3	93.8
	P. distinctus A. Benn.	51.1	55.7	100	100	100
Ethoxysulfuron (21g ai/ha^{-1})	*C. serotinus* Rottb.	83.3	96.7	69.3	72.3	81.7
	E. kuroguwai Ohwi.	100	88.9	96.9	99.3	98.2
	S. trifolia Rottb.	50.0	57.8	58.5	98.1	84.0
	P. distinctus A. Benn.	22.6	67.6	100	100	100

$$\text{* \% weed control: } \frac{\text{Dry weight of the untreated control - Dry weight of the treated control}}{\text{Dry weight of the untreated control}} \times 100$$

LITERATURE CITED

Im, I.B. et al. 1991. Study on control and tuberization inhibition of *Eleocharis kuroguwai* Ohwi. Korean J. of Weed Science 11(1):60-67.

Lee, H.K. et al. 1994. Biological properties of benfuresate and cinosulfuron for chemical control of *Eleocharis kuroguwai* Ohwi. Korean J. of Weed Science 14(4):272-279.

Lee, S.G. and J.Y Pyon. 2001. Effect of temperatures on emergence and early growth of perennial paddy weeds. Korean J. of Weed Science 21(1):42-48.

Pyon, J.Y. 1984. Effect of soil temperatures on emergence and early growth of

Eleocharis kuroguwai, Potamogeton distinctus, and *Sagittaria pygmaea*. Korean J. of Weed Science 4(2):125-129.

Pyon, J. Y. et al. 1990. Effect of soil temperature and planting depth on emergence and growth of perennial paddy weeds. Korean J. of Weed Science 10(3):197-201.

Suzuki, M. and T. Suto. 1975. Emergence of weeds in paddy rice fields. I. Relation between temperature and emergence. Weed Research (Japan) 20:105-109.

Crop Injury Caused by Herbicides

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Abstract: The experiment was carried out to survey the mechanism of herbicide action and examine the effects and injury on crops caused by herbicides. Soybean, Chinese cabbage and pumpkin showed high tolerance to alachlor and metolachlor, herbicides belonging to the acetanilide group. The main symptoms caused by these herbicides include stunting, local leaf spot and necrosis of growing points. Watermelon, melon and pumpkin were highly sensitive to dinitroanilines, ethalfluralin and pendimethalin than the other crops tested. Crop injury symptoms due to these herbicides were inhibited root growth and swollen hypocotyl. Watermelon, melon, onion, green onion and carrot were sensitive to napropamide, which caused abnormal growth of new leaves due to necrosis of growing points. The grass killer herbicides of the phenoxy group, fenoxaprop-p-ethyl, haloxyfop-R-methyl except 2,4-D, were highly tolerated by the broad-leaved crops. All crops were sensitive to hexazinone, herbicide of the triazine group. The herbicide caused chlorotic spots in the upper leaf that later turned brown. All crops were likewise relatively sensitive to the sulfonylureas, especially thifensulfuron-methyl.However, all crops showed high sensitivity to flazasulfuron. The phytotoxicity symptoms caused by the sulfonylureas were inhibited hypocotyls, reduced root growth and dwarfing of new leaf.

Key words: Herbicide, phytotoxicity, sensitive crop, tolerant crop

INTRODUCTION

In 2000, herbicides comprised 22.3% of the 5,822 Mt of pesticides used in Korea. This amount was decreased by 140 Mt compared to that in 1996. The reasons for the reduction in the total amount of herbicide were lesser amount of herbicides used as well as increased usage of high activity herbicides like sulfonylureas that require low dose of application.

Many farmers experienced phytotoxicity because of drift, inflow or irrigation of herbicides to neighboring crops, application of unregistered herbicides, misuse, presence of herbicide residues in soil affecting the succeeding crops, and application to unsuitable field conditions (Lee and Ryu 1993; Kwon et al. 1993). According to a survey in 1998, about 22% farmers experienced herbicide phytotoxicity caused by butachlor 5% GR, esprocarb · pyrazosulforon-ethyl 5.07% GR, bensulfuron · molinate 5.17% GR (Lee et al. 1998). This experiment was conducted to survey mechanisms of herbicide action and examine the injury caused and influence of herbicides on crops.

MATERIALS AND METHODS

A total of 17 herbicides were used in the experiment (Table 1). Seven groups comprised the test herbicides namely: acetanilides (alachlor and metolachlor), dinitroanilines (ethalfluralin and pendimethalin), amides (napropamide), phenoxies (2,4-D, fenoxapro-P-ethyl and haloxyfop-R-methyl), triazines (terbuthylazine, prometryn and hexazinone), carbamates (molinate and pyributicarb), and sulfonylureas (azimsulfuron, bensulfuron-methyl, cinosulfuron, pyrazosulfuron-ethyl, thifensulfuron-methyl and flazasulfuron). Various dosages including the recommended rates, 2 and 4 times the recommended rate were used to induce artificial phytotoxicity (Table 1). The crops tested were watermelon, cucumber, pumpkin, melon, potato, eggplant, red pepper, tomato, soybean, onion, green onion, lettuce, corn and Chinese cabbage (Table 2). Crop seeds were soaked in the herbicide solution for 24 h, washed with water and sown in the growth chamber at 28°C. Soil treatment was done prior to transplanting of the seedlings. Seedlings were raised in the greenhouse.

RESULTS AND DISCUSSION

Table 3 shows the relative response of crops to herbicides and the phytotoxic symptoms observed. These responses are affected by factors such as soil, wind, water, temperature and method of spray, and others. Soybean, pumpkin and Chinese cabbage showed high tolerance to alachlor and metolachlor, herbicides of the acetanilide group. The main symptoms caused by these herbicides were stunting, localized leaf spot and necrosis of growing points. The dinitroanilines (ethalfluralin and pendimethalin) were highly phytotoxic to watermelon, melon and pumpkin but not to the other crops tested.

Table 1. Herbicides tested and the dosages applied.

Group	Herbicide	Dosage (ai g $10a^{-1}$)		
Acetoalide	Alachlor	87.4	174.8	349.6
	Metolachlor	80.0	160.0	320.0
Denitroanine	Ethalfluralin	105.0	210.0	420.0
	Pendimethalin	95.1	190.2	380.4
Amide	Napropamid	150.0	300.0	600.0
Phenoxy	2, 4-D	28.0	56.0	112.0
	Fenoxaprop-P-ethyl	7.0	14.0	28.0
	Haloxyfop-R-methyl	6.0	12.0	24.0
Triazine	Terbuthylazine	400.0	800.0	1,600.0
	Prometryn	100.0	200.0	400.0
	Hexazinone	250.0	500.0	1,000.0
Carbamate	Molinate	150.0	300.0	600.0
	Pyributicarb	352.5	705.0	1,410.0
Sulfonylurea	Azimsulfuron	0.9	1.8	3.6
	Bensulfuron-methyl	2.4	4.8	9.6
	Cinosulfuron	2.4	4.8	9.6
	Pyrazosulfuron-ethyl	2.1	4.2	8.4
	Thifensulfuron	5.25	10.5	21.0
	Flazasulfuron	7.5	15.0	30.0

The symptoms of crop injury were inhibited root growth and swollen hypocotyl. The amides, represented by napropamide, were likewise highly phytotoxic to watermelon, melon, onion, green onion and carrot. The specific symptoms observed were abnormal growth of new leaves caused by necrosis of the growing points. The grass killer herbicides of phenoxy group, fenoxaprop-p-ethyl and haloxyfop-R-methyl except 2,4-D, were highly tolerated by all broad-leaved test crops. Red pepper, cucumber and melon were highly sensitive to 2,4-D. All crops were sensitive to hexazinone, (triazine group) and caused chlorotic spots that later turned brown in the upper leaves. All crops were highly sensitive to the sulfonylureas especially thifensulfuron-methyl and flazasulfuron. The crop injury main symptoms caused by the sulfonylureas were inhibited hypocotyls, reduced root growth and abnormal growth, of new leaves.

Table 2. The crops used and their cultivation methods.

Family	Crop	Cultivation Method
Cucurbitaceae	Water melon	Transplanting
	Cucumber	"
	Pumpkin	"
	Melon	"
Solanaceae	Potato	Seeding
	Egg plant	Transplanting
	Red pepper	"
	Tomato	"
Leguminosae	Soybean	Seeding
Liliaceae	Onion	"
	Green onion	"
Compositae	Lettuce	Transplanting
Gramineae	Corn	Seeding
Cruciferae	Chinese cabbage	"

Benzothiadiazole, bipyridylium and diphenyl ether groups caused local leaf spots. Isoxazolidinone, amino acid and triazine group herbicides caused phytotoxicity in the whole plant but did not inhibit crop growth. Isoxazolidinone, a herbicide representing clomazone, caused chlorosis, while glyphosate caused leaf wilting. Triazine (hexazinone) caused necrosis in the whole plant. The benzoic acid group (dicamba) and phenoxycarboxylic acid (2,4-D) caused malformation of leaves and twisting of stems. Root pruning was exhibited by crops sensitive to dinitroaniline. The imidazolinones caused malformation of above-aerial parts and the sulfonylureas caused rosette formation of above-aerial parts.

The responses of sensitive test crops to the herbicide groups are shown in Table 4. Local leaf spot was observed in leaves that came in contact with benzothiadiazole, bipyridylium, and diphenyl ether herbicides. The whole plant exhibited chlorosis and necrosis but growth of crops was not inhibited by the application of isoxazolidinone, amino acid and triazines. Clomazone treatment caused chlorosis while glyphosate caused leaf wilting. Hexazinone, a member of the traizine group, caused necrosis. Dicamba and 2, 4-D treated plants had twisted in stem while dinitroaniline sensitive plants exhibited root pruning.

Imidazolinones caused malformation of the above-aerial part, and the sulfonyureas inhibited of crop growth and rosette formation of above aerial parts.

Table 3. Relative response of crops to various herbicides and symptoms exhibited by the test crops.

Group[1)]	Herbicide[2)]	Relative response to herbicide treatment		Symptoms [4)]
		Sensitive Crop[3)]	Tolerant Crop[3)]	
Aceta.	Alachlor	Wm, on, me, le, ca, cu, go, co, eg, rp, to	so, pu, cc	a(cu), b(to), c, f, g, d
	Metola.	Wm, me, le, cu, pu, go, to, co	so, on, po, ep, rp, cc	e(cu, to), d, c, g
Dinitro.	Ethal.	Wm, me, pu	on, le, go, ca, cu, so, co, po, ep, to, cc, rp	h, g, c
	Pendi.	Wm, on, me, ca., pu, go	so, cu, on, le, co, po, ep, rp, to, cc	h, k, i
Amide	Napropa.	Wm, on, me, go, ca, cu, le, pu, so, co	po, ep, rp, to, cc	i, c, a(co), k
Carba.	Molinate	rp, cu	so	l, m
	Pyribu.		ep, rp, le, to, cu, co, on, go, me, so	n(ep, so), g, i
Phenoxy	2,4-D	le, to, go, so	rp, cu, me, co, on, ep	o, f, a(to), p
	Fenoxa.	co	ep, rp, le, to, cu, go, on, me, so	a(co)
	Haloxy.	co, so	ep, rp, le, to, cu, me, on, go	q, f, p(so)
Triazine	Terbuth.	to, go, me, cu, on, so, le	rp, co, ep	q, f(cu), p(me, cu), d
	Prome.	to, ep, so, go, on, co	rp	a(to, cu), q(ep, le)
	Hexazi.	ep, rp, le, cu, me, on, co, go, so, to		n, q, f, r
Sul.	Azim.	co, ca, go, ep, le, cu, me, rp	wm, pu, ca, co, to	s(wm), g
	Bensul.	co, go, to, cu, wm, pu, me, rp, ep, le	ca, on	t(wm), u(co), v(Cucurbitaceae)
	Cino.	ca, go, on, ep, le, cu, wm, me	co, pu, rp, to	g, i
	Pyrazo.	wm, ca, ro, on, le, cu, me, rp, ep	co, pu, to	v(Cucurbitaceae)
	Thifen.	co, me, pu, cu, wm, rp, ep, to, go, on, le, ca		k, t, o(rp)
	Flaza.	co, me, pu, cu, wm, rp, ep, to, go, on, le, ca		k, t

1. Group - Aceta.: Acetanilide, Dinitro.: Dinitroanilide, Carba.: Carbamate, Sul.: Sulfonylureas
2. Herbicide - Metola.: Metolachlor, Ethal.: Ethalfulalin, Pendi.: Pendimethalin, Napropa.: Napropamide, Pyribu.: Pyributicarb, Fenoxa.: Fenoxaprop-P-ethyl, Haloxy.: Haloxyfop-P-ethyl, Terbuth.: Terbuthylazin, Prome.: Prometryne, Hexazi.: Hexazinone, Azim.: Azimsulfuron, Bensul.: Bensulfuron, Cino.: Cinosulfuron, Pyrazo.: Pyrazosulfuron-ethyl, Thifen.: Thifensulfuron, Flaza.: Flazasulfuron
3. Crop - wm: water melon, on: onion, me: melon, le: lettuce, ca: carrot, cu: cucumber, go: green onion, co: corn, ep: egg plant, rp: red pepper, to: tomato, so: soybean, pu: pumpkin, cc: chinese cabbage, po: potato
4. Symptom - a: death of growing point, b: local leaf spot, c: stunting of new leaf, d: death of bottom leaf, e: death of growing point and new leaf, f: stunting of plant, g: growth inhibition of root, h: plumping of hypocotyl, i: inhibition of growth, j: stunting of leaf, k: growth inhibition of lateral root, l: local leaf spot, m: chlorotic spot of leaf edge, n: chlorosis and defoliation in bottom leaf, o: twist of stem, p: top-necrosis, q: browing of bottom leaf edge, r: completely dead, s: inhibition of radical, t: inhibition of hypocotyl, u: malformation of root, v: cluster of new leaf

Table 4. Herbicide phytotoxicity symptoms observed from the sensitive crops.

Symptom						Specific symptom		Group	Herbicide
root[1]	above[2]	discolor[3]		malfor.[4]					
		whole[5]	local[6]	leaf	stem				
			O	O		local[7]	not uni.[8]	Benzothi.[9]	bentazone
			O	O		local[7]	uniform	Bipyridylium	paraquat
			O	O		local[7]	soil sur.[10]	Diphenyl[11]	oxadiazone etc.
		O		O		whole[12]	chlorosis	Isoxazo.[13]	clomazone
		O		O	O	whole[12]	tender[14]	Amino acid	glyhposate
		O	O			whole[12]	necrosis[15]	Triazine	hexazinone etc.
	O			O		above-aerial part[16]		Acetanilide	meto.[17], ala.[18] etc.
	O	O		O	O	malfor[19]	cup[20]	Benzoic acid	dicamba
	O	O		O	O	malfor[19]	cup(turn)[21]	Phenoxy[22]	2.4-D
O	O		O		O	root pruning		Dinitroanilin e	ethal.[23], pendi.[24] etc.
O	O	O		O	O	malfor. of above[25]		Imidazo.[26]	imazethapyr etc.
O	O	O		O	O	shape of rosette		Sulfonylurea	bensulfuron etc.

1) inhibition of root, 2) inhibition of above-aerial- part, 3) discoloration of leaf, 4) malformation, 5) symptoms in whole plant, 6) local leaf spot, 7) local necrosis, 8) not uniform, 9) Benzothiadiazole, 10) soil surface, 11) Dipheny ether, 12) necrosis of whole plant, 13) Isoxazolidinone, 14) symptoms of tender leaf, 15) irregular necrosis, 16) inhibition of above-aerial part, 17) metolachlor, 18) alachlor, 19) malformation leaf, 20) shape of cup, 21) shape of cup(turnover), 22) Phenoxycarboxylic acid, 23) ethalfluralin,24) pendimethalin, 25) malformation of above-aerial part, 26) Imidazolidinone

LITERATURE CITED

Kwon, Y. W., H. S. Hwang and G. H. Kang. 1993. Herbicidal phytotoxicity under adverse environments and countermeasures. Korean J. of Weed Science. 13(4): 210-233.

Lee, J. O. and G. H. Ryu. 1993. Problems associated with farmer's use of herbicide and countermeasures. Korean J. of Weed Science. 13(4): 203-209.

Lee, I. Y., J. E. Park, T. S. Park, T. W. Kim and B. Y. Oh. 1998. Fact-finding survey on herbicide use in paddy rice at farmer's level. The Korean J.of Pesticide Science. 2(2): 119-125.

Evaluation on Herbicide Hazards to Rice

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Abstract: Herbicides belonging to 8 chemical families were used on both *Indica* and *japonica* rice at higher dosage than recommendation to estimate their harmfulness on rice. The chemical families were amides, thiocarbamates, sulfonylureas, heterocyclics, ureas and phenoxy carboxylic acids. Rice was planted by seedling throwing and directed-sowing. Herbicides were applied with moving liquid treatment and mist spray. The symptoms on rice plants caused by these herbicides were described and the difference of susceptibilities to the herbicides between *indica* and *japonica* were compared. Results also showed that the hazards in rice plants caused by some herbicides could be reduced by fertilizer application

Key words: hazard, herbicides, rice

INTRODUCTION

Herbicides are widely used in many countries in rice production. Herbicide hazards to rice sometimes occur due to application mistakes, variance of environmental factors and quantity of herbicides. This study was conducted to better understand the harmfulness of these herbicides on rice define the symptoms when herbicide injury in rice plants, and finally find some ways to reduce the injury.

MATERIALS AND METHODS

Greenhouse experiments were carried out during 2000~2001 in the Institute of Plant Protection, Jiangsu Academy of Agricultural Science. Rice plants at 5 to 6 leaf-stage were transplanted using seedling broadcasting and rice seeds were planted in plastic bucket of 20 cm × 17 cm. Rice cultivars were Shanyou 63 (*indica*) and Wuyugen 3 (*japonica*).

Herbicides:

60% Butachlor EC provided by Changzhou Pesticide Company;
50% Guinclorac WP provided by Qunshan Chemical Factory;
10% Clincher EC provided by Dow Chemical Pacific LTD;
25% Chlorsulfuron WP provided by Institute of Hormone Science at Jintan County, Jiangsu province
50% Isoproturon WP provided by Institute of Agricultural Science at Wuxuan County
96% Molinate EC provided by ICI

Herbicide hazards to *japonica* and *indica* transplanted by throwing and seedling

Rice at 5 to 6 leaf stage was transplanted by throwing. The seedlings were treated with herbicides at designated rates with drop injection while seed planted rice were treated by spray. The height and tillers were recorded at 7-day interval after treatment. The plant fresh weight, root length and dry weight were assessed at 28 days after treatment. Herbicides and rates of application are listed in Table 1.

Table 1.Treatments and time of application for herbicide hazards to *japonica* and *indica* .

No.	Treatment	Rate ha^{-1}	No of Rate
1	Butachlor	900	A
2	Butachlor	1800	B
3	Butachlor	2700	C
4	Clincher	75	A
5	Clincher	150	B
6	Clincher	225	C
7	Molinate	2880	A
8	Molinate	5760	B
9	Molinate	6840	C
10	Quinclorac	375	A
11	Quinclorac	750	B
12	Quinclorac	1125	C
13	Isoproturonc	225	A
14	Isoproturonc	450	B
15	Isoproturonc	675	C
16	Chlorsulfuron	7.5	A
17	Chlorsulfuron	15	B
18	Check	0	

Herbicide hazards to rice at different leaf stages

Japonica seeds were planted in plastic buckets tilled with 0.5 sm soil on August 20,24,28 and September 1. Seedlings were treated with herbicides at designated rates on September 3 with a pot spray tower at pressure of 10 lb $inch^{-2}$ and spray volume was 50ml m^{-2}. The height and tillers of seedlings were measured at 7-day interval after treatment Fresh and dry weight of the seedlings above soil, root length and root fresh weight were measured 28 days after treatment.

All treatments were repeated 3 times, and all buckets were arranged in completely randomized design. Inhibition rate and recovery was calculated as follows:

Inhibition (%) = Untreated (height, fresh weight)-treated (height, fresh weight)

Untreated (height, fresh weight)

$$\text{Recovery (\%)} = \frac{\text{Untreated (height, fresh weight)-treated (height, fresh weight)}}{\text{(Untreated (height, fresh weight)}}$$

The standard of the hazard degree and regrowth in rice are listed in Tables 2 and 3.

Table 2. Standard of the hazard degree to rice.

Hazard level	Inhibition	Status
0	≤0	Normal
I	0.1~20%	Slightly inhibition
II	20.1~40%	Inhibited
III	40.1~60%	Obviously inhibited
IV	60.1~80%	Seriously inhibited
V	≥80%	Completely inhibited

Table 3.Standard of the regrowth

Recovery level	Recovery	Status
A	>80%	Completely recover
B	50%~80%	Could recover
C	<50%	Recover

RESULTS AND DISCUSSION

Herbicide hazards to *japonica* and *indica* transplanted by rice seedling throwing

Effect of herbicides on fresh weight of rice seedling and root are shown in Figures 1 and 2. Clincher was safe to *japonica* and *indica,* even at high rates of up to three times more than the recommendation. Other herbicides at high rate caused injury on rice seedlings. There were significant differences among treatments on the rice performance. It was also seen that *japonica* was more sensitive than *indica*. Both japonica and *indica* were most susceptible to chlorsulfuron, *indica*ting that the chlorsulfuron is not safe when applied to rice.

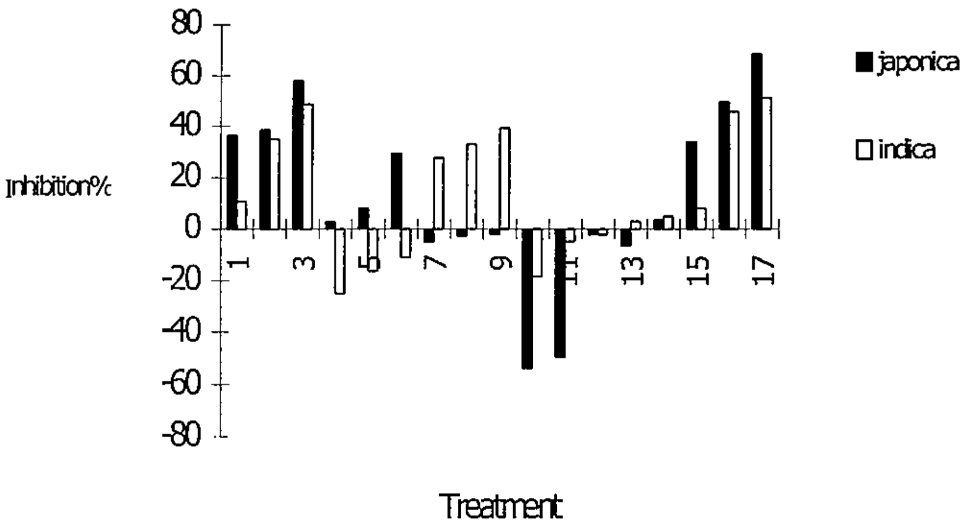

Figure.1 Inhibition of fresh weight on transplanted rice seedlings 30 days after treatment.

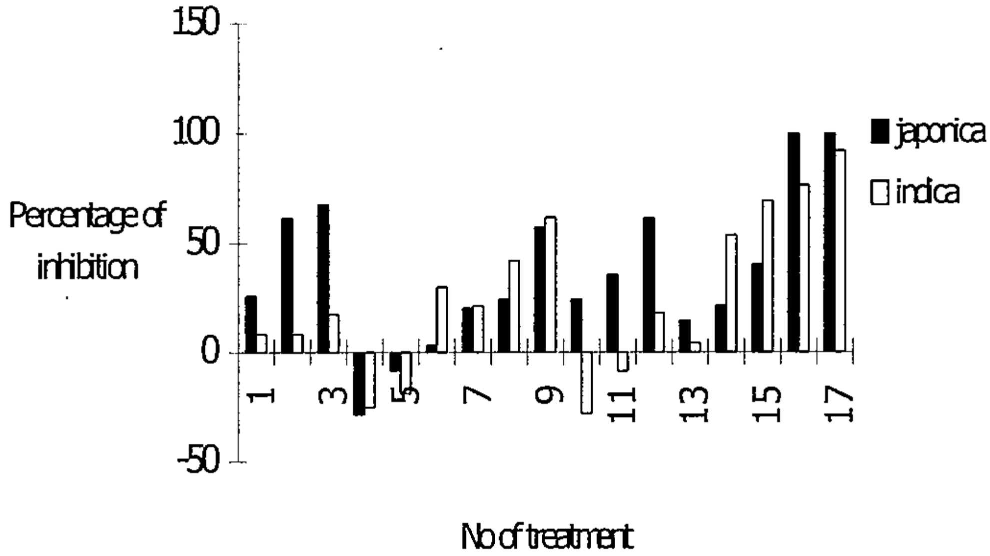

Figure 2.The percentage of inhibition of fresh weight on seed sowing rice at 30 days after treatment.

The symptoms of injured rice

Various symptoms observed after treatment, and all the symptoms were different from each other as shown in Table 4.

Table 4. Symptoms on direct-seeded rice caused by herbicides at 14 days after treatment.

Treatments	*Indica*			*Japonica*		
	NO of rate			NO of rate		
	A	B	C	A	B	C
Butachlor (60%EC)	No injury.	Short and small plants lightly. Dark green leaf.	Short and light in plant, dark green growth leaf and nature leaf change yellow.	Short and small lightly in plant. Dark green leaf	Short and small in plant. Dark green leaf.	Short and small growth seriously, inhibited Dark green leaf.
Quinclorac (50%WP)	No injury.	No injury.	The growing leaf in tuble in shape.	No injury.	leaf	The growing onion leaf in tuble in shape.
Clincher (10%EC)	No injury.	No injury.	No injury	No injury	No injury.	No injury.
Chlorsulfuron (25%WP)	Nature leaf change yellow.	Death		Nature leaf change yellow.	Death	
Isoproturon (50%WP)	leaf	Crimping leaf. Withered leaf	Reduced tillering Withered plants Seriously inhibited growth.	Crimping leaf withered plants lights.	Reduce tillering withered plants lightly. Growth slightly inhibited.	Reduced tillering Withered plants. Growth seriously inhibited.
Molinate (96%EC)	No injury.	No injury.	Somewhat green leaf	No injury.	Somewhat green leaf	Somewhat green leaf

Growth recovery of injured rice seedlings after applying urea

With the application of 150 kg ha^{-1} urea after herbicide treatment, the injured rice, except those treated with Chlosulfuron, started to recover 30 days after herbicide application. Except in rice treated with chlosulfuron whose recovery rate was below 10%, recovery rate in general was 50% to 100%. The results indicated that urea application can reduce injury caused by butachlor,quinolac and isoproturon but not on plants injured by chlorsulfuron.

Table 5. The degree of recovery height of rice at 30 days after high rate treatment.

Treatments	No of	Transpalnted		Direct-sown	
		Indica	japonica	Indica	japonica
Butachlor(60%EC)	1	B**	C	A	A
Butachlor(60%EC)	2	C	C	A	B
Butachlor(60%EC)	3	C	C	A	B
Clincher(10%EC)	4	*	A	*	*
Clincher(10%EC)	5	*	B	*	*
Clincher(10%EC)	6	*	B	*	A
Molinate(96%EC)	7	B	C	A	C
Molinate(96%EC)	8	B	C	A	C
Molinate(96%EC)	9	B	C	B	C
Guinclorac(50%WP)	10	*	*	*	A
Guinclorac(50%WP)	11	*	*	*	B
Guinclorac(50%WP)	12	A	*	C	B
Isoproturon(50%WP)	13	*	*	C	*
Isoproturon(50%WP)	14	A	A	C	C
Isoproturon(50%WP)	15	A	A	C	C
Chlorsulfuron(25%WP)	16	C	C	C	X
Chlorsulfuron(25%WP)	17	C	C	C	X

*: Percentage of recovery height below 10% comparing to CK

**: Degree of growth recovery of rice at 30days after treatment with high dose of herbicide

×: Died plant

LITERATURE CITED

Huang Chunyan, Chen Tiebao. 2000 The Symptom of herbicide hazard to maize. Weed Science. 2:290

Huang Jianzhong.1996 Injury of herbicides. Weeds Sciences. Agriculture Science and Technology Press of China, p151~156.

Yang Xianzhi,Yang Jianyuan,1999, Study on Weed Management and Its Application. Sustainable Weed management towards the 21th century in China p29~31.

Zhang Dianjing,Chen Renlin. 1992 Hazard to Plant Chemical Control of Weds in Field. Science and Technology Cited Press of Shanghai p254~364.

Zhang Zepu. 2000 Study on Butchlor hazard to Rice. Journal of Weed Science. 5: 2~8.

Performance of Diammonium Salt of 56.4% W/V AS for Weed Control in Rubber Crop

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Abstract: The study was conducted in a rubber plantation in Chanthaburi province, Thailand in 2000 to determine the efficacy of herbicide diammonium salt of glyphosate 56.4% W/V AS 0.53, 0.70, 0.88, 1.06, and 1.23 kg ai ha^{-1}. It was carried out in RCB with 3 replications with plot size 5 m x 13 m. Herbicides were applied between rows of rubber plants by using knapsack sprayer with spray volume 375 to 500 L ha^{-1}. The results showed that diammonium salt of glyphosate at 1.06 to 1.23 kg ha^{-1} had no phytotoxic eefect on rubber plants and a good efficacy to control narrowleaved weeds, eg., *Pennisetum polystachyon*, (L.) Schult., *Ottochloa nodosa* (Kunth) Dandy, *Digitaria adscendens* (H.B.K.) Henr., *Eleusine indica* (L.) Gaertn. and *Paspalum conjugatum* Berg., as well as broadleaved weeds eg. *Chromolaena odorata* Linn., *Borreria laevis* (Lamk.) Griseb., *Borreria alata* (Aubl.) DC., *Ageratum conyzoides* Linn., *Mimosa pudica* Linn. and *Synedrella nodiflora* (L.) Gaertn. Diammonium salt of glyphosate at higher rate gave better efficacy than lower rate, similar with glyphosate 48% SL at 1 kg ha^{-1}. However, diammonium salt of glyphosate 56% at every rate could decrease density and weed dry weight significantly but did not affect the growth rate of rubber plants.

Key words: diammonium salt of glyphosate, rubber crop, weed control

INTRODUCTION

Weeds are one of the major problems in rubber plantations. Weeds affect rubber growth especially in the early growth stage. The major weeds in rubber plantations are *Pennisetum polystachyon* (L.) Schult., *Ottochloa nodosa* (Kunth) Dandy, *Digitaria adscendens* (H.B.K.) Henr., *Eleusine indica* (L.) Gaertn., *Paspalum conjugatm* Berg., *Chromolaena odorata* Linn., *Ageratum conyzoides* Linn., *Mimosa pudica* Linn. (Aubl.), *Borreria laevis* (Lamk.) Griseb., *Borreria. alata* (Aubl.) DC., *Crassocephalum crepidioides* (Benth) S. Moore, and *Synedrella nodiflora* (L.) Gaertn. (Yongboonkead *et al.* 1984) There are various methods to control weeds. One method is manual weed control, but laborious and expensive (Leungapapong 1997). The Gardeners increasingly use herbicides in rubber crop especially glyphosate 48% SL which could inhibit the enzyme EPSPS (5-enolpyruvylshikimate 5- phosphate synthase). The weed cannot synthesize three amino acids: phenylanine, tryptophan and tyrosine, which inhibit the metabolism of weed and dead weeds, were eradicated (Franz *et al.* 1997). Glyphosate 48 % SL is one of the popular herbicide in rubber crop to control major weeds, such as grasses and broadleave weed. A popular method is to use herbicide. Therefore, this study, using various rates of herbicides, is important for rubber plantations. It evaluated the appropriate rates of diammonium salt of glyphosate 56.4% W/V AS in rubber plantations.

MATERIAL AND METHODS

The experiment was conducted in the gardener's field in Chanthaburi province during September to December 2000. It was laid out in randomized complete block design (RCB) with 3 replications and 8 treatments in plot size 5 m x 13 m, Knapsack sprayer with fan type nozzle spray volume 375 to 500 L ha^{-1}, applied between rubber row at 2 to 3 leaf stage of weeds, less than 30 cm height. The treatments comprised of diammonium salt of glyphosate at 0.53, 0.70, 0.88, 1.06, 1.23 kg ha^{-1} and glyphosate 48% SL at 1 kg ha^{-1}, hand weeding 3 times and weedy check. The number and species of weeds were collected by random from 2 points of 0.5 m x 0.5 m. Growth rate of rubber plants was recorded at 30, 60 and 90 days after application (DAA). Data were analyzed by using Duncan's New Multiple Range Test (DMRT). Phytotoxic of herbicides to rubber plants were evaluated by visual rating 0-10 at 15, 30 and 60 DAA. and efficacy of herbicide to control of weeds that classified by species and types were rating 0-10 by visual assessment at 30 DAA.

RESULTS AND DISCUSSION

Weed density

The experimental field was infested with high density of weeds (Table 1). Predominant weeds were *P. conjugatum* Berg., *D. adscendens* (H.B.K.) Henr., *O. nodosa* (Kunth) Dandy, *E. indica* (L.) Gaertn. and *P. polystachyon* Schult. with 72, 42, 24, 22 and 18 plants m^{-2} respectively. For broadleave weeds, *B. alata* (Aubl.) DC., *A. conyzoides* Linn., *B. laevis* (Lamk.) Griseb., *C. odorata* Linn., *S. nodiflora* (L.) Gaertn. and *M. pudica* Linn. average 150, 30, 28, 12, 10 and 6 plants m^{-2} respectively.

Table 1. Weed species occurance in rubber crop.

Weed species	No. plants m^{-2}	%
Grasses		
Paspalum conjugatum Berg.	72	17.4
Digitaria adscendens (H.B.K.) Henr.	42	10.1
Ottochloa nodosa (Kunth) Dandy	24	5.8
Eleusine indica (L.) Gaertn.	22	5.3
Pennisetum polystachyon (L.) Schult.	18	4.4
Broadleaves		
Borreria alata (Aubl.) DC.	150	36.2
Ageratum conyzoides Linn.	30	7.2
Borreria laevis (Lamk.) Griseb.	28	6.8
Chromolaena odorata Linn.	12	2.9
Synedrella nodiflora (L.) Gaertn.	10	2.4
Mimosa pudica Linn.	6	1.5
Total	414	100

Crop injury

There were crop injury at 15, 30, 60 DAA, after application (DAA) and not significantly different with hand weeding (Table 2).

Table 2. Injury of rubber at 15, 30 and 60 days after herbicide applications (DAA)*

Treatment	Rate (kg ai ha^{-1})	Crop injury		
		15 DAA	30 DAA	60 DAA
1. Diammonium salt of glyphosate 56.4% W/V AS	0.53	0	0	0
2. Diammonium salt of glyphosate 56.4% W/V AS	0.70	0	0	0
3. Diammonium salt of glyphosate 56.4% W/V AS	0.88	0	0	0
4. Diammonium salt of glyphosate 56.4% W/V AS	1.06	0	0	0
5. Diammonium salt of glyphosate 56.4% W/V AS	1.23	0	0	0
6. Glyphosate.48% W/V SL	1	0	0	0
7. Hand weeding	-	0	0	0
8. Weedy check	-	0	0	0

* DAA = days after herbicide application

Growth of rubber

Growth of rubber plants was measured by circumference at 170 cm height (Table 3). At 30 DAA, the treatments of diammonium salt of glyphosate at the rates of 0.70, 0.88 and 1.06 were not significantly different from glyphosate 48% SL and hand weeding, but differed from low rate of diammonium salt of glyphosate 0.53 and weedy check. At 60 DAA, the average circumference of rubber plants was the same as at 30 DAA. At 90 DAA, the diameter of weeds applied with diammonium salt of glyphosate at 0.70, 0.88, 1.06 and glyphosate 48% SL including hand weeding was not significantly different from that of the weedy check. However, the percentage of growth did not differ among treatments.

Table 3. Circumference of rubber at 170 cm height at 30, 60 and 90 days after application.

Treatment	Rate (Kg ai ha)$^{-1}$	Circumference[1]				Increased circumference (%)		
		DBA[2]	DAA[3]			DAA		
			30	60	90	30	60	90
1.Diammonium salt of glyphosate	0.53	21.7 ab	22.1b	22.5b	23.2ab	1.6a	1.8a	3.1a
2.Diammonium salt of glyphosate	0.70	23.2a	23.5a	23.9a	24.5a	1.5a	1.7a	2.3a
3.Diammonium salt of glyphosate	0.88	23.3a	23.6a	23.9a	24.7a	0.9a	1.6a	30.a
4.Diammonium salt of glyphosate	1.06	23.1a	23.4a	23.8a	24.4a	1.2a	1.6a	2.4a
5.Diammonium salt of glyphosate	1.23	22.2ab	22.6b	23.0b	23.6ab	1.6a	1.7a	2.9a
6.Glyphosate.48% W/V SL	1	24.0a	24.5a	24.8a	25.3a	1.8a	1.3a	2.1a
7.Hand weeding	-	23.1ab	23.5a	24.0a	24.4a	1.8a	2.2a	2.4a
8.Weedy check	-	21.3b	21.6b	22.0b	22.5b	1.4a	1.7a	2.3a
C.V. (%)		18.8	16.4	15.5	15.7	28.5	32.4	33.4

[1] Means within a column followed by the same letter are not significantly different at the 95% level by DMRT.

[2] DBA = day before application. [3] DAA = day after application.

Grass control efficiency

At 30 DAA, diammonium salt of glyphosate 56.4% at every rates had a good grass control like glyphosate 48% SL (Table 4). The rates of diammonium salt of glyphosate 56.4% at 1.06 and 1.23 kg ha^{-1} could decrease the population of *P. conjugatum* Berg., *D. adscendens* (H.B.K.) Henr., *O. nodosa* (Kunth) Dandy, *E. indica* (L.) Gaertn. and excellent control of *P. polystachyon* (L.) Schult. These results were similar with the Botany and Weed Science Division recommendation (1995).

Table 4. Grass control efficiency with visual assessment at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Grass control efficiency				
		PAS	DIG	OTT	ELE	PEN
1. Diammonium salt of glyphosate	0.53	7.8	7.5	7.2	7.7	8.1
2. Diammonium salt of glyphosate	0.70	8.2	8.3	8.3	8.2	8.0
3. Diammonium salt of glyphosate	0.88	9.2	9.3	9.3	9.5	9.8
4. Diammonium salt of glyphosate	1.06	9.5	9.5	9.7	9.7	10.0
5. Diammonium salt of glyphosate	1.23	9.8	9.7	9.8	9.8	10.0
6. Glyphosate 48% SL	1	9.8	10.0	9.8	10.0	10.0
7. Hand weeding	-	6.0	7.0	7.0	7.0	5.0
8. Weedy check	-	0.0	0.0	0.0	0.0	0.0

Weed species
PAS = Paspalum conjugatum *Berg.*
DIG = Digitaria adscendens *(H.B.K.) Henr.*
PEN = *Pennisetum polystachyon* (L.) Schult.
OTT = *Ottochloa nodosa* (Kunth) Dandy
ELE= *Eleusine indica* (L.) Gaertn.

Broadleave control efficiency

At 30 DAA, broadleaved weeds were eliminated by all rates of chemical control, and hand weeding had lower control efficiency (Table 5). *A. conyzoides* Linn. was completely controlled by the high rate of diammonium salt of glyphosate 56.4% at 1.23 kg ha^{-1} and glyphosate 48% SL.

Table 5. Broadleave control efficiency with visual assessment at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Broadleave control efficiency					
		BORL	BORA	AGE	CHR	SYN	MIM
1.Diammonium salt of glyphosate	0.53	6.8	6.8	7.2	6.7	6.8	7.0
2.Diammonium salt of glyphosate	0.70	7.2	7.2	7.8	7.3	7.5	7.7
3.Diammonium salt of glyphosate	0.88	8.2	8.2	8.5	7.8	8.2	8.3
4.Diammonium salt of glyphosate	1.06	8.8	8.8	9.8	9.2	9.2	9.5
5.Diammonium salt of glyphosate	1.23	9.5	9.5	10.0	9.3	9.3	9.7
6.Glyphosate 48% SL	1	9.3	9.7	10.0	9.5	9.5	9.8
7.Hand weeding	-	5.0	7.0	7.0	5.0	6.0	5.0
8.Weedy check	-	0	0	0	0	0	0

Weed species
BORL = *Borreria laevis* (Aubl.) DC.
BORA = *Borreria alata* (Lamk.) Griseb.
AGE = *Ageratum conyzoides* Linn.
CHR = *Chromolaena odorata* Linn.
SYN = *Synedrella nodiflora* (L.) Gaertn.
MIM = *Mimosa pudica* Linn.

Number and dry weight of grasses

At 30 DAA, the number of grass weeds was significantly reduced in four-weed species spray with chemicals (Table 6). Only *E. indica* (L.) Gaertn. had the same density as a result of chemical and hand weeding. All weed control treatments were significantly different from the untreated plots. Narrow-leaved weeds had higher weight in the weedy check treatment than weed control treatments, except *P. polystachyon* (L.) Schult., which had high density in the hand weeding treatment (Table 7).

Table 6. Number of grass weeds in rubber crop at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Grasses *(no. plants m^{-2})				
		PAS	DIG	OTT	ELE	PEN
1.Diammonium salt of Glyphosate	0.53	8.7c	6.0bc	9.3c	6.0b	4.0a
2.Diammonium salt of glyphosate	0.70	10.0c	8.0c	8.6c	5.3b	5.0a
3.Diammonium salt of glyphosate	0.88	4.7b	4.7bc	5.3b	2.0a	0.7a
4.Diammonium salt of glyphosate	1.06	3.3ab	4.7bc	4.0b	2.0a	0.0a
5.Diammonium salt of glyphosate	1.23	1.3a	2.7ab	1.3a	2.0a	0.0a
6.Glyphosate 48% SL	1	1.3a	0.0a	1.3a	0.0a	0.0a
7.Hand weeding	-	14.7d	12.7d	14.0d	6.7a	11.3b
8.Weedy check	-	26.7e	27.3e	27.0e	30.7	20.7c
C.V. (%)		7.5	26.8	15.3	24.3	63.8

* Means within a column followed by the same letter are not significantly different at the 95% level by DMRT.

Weed species

PAS = Paspalum conjugatum *Berg.* OTT = *Ottochloa nodosa* (Kunth) Dandy

DIG = Digitaria adscendens *(H.B.K.) Henr.* ELE = *Eleusine indica* (L.) Gaertn.

PEN = *Pennisetum polystachyon* (L.) Schult.

Table 7. Dry weight of grass weeds in rubber crop at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Grass weight* (g m^{-2})				
		PAS	DIG	OTT	ELE	PEN
1. Diammonium salt of glyphosate	0.53	8.9c	7.8c	9.8c	5.2c	26.1c
2. Diammonium salt of glyphosate	0.70	9.3c	7.7c	10.0c	5.1c	28.8c
3. Diammonium salt of glyphosate	0.88	3.9b	3.6b	4.8b	1.8b	17.1b
4. Diammonium salt of glyphosate	1.06	3.9b	2.4b	4.7b	1.9b	0a
5. Diammonium salt of glyphosate	1.23	2.9ab	1.7ab	2.2ab	1.4ab	0a
6. Glyphosate 48% SL	1	1.9a	0a	0.8a	0a	0a
7. Hand weeding	-	14.7d	13.6d	15.0d	8.1d	85.9e
8. Weedy check	-	28.3c	27.7e	30.9e	33.3e	67.4d
C.V. (%)		10.8	15.9	16.5	13.1	14.0

* Means within a column followed by the same letter are not significantly different at the 95% level by DMRT.

Weed species

PAS = Paspalum conjugatum *Berg.* OTT = *Ottochloa nodosa* (Kunth) Dandy

DIG = Digitaria adscendens *(H.B.K.) Henr.* ELE = *Eleusine indica* (L.) Gaertn.

PEN = *Pennisetum polystachyon* (L.) Schult.

Number and dry weight of broadleaves

At 30 DAA, four weed species, *B. laevis* (Lamk.) Griseb., *B. alata* (Aubl.) DC., *C. odorata* Linn. and *S. nodiflora* (L.) Gaertn. were reduced when sprayed with every chemical treatments and differed from hand weeding and untreated check plots (Table 8). Chemical treatments significantly decreased the density of broadleaves like, *C. odorata* Linn. and *M. pudica* Linn., The weight of broadleaved weeds were different in untreated check treatment from weeded treatments in 4 species: *B. alata* (Aubl.) DC., *C. odorata* Linn., *S. nodiflora* (L.) Gaertn. and *M. pudica* Linn. (Table 9). These results showed that diammonium salt of glyphosate 56.4% have a good efficiency in grasses.

Table 8. Number of broadleaved weeds in rubber crop at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Broadleaves number * (no. plants m^{-2})					
		BORL	BORA	AGE	CHR	SYN	MIM
1.Diammonium salt of glyphosate	0.53	45.3d	65.3c	10.0c	7.3c	6.7c	7.3d
2.Diammonium salt of glyphosate	0.70	36.7cd	62.7c	7.3bc	6.7c	5.3bc	6.0d
3.Diammonium salt of glyphosate	0.88	25.3bc	56.7bc	4.7ab	3.3b	3.3ab	4.0c
4.Diammonium salt of glyphosate	1.06	16.0ab	44.7b	2.0a	3.3b	2.7a	3.3bc
5.Diammonium salt of glyphosate	1.23	10.0ab	26.7a	2.0a	2.7b	2.0a	2.0b
6.Glyphosate 48% SL	1	9.3a	26.7a	2.0a	0a	1.3a	0a
7.Hand weeding	-	70.0e	82.7d	6.7bc	13.3d	15.3d	7.3d
8.Weedy check	-	75.3e	87.3d	8.0bc	15.3d	17.3d	12.7e
C.V. (%)		13.2	14.2	36.7	19.6	20.1	18.3

* Means within a column followed by the same letter are not significantly different at the 95% level by DMRT.

Weed species

BORL = *Borreria laevis* (Aubl.) DC.
BORA = *Borreria alata* (Lamk.) Griseb.
AGE = *Ageratum conyzoides* Linn.
CHR = *Chromolaena odorata* Linn.
SYN = *Synedrella nodiflora* (L.) Gaertn.
MIM = *Mimosa pudica* Linn.

Table 9. Dry weight of broadleaved weeds in rubber crop at 30 DAA.

Treatment	Rate (Kg ai ha^{-1})	Broadleaves weight * (g m^{-2})					
		BORL	BORA	AGE	CHR	SYN	MIM
1. Diammonium salt of glyphosate	0.53	16.9c	142.7c	83.2c	7.5d	5.8c	2.5d
2. Diammonium salt of glyphosate	0.70	12.2b	115.8bc	79.9a	7.3d	5.7c	2.1cd
3. Diammonium salt of glyphosate	0.88	11.9b	98.2b	43.7b	5.7c	4.9bc	1.9c
4. Diammonium salt of glyphosate	1.06	4.2a	92.8b	18.9a	5.4c	4.7b	2.2cd
5. Diammonium salt of glyphosate	1.23	3.8a	57.9a	11.6a	3.5b	2.2a	0.9b
6. Glyphosate 48% SL	1	3.7a	58.6a	11.4a	0a	1.9a	0a
7. Hand weeding	-	15.6c	216.3d	86.8c	12.3c	16.8d	3.1e
8. Weedy check	-	15.9c	228.0d	88.7c	16.2f	17.6d	6.2f
C.V. (%)		12.5	13.0	9.3	12.2	6.9	11.4

* Means within a column followed by the same letter are not significantly different at the 95% level by DMRT.

Weed species

BORL = *Borreria laevis* (Aubl.) DC.
BORA = *Borreria alata* (Lamk.) Griseb.
AGE = *Ageratum conyzoides* Linn.
CHR = *Chromolaena odorata* Linn.
SYN = *Synedrella nodiflora* (L.) Gaertn.
MIM = *Mimosa pudica* Linn.

CONCLUSION

Diammonium salt of glyphosate 56.4% W/V AS can be applied from 1.06 to 1.23 kg ha^{-1}. It was an excellent of grass control such as *Pennisetum polystachyon* (L.) Schult., *Paspalum conjugatum* Berg., *Digitaria adscendens* (H.B.K.) Henr. and control of broadleaved weeds such as *Borreria laevis* (Lamk.) Griseb., *Chromolaena odorata* Linn. and *Synedrella nodiflora* (L.) Gaertn. Similarity with glyphosate 48% SL at the recommended rate provided a good control of grasses and broadleaved weeds. In addition, diammonium salt of glyphosate 56.4% had no effect and injury of growth and development of rubber resulting in the high yield of rubber production.

LITERATURE CITED

Anonymous, 1995. Weed Control Recommendation. Botany and Weed Science Division, Department of Agriculture, Bangkok, Thailand, 144 p.

Franz, J.E., K.M. Michael and A.S. James. 1997. Glyphosate: A Unique Global Herbicide. American Chemical Society. Washington DC., 653 p.

Leung-apapong, P. 1997. Weed Science. Bangkok, 585 p.

Yongboonkead, A., S. Suteeson and J. Sadakon. 1984. Weed in Rubber Plantation. Weed Science Society of Thailand, Technical bulletin no.3, 171 p.

New Herbicides

Potential Use of Mesotrione in Controlling Annual Weed Species in Maize (*Zea Mays*)

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Abstract. Mesotrione [2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone] is a relatively new pigment inhibitor herbicide that is labeled for use in maize (*Zea mays* L.). This herbicide controls susceptible weed species by inhibiting the 4-HPPD enzyme. Field studies were conducted using typical, replicated, small-plot research techniques at our research farm since 1999. Mesotrione was applied PRE or POST, either alone or in combination with acetochlor, s-metolachlor, or atrazine. Mesotrione applied PRE provided excellent control (>95%) of *Chenopodium album*, *Amaranthus retroflexus*, while mesotrione treatments controlled 82% of *Ambrosia artemisiifolia*. Mesotrione applied POST provided excellent control of these species. In general, mesotrione in combination with acetochlor or s-metolachlor provided excellent control of *Digitaria sanguinalis* and *Setaria glauca*. None of the treatments with mesotione had any adverse effects on silage or grain yield of maize. The pre-mix combination of mesotrione plus acetochlor (2.45 kg ha^{-1}), paraquat (0.7 kg ha^{-1}) and atrazine (1.12 kg ha^{-1}) resulted in highest silage (71000 kg ha^{-1}) and grain (12950 kg ha^{-1}) yields of maize under minimum-tillage system. Under conventional-tillage system, the treatment combination of metolachlor plus atrazine II Magnum and mesotrione (3.24 and 0.11 kg ha^{-1}) resulted in highest maize silage (84448 kg ha^{-1}) and grain (11751 kg ha^{-1}) yields as compared to maize yields of silage (19264 kg ha^{-1}) and grain (24451 kg ha^{-1}) from the untreated check.

Key words: *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Digitaria* sanguinalis, *Setaria glauca*, *Zea mays*, ZA-1296

INTRODUCTION

For many years, maize growers all over the world have relied on triazines, chloroacetamides and thiocarbamates for pre-mergence weed control, and imidazolinones and sulfonylureas for post-emergence weed control. The continuous use of these herbicides has resulted in contamination of groundwater and related environmental concerns. The development of weed resistance to triazines and sulfonylureas has been a result of the use of these herbicides annually, primarily on a continuous basis. The resistant weeds have created major challenges for current weed management systems. Therefore, the introduction of a new pre-mergence herbicide will become an important tool for weed management strategy in maize production.

Mesotrione [2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone] is a systemic herbicide that belongs to triketone herbicide, which is known as HPPD inhibitor. Mesotrione is absorbed by root and foliage. The molecular target for mesotrione is the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD). This enzyme is involved in the pathway that converts the amino acid tyrosine to plastoquinone. Mesotrione is structurally similar to the substrate p-hydroxyphenylpyruvate and acts by competitive inhibition, which results in the blockage of carotenoid synthesis (Lackey et al. 2000). The lack of carotenoid synthesis in meristematic tissues gives rise to bleaching symptoms.

Mesotrione applied PRE selectively controls both grass and broadleaf weeds in maize (Armel et al. 2000; Bhowmik 2000; Bhowmik and Lackey 1999; Bhowmik and McGlew 2001; Lackey and Brownell 2001). Mesotrione applied preemergence at 0.16 kg a.i. ha^{-1} controls important broadleaf weeds such as *Abutilon theophrasti* Medic., *A. retroflexus* L., *A. artemisiifolia* L., *C. album* L. and grass weeds such as *D. sanguinalis* (L.) Scop., *Echinochloa crus-galli* (L.) Beauv., *Seatria faberi* Herrm., *Setaria viridis* (L.) Beauv., and *Panicum dichotomiflorum* Michx (Armel et al. 2000; Bhowmik 2000; Bhowmik and Lackey 1999; Bhowmik and McGlew 2001). Mesotrione at 0.105 kg ha^{-1} when applied postemergence controls *A. retroflexus*, *A. artemisiifolia*, *C. album* (Bhowmik and Lackey 1999; Bhowmik and McGlew 2001) and *Abutilon theophrasti*, *Xanthium strumarium* L. and *Datura stramonium* L. (Lackey and Browell 2001).

Limited information is available on the effectiveness of mesotrione and their combinations with grass control herbicides. The objective of our studies was to evaluate various PRE and/or POST treatments under three tillage systems.

MATERIALS AND METHODS

General experiments were conducted at the University of Massachusetts Experiment Station, South Deerfield, MA. The soil was a Hadley fine sandy loam (Typic Udifluvents) containing sand, silt and clay in 50.2, 44.6 and 5.1%, respectively. The soil had a pH of 6.3 with an organic matter content of 1.5% and a cation exchange capacity (CEC) of 6.8-meq/100 g. The area was heavily infested with *D. sanguinalis*, *S. glauca*, *C. album*, and moderately infested with *A. artemisiifolia* and *A. retroflexus*. Plots were 3 by 6 m and maize was planted in 75 cm rows at a density of 62,800 plants ha^{-1}. Treatments were applied with a CO_2 backpack sprayer at a pressure of 152 kPa in 189 l ha^{-1}, using 8002 flat fan nozzles. Weed control was visually estimated on a scale of 0% to 100%, where 0% = no control and 100% = complete control. Weed control was estimated 2, 3, 5, 10, and 12 weeks after treatment (WAT). Maize was harvested from the center row of each plot. Grain yield was adjusted to 15% moisture.

General linear model (GLM) program of SAS (SAS Institute Inc., 1995) was used to analyze the data. All data were subjected to analysis of variance (ANOVA) and appropriate mean separation techniques were used. ANOVA was used to determine significant treatment effects and the error terms were obtained by calculating the expected mean squares (Damon and Harvey 1987). Data were subjected to analysis of variance and means were separated by Student-Newman-Keuls test at $p = 0.05$, using the appropriate error term.

Minimum-tillage system, 1999 Minimum-tillage area consisted of maize stubbles from 1998 season and was disked once in the spring. Prior to planting, fertilizer was applied broadcast at 560 kg ha^{-1} of 15-8-12 (N-P-K). The minimum-tillage area had also moderate infestations of horseweed (*Conyza canadensis* L.) and catchweed bedstraw (*Galium aparine* L.). 'Max-21' maize was planted with a no-till planter on May 12, 1999. The PRE treatments were applied on May 13, 1999. The experiment was a randomized block design with three replications. Maize was harvested on October 7, 1999.

No-tillage system, 2000 No-tillage area was maintained with maize stubbles from 1999 season. Fertilizer was applied broadcast at 560 kg ha^{-1} of 15-8-12 (N-P-K). 'Max-21' maize was planted with a no-till planter on May 11, 2000. Maize was also side-dressed with 50 kg ha^{-1} of 34-0-0 (N-P-K) on July 18, 2000. All treatments were applied one week prior to maize planting (PTP). This site had an infestation of rough fleabane (*Erigeron strigosus* Muhl. ex Willd.) and white campion [*Silene alba* (Mill.) E.H.L. Krause]. The experiment was a randomized block design with three replications. No yield data was recorded due to heavy bird damage.

Conventional-tillage system, 2001 Conventional tillage consisted of moldboard plowing followed by two diskings in the spring. Fertilizer was applied broadcast at 560 kg ha^{-1} of 15-8-12 (N-P-K). 'Max-21' maize was planted with a no-till planter on May 7, 2001. Maize was also side-dressed with 50 kg ha^{-1} of 46-0-0 (N-P-K) on July 2, 2001. The PRE treatments were applied on May 9, 2001, while POST treatments were applied at the 3- to 4-leaf stage of *D. sanguinalis* species when *C. album* was at the 6- to 7-leaf stage and *A. artimisiifolia* was at the 5- to 6-leaf stage. The experiment was a randomized block design with three replications. Maize was harvested on October 2001.

RESULTS AND DISCUSSION

Minimum-tillage system, 1999 The pre-mix combinations of mesotrione plus acetochlor either with glyphosate or paraquat controlled *C. album*, *A. artemisiifolia* and *A. retroflexus* 5 and 12 WAT, and *Galium aparine* and *C. canadensis* 12 WAT (Table 1). The addition of 2,4-D or atrazine to these treatments did not improve any control of these species. The PRE treatment of acetochlor or a pre-mix combination of acetochlor plus atrazine, when combined with isoxaflutole provided excellent control of these species 5 and 12 WAT. These treatments provided excellent (>95%) control of *G. aparine* and *C. canadensis* 12 WAT. Similar broadleaf weed control with isoxaflutole has been reported earlier (Bhowmik et al. 1996; Bhowmik et al. 1999).

All treatments provided excellent burn-down control of all grass and broadleaf weed species present at the time of application (Table 2). These treatments controlled *S. glauca* and *D. sanguinalis* up to 12 WAT. The PRE treatments of acetochlor or a pre-mix combination of acetochlor plus atrazine, when combined with isoxaflutole provided excellent control of *S. glauca* and *D. sanguinalis* up to 12 WAT. The pre-mix combination of mesotrione plus acetochlor (2.45 kg ha^{-1}), paraquat (0.7 kg ha^{-1}) and atrazine (1.12 kg ha-1) resulted in highest silage (71000 kg ha^{-1}) and grain (12950 kg ha^{-1}) yields of maize.

Table 1. Broadleaf weed control as affected by various PRE treatments in maize under minimum-tillage system in 1999, South Deerfield, MA.

Treatment	Rate		Control, WAT[1]							
			Chenopodium album		*Ambrosia artemisiifolia*		*Amaranthus retroflexus*		*Galium aparine*	*Conyza canadensis*
			5	12	5	12	5	12	12	12
	Kg ha^{-1}		%							
Mesotrione plus acetochlor[2] + Glyphosate + AMS	2.45 + 0.70	PRE	100 a[3]	100 a	99 a	93 a	100 a	99 ab	98 a	99 a
Mesotrione plus acetochlor + Glyphosate + 2,4-D + AMS[4]	2.45 + + 0.70 + 0.53	PRE	100 a	99 b	100 a	94 a	99 a	96 b	96 a	99 a
Mesotrione plus acetochlor + Paraquat + NIS[5]	2.45 + 0.70	PRE	100 a	99 ab	99 a	93 a	98 a	97 ab	98 a	100 a
Mesotrione plus acetochlor + Paraquat + Atrazine + NIS[5]	2.45 + 0.70 + 1.12	PRE	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Mesotrione plus acetochlor + Paraquat + 2, 4-D + NIS[5]	2.45 + 0.70 + 0.53	PRE	100 a	100 a	100 a	99 a	100 a	100 a	96 a	100 a
Acetochlor + isoxaflutole	2.24 + 0.08	PRE	100 a	100 a	100 a	98 a	99 a	99 ab	97 a	100 a
Acetochlor plus atrazine+ Isoxaflutole	2.52 + 0.08	PRE	100 a	100 a	100 a	99 a	100 a	99 ab	100 a	100 a

[1]WAT = weeks after initial treatment application
[2]Mesotrione plus acetochlor is a pre-mix product
[3]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.
[4]AMS = ammonium sulfate was used at 2% (v/v)
[5]NIS = non-ionic surfactant, X-77 was used at 0.25% (v/v)

Table 2. Total burn-down control of existing weed species and subsequent PRE control of grass species control and maize yields as affected by various treatments under minimum-tillage system in 1999, South Deerfield, MA.

Treatment	Rate	Timing	Control, WAT[1]					Maize yield	
			All species	*Setaria glauca*		*Digitaria sanguinalis*		Silage	Grain
			1	2	12	2	12		
	Kg ha^{-1}		%					Kg ha^{-1}	
Mesotrione plus acetochlor[2]+ Glyphosate + AMS[4]	2.45 + + 0.70	PRE	100 a[3]	100 a	99 a	98 a	98 a	59808 ab	9005 ab
Mesotrione plus acetochlor + Glyphosate + 2,4-D + AMS[4]	2.45 + + 0.70 + 0.53	PRE	100 a	100 a	99 a	98 a	94 a	50400 bc	8127 bc
Mesotrione plus acetochlor+ Paraquat + NIS[5]	2.45 + 0.70	PRE	100 a	100 a	99 a	100 a	96 a	51072 bc	7789 bc
Mesotrione plus acetochlor + Paraquat + Atrazine + NIS[5]	2.45 + + 0.70 + 1.12	PRE	100 a	100 a	99 a	100 a	98 a	71008 a	12950 a
Mesotrione plus acetochlor + Paraquat + 2, 4-D + NIS[5]	2.45 + 0.70 + 0.53	PRE	100 a	100 a	100 a	100 a	98 a	55328 ab	8616 bc
Acetochlor + isoxaflutole	2.24 + 0.08	PRE	95 ab	97 ab	99 a	100 a	97 a	4741 bc	7080 bc
Acetoclor plus atrazine + Isoxaflutole	2.52 + 0.08	PRE	97 a	99 ab	100 a	100 a	95 a	4547 bc	6447 bc

[1]WAT = weeks after initial treatment application
[2]Mesotrione plus acetochlor is a pre-mix product
[3]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.
[4]AMS = ammonium sulfate was used at 2% (v/v)
[5]NIS = non-ionic surfactant, X-77 was used at 0.25% (v/v)

No-tillage system, 2000 The pre-mix combination of mesotrione plus acetochlor and glyphosate or paraquat applied PRE controlled *C. album, Erigeron strigosus, Silene alba, D. sanguinalis* and *Elytrigia repens* up to 10 WAT (Table 3). Similar weed control with mesotrione treatments under no-tillage system has been reported (Armel et al. 2000; Bhowmik et al. 2000). There were no differences in weed control due to various rates (1.96, 2.20 and 2.45 kg ha^{-1}) of mesotrione plus acetochlor treatments in our trial in 2000. The premix combination of acetochlor plus atrazine plus glyphosate at 4.76 kg ha^{-1} controlled *C. album, Erigeron strigosus, S. alba, D. sanguinalis* and *E. repens* 10 WAT.

Conventional-tillage system, 2001 Treatment of metolachlor II Magnum and mesotrione at 1.78 kg ha^{-1} + 0.21 kg ha^{-1} alone or with an application of atrazine (1.12 kg ha^{-1}) applied PRE controlled *C. album, A. artemisiifolia, S. glauca* and *D. sanguinalis* 10 and 16 WAT (Table 4). The pre-mix combination of metolachlor plus atrazine II Magnum PRE alone or followed by a POST application of mesotrione at 0.11 kg ha^{-1} provided excellent control (>95%) of all of these species up to 16 WAT. The PRE application of metolachlor II Magnum followed by an application of mesotrione and atrazine (0.11 and 0.28 kg ha^{-1}) or primisuilfuron at 0.04 kg ha^{-1} POST controlled *C. album, A. artemisiifolia, S. glauca* and *D. sanguinalis* up to 16 WAT. Similar broadleaf weed control with mesotrione has been reported earlier (Armel et al. 2000; Bhowmik 2000; Bhowmik and Lackey 1999; Creech and Evans 2002).

None of the treatments with mesotione had any adverse effects on silage or grain yield of maize. The tolerance of maize and sensitivity of weed species to mesotrione is explained by differences in uptake and metabolism (Lackey et al., 2000). The treatment combination of metolachlor plus atrazine II Magnum and mesotrione at 3.24 + 0.11 kg ha^{-1} resulted in highest maize silage (84448 kg ha^{-1}) and grain (11751 kg ha^{-1}) yields as compared to maize yields of silage (19264 kg ha^{-1}) and grain (24451 kg ha^{-1}) from the untreated check.

These studies demonstrate the importance of mesotrione in annual broadleaf weed control. This type of weed control would be of great importance in designing weed management practices in maize around the world, especially for the control of triazine resistant broadleaf weeds.

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Table 3. Weed control as affected by various treatments applied one week prior to planting maize under no-tillage system in 2000, South Deerfield, MA.

Treatment	Rate	Timing	Control, WAT[1]									
			Chenopodium album		*Erigeron strigosus*		*Silene alba*		*Digitaria sanguinalis*		*Elytrigia repens*	
			3	10	3	10	3	10	3	10	3	10
	Kg ha^{-1}		%									
Mesotrione plus acetochlor[2]+ Glyphosate + AMS[4]	1.96 + 0.84	PTP	100 a[3]	95 a	100 a	98 a	100 a	99 a	100 a	98 a	98 a	98 a
Mesotrione plus acetochlor + Glyphosate + AMS[4]	2.20 + 0.84	PTP	100 a	96 a	100 a	100 a	100 a	100 a	100 a	95 a	99 a	96 ab
Mesotrione plus acetochlor + Glyphosate + AMS[4]	2.45 + 0.84	PTP	100 a	96 a	99 a	100 a	100 a	100 a	100 a	95 a	98 a	96 ab
Mesotrione plus acetochlor + Paraquat + NIS[5]	1.96 + 0.70	PTP	100 a	95 a	100 a	98 a	97 a	88 a	100 a	93 a	99 a	96 ab
Mesotrione plus acetochlor + Paraquat + NIS[5]	2.20 + 0.70	PTP	100 a	93 a	100 a	99 a	97 a	88 a	100 a	93 a	98 a	87 bc
Mesotrione plus acetochlor + Paraquat + NIS[5]	2.45 + 0.70	PTP	100 a	93 a	100 a	100 a	100 a	96 a	100 a	95 a	96 a	83 c
Acetochlor plus atrazine[2] plus Glyphosate (PM) + AMS[4]	4.76	PTP	100 a	99 a	99 a	100 a	99 a	100 a	100 a	93 a	95 a	96 ab

[1]WAT = weeks after initial treatment application prior to planting (PTP)
[2]Mesotrione plus acetochlor and acetochlor plus atrazine are pre-mix products
[3]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.
[4]AMS = ammonium sulfate (21%) was used at 2 lb/A
[5]NIS = non-ionic surfactant, X-77 was used at 0.25% (v/v)

Table 4. Weed control and maize yields as affected by various treatments under conventional tillage system in 2001, South Deerfield, MA.

Treatment	Rate	Timing	Control, WAT[1] *Chenopodium album* 10	*Chenopodium album* 16	*Ambrosia artemisifolia* 10	*Ambrosia artemisifolia* 16	*Setaria glauca* 10	*Setaria glauca* 16	*Digitaria sanguinalis* 10	*Digitaria sanguinalis* 16	Yield Silage	Yield Grain
	kg ha^{-1}		(%)								Kg/ha	
Metolachlor II Mgn + Mesotrione	1.78 + 0.21	PRE	93 b[4]	90 b	99 a	92 b	94 b	90 c	97 a	88 c	51520 a	6867 a
Metolachlor II Mgn +	1.78 + 0.21	PRE										
Mesotrione + Atrazine	+ 1.12	PRE9	8 a	94 ab	99 a	94 ab	96 ab	95 abc	97 a	93 b	74592 a	10893 a
Metolachlor II Mgn +	1.78 + 0.21	PRE										
Mesotrione + UAN[2] + COC[3]		POST	100 a	96 ab	100 a	98 a	98 ab	95 abc	98 a	95 b	75488 a	10836 a
Metolachlor plus atrazine II Mgn	3.24	PRE	97 a	92 ab	99 a	93 ab	96 ab	93 bc	93 b	92 b	70784 a	9783 a
Metolachlor plus atrazine II Mgn	3.24 + 0.11	PRE										
Mesotrione + UAN[2] + COC[3]		POST	100 a	100 a	100 a	100 a	100 a	99 a	100 a	99 a	84448 a	11751 a
Metolachlor II Mgn +	1.78 +	PRE										
Mesotrione + Atrazine + UAN[2] + COC[3]	0.11 + 0.28	POST	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	72576 a	10372 a
Metolachlor II Mgn +	1.78 +	PRE	100 a	99 a	100 a	99 a	99 a	95 abc	96 ab	93 b	71680 a	10002 a
Primisulfuron + UAN[2] + COC[3]	0.04	POST										
Untreated check			0 c	0 c	0 b	0 c	0 c	0 d	0 c	0 d	19264 b	2445 b
Cultivated check			100a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	75040 a	11256 a

[1]WAT = weeks after treatment
[2]UAN = urea nitrogen was used at 2% (v/v)
[3]COC = crop oil concentrate was used at 1% (v/v)
[4]Means followed by the same letter within a column are not significantly different at the 5% level according to Student-Newman-Keuls test.

LITERATURE CITED

Armel, G. R, H. P. Wilson and T. E. Hines. 2000. Annual weed and grass control in corn with ZA-1296. Proceedings of the Northeastern Weed Science Society, 54:29.

Bhowmik, P.C. 2000. Burndown activity and residual weed control with ZA 1296/acetochlor with touchdown or gramoxone. Massachusetts Weed Science Research Results, 19:47-55.

Bhowmik, P.C. and B.A. Lackey. 1999. Performance of various treatments in weed control in minimum-tillage corn. Massachusetts Weed Science Research Results, 18:49-55.

Bhowmik, P.C. and E. McGlew. 2001. Comparison of various pre and post treatments for weed control in corn. Massachusetts Weed Science Research Results, 20:27-32.

Bhowmik, P. C., S. Kushwaha and S. Mitra. 1999. Response of various weed species and corn *(Zea mays)* to RPA 201772. Weed Technology, 13:504-509.

Bhowmik P.C., T. E. Vrabel., R. Prostak and J. Carter. 1996. Activity of RPA 201772 in controlling weed species in field corn. Proceedings of the Second International Weed Control Congress, 2:807-812.

Creech, J.E. and J.O. Evans. 2002. Performance of mesotrione for annual weeds in corn under semi-arid conditions of the West. Weed Science Society of America Abstracts, 42:1.

Damon R. A. Jr. and W. R. Harvey. 1987. Experimental Design, ANPVA and Regression. Harper and Row, Publishers, Inc. NY, 508 p.

Lackey, B.A., R.A. Wichert, D.W. Bartlett, and J.K. Townson. 2000. Mode of action, absorption, translocation and metabolism of mesotrione in weeds and corn. Proceedings of the Northeastern Weed Science Society, 54:55.

Lackey, B.A. and K.H. Brownell. 2001. ZA1296: Preemergence and postemergence performance in weed control programs. Proceedings of the Northeastern Weed Science Society, 55:8.

[SAS] Statistical Analysis Systems. 1995. SAS/STAT User's Guide. Version 6, 4th ed., Vol. 2. Cary NC: Statistical Analysis Systems Institute, 846 p.

LGC-42153: A New Generation Sulfonylurea Herbicide

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Abstract: LGC-42153 is a new sulfonylurea herbicide being developed by LG Life Sciences Ltd., Korea. This new herbicide can be used for rice and cereal crops. In rice, the herbicide provides excellent control of *Echinochloa crus-galli*, which is not controlled or only marginally controlled by the commercial sulfonylurea herbicides such as bensulfuron-methyl or pyrazosulfuron-ethyl. In addition to *E. crus-galli* control, the herbicide provides excellent control of annual broad-leaved, sedges and perennial weeds of rice with similar efficacy to other sulfonylurea rice herbicides. For rice, the herbicide can be applied both to soil and as foliar, and its use rate is 15 to 30 g a.i. ha^{-1}. The herbicide can also control broadleaved weeds including *Galium* spp. with good safety to cereal crops when foliar-applied. In this report, we introduce basic physicochemical, toxicological, and biological properties of the herbicide, and its development status.

Key words: LGC-42153, sulfonylurea, acetolactate synthase, rice, winter cereals

INTRODUCTION

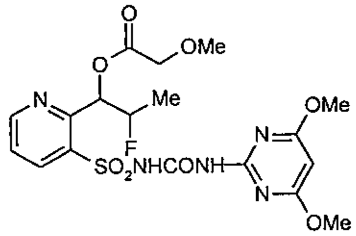
Sulfonylurea rice herbicides such as bensulfuron-methyl and pyrazosulfuron-ethyl were first introduced in the early to mid 80s, and have contributed greatly in weed control of all rice systems in the world. Typical characteristics of these sulfonylurea herbicides include low use rates, low mammalian and eco-toxicity, and high efficacy and good rice safety. In terms of biological efficacy, these herbicides control various annual and perennial weeds, but are weak on *Echinochloa crus-galli*, the primary grass weed in rice. Due to this weakness, these herbicides have been used as mixtures with grass killer partners, as so called "one-shot" herbicides in various countries, particularly Korea and Japan.

In the new herbicide discovery with sulfonylurea chemistry, a new molecule that can control *Echinochloa* in addition to sedge and broadleaved weeds has been a major goal for over 15 years; however, there has been no real success.

LGC-42153 [N-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-2-[2-fluoro-1-(methoxy methylcarbonyloxy) propyl]-3-pyridinesulfonamide] is a new sulfonylurea herbicide discovered by LG Life Sciences Ltd. jointly with Korea Research Institute of Chemical Technology. This new herbicide controls *E. crus-galli* very effectively as well as the other various rice weeds by soil and foliar application, demonstrating a significant advancement in this chemistry.
In this report, we introduce basic chemical and biological information of the herbicide LGC-42153.

CHEMICAL AND PHYSICAL PROPERTIES

Structure:



Code Name:	LGC-42153
Common Name:	Flucetosulfuron (ISO-proposed)
Chemical Name:	N-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-2-[2-fluoro-1- (methoxymethylcarbonyloxy) propyl]-3-pyridinesulfonamide
Chemical Formula:	$C_{18}H_{21}FN_5O_8S$
Molecular Weight:	487.3
CAS RN:	412928-75-7
Appearance:	Odorless, solid white powder at 25□
Melting Point:	178~182□
Solubility (water at 25□):	114 mg L^{-1}
Partition (octanol/water) coefficient:	logP = 1.05
Vapour pressure :	<1.86×10^{-5} Pa at 25□
pKa value :	3.5

TOXICOLOGICAL PROPERTIES

Rat acute oral toxicity	LD50: >5000 mg
Mouse acute oral toxicity	LD_{50}: >5000 mg kg^{-1} (♂♀)
Dog acute oral toxicity	LD_{50}: >2000 mg kg^{-1} (♂♀)
Rat 13 weeks oral toxicity	NOAEL 200 ppm (dietary)
Ames test	Negative
Chromosome aberration test	Negative
Micronucleus test	Negative

ENVIRONMENTAL SAFETY

Fish acute toxicity (carps)	LC_{50} >10 ppm
Algae acute toxicity	EC_{50} >10 ppm
Daphnia acute toxicity	LC_{50} >10 ppm

FORMULATION

For soil application, LGC-42153 is formulated as 0.07 and 0.1% GR in Korea and Japan, respectively. For foliar application, it is formulated as a water dispersible granule of various contents from 10 to 50%. We also developed self-dispersible laborsaving granules (Kim et al. 2003).

MODE OF ACTION

LGC-42153 is a sulfonylurea and, thus, inhibits acetolactate synthase (ALS) (Hwang et al. 2003), the first committed enzyme for the biosynthesis of branched-chain amino acids, valine, leucine, and isoleucine. This compound can be absorbed via roots, stem and leaf, and its translocation via leaf is faster than that of glyphosate and pyribenzoxim (Lee et al. 2003). The symptoms of herbicidal action include growth cessation, chlorosis, death of apical meristems, and subsequently whole plant death in 2~3 weeks. The selectivity mechanism to this herbicide is assumed to be due mainly to the rapid recovery of ALS activity in rice as compared with weeds (Hwang et al. 2003).

BIOLOGICAL PROPERTIES

Weed Control Spectrum

LGC-42153 applied to the soil or foliarly provides broad weed control spectrum including annual broad-leaved weeds, sedges, some grasses such as *Echinochloa* spp., and perennial weeds (Table 1). Particularly in rice, its weed control spectrum is very similar to conventional sulfonylurea rice herbicides such as pyrazosulfuron-ethyl but the advanced characteristics of LGC-42153 is its capacity to control of *Echinochloa* spp. Therefore, the efficacy of LGC-42153 alone is similar to that of the conventional one-shot mixtures in Korea and Japan. Based on this advantage in biological efficacy, LGC-42153 can be developed either as a solo product or mixture with new concepts or simpler composition.

Table 1. Weed control by LGC-42153 at a range of application doses in comparison with pyrazosulfuron-ethyl applied to soil at 7 days after rice transplanting. Results from pot trials.

Herbicide	Dose g a.i. ha⁻¹	Rice safety	Weed control (%; 0: no control, 100: complete kill)							
			ECHCG	MOOVA	SCPJU	ROTIN	LIDPY	CYPSE	ELOKU	SAGTR
LGC-42153	5	0	40	50	50	70	60	40	30	40
	10	0	80	90	70	100	90	65	60	50
	15	0	95	100	95	100	95	80	70	60
	20	10	100	100	100	100	100	95	80	70
	40	20	100	100	100	100	100	95	90	80
Pyrazosulfuron-ethyl	5	0	0	80	70	90	60	60	30	40
	10	0	20	90	80	100	90	70	60	60
	15	0	30	100	100	100	100	80	80	70
	20	10	40	100	100	100	100	95	90	80
	40	10	40	100	100	100	100	95	90	90

ECHCG: *Echinochloa crus-galli*, MOOVA: *Monochoria vaginalis*, SCPJU: *Scirpus juncoides*, ROTIN: *Rotala indica*, LIDPY: *Lindernia pyxidaria*, CYPSE: *Cyperus serotinus*, ELOKU: *Eleocharis kuroguwai*, SAGTR: *Sagittaria trifolia*

Echinochloa crus-galli Control

When applied to the soil, LGC-42153 controlled *E. crus-galli* completely even at 10g a.i. ha^{-1} (Figure 1A). When applied as foliar spray, LGC-42153 controlled *E. crus-galli* at 20g a.i. ha^{-1}, and the overall efficacy was similar to that of pyribenzoxim (Figure 1B).

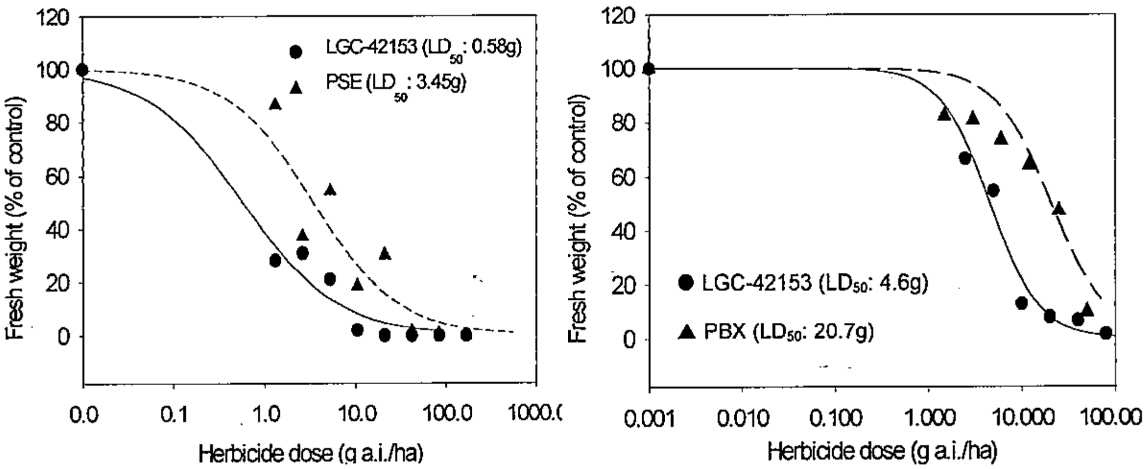


Figure 1. Dose responses of *E. crus-galli* to soil-applied LGC-42153 and pyrazosulfuron-ethyl (PSE) (A), and to foliar-applied LGC-42153 and pyribenzoxim (PBX) (B). The growth stage of *E. crus-galli* at the time of application was three leaf stage for both soil and foliar application.

Residual Efficacy

Residual period of *Echinochloa* spp. control by LGC-42153 was between 30~40 days after application, which was significantly longer that those of molinate and molinate+pyrazosulfuron-ethyl mixture (Fig. 2). Therefore, it is expected that season-long control of *Echinochloa* spp. is assured by one application of LGC-42153. Carry-over potential to succeeding crops was also evaluated in pots and field conditions; however, there was no injury to cereals and the various vegetables (data not shown).

Crop Safety

LGC-42153 offers good selectivity to rice and cereal crops. The dose-response study of rice to LGC-42153 revealed that the LD_{50} value of LGC-42153 was 182.9g a.i. ha^{-1}, while that of pyrazosulfuron-ethyl was 323.1g a.i. ha^{-1} (Fig. 3). As recommended application dose range of LGC-42153 is 20~30g a.i. ha^{-1}, it can be concluded that LGC-42153 is very safe to rice.

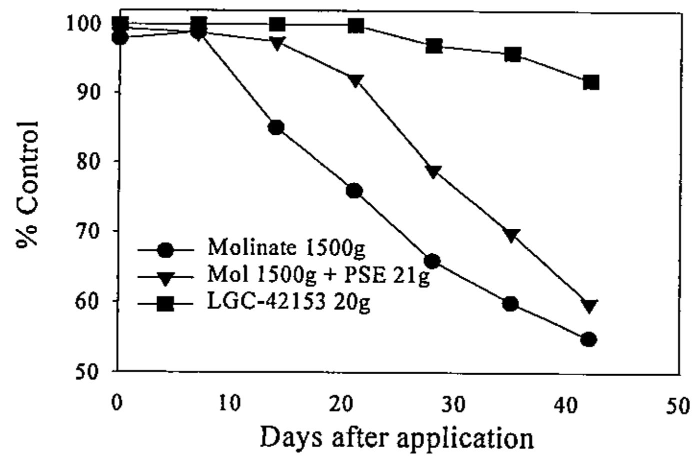


Figure 2. Residual efficacy of LGC-42153 in controlling *E.crus-galli* in comparison with molinate (●) and molinate + pyrazosulfuron-ethyl (▲). The test was conducted in a pot condition.

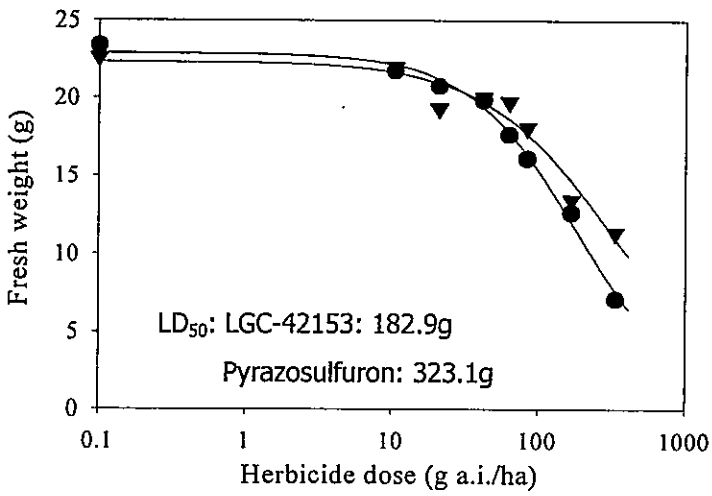


Figure 3. Dose responses of rice to LGC-42153 (●) and pyrazosulfuron-ethyl (▲) which were applied to paddy water at 12 days after rice-transplanting. The fresh weight was recorded at 30 days after herbicide application.

CONCLUSIONS

The herbicide LGC-42153 is a new highly active herbicide to *E. crus-galli*, and other annual and perennial rice weeds. Due to its excellent activity to *E. crus-galli*, the herbicide does not require a grass-killer partner in the typical one-shot mixture in Korea and Japan. This advantage opens possibilities to develop a solo product and also new mixtures with low environmental input of chemicals. As the herbicide is effective by soil as well as foliar application, flexibility to develop diversified products in all rice culture system is very high.

LITERATURE CITED

Hwang, K. H., D. S. Kim, J. N. Lee, and S. J. Koo, 2003. Crop and weed selectivity to a new sulfonylurea herbicide LGC-42153. Proceedings of the 19th Asian-Pacific Weed Science Society Conference, 17-21 March 2003, Manila, Philippines.

Lee, J. N., D. S. Kim, K. H. Hwang, and S. J. Koo. 2003. Rapid and cost-effective measurement of herbicide translocation. Proceedings of the 19th Asian-Pacific Weed Science Society Conference, 17-21 March 2003, Manila, Philippines.

Kim, T. Y., K. G. Kang, D. S. Kim, J. N. Lee, and S. J. Koo. 2003. Formulation factors influencing effective action of LGC-42153 laborsaving GR formulation. Proceedings of the 19th Asian-Pacific Weed Science Society Conference, 17-21 March 2003, Manila, Philippines. pp. 682-698

Efficacy and Crop Tolerance of DE-638 in Indica Rice in ASEAN Countries

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Abstract: DE-638, common name penoxsulam, is a new rice herbicide belonging to triazolopyrimidine sulfonamides that is being developed by Dow AgroSciences for weed control in rice growing countries around the world. It is a broad-spectrum herbicide with post-emergence and residual activity. This paper presents the efficacy and crop tolerance field trial results for DE-638 in direct-seeded and transplanted indica rice across 5 ASEAN countries (Malaysia, Philippines, Indonesia, Thailand &Vietnam) from 1998 to 2002. Multi-location field studies across ASEAN over 5 years demonstrated that when applied as a post-emergence application at 3 to 16 days after sowing in direct wet-seeded rice, 10 to 15 g ai ha^{-1} provided >90% control of ECHCG, FIMMI, CYPIR, CYPDI, MOOVA and SPDZE. When applied post-emergence at 10 to 16 days after transplanting in transplanted rice, 10 to 15 g ai ha^{-1} provided >90% control of ECHCG, FIMMI, CYPIR, CYPDI, MOOVA and SPDZE. The highest rate of DE 638 alone tested (60 g ai ha^{-1}) did not provide commercial control of LEFCH. However, DE-638 + cyhalofop-butyl tankmix at 10 +50 to 12.5 + 62.5 g ai ha^{-1}, respectively, can be used to attain >90% control of LEFCH and to enhance the performance of DE-638 on ECHCG. DE-638 is very safe to indica rice when applied to direct-seeded and transplanted rice at tested rates of 2.5 to 60 g ai ha^{-1} with application timing of 3-16 days after sowing or transplanting.

Key words: DE-638, efficacy, direct seed rice, grasses, sedges, broadleaf weeds

INTRODUCTION

Rice is a major staple food with a planted area of 29 MM ha in ASEAN countries in 2001, which provides for 520 million people. Rice production should be increased to meet the demand of the increasing population. One of the most important methods to increase rice production is to minimize the loss caused by weed competition in rice fields. These weeds not only reduce rice production but also affect rice seed quality. Since the beginning of agriculture, growers have tried to control rice weed by any means. One of the means is using synthetic herbicides. Many different herbicides have been commercialized, but farmers still prefer a one shot treatment that will provide broad-spectrum weed control.

DE-638, common name penoxsulam, has been in development by Dow AgroSciences since 1998. It is a new rice herbicide belonging to triazolopyrimidine sulfonamides that could be a suitable herbicide for broad-spectrum weed control in rice. Field studies with DE-638 were started in ASEAN countries in 1999.Since then, further field studies to fine tune the dosage were initiated in Malaysia, Philippines, Indonesia, Thailand & Vietnam.

This report summarizes the result of 66 efficacy and rice crop tolerance trials conducted in these countries in 1998-2002.

MATERIALS AND METHODS

Field studies were conducted on field stations of Rice Research Institutes in Malaysia, Philippines, Indonesia and Vietnam and on farmer fields in Thailand. Trials were done in randomized complete block design (RCBD) with 3 or 4 replications with plots of 16-25 m^2. Target crop was *Oryza sativa (Indica)* cultivated by direct seeding or transplanting. In wet seeded rice, water was partially drained from the field. Post emergence foliar application was made to exposed weeds, and the paddy was reflooded within 48 h after application. Other cultivation followed local farming practices.

DE-638 formulation (formulation code GF-237) tested in these trials contained 25g ai L^{-1} penoxsulam, with the tested rates ranging from 5 to 60 g ai ha^{-1}. Each tested rate was diluted in a spray volume of 300 - 400 L water ha^{-1} and applied by knapsack sprayer with fan nozzle. No additional adjuvant tankmix was required for use with GF-237.

Weed control evaluations were made at 14 DAA (days after application), 28 DAA and 42 DAA by visual observation on biomass reduction of weed compared with untreated plot as percentage of control. Phytotoxycity was recorded at 3, 7 and 14 DAA by visual assessment of percent injury.

Collected data were statistical analyzed by PRM5 (owned by Dow AgroSciences) and Minitab software

RESULTS AND DISCUSSIONS

Rice crop phytotoxicity

The tested rate 5-60 g ai ha^{-1} DE-638, did not cause any injury symptom on indica rice when applied at 3-16 days after sowing or transplanting.

Efficacy of DE-638 on *Echinocloa crus -galli* (ECHCG)

Table 1 demonstrates that all tested rates provided herbicidal activity on ECHCG with wide application window of 3-16 DAS. DE-638 at 10-15 g ai ha^{-1} provided more than 85% control, except in 2001 trials in Philippines where12.5 g ai ha^{-1} were applied at 10-15 DAS.

Table 1. Efficacy of DE-638 on ECHCG at 28 DAA (% biomass reduction).

Days after Sowing	Country	No. of observation	DE-638 (g ai ha^{-1})			
			7.5	10	12.5	15
3 -5 DAS	Philippines	n = 4	78.8	86.3	94.5	95.5
	Vietnam	n = 8	78.8	94.4	96.3	96.9
6 -9 DAS	Malaysia	n = 4	70.0	85.0	85.0	90.0
	Philippines	n = 4	83.8	96.0	98.0	98.3
	Thailand	n = 8	60.0	93.4	95.9	98.4
	Vietnam	n = 8	65.0	84.4	88.2	96.4
10 –12 DAS	Malaysia	n = 4	-	90.0	-	96.0
	Philippines	n = 12	-	71.8	86.0	93.8
	Thailand	n = 8	-	97.5	97.5	96.3
	Vietnam	n = 12	-	93.3	95.0	98.3
13-16 DAS	Malaysia	n = 12	-	88.0	87.5	93.3
	Philippines	n = 24	83.25	77.7	92.4	95.8
	Thailand	n = 8	-	91.7	95.0	98.4
	Vietnam	n = 24	78.8	89.0	92.5	97.5
	Indonesia *	n = 4	-	85.7	-	98.4

* Transplanted rice

Efficacy of DE-638 on *Fimbristylis miliacea* (FIMMI)

Table 2 demonstrates that 12.5 g ai ha^{-1} DE-638 provided >87.5% control of FIMMI with an application window of 3-16 DAS across ASEAN countries.

Table 2. Efficacy of DE-638 on FIMMI at 28 DAA (% biomass reduction).

Days after Sowing	Country	No. of observation	DE-638 (g ai ha^{-1})			
			7.5	10	12.5	15
3 -5 DAS	Vietnam	n = 8	85	95.7	96.3	98.8
6 -9 DAS	Malaysia	n = 4	80.0	95.0	95.0	100.0
	Vietnam	n = 8	81.3	87.6	88.75	98.2
10 –12 DAS	Malaysia	n = 4	-	80.0	-	98.0
	Thailand	n = 4	-	98.8	100.0	100.0
	Vietnam	n = 12	-	89.6	93.8	96.7
13-16 DAS	Malaysia	n = 12	-	87.0	-	100.0
	Vietnam	n = 20	82.5	87.5	91.7	96.8

Efficacy of DE-638 on *Cyperus spp* (CYPSS)*

Table 3 demonstrates that 10 g ai ha^{-1} DE-638 provided more than 85% control of CYPSS with an application window of 3-12 DAS. DE-638 applied at 13-16 DAS required12.5 g a.i./ha.

Table 3. Efficacy of DE-638 on CYPSS* at 28 DAA (% biomass reduction).

Days after Sowing	Country	No. of observation	**DE-638 (g ai ha^{-1})**			
			7.5	**10**	**12.5**	**15**
3 -5 DAS	Philippines	n = 4	100.0	100.0	100.0	100.0
	Vietnam	n = 4	85.0	91.3	92.5	97.5
6 -9 DAS	Malaysia	n = 4	80.0	95.0	95.0	100.0
	Philippines	n = 8	-	100.0	100.0	100.0
	Thailand	n = 4	93.3	96.7	100.0	100.0
	Vietnam	n = 8	81.3	89.4	88.2	96.9
10 –12 DAS	Malaysia	n = 4	-	85.0	-	100.0
	Philippines	n = 8	-	100.0	100.0	100.0
	Thailand	n = 4	-	85.0	93.3	95.0
	Vietnam	n = 12	-	89.2	93.2	96.3
13-16 DAS	Malaysia	n = 12	-	85.0	93.0	88.3
	Philippines	n = 20	100.0	100.0	100.0	100.0
	Thailand	n = 8	-	88.3	90.0	100.0
	Vietnam	n = 20	77.5	87.0	91.3	96.3

* CYPSS in Malaysia and Vietnam was CYPIR and in Philippine and Thailand was CYPDI

Efficacy of DE-638 on *Sphenoclea zeylanica* (SPDZE)

Table 4 demonstrates that 10 g ai ha^{-1} DE-638 provided more than 85% control of SPDZE with an application window of 3-16 DAS.

Table 4. Efficacy of DE-638 on SPDZE at 28 DAA (% biomass reduction).

Days after Sowing	Country	No. of observation	DE-638 (g ai ha^{-1})			
			7.5	10	12.5	15
3 -5 DAS	Philippines	n = 4	100.0	100.0	100.0	100.0
	Vietnam	n = 8	77.5	96.9	96.3	98.2
6 -9 DAS	Philippines	n = 4	100.0	100.0	100.0	100.0
	Thailand	n = 8	100.0	100.0	100.0	100.0
	Vietnam	n = 8	77.5	86.9	94.4	95.7
10 –12 DAS	Philippines	n = 8	-	100.0	100.0	100.0
	Thailand	n = 8	-	100.0	100.0	100.0
	Vietnam	n = 12	-	92.5	95.7	97.9
13-16 DAS	Philippines	n = 12	-	100.0	100.0	100.0
	Thailand	n = 12	-	100.0	100.0	100.0
	Vietnam	n = 20	-	90.9	93.0	96.9

Efficacy of DE-638 on *Monochoria vaginalis* (MOOVA)

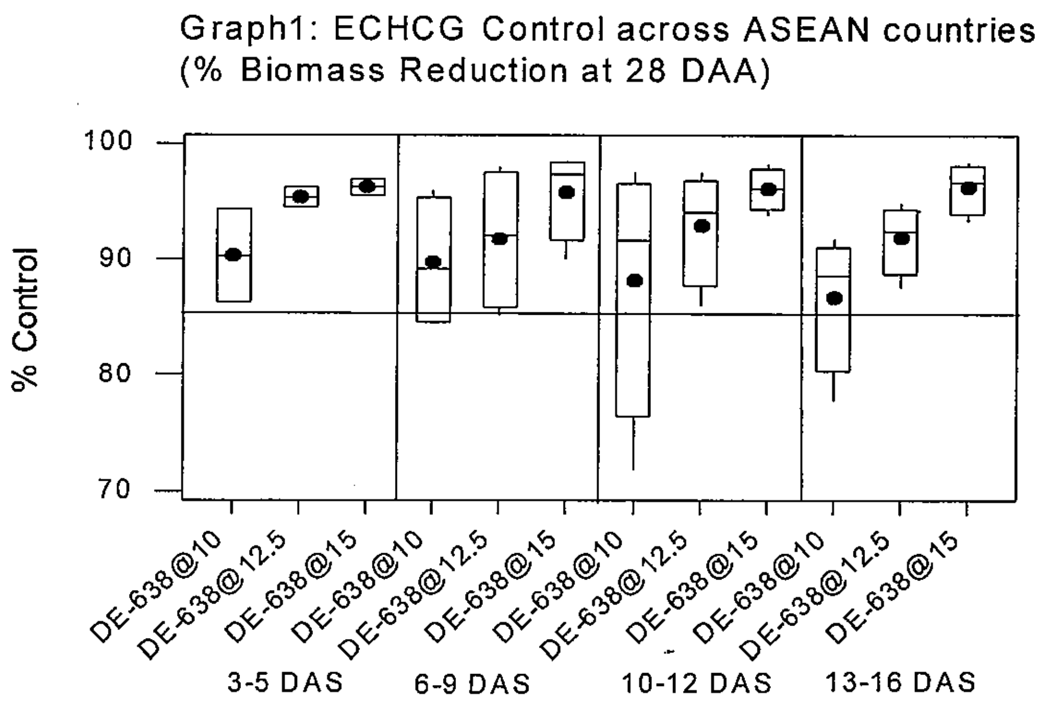
Table 5 demonstrates that 7.5 g ai ha^{-1} DE-638 provided more than 90% control of MOOVA with an application window of 3-16 DAS across ASEAN countries.

Table 5. Efficacy of DE-638 on MOOVA at 28 DAA (% biomass reduction).

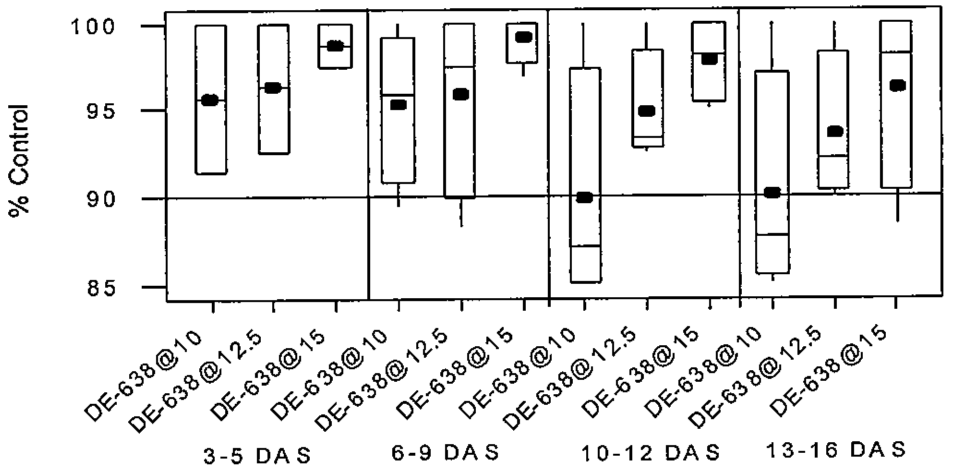
Days after Sowing	Country	No. of observation	DE-638 (g ai ha^{-1})			
			7.5	10	12.5	15
3 -5 DAS	Philippines	n = 4	100.0	100.0	100.0	100.0
6 -9 DAS	Malaysia	n = 4	90.0	95.0	95.0	100.0
	Philippines	n = 4	-	100.0	100.0	100.0
10 –12 DAS	Philippines	n = 12	-	100.0	100.0	100.0
	Vietnam	n = 4	-	98.8	100.0	100.0
13-16 DAS	Malaysia	n = 4	-	100.0	100.0	100.0
	Philippines	n = 20	100.0	100.0	100.0	100.0
	Vietnam	n = 4	-	100.0	100.0	100.0
	Indonesia	n = 4	-	100.0	100.0	100.0

Summary on efficacy of DE-638 on common weeds listed.

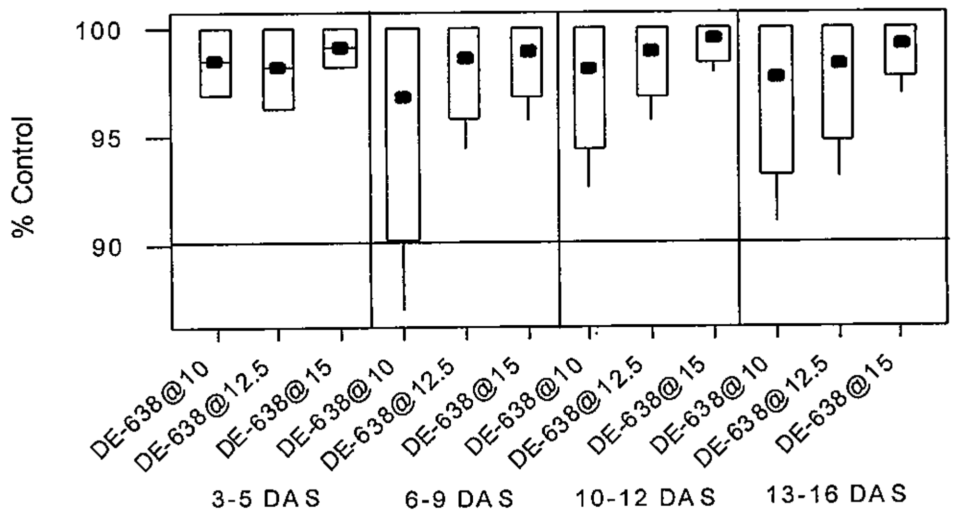
Overall, the trial results shown in Tables 1-5 and illustrated in Figures1-3 demonstrate that DE-638 was very efficacious on common weeds in rice. Farmers can apply a very low use rate of 10-15 g ai ha^{-1} to attain more than 85% control of key important weeds. This product will provide farmers a very flexible timing of application when compared with other current products that at certain rate provided one of two choices, either early post application of 3-9 DAS or late post application of 12-15 DAS.



Graph 2: CYPSS Control across ASEAN countries
(% Biomass Reduction at 28 DAA)



Graph 3: SPDZE Control across ASEAN countries
(% Biomass Reduction at 28 DAA)



Efficacy of mixture of DE-638 + Cyhalofop on *Leptochloa chinenses* (LEFCH)

DE-638 at10-30 g ai ha^{-1} did not provide commercial control of LEFCH. However, cyhalofop-butyl made an excellent tank mix partner for control of LEFCH without antagonizing the control of ECHCG and other weeds listed. The result of this mixture as shown in Table 6 demonstrates that 10+50 to 12.5+62.5 g ai ha^{-1} of DE-638 + cyhalofop-butyl, respectively, provided more than 85% control of this weed without antagonizing ECHCG control.

Table 6. Efficacy of DE-638 + Cyhalofop on LEFCH at 28 DAA (% biomass reduction).

Days after Sowing	Country	No. of observation	DE-638 + Cyhalofop (g ai ha^{-1})		
			10 + 50	12.5 + 62.5	15 + 75
3 -5 DAS	Vietnam	n = 12	90.9	94.2	99.6
6 -9 DAS	Thailand	n = 12	94.4	100.0	100.0
	Vietnam	n = 12	89.6	94.2	98.0
10 –12 DAS	Philippines	n = 12	96.4	99.7	95.0
	Thailand	n = 8	89.2	91.7	95.0
	Vietnam	n = 12	90.7	94.2	97.5
13-16 DAS	Philippines	n = 20	96.2	100.0	98.4
	Thailand	n = 8	70.0	85.0	91.7
	Vietnam	n = 16	95.3	95.0	99.1

In summary, low use rates of 10-15 g ai ha^{-1} DE-638 provided more than 85% control of above common weeds in direct seeded and transplanted rice in tropical rice culture. DE-638 +cyhalofop-butyl is an efficacious mixture for truly broad-spectrum grass, broadleaf and sedge control in ASEAN rice.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Mann, R.K. et al. DE-638 Technical Bulletin. Dow AgroSciences internal document

Sujitno, S et al. XDE-638 Postemergence Foliar Applications at 3 to 27 Days After Seeding in ASEAN Direct-Seeded Indica Rice in 2000 – 2001. DERBI DowAgroSciences internal report.

Glyphosate SP (Feida) and its Use Techniques

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Abstract: A new herbicide product, Glyphosate Soluble Powder, was invented and produced by Nantong Feitian Chemical industrial Co. Ltd. This product was registered in the end of 1997 and patent right was obtained in 2001 in China. This paper provides the weed control spectrum, weed control in peach, fellow after wheat, and rubber plantation and the advanced techniques and directions for use of this new product.

Key words: Glyphosate SP, new herbicide

INTRODUCTION

Glyphosate has been been used as a major non-selective herbicide with the widest application area in the world for its "wide spectrum, high efficacy and low toxicity". However, due to its low solubility in water, this compound must be made into salt by combination reaction with other compositions such as ammonia before field use.

Glyphosate SP (commercial name Feida), was studied and produced only by Nantong Feitian Chemical Industrial Co. Ltd. The product was registered in the end of 1997. This product can dissolve in water at a high-speed. The unique characteristic of this product is that it is mixture between glyphosate and SDP, a natural adjuvant extracted from plants. The production process is easy to operate and avoids the use of materials that have high toxicity, easy to explode and burn.

Spectrum of weeds controlled by Glyphosate SP

Trials in field borders, mulberry fields, fruit gardens, tea gardens, and other non-tilled fields were conducted from 1996 to 1999 in Jiangsu and Zhejiang province. The dose used for 30% Glyphosate SP were 0.75, 1.125, 1.5, 1.875, 2.25, 2.625, 3.00, and 7.5kg ha^{-1}. The herbicide spray volume used was 450 li ha^{-1}.

Following are the weed control efficacy parameters gathered:

1. Percent weed control by species = – number of plants/species in plots with herbicide treatment)/ (number of plants/species in the check plots (CK)
2. Fresh weight weed control = (fresh weight of weeds/species in the CK - fresh weight of weeds/species in herbicide treated plots)/ fresh weight of weeds/species in the CK

RESULTS AND DISCUSSION

The results obtained showed that almost all annual, biennial, or perennial weed species were sensitivity to 30% Glyphosate SP at the dose of 0.75kg ha^{-1} to 3.00 kg ha^{-1}, although the herbicide efficacy to weeds varied with the change of weed species and herbicide dose. Integrating the results obtained in different areas and in different years, weed species investigated and the weed control over them are listed as follows:

Table 1. Weed species and weed control of Glyphosate SP at 1.5 and 3.0kg ha^{-1} application rate.

Weed species		Weed control*	
		.5 $kgha^{-1}$	3 kg ha^{-1}
Annual and biennial weeds	Common Crabgrass (*Digitaria sanguinalis*)	5	5
	Goosegrass Wiregrass (*Eleusine indica*)	5	5
	Green Bristlegrass (*Setaria vividis*)	5	5
	Annual Bluegrass (*Poa annua*)	4	5
	Barnyardgrass (*Echinochloa crusgalli*)	4	5
	Equal Alopecurus (*Alopecurus aequalis)*	5	5
	Common Chickweed (*Stellaria media*)	5	5
	Chinese Pennisetum (*Pennisetum alopecuroides*)	4	4
	Lambsquarters Goosefoot (*Chenopodium album*)	5	5
	Hairy Bittercress (*Cardamine hirsuta*)	5	5
	Spiny Amaranth (*Amarabthus spinosus*)	5	5
	Slender Catchweed Bedstraw (*Galium aprine*)	5	5
	Annual Fleabane (Erigeron annus)	4	5
	Ternate Pinellia (*Pinellia ternata*)	5	5
	Perfoliate Knotweed (*Polygonum perfoliatum*)	5	5
	Flower Gentle (*Amaranthus tricolor*)	5	5
	Arista Goosefoot (*Chenopodium aristatum*)	5	5
	Sunspurge (*Euphorbia helioscopia*)	3	4
	Canton Buttercup (*Ranunculus cantoniensis*)	4	5
	Common Dayflower (*Commelina communis*)	4	4
	Scabrous Hedgeparsley (*Torilis scabra*)	4	5
	Japanese Mazus (*Mazus japonicus*)	5	5
	Japanese Paspalun (*Paspalum thunbergii*)	3	4
	Flowerofanhoux (*Hibiscus trionum*)	5	5
	Common Ragweed (*Ambrosia artemisifolia*)	3	4
	Japanese Hop (*Humulus scandens*)	5	5
	Common Knotweed (*Polygonum aviculare*)	3	3
	Prostrate Yerbadetojo (*Eclipta prostrata*)	4	5
Perennial weed	Bermudagrass (*Cynodon dactylon*)	3	4
	Nutgrass Galingale (*Cyperus rotundus*)	4	5
	Common Cephalanoplos (*Cephalanoplos segetum*)	4	5
	Lalang Grass (*Inperata cylindria*)	4	5
	Amur Silvergrass (*Miscanthus sacchariflorus*)	4	5
	Common Reed (*Phragmittes communis*)	3	4
	Chinese Silvergrass (*Miscanthus sinesis*)	4	5
	Mugwort Wormwood (*Artemisia argyi*)	4	5
	Alligator Alternathera (*Alternanthera philoxeroides*)	5	5
	Sibirian Cocklebur (*Xanthium sibiricum*)	4	5
	Indian Hemp (*Abutilon theophrasti*)	4	5

- In the above table, "5" means more than 95% weed control, "4" means more than 85% and less than 95% weed control, "3" means less than 85% weed control.

and tea due to herbicide by-products and its residues. The 30% Glyphosate SP contains minimum by-products, and its active ingredient can be rapidly decomposed in soil and water into materials that do not affect plants and environment. This product has more merit on weed control and environment protection compared with 10% glyphosate and other kinds of herbicides.

It was found in the field experiments that some perennial weeds such as bermudagrass (*Cynodon dactylon*) and common reed (*Phragmittes communis*) and some others can not be effectively controlled by Feida at less than 3kg ha^{-1} so it is necessary to raise the herbicide dose to increase its efficacy.

Weed control in peach garden, fellow after wheat and rubber plantation

1. Peach garden (Nanjing)

Field trials were conducted in June in a peach garden in Jiangsu Academy of Agricultural Science. The experimental land area was 9650 m^{-2}. The main weeds in the field are *Erigeron annuus, Setaria vividis, Leonurus japonica, Commelina communis, Humulus scandens, Amaranthus spinosus, Convolvulus arvensis, Cyperus rotundus,* and *Cayratia japonica* etc. Herbicide treatment was carried on 10th June, and weed control was investigated on 24th July.

Herbicide dose: 30% Glyphosate SP: 150, 200, 250 g mu^{-1}
41% Glyphosate ammonia salt: 200g mu^{-1}

Result obtained showed that 30% Glyphosate SP could give excellent weed control in peach garden (Table 2). It is suggested that the appropriate dose of this herbicide is 150g mu^{-1} for annual weeds and 200~250g mu^{-1} for perennial weeds.

2. Fellow after wheat (Shijiazhuang)

The experimented field was located at the Houbei Food and Oil Crops Research Institute. The main weeds were common crabgrass (*Digitaria sanguinalis*), goosegrass wiregrass (*Eleusine indica*), green bristlegrass (*S. vividis*), and lambsquarters, and goosefoot (*Chenopodium album*). Herbicide treatment was conducted on July 11th 1997. Table 3 shows the individual weed control at 20 days after herbicide spraying

Further investigation showed that the fresh weight reduction of the above treatment at doses of 100, 150, 200, 250, 300g mu^{-1} (30% Glyphosate SP), 100, 200, and 300 ml mu^{-1} (41% Glyphosate ammonia salt) are 63.8%, 79.6%, 90.9%, 97.0%, 97.6%, 64.2%, 88.1%, and 97.4%, respectively.

It can be concluded that the herbicide 30% glyphosate SP was as effective or even more effective than glyphosate ammonia salt while the weed death was 2 to 3 days earlier.

Table 2. Weed control in peach garden (Nanjing, 1997).

Weed species	Weed individuals before spraying (plants/0.2 m^{-2})				
	CK	G 150g mu^{-1}	G200g mu^{-1}	G250g mu^{-1}	R200g mu^{-1}
Erigeron annuus	20.3	21.8	20.3	21.5	20.3
Setaria vividis	13.8	13.8	14.0	14.3	13.5
Leonurus japonica	5.8	6.0	5.5	4.8	5.3
Commelina communis	4.3	4.8	5.5	3.8	5.0
Humulus scanden	15.5	13.5	14.0	14.3	13.8
Amaranthus spinosus	3.8	4.3	3.8	3.5	3.5
Convolvulus arvensis	3.3	3.3	3.8	4.0	3.5
Cyperus rotundu	4.5	4.8	5.3	5.0	4.3
Cayratia japonica	3.0	3.5	4.3	4.0	3.8
Dead rate (25d)	0	100	100	100	100
Fresh weight weed control (%)	0	97.5	98.8	99.8	98.5

- In the above table, "G" means 30% Glyphosate, "R" means 41% Glyphosate ammonia salt.

Table 3. Individual weed control (%) in fellow after wheat (Shijiazhuang, 1997).

Treatment	30% Glyphosate SP					41% Glyphosate salt		
(g or ml mu^{-1})	100	150	200	250	300	100	200	300
Grass weeds								
Digitaria sanguinali	31.7	65.6	83.9	100	100	39.1	73.0	100
Eleusine indic	43.3	78.0	80.0	100	100	44.7	82.0	100
Setaria vividis	74.8	95.3	100	100	100	74.2	95.1	100
Echinochloa crusgalli	11.7	36.7	31.6	100	100	40.0	65.0	100
Total	50.2	77.3	85.6	100	100	54.8	85.2	100
Broadleaf weeds								
Portulaca oleracea	40.5	61.4	85.8	89.3	90.3	42.1	58.7	87.3
Amaranthus retroflexus	93.3	91.7	100	100	100	91.7	91.7	100
Acalypha australis	40.0	40.0	45.4	81.8	81.8	89.1	45.5	98.1
Abutilon theophrasti	0	41.7	28.3	83.3	81.7	0	0	70.5
Convolvulus arvensis	55.5	55.5	100	100	100	0	66.7	100
Pharbitis nil	0	16.3	12.5	32.3	60.8	0	0	26.6
Chenopodium album	37.2	65.1	100	100	100	18.6	32.6	100
Polygonum aviculare	0	0	6.7	0	0	6.7	43.3	16.7
Erigeron annuus	0	0	100	100	100	0	0	100
Humulu scandens	33.3	33.3	33.3	100	100	0	33.3	100
Total	35.5	69.0	78.2	86.4	86.7	30.9	50.3	87.0
Final weed control	**35.4**	**60.5**	**81.6**	**92.5**	**92.7**	**41.8**	**69.9**	**92.4**

It is suggested the appropriate dose of herbicide 30% Glyphosate SP are 200g mu^{-1} for annual weeds and 200 to 250g mu^{-1} for perennial weeds in fellow.

3. Rubber plantation

The perennial weed *Imperata cylindria* is the major malignant weed in rubber plantation in Hainan province. Trials to control this weed with 30% Glyphosate SP and 41% Glyphosate ammonia salt were conducted in August 1998 in Haikou. Results (the data is omitted here) obtained showed that 500 g Glyphosate mu^{-1} provided the same control of *I. cylindrcia* compared to 500 ml Glyphosate ammonia salt mu^{-1}. The application of 300 g Glyphosate mu^{-1} gave approximately the same control as that of 300ml Glyphosate ammonia salt mu^{-1}. This result indicates that 30% Glyphosate SP at the dose of 300~500g mu^{-1} can provide good control of *I. cylindrica.*

The advanced techniques of Glyphosate SP

Glyphosate SP has a pale yellow to yellowish brown powder color. The main technique targets are as Table 4.

Table 4. Physical and chemical targets.

Item	Targets
Glyphosate content, %(m/m)≥	30.0
No-dissoved matter in water, %(m/m)≤	0.5
pH	2.0-4.0

The most important and advanced characteristic of the product is the initiated application of SDP, the new and high effective adjuvant isolated from plants. The mixture of glyphosate and SDP is easy to produce and provides an excellent solution the problem of glyphosate being hard to disslove in water, avoids the use of materials that are highly toxic, easy to explode and burn. The four years of weed control experiments and practice in 16 provinces in China showed that Feida could give better control over weeds with about 20% less cost compared with the herbicides of glyphosate salts. The five major merits of this product are as follows:

1. Feida was produced through a physics process, thus avoiding the second pollution in traditional craft in which some materials with high toxicity, easy to explode and burn were used. SDP, the natural adjuvant from plants used in the product has no toxicity, and residue, does not pollute the environment and has synergistic action to herbicides, with high stability. The efficacy of Feida is equal or better compared with the herbicides of glyphosate salts at the same dose.

2. The toxicity of Feida is lower. The adjuvant used in Feida is SDP that does not have other toxic compositions, so the toxicity of Feida is lower. The inspection report by the Jiangsu Provincial Sanitary & Anti-Epidemic Station showed that the oral LD_{50} of the male and female rats were 9260 mg kg^{-1} and 7940 mg kg^{-1}, respectively, and the score of the acute dermal irritation test and the integral index of the acute eye irritation test on rabbit were all zero.

3. Feida is easy to dissolve in water, easy to use, easy to pack and transport.
4. The cost of Feida is lowest of all the same kinds of herbicides.
5. The efficacy of Feida would not decline when there is a rain 3 hours after spraying.

Directions for use

Use category

As a non-selective herbicide, Feida can be used in field borders, irrigation canals, sides of highways or railways, airports, oil depositories, and other non-tilled fields, or used in mulberry fields, fruit gardens, tea gardens, bamboo fields, and rubber gardens.
Otherwise, Feida can be used by directional spraying in maize, soybean, sorghum, cotton, and sugarcane fields. In this case, the spray nozzle must be low to avoid crop leaves from coming into contact with the herbicide. Feida can be also used for stubble cleaning in wheat, rape, sugarcane, and other crop fields to control weeds before crop planting. Reports from Guangdong province showed that 10% glyphosate used for stubble cleaning would decrease emergence rate of sugarcane by 20% while Feida would not affect sugarcane emergence.

Dose

In general, Feida is used in fields at the dose of 3 kg ha^{-1}. But due to the various situations in fields, users should select application doses according to weed populations and their growth states. Some common weed species and herbicide dose involved recommended are as follows:

Table 5. Common weed species and herbicide dose.

Weed species		Dose (kg ha^{-1})
Annual weeds	Common Crabgrass (*Digitaria sanguinalis)* Goosegrass Wiregrass (*Eleusine indica*) Green Bristlegrass (*Setaria vividis*) Annual Bluegrass (*Poa annua*) Equal Alopecurus (*Alopecurus aequalis*) Common Chickweed (*Stellaria media*) Lambsquarters Goosefoot (*Chenopodium album*) Hairy Bittercress (*Cardamine hirsuta*) Spiny Amaranth (*Amarabthus spinosus*) Slender Catchweed Bedstraw (*Galium aprine*)	3
Perennial weeds	Bermudagrass (*Cynodon dactylon*) Nutgrass Galingale (*Cyperus rotundus*) Common Cephalanoplos (*Cephalanoplos segetum*) Lalang Grass (*Inperata cylindria*) Common Reed (*Phragmittes communis*) Mugwort Wormwood (*Artemisia argyi*) Alligator Alternathera (*Alternanthera philoxeroides*)	3-7.5

Dilution

Four hubdred fifty to 750 kg ha^{-1} water is suitable for diluting Feida when it is for field use. According to weed density and growth stand, users can decide whether to use low or high water volume of herbicide solution but should be within the limits recommended.

Important Notes

1. Glyphosate SP is a non-selective herbicide, so users should avoid the herbicide from coming into contact with crop leaves.
2. This herbicide is only effective for controlling weeds by stem and leaf spraying, and not effective for controlling weeds when sprayed on the soil.
3. No re-spraying or missing spraying when the herbicide is used in the fields.
4. Use clear water to dilute the herbicide. Murky water would decrease the weed control efficacy.
5. Use this herbicide at proper dose and spray volume to bring the herbicide efficacy into full effect.
6. Take care in mixing Feida with other herbicides. This product contains high effective natural adjuvant, so other adjuvants are not needed when it is used.
7. Wash sprayer thoroughly to avoid damage to crops when the sprayer is used in crop fields later.
8. Quality guarantee period: 2 years.

Formulation Factors Influencing Effective Herbicidal Action of a Floating Granule of LGC-42153

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Abstract: The novel sulfonylurea herbicide LGC-42153 can control *Echinochla crus-galli* and a wide range of annual and perennial weeds in paddy rice field at 20~30 g ai ha^{-1}. Since the herbicide provides broad weed control spectrum as a solo product, development of laborsaving formulations can be easier and more flexible than the conventional mixture herbicides in Korea and Japan. A series of experiments was conducted to optimize formulation factors of a self-dispersible floating granule that can be used at a low application volume (5 kg ha^{-1}). In this report, we describe the classified main characteristics that an ideal formulation has to possess, selection of appropriate media based on established critical level of each character, and the physico-chemical properties and biological performance of the selected formulation.

Key words: LGC-42153, laborsaving, floatation, dispersal, granule

INTRODUCTION

Recently many laborsaving formulations and application systems have been developed to reduce labor input, particularly for the weed control in paddy-rice field. Since the introduction of "1 kg" (used at the application volume of 1 kg $10a^{-1}$) granules in Japan, laborsaving formulations such as flowable, jumbo formulations, and spreading oil have been used widely in Japan (Takefumi et al. 2001). These formulations enable farmers to apply the formulation from levee by hand or application equipment (Noritake 1993).

LGC-42153 is a new sulfonylurea herbicide that controls *E. crus-galli* effectively as well as other annual and perennial weeds at 20~30g ai ha^{-1}, indicating that LGC-42153 alone can be an one-shot rice herbicide without other grass-killer partners such as carbamates and aryloxyphenoxy propionic acids. This biological property has been waited for long in sulfonylurea chemistry since the introduction of bensulfuron-methyl.

In this paper, we describe a floating self-dispersible "500 g" (used at the application volume of 500 g/10a) granule of LGC-42153 without water-soluble pack as a type of laborsaving formulation. This floating granule submerges down to the soil surface immediately after application, then floats up onto the water surface, and releases the active ingredient as the granule disperses rapidly across a long distance. This study was conducted to select appropriate formulation ingredients for each characteristic, floating capacity or dispersibility, and to make the granule physico-chemically more self-dispersible and biologically stable.

MATERIALS AND METHODS

The self-dispersible "500 g" granule containing LGC-42153 of 2.1 g (0.42% w/w) requires specific characteristics; submerging after being applied, floating within a few minutes, dispersing to a long distance within a short period of time (long distance dispersal) and finally releasing or diffusing active ingredients (short distance dispersal). To optimize these characteristics, this study was conducted stepwise by examining formulation ingredients (Table 1) and granule size involved in each characteristic.

Table 1. Formulation ingredients used in this study.

Category	Formulation ingredients
Binders	Aaravia gum, Dextrin, Gellan gum, Guar gum, Locust bean gum, PEG6000 (polyethyleneglycol), Sodium alginate, Tara gum, Xanthan gum, Na-CMC (sodium carboxymethylcellulose), PVP (polyvinylpyrrolidone), SPA (sodium polyacrylate), PVA (polyvinyl alcohol)
Water-soluble fillers	Potassium chloride, Ammonium chloride, Sodium sulfate, Ammonium sulfate, Urea, Sodium benzoate, Glucose, Lactose
Wetters/ Dispersants	EP4C (sodium di-ethylhexylsulfosuccinate), Surfynol 440 (ethoxylated 2,4,7,9-tetramethyl-5-decyn-4,7-diol), Q2-5211 (polyoxyethylene modified polydimethyl siloxane)

Formulation preparation

To evaluate the effects of binders on granulation and floatation, various types of polymers, listed in Table 1, were added into a template granule formulation, which contains the same amount of potassium chloride, EP4C, and so on. Samples were prepared at a 200 g scale. The dry ingredients (Table 1) including LGC-42153 were blended using a Ken mixer (KM-600, AICOH Co, Japan), followed by a wet blending step in which sufficient water (10~13% w/w) was added. Blended samples were then transferred to a laboratory extruder (KAR-75, Tsutsui Co, Japan) for granulation by extruding them through the screen with holes of different sizes. The extruded granules were dried at 50□ for about 3 min in a laboratory fluid bed drier (TG100, Retsch Co, Germany).

Measurement of floatation

To investigate the influence of ingredients and granule sizes on floatation, experimental granules containing different binders or water-soluble fillers were prepared with different sizes as described above. Five granules of a uniform size in each granule sample were placed gently into a 500 ml beaker containing tap water at 5 cm depth. Then, the floating time of each granule was recorded.

Measurement of dispersal of LGC-42153

To evaluate the influence of the wetters/dispersants and granule sizes on dispersal of LGC-42153, granule samples were formulated at different sizes. The granule samples contained the same binder and filler selected on the basis of floating time in the above study. For simple quantification of dispersed LGC-42153 without clean-up process, application dose rate of LGC-42153 was 90 g a.i. ha^{-1}, about four times higher than the recommended dose. Accurately weighed granules containing about 18.9 mg of LGC-42153 were spot-applied at the top front of a specially designed Styrofoam box (0.3 m×10 m) laid black vinyl sheet and containing 150 L of tap water (5 cm in depth). At different time intervals, aliquots of 1 mL were sampled at three points at 7 m away from the treated spot. Three aliquots were filtered and analyzed using a high performance liquid chromatography (HPLC) to quantify LGC-42153.

Biological evaluation

An indoor paddy field test was conducted to evaluate the biological performance of the floating granule selected in the above studies as compared with a conventional "3 kg" (used at the application volume of 3 kg/10a) granule formulation. The indoor paddy field system was designed to be 0.32 m deep x 0.3 m wide x 13 m long using Styrofoam box filled with paddy soil up to 20 cm. Top soil was puddled after top irrigation and seeds of *E. crus-gall* were sown at 3 days after puddling. As soon as the seedlings emerged, water depth was maintained at 5 cm throughout the experiment period. At the 3 -leaf stage of *E. crus-galli*, the selected floating "500 g" granule and a conventionally formulated "3 kg" granule, at the LGC-42153 dose rate of 21 g ai ha^{-1}, were applied at the front of each system by hand. Efficacy was visually assessed at 30 days after application.

RESULTS AND DISCUSSION

Selection of binder

All the granules containing different binders listed in Table 1 did not float (collapsed or soluble in water) except for those with SPA (sodium polyacrylate) and xanthan gum (Table 2). It is thought that the reason for floating capacity of these granules using SPA and xanthan gum may be due to the capacity of keeping airs in the network or matrix structure within a granule (Mikio et al. 1991). Although both xanthan gum and SPA made the granule to float readily, dispersal after floatation was different. While the granules containing 5% xanthan gum disintegrated slowly, that with 5% SPA disintegrated rapidly although its shape in water was somewhat distorted. These results imply that blending of SPA and xanthan gum could optimize granulation, floating capacity, and disintegration property of the granule. When they were blended, floating capacity improved slightly with increasing xanthan gum at a constant content of SPA, but floatation was delayed with increasing SPA at a constant content of xanthan gum (Figure 1). Based on this result, the contents of SPA and xanthan gum were fixed at 0.8 and 0.5%, respectively, for further studies.

Table 2. Granulation and floatation properties of the granules as affected by binders.

Binders	Degree of granulation	Floating time (second)
Na-CMC	Intermediate	Not detected (soluble in water)
Dextrin	Good	Not detected (soluble in water)
PVA	Intermediate	Not detected (soluble in water)
SPA	Poor	239
PVP	Good	Not detected (soluble in water)
PEG6000	Good	Not detected (soluble in water)
Sodium alginate	Good	Not detected (soluble in water)
Xanthan gum	Poor	117
Gellan gum	Poor	Not detected (soluble in water)
Tara gum	Poor	Not detected (soluble in water)
Locust bean gum	Good	Not detected (soluble in water)
Arabic gum	Intermediate	Not detected (soluble in water)
Guar gum	Good	Not detected (soluble in water)

* These GRs were made using the same template containing LGC-42153, EP4C, and potassium chloride and varying binders (5.0%).

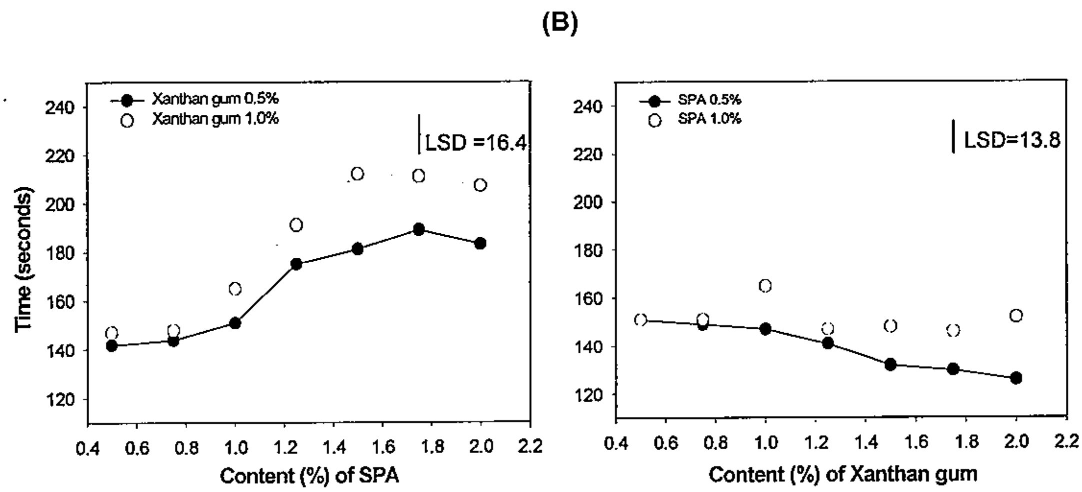

Figure 1. Floating time of the granules as affected by the contents of SPA (A) and xanthan gum (B). These granules were made using the same template containing LGC-42153, EP4C, and potassium chloride and varying the relative content of SPA and xanthan gum.

Selection of water-soluble fillers

To examine the suitability of potassium chloride in comparison with other fillers, potassium chloride was replaced with other soluble fillers. For granulation, most fillers were good except Na_2SO_4 and sodium benzoate (Table 3). Regarding floating time, the granule containing urea floated most quickly in 122 seconds, followed by those containing KCl, sodium benzoate, and glucose, while those containing $(NH_4)_2SO_4$ and lactose failed to float. After floatation, the disintegration property was best with KCl, disintegrating immediately. Therefore, this result suggest that KCl is the most

appropriate filler among the tested.

Table 3. Physical properties of the granules as affected by fillers.

Filler	Degree of granulation	Floating time (second)	Disintegration of granules after floatation
KCl	Good	142	Disintegrated immediately
NH_4Cl	Good	357	Disintegrated 2~3 seconds later
Na_2SO_4	Intermediate	792	Disintegrated 2~3 seconds later
$(NH_4)_2SO_4$	Good	Not floated	Not applicable
Urea	Good	122	Not disintegrated
Sodium benzoate	Poor	154	Disintegrated 2~3 seconds later
Glucose	Good	230	Disintegrated 2~3 seconds later
Lactose	Good	Not floated	Not applicable
$LSD_{0.05}$		81.5	

Template formulation: LGC-42153, EP4C, SPA, xanthan gum and fillers to 100%.

Selection of wetter/dispersants

It is thought that the spread and dispersal of an active ingredient after floatation is firstly due to the driving force generated during the disintegration of granules on the water surface. In generating such a driving force, surfactants such as wetters and dispersants can contribute as a spreading agent.

Dispersal capacity was assessed by measuring LGC-42153 concentration at 7 m apart from the applied spot at different time intervals. In all the formulations, the maximum concentration was reached at about 72 h after application; however, the concentration of LGC-42153 was almost instantaneously (within 1 h) 60 to 80% of the maximum in the formulations containing EP4C, Surfynol 440, or Q2-5211 (Fig. 2). By comparison, in the conventional 1 ~3 kg" granule, the concentration of LGC-42153 distant from the applied spot was initially very low and increased linearly until 72 h, suggesting dispersal [illegible] dependent on diffusion of the active ingredient. Therefore, it was evident [illegible] surfactants contribute greatly in dispersion of the disintegrated particl[illegible] [illegible]face. Among the surfactants, Surfynol 440 gave the GR the be[illegible]

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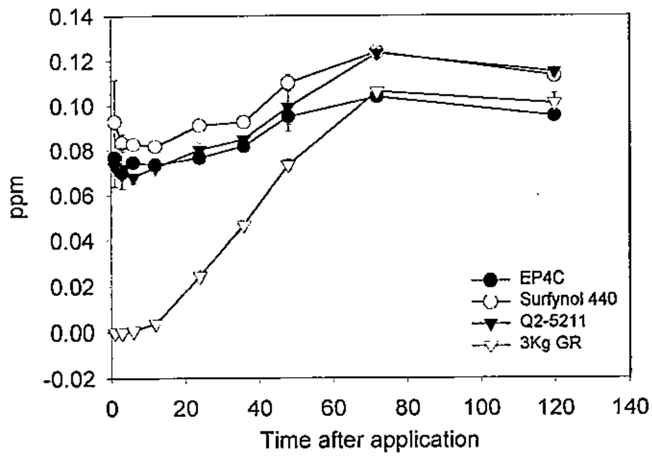

Figure 2. Time-course change in LGC-42153 concentration at 7 m apart from the applied point of the various granules containing different wetters/dispersants.

Template formulations for the GRs: LGC-42153, wetter/dispersant, SPA, xanthan gum, KCl to 100%, and for the conventional 3kg GR: LGC-42153, EP4C, sodium lignosulfonate, dextrin, Na-bentonite, talc to 100%. Theoretical maximum concentration of LGC-42153: 0.126ppm.

Effects of granule size on floatation and dispersal

It was assumed that granule size would affect the floatation and dispersal of granules. As the granule size of the conventional "3 kg" granules ranges from 0.7 to 1.2 mm in diameter, we tested the experimental granules range from 0.5 to 1.5 mm in diameter. Figure 3 shows that the smaller the granule size, the faster its floating time; and dispersal of the granule also varied with its granule size. To disperse an active ingredient to a long distance in a short time, our results suggest that smaller granules are more appropriate. However, a small-sized granule is difficult to broadcast and can be influenced by wind. In addition, the dispersal of LGC-42153 formulated in 1.0 mm granule was similar to that of 0.7 mm granule. Therefore, we determined the optimal diameter of the granule to be 1.0 mm for the biological efficacy test.

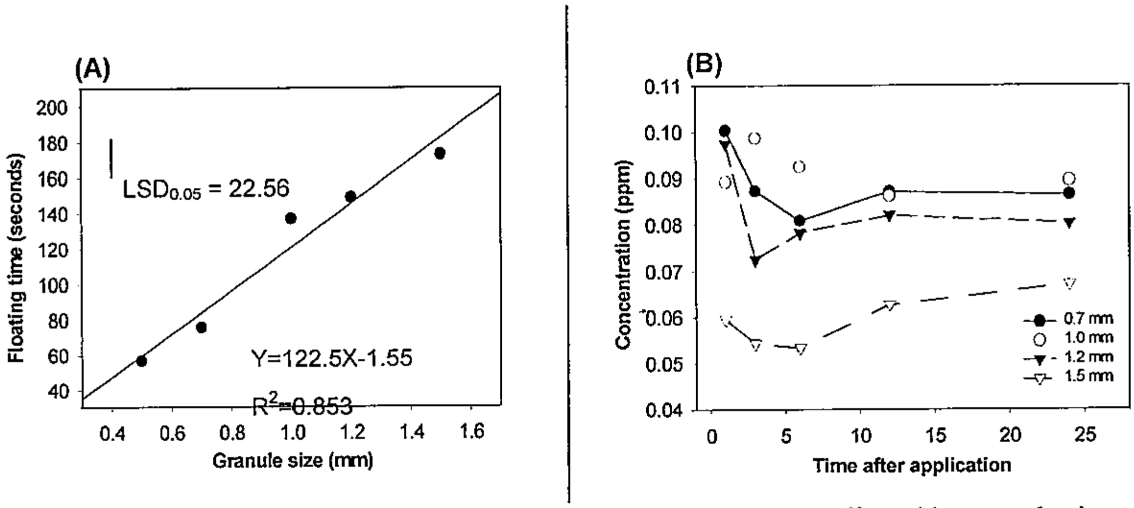

Fig. 3. Floatation of granules (A) and dispersal of LGC-42153 (B) as affected by granule size. Template formulation: LGC-42153, Surfynol 440, SPA, xanthan gum, fillers to 100%.

Biological performance of the floating granule

When the conventional "3 kg" granule was spot-applied, *E. crus-galli* was controlled up to about 6 m away from the treated spot; however, control decreased dramatically in a farther distance, showing no control from 9 m. By comparison, efficacy of the floating granule was complete up to about 9 m and evident in the region farther than 9 m (Fig. 4). This result suggests that LGC-42153 has a good intrinsic diffusion potential and floating granule technology increases the dispersal of LGC-42153 greatly.

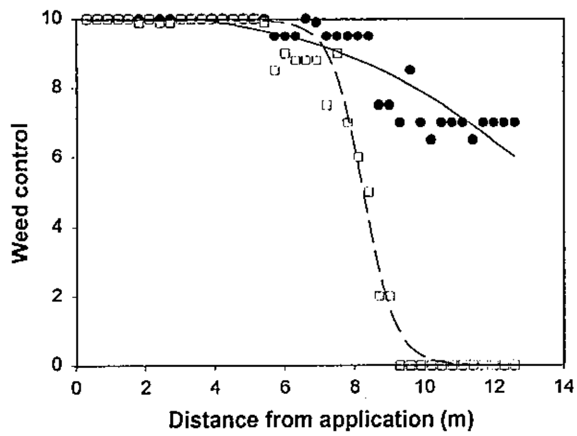

Fig. 4. Biological performance of the floating granules (●) in comparison with the conventional "3 kg" GR (□) in controlling *Echinochloa crus-galli.*

In conclusion, the results of this study suggest that the floating granule we developed offers very good dispersibility and can be applicable to current rice cropping systems. Under the field condition, performance of the new granule was equivalent to that of the conventional "3 kg" formulation (data not shown). The floating granules can also save labor very significantly as compared with the conventional granules.

LITERATURE CITED

Mikio Sekiguchi, Iwao Takahashi, Akio Masui. 1991. Formulation technique of granules resurfacing after submerged application. Journal of Pesticide Science 16: 325-334.

Noritake, K. 1993. Labour saving application of jumbo pellet type herbicides. Agrochemicals Japan 63: 5-6.

Takefumi T, Koji N. 2001. Development and promotion of labor-saving application technology for paddy herbicides in Japan. Weed Biology and management 1: 61-70

Crop and Weed Selectivity to the New Sulfonylurea Herbicide LGC-42153

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Abstract: LGC-42153 is a new sulfonylurea herbicide being developed by LG Life Sciences Ltd., Korea. This new herbicide can control barnyard grass (*Echinochloa crus-galli)*, broad-leaved and sedge weeds with a high level of crop safety. At the whole plant level, LD_{50} values for rice and barnyard grass were 83.8 g a.i. ha^{-1} 4.5 g a.i. ha^{-1}, and I respectively. *In vitro* acetolactate synthase (ALS) enzyme assay revealed that ALS I_{50} values of LGC-42153 were very little different between rice and barnyard grass (8.38×10^{-5} vs. 9.38×10^{-5} M). In an *in vivo* ALS assay, rice treated with LGC-42153 showed initial decrease in acetolactate accumulation within 6 h, but showed recovery afterward reaching about 70% of the untreated control at 96 h. However, this recovery did not occur in barnyard grass. In conclusion, this study suggests that LGC-42153 inhibits ALS and its selectivity mechanism between rice and barnyard grass is due to differential recovery mechanism of ALS in rice.

Key words: acetolactate synthase, *Echinochloa*, selectivity, LGC-42153, sulfonylurea

INTRODUCTION

LGC-42153 is a new sulfonylurea herbicide being developed by LG Life Sciences Ltd., Korea. This new herbicide can control the major grass weeds such as *Echinochloa* spp. and *Digitaria* spp, various broad-leaved weeds including *Galium* spp., and sedges, while it is very safe to rice and cereal crops. Like other sulfonylurea herbicides, LGC-42153 was assumed to inhibit ALS in plants. In this study we conducted whole plant assay, and *in vitro* and *in vivo* ALS assays to investigate the mode of action of LGC-42153 and determine the basis of selectivity between rice and barnyard grass.

MATERIALS AND METHODS

Whole plant assay

For foliar application, each 10 plants of rice (*Oryza sativa* cv. Chucheong) and barnyardgrass (*Echinochloa crus-galli*) were grown in a plastic pot (100 cm^2) in a glasshouse maintained at 30/23 (day/night) ± 3°C. At the 4-leaf stage, the herbicide was sprayed using a CO_2-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8002E flat fan nozzle (Spraying System Co., USA) adjusted to deliver 300 L ha^{-1}. Application rates of LGC-42153 (50% WG) were 10~320 g a.i. ha^{-1} for rice and 2.5~80 g ai ha^{-1} for barnyardgrass. Treated plants were then returned to the glasshouse and watered by sub-irrigation as needed. Fresh weight was measured at 20 days after application.

For soil application, 6 plants of 14 days-old rice seedlings were transplanted, and 20 seeds of barnyard grass were sown in separate plastic pots (200 cm^2) containing paddy soil. The plants were grown in a submerged paddy condition at 3 cm water depth in the glasshouse. At 3-leaf stage of barnyard grass, LGC-42153 (0.07% GR) and pyrazosulfuron-ethyl (0.07% GR) were applied onto the paddy water in the pots at 1.25, 2.5, 5, 10, 20, 40, and 80 g ai ha^{-1} for barnyard grass, and 10.5, 21, 42, 63, 84, 168, and 336 g ai ha^{-1} for rice. At 34 days after application, fresh weight was measured.

The experiment was conducted in a completely randomized design with three replications.

In vitro ALS assay

ALS was extracted from green seedlings of rice and barnyard grass at 3-leaf stage. The enzyme extraction and assay were conducted using a modified protocol of Ray (1984).

In vivo ALS assay

Rice and barnyard grass seedlings at 3-leaf stage were prepared, and LGC-42153 was sprayed at 30 g a.i. ha^{-1} as described in the whole plant assay. After various periods of time (6, 12, 24, 48, and 96 h), the plants were harvested and ALS activity was measured. The amount of acetolactate accumulation *in vivo* was determined by the method of Simpson *et al.* (1995) using cyclopentanecarboxylic acid (CPCA) as the inhibitor of keto acid reductoisomerase (KARI), the next enzyme to ALS in the pathway of the branched amino acid synthesis. The rate of CPCA used in this experiment was 1 kg ha^{-1}, and the period between CPCA and LGC-42153 application was 96 h.

Statistical analysis

All measurements were initially subjected to analysis of variance (ANOVA). For data from whole plant and *in vitro* assays, the standard dose-response model (Streibig, 1980) was fitted to estimate dose rates or concentrations causing 50% inhibition (LD_{50} or I_{50} value) relative to untreated controls. All statistical analyses were conducted using Genstat 5.

RESULTS AND DISCUSSION

When applied foliarly in the glasshouse condition, the whole plant LD_{50} values were 4.5 g a.i. ha^{-1} and 83.8 g a.i. ha^{-1} for barnyard grass and rice, respectively, demonstrating good selectivity between the weed and crop (Fig. 1). Similarly when applied to the soil, the whole plant LD_{50} values were 0.58 g a.i. ha^{-1} and 182.9 g ai ha^{-1} for barnyard grass and rice, respectively. Therefore, selectivity of LGC-42153 was 315-fold between rice and barnyard grass. In the same condition, LD_{50} values of pyrazosulfuron-ethyl were 3.45 and 323 g a.i. ha^{-1} for barnyardard grass and rice, respectively, showing 94-fold selectivity. This comparison demonstrates that selectivity of LGC-42153 is more than 3-times greater than that of pyrazosulfuron-ethyl, mainly due to the stronger activity to barnyard grass (3.45/0.53 = 6-fold).

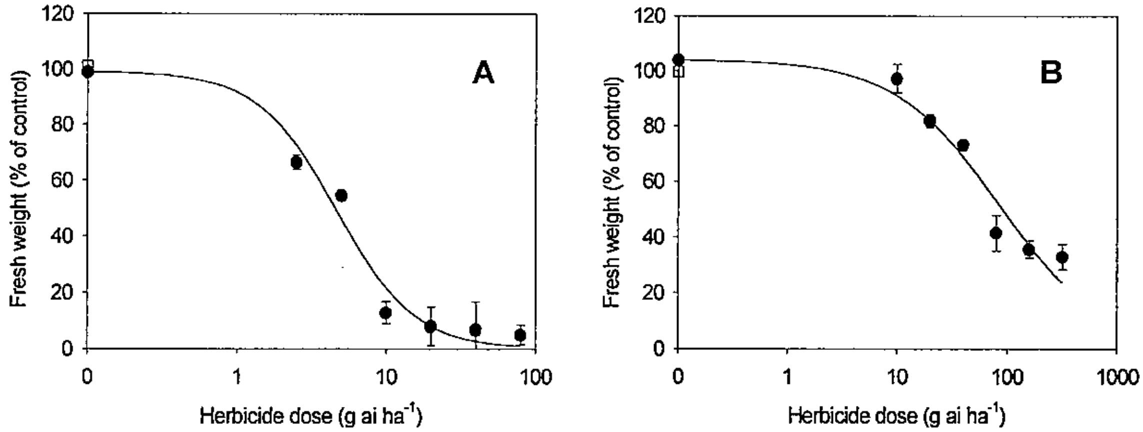

Figure 1. Dose responses of barnyard grass (A) and rice (B) to LGC-42153, which was foliar-applied. The bar represents the standard error of three replicates and the solid line represents fitted values.

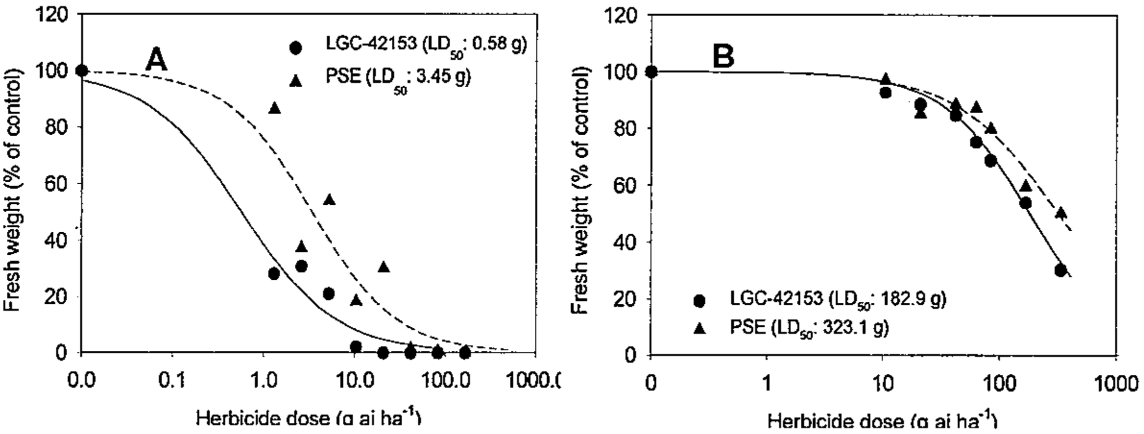

Figure 2. Dose responses of barnyard grass (A) and rice (B) to LGC-42153 and pyrazosulfuron-ethyl, which were directly applied to the soil.

To determine if differential ALS sensitivity has a role in rice selectivity, *in vitro* enzyme assay using ALS extracted from rice and barnyard grass was conducted. ALS I_{50} values of LGC-42153 to rice and barnyard grass were 8.38×10^{-5} and 9.38×10^{-5} M, respectively. However, ALS I_{50} values of pyrazosulfuron-ethyl were 2.29×10^{-7} M for rice and 1.17×10^{-7} M for barnyard grass (Fig. 3 and Table 1). The I_{50} values of LGC-42153 were about 100 ~ 1000 times greater than those of pyrazosulfuron-ethyl. Other sulfonylurea herbicides such as chlorsulfuron, sulfometuron-methyl, and chlorimuron-ethyl had similar I_{50} values to that of pyrazosulfuron-ethyl (Beyer et al. 1988; Gerwick et al. 1990). It was unique that, despite this marked weakness in *in vitro* ALS inhibition activity, LGC-42153 had stronger whole plant activity than pyrazosulfuron-ethyl in barnyard grass as shown in Figure 1. Therefore, in the herbicidal action at the whole plant level, LGC-42153 is likely to involve some unknown physiology in the potent herbicidal action. In terms of selectivity, the difference in *in vitro* ALS I_{50} values between rice and barnyardgrass was not great; therefore, differential ALS sensitivity did not seem to contribute significantly.

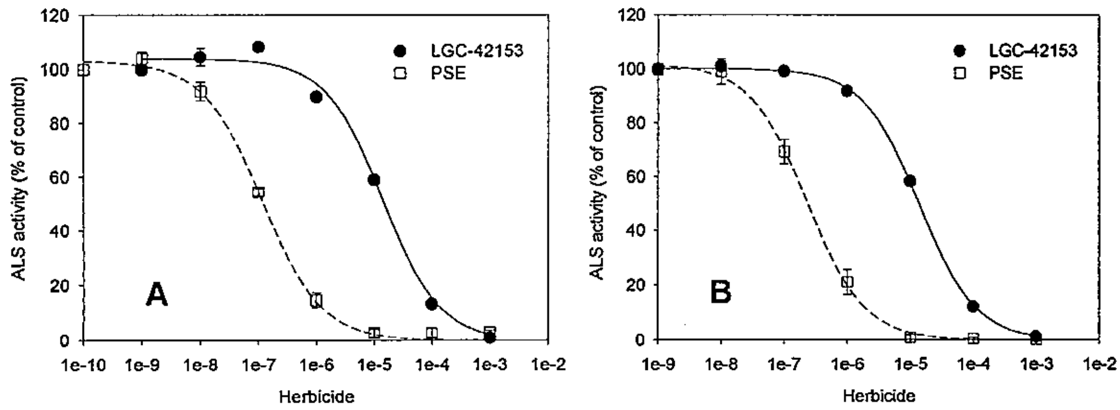

Figure 3. Dose response of ALS extracted from barnyard grass (A) and rice (B) to LGC-42153, and pyrazosulfuron-ethyl. The bar represents the standard error of three replicates.

Table 1. *In vitro* ALS I_{50} values of LGC-42153 and pyrazosulfuron-ethyl to rice and barnyard grass.

Herbicide	I_{50} (M)	
	Rice	Barnyardgrass
LGC-42153	8.38×10^{-5}	9.38×10^{-5}
Pyrazosulfuron-ethyl	2.29×10^{-7}	1.17×10^{-7}

In the *in vivo* assay, ALS activity was rapidly inhibited soon after application reaching about 70% to 80% at 6 h in the both plants. After 12 h, rice ALS activity began to recover and reached about 70% of that of the untreated plant at 96 h. However, this recovery did not occur in barnyard grass (Fig. 4). This comparison demonstrates that rice tolerance is mainly based on a recovery mechanism.

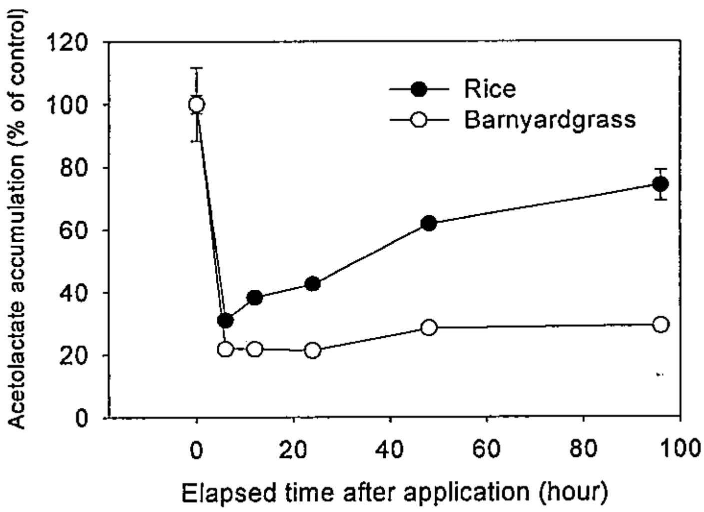

Figure 4. Acetolactate accumulation in rice and barnyard grass after application of LGC-42153. The bar represents the standard error of three replicates.

In conclusion, LGC-42153 inhibited ALS of plants similar to other sulfonylurea herbicides, but at a significantly higher concentration than pyrazosulfuron-ethyl. Further investigation is needed to understand the relationship of the weak *in vitro* activity and the potent whole plant activity. Selectivity at the target site was not apparent between rice and barnyard grass, indicating that the selectivity mechanism of LGC-42153 will involve other mechanisms. *In vivo* assay showed that ALS activity was rapidly inhibited in the both plants. This inhibition was recovered in rice, but not in barnyard grass, indicating that this differential recovery is involved in rice selectivity. The specific biochemical mechanism conferring this recovery in rice is yet to be investigated.

LITERATURE CITED

Beyer, E. M. Jr., M. J. Duffy, J. V. Hay, and D. D. Schueter. 1988. Sulfonylureas. In *Herbicides – Chemistry, Degradation, and Mode of Action*, P. C. Kearney and D. D. Kaufman, eds. New York: Marcel Dekker. pp. 117-189.

Gerwick, B. C., M. V. Subramanian, V. I. Loney-Gallant, and D. P. Chandler. 1990. Mechanism of action of 1,2,4-triazlo[1,5-α]pyrimidines. Pesitic. Sci., 29: 357-364.

Koeppe, M. K., A. C. Barefoot, C. D. Cotterman, W. T. Zimmerman, and D. C. Leep. 1998. Basis of selectivity of the herbicide flupyrsulfuron-methyl in wheat. Pestic. Biochem. physiol. 59: 105-117.

Koeppe, M. K., C. M. Hirata, H. M. Brown, W. H. Kenyon, D. P. O'Keefe, S. C. Lau, W. T. Zimmerman, and J. M. Green. 2000. Basis of selectivity of the herbicide rimsulfuron in maize. Pestic. Biochem. physiol. 66: 170-181.

Ray, T. B. 1984. Site of action of chlorsulfuron: Inhibition of valine and isoleucine biosynthesis in plants, Plant Physiol. 75: 827-831.

Simpson, D. M., E. W. Stoller, and L. M. Wax. 1995. An in vivo acetolactate synthase assay. Weed Technol. 9: 17-22.

Streibig, J.C.1980. Models for curve fitting herbicide dose response data. Acta Agriculturae Scandinavica 30:59-64.

Synthesis of Novel Glycol Benzyl Ethers and Their Herbicidal Activities

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Abstract: Novel tricyclic glycol benzyl ether compounds, having chiral 3, 3a, 5, 9b-tetrahydro-2*H*-furo[3,2-c][2]benzopyran (TFB) skeleton, were synthesized and their herbicidal activities against paddy weeds were examined. TFBs were synthesized from D-glucose, which was used as a natural chiral source. Herbicidal activity was mainly influenced by the substituents at the 6- and 7- position of the TFB ring system. Introduction of oxygen carrying small alkyl groups (e.g. methoxy group) at these positions increased the selectivity between rice and annual paddy weeds such as *Echinochloa crus-galli*. Further experiments indicated that (2*R*,3*S*,3a*S*,9b*R*)-2-ethyl--3-(2fluorobenzyloxy)-6,7-methylenedioxy-3,3a,5,9b-tetrahydro-2*H*-furo[3,2-C][2] benzopyran (MT-147) has the most suitable herbicidal properties for paddy rice among these compounds. MT-147 exhibited excellent herbicidal activity against annual paddy weeds including barnyardgrass with pre- and post-emergence applications at 300 to 1000 g a.i. ha^{-1}. No phytotoxicity was observed against rice plants at these dosages.

Key words: chiral 3,3a,5,9b-tetrahydro-2*H*-furo[3,2-c][2]benzopyran (TFB) skeleton, D-glucose, *Echinochloa crus-galli*, glycol benzyl ether, herbicidal activity, MT-147

INTRODUCTION

In the late 1960s, a variety of herbicidal glycol benzyl ethers, which were effective for weed control in cereals, were discovered by Shell, and many other cyclic benzyl ether herbicides were found out (Isaac et al. 1975, Grayson et al. 1987). The structural features of these compounds were oxygen containing heterocyclic systems with substituted benzyloxy groups or equivalent substituents (Fig. 1). We hypothesized that the orientation of the oxygen in the heterocyclic system and that of benzyl ether were closely related to herbicidal activity. If the orientation of these oxygen atoms was fixed at favorable direction for the active site interaction by the rigid cyclic system, herbicidal activity could be increased. Based on this hypothesis, various cyclic benzyl ethers, which had rigid cyclic systems, were synthesized and their herbicidal activities were elucidated. As a result, it was revealed that the tricyclic tetrahydro2*H*--furo[3,2-c][2]benzopyrans (TFBs, Figure 1) had good herbicidal properties against annual paddy weeds (Arai et al. 1993). The reports on the synthesis of compounds with TFB ring were very few, and there were no reports, which revealed that such compounds have herbicidal activity. The only TFB derivative that reportedly exhibited a biological activity was monocerin (Fig. 1). This compound was reported in 1970 as an antifungal metabolite of *Heminthosporium monoceras* for the first time (Aldridge et al. 1970).

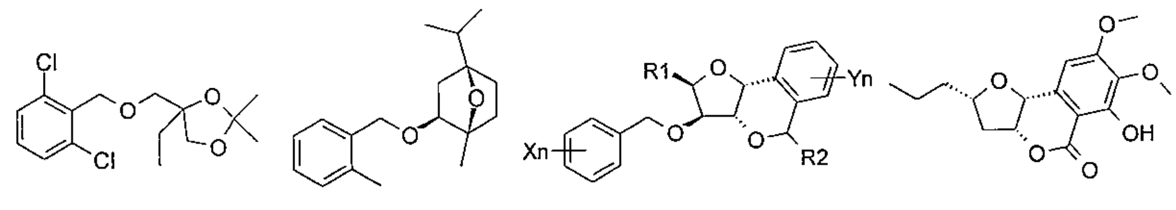


Figure 1. Chemical structures of glycol benzyl ether herbicide, TFBs, and monocerin.

In the last two decades, the combination products of herbicides have been developed and dominating the market of rice weed control in Japan. The most popular combination products are so-called "one-shot" herbicides, which consist of graminicides and compounds effective on broad leaf or sedge weeds. "One-shot" herbicides exhibit a broad spectrum by a single application, providing farmers with laborsaving application methods. We have found, through our intensive discovery program to develop a graminicide for "one-shot" herbicide, that several TFBs had excellent herbicidal activity on *Echinochloa* species.

In this study, several TFBs were synthesized, and structure-activity relationships were examined. Herbicidal properties of the selected compounds, MT-147, were also described.

MATERIALS AND METHODS

1. Synthesis of TFBs

Figure 2 shows the synthetic route for TFBs **9**. The hydroxyl groups of *1*-, *2*-, *5*- and *6*-position of D-glucose were protected by isopropylidene group to yield diacetonide **1**. The diacetonide **1** was benzylated with benzyl chloride and NaH in the presence of phase transfer catalyst to give benzyl ether **2**. The 5,6-isopropylidene group of benzyl ether **2** was selectively hydrolyzed in 70% acetic acid to afford 5,6-vicinal diol **3**. Oleffination of vicinal diol **3** was furnished via 5,6-cyclic orthoester **4** to give olefin **5**. Olefin **5** was treated with 5% palladium on carbon under hydrogen atmosphere to afford xylohexofuranoside **6**. Methanolysis of 1,2-isopropylidene group of xylohexofuranoside **6** with *p*-TsOH gave the anomeric mixtures of methyl glycoside **7**. The 2-hydroxyl group of methyl glycoside **7** was reacted with benzyl chloride under similar condition as etherification of **2** to yield anomeric mixtures of 2,3-dibenzylglucofranoside **8**. These anomeric mixtures such as **7** and **8** were easily separated into single isomers, for example by silica gel column chromatography, and could be used for the subsequent reaction without separation of each isomer. Treatment of the anomeric mixtures of 2,3-dibenzyl glucofranosides **8** with $BF_3{\bullet}OEt_2$ afforded TFBs **9**.

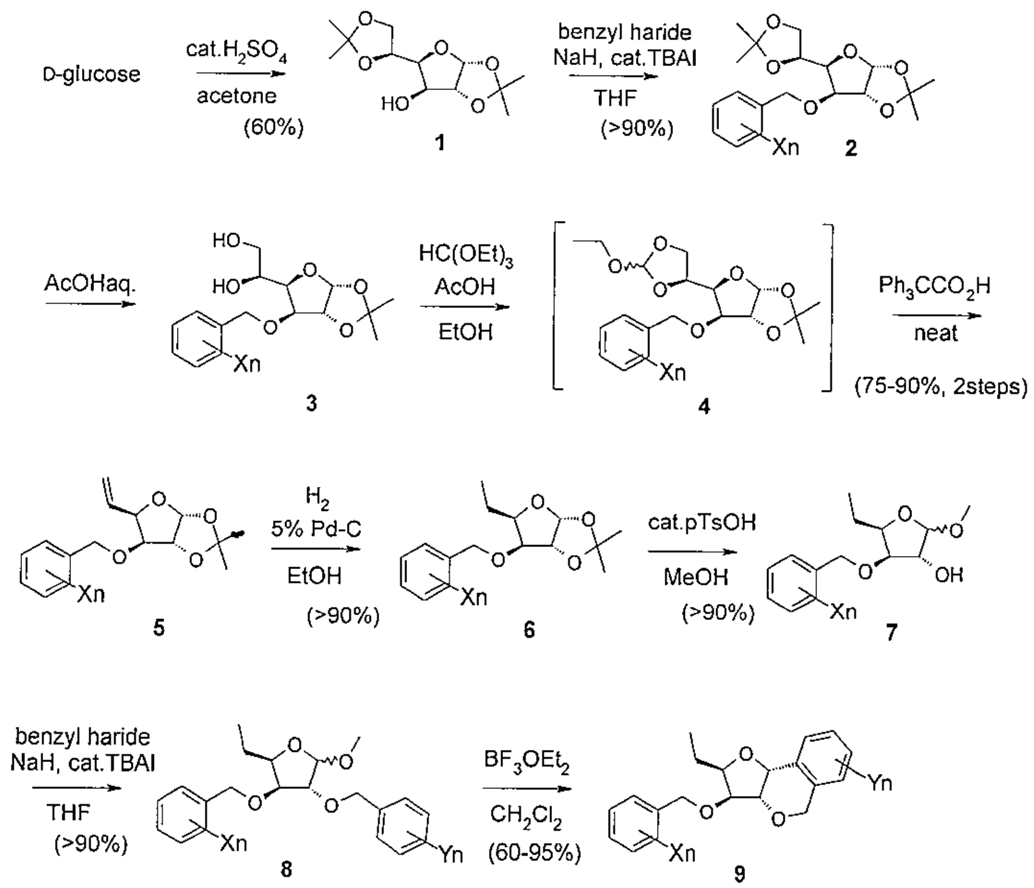


Figure 2. Synthesis of TFBs.

2. Basic herbicidal activities of TFBs

Germinated seeds of barnyardgrass (*Echinochloa crus-galli*) were placed in 5 ml of deionized water containing various concentrations of test compounds in a cylindrical grass vessel. The final concentration of acetone was less than 0.5 % (v/v), and this concentration of acetone did not affect the growth of *E. crus-galli*. The grass vessels were covered with plastic caps, and the plants were grown at 25 °C under light (14 h, 72 $\mu E\ m^{-2}\ sec^{-1}$) and dark conditions (10 h) for 7 days. Then the plants were visually observed and minimum concentration necessary for the complete inhibition of shoot growth (MIC, minimum inhibitory concentration) was examined.

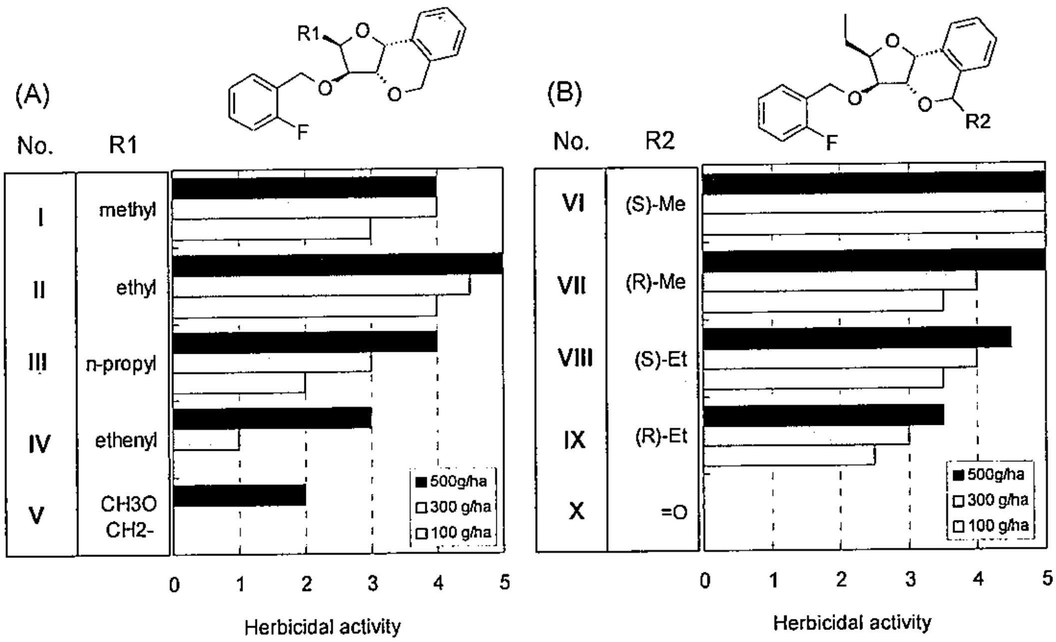
3. Post-emergence herbicidal activities of TFBs

Wagner's pots were filled with soil, seeded with *E. crus-galli* and then flooded with water. Two plants of paddy rice (*Oryza sativa*, cv Koshihikari) were transplanted to these pots and were grown in a green house. At the 2nd leaf stage of *E. crus-galli*, the test compounds dissolved in acetone were applied onto the paddy water at 100, 300, 500 and 1,000 g a.i. ha^{-1}. Thirty days after treatment, the degree of weed control and injury on the paddy rice by test compounds were visually investigated (0; no damage, 1; slight damage, 2; small damage, 3; medium damage, 4; severe damage, 5; dead).

RESULTS AND DISCUSSION

Figure 3(A) represents effects of substituent R1 on herbicidal activities of TFBs. Among these TFBs (**I–V**), ethyl derivative **II** showed the highest herbicidal activity against *E. crus-galli*. It was noteworthy that ethyl group made a contribution to produce more potent herbicidal activity, and methyl, *n*-propyl, ethenyl, methoxymethyl group decreased herbicidal activity. Other substituents (e.g. -CHO, CH_2OH, $-CH_2SCH_3$, $-CH=CHCH_3$: data not shown) also decreased herbicidal activity.

Figure 3(B) shows effects of substituent R2 on herbicidal activities of TFBs. In a series of R2-substituted analogs, TFB **VI** showed the highest activities. The (*S*)-isomers, **VI** and **VIII**, were more active than their corresponding (*R*)-isomers, **VII** and **IX**, respectively. Lengthening of carbon chain of R2 seems to reduce the herbicidal activity.



The carbonyl analog **X** was inactive.

Figure 3. Effects of substituent R1and R2 on herbicidal activities of TFBs.

Figure 4 summarizes the results of effects of substituent Yn on herbicidal activities of TFBs. TFBs **XI** and **XII**, which had methyl group at 6- or 7-position of TFB ring system, exhibited more potent activity than non-substituted one. But 8- or 9-substituted TFBs **XIII** and **XIV** were less active than non-substituted one. It seemed that the substituents at 6- or 7-position of TFB ring system contributed to increase herbicidal activities of TFBs. For the purpose of obtaining further information about structure-activity relationships, TFBs that had various substituents at 6- and/or 7-position were synthesized and their herbicidal activities were examined. 7-methoxy analog **XV** and 6,7-methylenedioxy analog **XXI** showed good herbicidal activities, and the activity of 6-ethyl analog **XIX** was as active as non-substituted analog **II**. 7-chloro analog **XVII**, 7-bromo analog **XVIII**, and 6,7-dimethyl analog **XX** showed moderate to weak activities (Fig. 4).

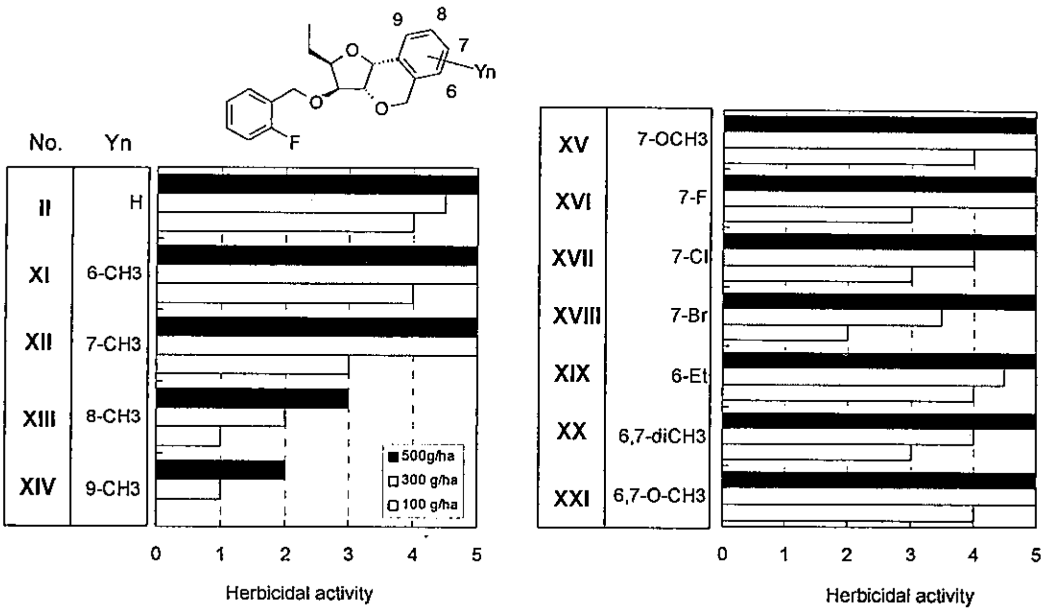
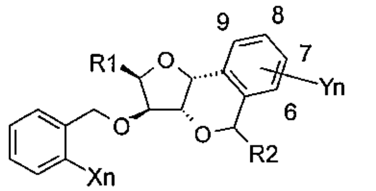


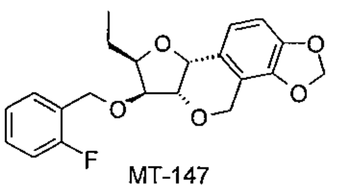
Figure 4. Effects of substituents Yn on herbicidal activities of TFBs.

As for the substituent Xn, compact substituents such as methyl, fluoro, chloro etc., at *ortho*-position were suitable, and thiophene ring could be used instead of substituted benzene ring to obtain the same levels of herbicidal activity (data not shown).

On the basis of the information about structure-activity relationships obtained by above-mentioned experiments, TFBs **10-14** were found out. Table 1 shows herbicidal properties of TFBs **10-14**. When methyl group was introduced at 5-position of TFB ring system, more potent herbicidal activity was obtained, but rice injury was increased (**12**, **13**). The oxygen-containing small alkyl group at 7-position of TFB ring system improved both herbicidal activity and selectivity on the paddy rice (**10**, **13**, **14**).

Table 1. Herbicidal properties of TFBs **10-14**.





MT-147
compound **14**

Herbicidal activities / Rice injury		
Rate	Growth rate	Extent of damage
5	0 - 5 %	dead
4	6 - 10 %	severe
3	11 - 40 %	medium
2	41 - 70 %	small
1	71 - 90 %	slight
0	91 - 100 %	no damage

No	Ssubstituents				MIC (ppm)	Herbicidal activity			Rice injury	
	R1	R2	Xn	Yn		$10\ g\ a^{-1}$	$3\ g\ a^{-1}$	$1\ g\ a^{-1}$	$30\ g\ a^{-1}$	$10\ g\ a^{-1}$
II	C_2H_5	H	F	H	0.20	5	4	3	3	1
10	C_2H_5	H	CH_3	7-OCH_3	0.13	5	5	4	0	0
11	C_2H_5	H	F	6-C_2H_5	0.20	5	4.5	4	3	1
12	C_2H_5	(*S*)-CH_3	F	H	0.10	5	5	5	5	3
13	C_2H_5	(*S*)-CH_3	Cl	7-OCH_3	0.04	5	5	5	3	0
14	C_2H_5	H	F	6,7-methylene -dioxy	0.05	5	5	4	0	0

It was revealed that TFB **14** (MT-147) had the most suitable herbicidal properties for paddy rice among these compounds. MT-147 controlled *E. crus-galli* at 300 g a.i. ha^{-1} by pre- and post-applications, and showed relatively good control for *Scirpus juncoides* and *Monochoria vaginalis* at 1,000 g a.i. ha^{-1}. But, it had no herbicidal activity on *Sagittaria pygmaea*. Application of MT-147 caused no injury on rice at 1,000 g a.i. ha^{-1} (Table 2).

Table 2. Herbicidal activity and phytotoxicity of MT-147.

Application timing	Dose (g a.i. ha^{-1})	Herbicidal activities **				Rice injury
		EC	SJ	MV	SP	
Pre-emergence	100	4	2	2	0	0
	300	5	4	4	0	0
	1,000	5	5	5	0	0
Post-emergence*	100	2.	1	3	0	0
	300	5	3	5	0	0
	1,000	5	4	5	0	0

* post: 2nd leaf stage of EC

** EC: *Echnochloa crus-galli*, SJ: *Scirpus juncoides*, MV: *Monochoria vaginalis*, SP: *Sagittaria pygma*

The herbicidal efficacy of MT-147 on *E. crus-galli* was enhanced by the simultaneous application with bromobutide or daimuron. When MT-147 (200 g a.i.ha^{-1}) was applied to the weed at 2.5 leaf stage with bromobutide (500 g a.i. ha^{-1}) or daimuron (800 a.i. g ha^{-1}), the combination completely killed the weed (Figure 5). Single application of MT-147 (200 g a.i. ha^{-1}) had a moderate efficacy on *E. crus-galli.* Single application of bromobutide (500 g a.i. ha^{-1}) or dimuron (800 g a.i. ha^{-1}) did not show herbicidal activity against *E. crus-galli.*

Based on this result, several "one-shot" herbicides were formulated as granules or suspension concentrates containing MT-147, bromobutide (or daimuron) and benzofenap (or imazosulfuron). These "one-shot" herbicides exhibited excellent herbicidal properties controlling wide range of annual and perennial paddy weeds without injury on rice (Data not shown).

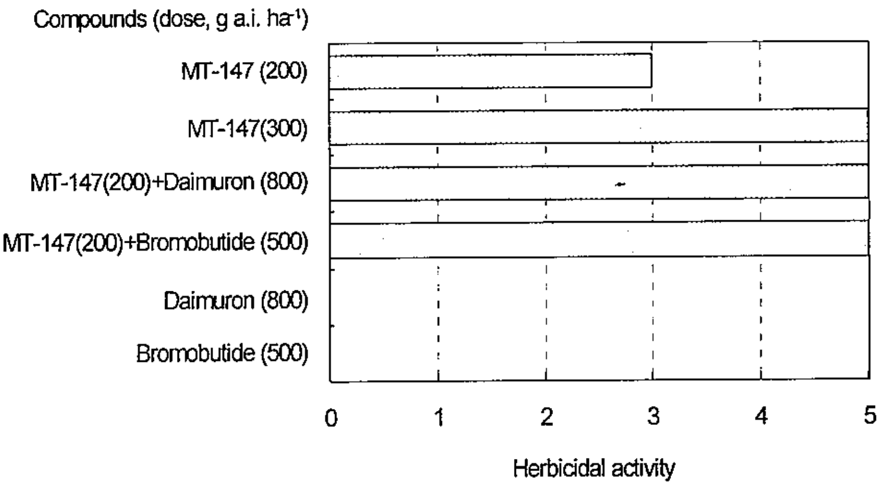


Figure 5. Herbicidal activity of MT-147 and its combination with bromobutide or daimuron.

In conclusion, it is very interesting that unique TFBs exhibited remarkable herbicidal activities. Several TFBs were effective for weed control in paddy rice. An object of our investigation was to find novel herbicides. Thus, various TFBs were synthesized, and their herbicidal activities were examined. As a result, it was revealed that MT-147, representative compounds of TFBs, exhibited excellent herbicidal properties for "one-shot" herbicide in paddy rice.

LITERATURE CITED

Arai, K. et al. 1993, Furobenzopyran Derivatives, Process for Preparation of some and Herbicides Containing same as Active Components. European Patent 0 572 001.

Aldridge, D.C. et al. 1970, Metabolites of *Helminthosporium monoceras*: Structure of Monocerin and Related Benzopyrans. *J Chem Soc (C)*, 2598-2600.

Grayson, B.T. et al. 1987, The Physical and Chemical Properties of the Herbicide Cinmethlyn. *Pestic Sci*, **21**: 143-153

Isaac, E.R. et al. 1975, Trihydric Alcohol Derivertives. US Patent 3 919 251 (1975)

Herbicide Application

Very-Low-Volume Basal Bark Applications of Triclopyr for Woody Plant Control

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Abstract: Conventional basal bark treatments of woody plants require the application of a rather large volume of a 2 to 4% herbicide product in oil solution (vv^{-1}). Portaging adequate amounts of the oil carrier into remote pastures and forests limited the utility of basal bark applications in such areas. In order to reduce the amount of oil carrier needed, a very-low-volume (VLV) basal bark method with a higher herbicide concentration was evaluated on several noxious woody plant species. In the VLV method a sprayer was fitted with an orifice disk instead of an atomizing nozzle. Under pressure, a fine stream of a concentrated herbicide solution was ejected through the orifice that was directed at the basal stem of the target plant. A 20% Remedy™ (butoxyethyl ester of triclopyr, Dow AgroSciences) in crop oil (vv^{-1}) or the ready-to-use Pathfinder II™ (Dow AgroSciences), which was equivalent in triclopyr concentration, was applied in either horizontal streaks from ground level to 45 cm high or in vertical streaks, from ground level to 90 cm high, on opposite sides of the basal stems. Horizontal streaking would be useful for treating stands of woody weeds. Vertical streaking would be useful for precise treatment of individual woody weeds. *Acacia confusa*, *Prosopis pallida*, and *Psidium cattleianum* were all susceptible to VLV applications applied in horizontal streaks. *Ardesia elliptica, Casuarina equisetifolia, Corynocarpus laevigatus, Psidium cattleianum,* and *Psidium guajava* were susceptible to vertical streak treatments. The volume of herbicide solution expended by this method was 8 to 17 mL $plant^{-1}$. This method should be useful to ranchers and foresters who must control these problem species in remote areas. Work continues to further assess this method on other species and on larger plants that will require higher doses (more or longer streaks).

Key words: basal bark application, invasive plants, very-low-volume method, woody plant control

INTRODUCTION

An effective method of killing many species of woody plants is the basal bark method wherein a lipophyllic herbicide in an oil carrier is applied to the basal stem up to the 40-

cm height level of the target plant. The concentration of herbicide is usually 2 to 4% of product (vv^{-1}) and the amount of solution applied is 15 mL cm^{-1} of basal stem diameter (Klingman and Ashton 1975). A 15 cm diameter stem would therefore require 225 ml of the herbicide solution. This is a rather large amount of material. In many remote ranches and forests, the terrain is rough and impassable with vehicles. In such areas, it would be difficult and unsafe to transport large quantities of material in addition to equipment. Walking on rough ground with heavy load risks severe injuries in falls. That danger is compounded by fatigue induced by carrying heavy loads for an extended period of time. The authors of this report have been working with a very-low-volume (VLV) foliar application method developed by Mr. Shigeo Uyeda, weed control superintendent (retired) of the McBryde Sugar Company in Hawaii. This method, originally called the "magic wand" method, now called the "Uyeda drizzle method" or more simply the "drizzle method", employs a conventional sprayer with the nozzle replaced by an orifice disk that ejects a fine stream of a concentrated herbicide solution or emulsion. By waving the wand and applying the herbicide in foliar applications at 15 L ha^{-1}, effective control of many species was obtained (Motooka *et al.* 1983). The drizzle applicator was used in basal bark applications to determine if VLV applications would be effective in the control of invasive woody plants. The VLV basal bark applications were made on opposite sides of the basal stem either in horizontal streaks from ground level to 45 cm high or in vertical streaks from ground level to 90 cm high. The horizontal streak method would be more efficient in treating stands of weeds where there is no danger of injuring desirable dicots. In such areas the wand can be waved to cover a wide swath. The vertical streak method would be more useful in natural areas where preservation of native plants is important. The fine stream can be applied to an invasive woody plant very precisely without injuring nearby plants.

MATERIALS AND METHODS

Sprayers pressurized with CO_2 and equipped with a disk with a 0.5 mm diameter orifice and discharging 3 mL sec^{-1} at 207 kPa were used to make VLV basal bark applications. Remedy™ (butoxyethyl ester of triclopyr, Dow AgroSciences) at 20% in crop oil or the ready-to-use Pathfinder II™ (Dow AgroSciences), which contain the equivalent triclopyr concentration, was applied to the bases of woody plants in these trials. The woody plants treated were severe pests in pastures, forests or both (Table 1). Data in percentage reported herein were converted by the arcsin transformation if appropriate for analysis (Steel and Torrie 1960).

Horizontal streak application

The following three trials employed the horizontal streak method. Four streaks were made on each of two opposite sides of the basal stem. This amounted to 7.6 mL $plant^{-1}$ including the material that missed the stems (Unpubl. data, University of Hawaii). Except Formosan koa, all plants were treated individually.

Table 1. Invasive woody plant species treated by very-low-volume basal bark applications (Wagner *et al.* 1999).

Species	Comment
Formosan koa (*Acacia confusa* Merr.)	A small, common ornamental tree, prolific seeder, invades pastures and disturbed areas in mesic to wet climates.
Shoebutton ardesia (*Ardesia elliptica* Thunb.)	A tall ornamental shrub, prolific seeder, spread by birds, forms dense stands in high rainfall pastures and disturbed areas in forests.
Australian pine (*Casuarina equisetifolia* L.)	A large, hardy tree, an ornamental and windbreak, especially in coastal areas. Spread by seed and rhizomes in mesic areas.
Karakanut (*Corynocarpus laevigatus* J.K. Forster & G. Forster)	A serious problem in Kauai forests where it was aerially seeded in 1929 in a reforestation project. Spread by feral pigs, deer and goats that feed on the fruits.
Olive (*Olea europaea* L. subsp. *africana*)	Planted as an ornamental, windbreak and for reforestation, olive flowers at elevations above ca. 1000 m. and has become a pest is such areas. Spread by birds and feral mammals that feed on the little fruits.
Kiawe (*Prosopis pallida* [Humb-Bonpl. ex Willd.] Kunth.)	An early introduction into Hawaii. Kiawe pods were valued as cattle feed. However kiawe is thorny with a large canopy that shades a large area in arid zones.
Waiawi (*Psidium cattleianum* Sabine var. *littorale*)	One of three varieties of *P. cattleianum* in Hawaii, waiawi bears edible, small yellow fruits with many hard seeds, used to make fruit drinks. Spread by birds and mammals that feed on the fruit. A serious pest in high rainfall pastures and forests where it forms dense stands. Only variety capable of growing into a substantial tree.
Guava (*Psidium guajava* L.)	Guava is a serious pest in mesic to high rainfall pastures and forests. The fruit is used to make juice and jelly. The fruit is yellow with many hard seed that is spread by birds and mammals, including cattle.

1. Formosan koa. At Wailua, Kauai, a single large plot was treated by walking through a grove of Formosan koa and back in the opposite direction while waving the wand to treat the basal stem of each plant in a 3 m by 15 m area. Each stem was treated with four horizontal streaks of triclopyr (20% in crop oil) on opposite sides although with this methodology the number of streaks per plant was not precise. Evaluations were made at 19 months after treatment (MAT) by visual estimates of defoliation in all plants within the center 1.5 m by 5 m of the plot and by counts of dead plants.

2. Kiawe. At Kohanaiki, Hawaii, stems of 48 plants in two class sizes, ≤10 cm and 11 to 15 cm in basal diameter, were treated with Pathfinder II™ and evaluated by visual estimates of defoliation at 2 MAT.

3. Waiawi. On the Waihee Ridge Trail, Maui, stems of waiawi, each 5 to 20 cm in basal diameter, were treated with triclopyr ester in crop oil. Visual evaluations of defoliation were made along with counts of dead plants at 27 MAT.

Vertical streak application

In the following six trials, Pathfinder II™ was applied in vertical streaks, one on each of two opposite sides of the basal stem from ground level to 90 cm high. The volume applied to each stem ranged from 9.4 to 17 mL plant^{-1}.

4. Shoebutton ardesia. At Princeville, Kauai stems of 96 saplings of shoebutton ardesia, 2.5 to 5 cm in basal diameter, were treated. Evaluations were made by visual estimates of defoliation at 4 MAT.

5. Australian pine. At Kalaheo, Kauai, the stems of 10 plants, 5 to 10 cm in basal diameter, were treated. The plants were evaluated by visual estimates of defoliation at 3 MAT.

6. Karakanut. At a high rainfall site at Kokee, Kauai, the stems of 10 plants, 5 to 12 cm in basal diameter, were treated. Evaluations were made by visual estimations of defoliation and a count of dead plants at 12 MAT.

7. Olive. At Kokee, Kauai, the stems of 10 plants, 6 to 12 cm in basal diameter, were treated. Evaluations were made at 3 MAT by visual estimations of defoliation at 3 MAT.

8. Waiawi. At Kokee on Kauai, stems of 10 plants, 7.5 to 12.5 cm in basal diameter, were treated. Visual estimates of defoliation and a count of dead plants were made at 13 MAT.

9. Guava. At Princeville, Kauai, stems of 21 guava plants, 15 cm basal diameter, were treated. Evaluation was made by visual estimates of defoliation at 4 MAT.

RESULTS AND DISCUSSIONS

Horizontal streak applications

Formosan koa was slow to succumb but by 17 MAT, the trees were almost completely defoliated and 73% of the treated plants were dead (Table 2). Kiawe of both size classes were severely defoliated at 2 MAT. There was significantly greater injury in the smaller plants (ANOVA, F=11.93, p=0.01), though defoliation was high for both size classes (Table 2). Unfortunately, this trial was prematurely terminated because volunteers hand-cleared the trial area. These results offer hope that the method would also be effective on a more recent introduction, mesquite (*Prosopis juliflora* [Sw.DC), a growing problem on Kauai and Oahu and recently found on Hawaii. Waiawi, a thin-barked species, demonstrated injury very quickly, and recovery was very poor. By 27 MAT, there was 96% defoliation and 70% of treated trees were killed (Table 2).

Table 2. Efficacy of very-low-volume basal bark applications of triclopyr in horizontal streaks on woody plants.

Species	Defoliation (%)	n	sd	Kill (%)	MAT[1]
Formosan koa	98	41	8.0	73	17
Kiawe[2]	99	32	2.0	-	2
Kiawe[3]	94	16	9.0	-	2
Waiwi	96	17	10.3	70	27

[1]Months after treatment. [2]Basal diameter ≤10 cm. [3]Basal diameter 11-15 cm.

Vertical streak applications

Shoebutton ardesia was severely defoliated at 4 MAT (Table 3). Australian pine was severely injured at 3 MAT with 94% defoliation (Table 3). Karakanut was severely injured at 12 MAT, but only 40% of the treated plants were killed (Table 3). A higher dose, more streaks or streaks longer than 90 cm may provide complete kill. Olive showed no injury at 3 MAT (Table 3) consistent with earlier conventional basal bark application trials (Unpubl. data, University of Hawaii). Apparently the bark of the plant is somewhat impervious to oil treatments. Other tolerant species encountered were the kahili flower tree (*Grevillea banksii* R.Br.), and paperbark (*Melaleuca quinquenerva* [Cav.] S.T. Blake) (Unpubl. data, University of Hawaii). On the other hand, the green-stemmed species catsclaw (*Caesalpinia decapetala* [Roth] Alston) and gorse (*Ulex europaeus* L.) were susceptible to VLV basal bark applications of triclopyr (Motooka *et al.* 1999). Waiwi was very susceptible to vertical streak treatment, with 99% defoliation and 90% kill at 13 MAT (Table 3). Guava was quickly and severely defoliated at 4 MAT (Table 3).

As with conventional basal bark treatments, large trees will probably not succumb to VLV basal bark treatments. Furthermore, some species seem to be tolerant to basal bark application. Higher doses will probably be required for trees larger than those targeted in these studies. Karakanut at 20 cm basal diameter for example, was little damaged by horizontal applications of triclopyr in oil (Unpubl. data, University of Hawaii). Control of large waiwi, a thin-barked species and sensitive to triclopyr, may be feasible. A tree 6 m in basal diameter was killed by applications of eight streaks completely around the trunk circumference (trees this size were rare so replication was not possible). Further work is planned to determine the species susceptible to VLV basal bark application as well as adjustments to the method to improve efficacy such as higher doses, i.e. more and longer streaks and follow-up applications after recovery begins. Follow-up will be required in any case to tend to missed target plants and re-infestations. A formula needs to be developed that provides guidance on the proper dose, i.e., number of streaks relative to the size of the target plant, i.e. the diameter of the basal stems. Even if utility of the VLV basal bark method is restricted to smaller woody plants, it will be a useful tool for vegetation managers who have to manage woody invasive plants in remote areas.

Table 3. Efficacy of very-low-volume applications of triclopyr made in vertical streaks on woody plants.

Species	Defoliation	n	sd	Kill	MAT[1]
	(%)			(%)	
Shoebutton ardesia	96	89	12.9	-	4
Australian pine	94	10	14.9	-	3
Karakanut	90	10	11.9	40	12
Olive	0	10	-	-	3
Waiawi	99	10	0.3	90	13
Guava	99	21	1.8	-	4

[1]MAT = months after treatment.

CONCLUSIONS

The VLV basal bark application method was effective on several woody invasive plant species. It provides an efficient and effective means to manage invasive plants in remote areas where the reduced portage will offer greater worker-safety than conventional methods. Additional research will further define the potential of method.

LITERATURE CITED

Klingman, Glenn C. and Floyd M. Ashton. 1975. Weed Science: Principles and Practices. John Wiley & Sons, New York. 431p.

Motooka, Philip, Guy Nagai and Lincoln Ching. 1983. The Uyeda "Magic Wand" method of herbicide application. Proc. Asian-Pacific Weed Sci. Soc. Suppl. vol.: 550-553. Manila.

Motooka, Philip, John Powley, Michael DuPonte, Lincoln Ching, Guy Nagai, and Galen Kawakami. 1999. Drizzle herbicide application for weed management in forests. Proc. West. Soc. Weed Sci. 52: 136-139. Colorado Springs.

Steel, Robert G.D. and James H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York. 481p.

Warren L. Wagner, Derral R. Herbst and S.H. Sohmer. 1999. Manual of the Flowering Plants of Hawaii. 2nd ed. University of Hawaii Press and Bishop Museum Press, Honolulu. 2 vol., 1854 p.

Efficacy of Selective Postplant Application Methods of Glyphosate Against Weeds in Onion (*Allium cepa* L.)

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Abstract: Field studies were conducted in Bongabon, Nueva Ecija during the 2001 and 2002 dry seasons to determine efficacy of selective postemergence application of glyphosate control against purple nutsedge (*Cyperus rotundus* L.) and other weeds infesting onion (*Allium cepa* L.) grown after rice (*Oryza sativa* L.).These application methods included use of shielded sprayer, shielded nozzles and paintbrush in directed herbicide applications. Shielded nozzle or shielded sprayer provided adequate season-long weed control and yielded comparably with plots treated with preplant treatments like stale-seedbed techniques and better than those of preplant applications of glyphosate or those treated with farmers' practice of tillage and interrow cultivation. Among the postplant methods, it took less time to apply with shielded nozzle than with shielded sprayer or paintbrush.

Key words: weed management, rice-based cropping systems, postemergence herbicides

INTRODUCTION

As much as 90% of potential yields in onion crops are lost due to competition when weeds are left uncontrolled. In farmers' fields, the amount that onion growers spend annually in weedling labor and herbicides, $400/ha or 20% of total production costs, reflects the critical role of weed control in onion production. Purple nutsedge, *Cyperus. rotundus* L., the most destructive weed in onion cannot be controlled selectively with herbicides once the onion crop has emerged because herbicides that can control this weed are not selective to onion. Glyphosate, which is effective against this weed, is not selective to onion and can only be used as a preplant application to avoid crop injury. Preplant applications however are too early in the season to control escapes and subsequent regrowths at mid-season and thus fail to provide adequate season long control. If glyphosate can be applied postplant without injury to onion, its efficacy can be increased and season-long control can be obtained. Thus, there is a need to determine safe and effective methods of applying herbicides postemergence which are safe tot he crop to obtain adequate season-long control of weeds in onion.The objectives of this study are to determine selective postplant application methods of glyphosate for control of purple nutsedge and other weeds in onion grown after rice.

MATERIALS AND METHODS

Site description. The studies were conducted at the IPM CRSP Asian site in Bongabon, Nueva Ecija, Philippines, lat 15-16°N and long 121°E, 50 to 100 m above sea level. The monthly average day and night temperatures range between 20°C and 32°C, with a yearly mean average of 27°C. The soil is clay loam, classification Inceptisols (Eutropepts with Dystropepts). The stale-seedbed studies were conducted in a farmer's field in Palestina village in San Jose, Nueva Ecija while the postplant herbicide application studies were conducted at the NOGROCOMA/IPM CRSP Demo Farm in Bongabon, Nueva Ecija.

The studies were conducted during the 2001 dry season (January to April 2001) and 2002 dry season from November 2001 to April 2002. The area was plowed twice and harrowed thrice. Forty-five day old seedlings of onion cultivar Red Pinoy were transplanted on January 11, 2001 and January 11, 2002 into 4 x 5 m plots in raised furrows, with three rows in each furrow. Complete fertilizer (14-14-14 NPK) was applied at 14, 34 and 49 days after transplanting (DAT). Chlorpyrifos and NPV were applied once to manage insect pests and benlate and captan also applied once to manage diseases. Plots were irrigated as needed with a deep-well pump and soil moisture maintained at field capacity. The treatments were: 1) glyphosate application with paintbrush; 2) glyphosate application with a sprayer with nozzle shield; 3) glyphosate application with a shielded sprayer; 4) preplant glyphosate at 2 weeks before transplanting followed by one handweeding; 5) chemical stale-seedbed (harrowing followed after 2 weeks by glyphosate); 6) mechanical stale-seedbed (harrowing followed after 2 weeks by another harrowing); 7) farmers' practice (tillage and interrow cultivation); 8) unweeded control; and 9) weed-free control (3x handweeding). Glyphosate was applied at 1.0 kg a.i. ha^{-1} at 3 weeks after transplanting in 200 L ha^{-1} water. The following data were recorded: 1) weed density (number of weeds by species per square meter), 2) weed fresh weights (g m^{-2}), 3) time spent in applying glyphosate (mandays ha^{-1}); 4) time spent in handweeding (mandays ha^{-1}); 5) crop injury rating (0-no injury; 100- dead plant); 6) weed control rating (0-no control; 100-complete control) and 7) bulb fresh weight at harvest. Onion bulbs were harvested at 81 DAT from a 2 x 3 m area on April 1, 2001 and April 1, 2002. Treatments were replicated four times in a Randomized Complete Block Design. Data were subjected to analysis of variance (ANOVA) using Proc GLM (SAS) and treatment means were compared using LSD (0.05).

RESULTS AND DISCUSSION

Dry season 2001. Weed growth in plots treated with postplant application methods (paintbrush, rollerbrush, and shielded sprayer) were significantly lower than weed growth in plots treated with preplant application methods and in unweeded plots (Figure 1). Weed growth in all the postplant application methods were further reduced when these were followed by one handweeding. Of the three postplant methods, shielded spreyaer was most efficient, followed by paintbrush, and rollerbrush. Weed growth in plots treated with preplant application methods was lower than weed growth in unweeded plots. Yields in plots treated with postplant application methods were higher than those in plots treated with preplant application and in unweeded plots but lower than those in weed-free plots (Table 1). Plants treated with shielded sprayer had the highest yields, followed by yields in plants treated with paintbrush and rollerbrush. Yields were further increased when followed by one handweeding.

The shortest time to apply the herbicide was obtained with the shielded sprayer (4 man-days ha^{-1}) while it took longer to apply both the paintbrush and rollerbrush (12 to 17 mandays ha^{-1}). The amount of time needed to handweed after herbicide application was fastest with the shielded sprayer, indicating less weed growth. Without herbicide application, it took about 60 man-days to handweed; this was twice longer than for the time it took to handweed the plots when a postplant application was made prior to handweeding (Table 1).

Table 1. Weed density, bulb yield and handweeding times in onion cv. Red Pinoy treated with different postplant application methods of glyphosate during in Bongabon, Nueva Ecija during the 2001 dry season.

Treatment[1] time	Weed density (no. m^{-2})	Bulb yield (t ha^{-1})	Handweeding (md ha^{-1})
Preplant	120 b	1.0 f	
Paintbrush	66 d	4.0 c	
Paintbrush/handweeding	37 f	6.0 c	28
Rollerbrush	89 c	2.3 e	
Rollerbrush/handweeding	43 ef	4.2 d	42
Shielded sprayer	60 de	4.7 d	
Shielded sprayer/handweeding	36 f	7.4 b	29
Unweeded control	143 a	0.1 g	
Weed-free control	0 g	9.2 a	64

[1]Average of 4 replications

Dry season 2002. At 30 DAT, weed fresh weights in stale seedbed, farmers' practice, and postplant treated plots were all lower than those of plots treated with preplant applications and the unweeded control plots (Table 2). At harvest, weed weights in the stale-seedbed treated plots were lowest among all the treatments, indicating that these methods provided adequate season-long weed control. Postplant application methods (nozzle shield and sprayer shield) and the farmers' practice plots also had low weed weights indicating adequate weed control from these treatments. Highest weed weights were obtained from the paintbrush application method and the preplant applications.

Good weed control obtained from stale seedbed plots and shielded nozzle treatments are reflected in the high yields obtained from these treatments (Table 3). Lower yields were obtained from farmers' practice-treated plots and those treated with shielded sprayer. Plants with preplant glyphosate applications and those applied with paintbrush gave the lowest yields among the treated plants. Lowest yields were from unweeded plots and highest yields from weed-free plots.

Table 2. Weed fresh weights at 30 DAT and at harvest and yield of onion cv. Red Pinoy with preplant or postplant treatments in Bongabon, Nueva Ecija during the 2002 dry season.

Treatment [1]	Weed fresh weight $g\ m^{-2}$		Yield $t\ ha^{-1}$
	30 DAT	Harvest	
Preplant glyphosate	146 b	926 b	13.4 de
Stale-seedbed, chemical	53 c	682 c	19.5 ab
Stale-seedbed, mechanical	48 c	694 c	19.1 ab
Farmer's practice	52 c	862 bc	15.1 cd
Paintbrush	59 c	924 b	10.9 e
Shielded nozzle	54 c	842 bc	17.8 bc
Shielded sprayer	52 c	817 bc	13.9 de
Weed-free control (3 hw)	0 d	0 d	21.7 a
Unweeded control	546 a	1188 a	0.8 e

[1] Average of four replications.

Among the postplant application methods, the shielded nozzle provided the highest yields, followed by yields from plants applied with the shielded sprayer and lowest yields were obtained from plants applied with paintbrush (Table 2). The shielded nozzle also took the least time to apply (about 1.2 mandays $^{-1}$ha) compared with 4 mandays ha^{-1} for the shielded sprayer and 17 mandays ha^{-1} for the paintbrush.

Results indicate that postplant application methods using a shielded nozzle or a shielded sprayer can control emerged weeds in onion with adequate selectivity to the crop. Onion treated with postplant applications yielded comparably with those treated with preplant application methods like stale seedbed techniques. Among the two postplant methods, plants applied with shielded nozzle gave higher yields than those applied with shielded sprayer. Preplant application of glyphosate without stale seedbed treatments did not provide adequate season-long control of weeds and provided yields lower than those in stale seedbed plots and those in plots applied with shielded nozzles.

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Allelopathy

Practical Weed Suppression by Using Allelochemical from Hairy Vetch

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Abstract: Screening of allelopathic plants by specific bioassays and field tests showed that Hairy vetch (*Vicia villosa*) is the strongest allelopathic plant. Hairy vetch was found to be the most promising cover plant for weed control in orchard, vegetable and rice production and even for landscape amendment in abandoned field in Japan. We have isolated "cyanamide", a well-known nitrogen fertilizer, from hairy vetch. This is the first finding of natural cyanamide in the world.

Key words: allelochemicals, hairy vetch, *Vicia villosa*, weed suppression

INTRODUCTION

Hairy vetch is a well-known green manure and cover crop in the United State and Europe. The origin of hairy vetch was estimated to the area from west Asia to east Mediterranean coast. It was cultivated in England and Germany in the early 19th then introduced to USA in the middle of 19th century, had a good reputation from USDA, and now widely distributed in the southern part of the USA. Hairy vetch was introduced into Japan in the early 20th, and had a good result by the Agricultural Experimental Station, but did not distribute until now.

There are some reports on the allelopathy and weed control by hairy vetch. Lazauskas and Balinevichiute (1972) tested the inhibitory activity of extract of seeds to barley, and found that hairy vetch showed the strongest activity. White et al. (1989) reported that the incorporation of residue of hairy vetch and crimson clover reduced the emergence Solanaceae weeds to 60 to 80 %, and water extract of hairy vetch had the stronger inhibitory activity. Johnson et al. (1993) reported that the mulch made from hairy vetch or rye completely inhibited the weed in non-tillage systems by killing them by herbicide or mechanical cutting. Teasdale and Daughtry (1993) reported that the living mulch of hairy vetch showed better inhibitory activity than the desiccated one. Abdul-Baki and Teasdale (1993) reported a unique system using hairy vetch mulch to compensate for the vinyl plastic film mulch in tomato production. There are so many reports and field observation about the weed suppression of hairy vetch, but still now the contribution of allelopathy and its allelochemicals are unknown. We are now going to separate allelochemicals from hairy vetch and will report about the chemical nature.

Hairy vetch is utilized as a winter cover crop in the United States (Greshenzon 1998) and Japanese orchards and abandoned paddy fields (Fujii 2001). In addition to providing a great amount of nitrogen to soils and preventing evaporation of water and soil erosion, hairy vetch effectively suppresses weed growth (Hoffman et al. 1993). Weed growth inhibition by chemicals contained in this plant (allelopathy) has been supposed to be an important factor for weed suppression, together with competition for light, nutrients, and water. The extracts of the leaves of hairy vetch show phytotoxicity stronger than those of many other plants. However, none of plant growth inhibitors in hairy vetch has been identified.

MATERIALS AND METHODS

Origin of seeds of cover crops and definition of hairy vetch

Most of the seed used in this experiments are from "Kaneko Seed Co.", "The Takii Seed Co.", "The Yukijirushi Seed Co.", and "Sakta no Tane, Sakata Seed Co.". The term 'hairy vetch' was used in Japan both for hairy vetch and woolly pod vetch. All seeds of hairy vetch was imported from USA, and some seed company deny to tell the origin, we call them as hairy vetch in a whole. But there are difference as *Vicia villosa* and *Vicia dasycarpa* (=syn. *V. villosa* subsp. *varia*). The allelopathic activity and inhibitory activity on the field of both is almost the same.

Screening of Allelopathic Cover Crops by the Plant Box Test

Primary selection of cover crops for allelopathy was done using "Plant Box Method", developed for the assessment of allelopathy (Fujii and Shibuya, 1991a). Young plants were cultivated for one to two months in a sand culture, in standing water containing a nutrient solution. The receiver plant used for bioassay was lettuce (Great Lakes 366), because it is high sensitivity to bioactive substances.

Extraction of Inhibitory Chemicals from Cover Crops

Plant growth inhibitory activities of some cover crops were tested by water and methanol extraction. Dried leaves and shoots (60 C, over night, forced air dry) were extracted by 150 times of water and 40 times of methanol. These extracts were mixed with agar (0.5%) to make a stuff bed to support lettuce seeds. After three days in a dark condition at 20 C, radicle and hypocotyl length were measured.

Screening of Cover Crops for Weed Control in the Experimental Fields

To know the practical weed suppression ability by cover crops, field tests for weed suppression were designed. All field trial for screening were done in the experimental field of Shikoku National Agricultural Experimental Station. For the first screening for spring seeding cover crops, 46 plants were sown on 1m x 1 m quadrates on May 23, 1992, then after three months of no weeding, weed density and dry weights were measured on August 25, 1992. For the preliminary screening for fall seeding plants, 39 candidates were sown on 2 x 2 m quadrates on November 5, 1992, and after no weeding for 6 months, weed dry weight of each plot was measured on April 20, 1993. For selected 21 cover crops of fall sown, 4 times replications by complete randomized bloc design were designed and seeded on 2 x 2 m quadrates on November 5, 1992, and weed and crop dry weighs were measured on April 20, 1993.

Application of Hairy Vetch to Abandoned Paddy Field in the Experimental Station

From the results of screening of cover crops, we focused on hairy vetch, and four trial was conducted on the experimental field in Shikoku National Agricultural Experiment Station. 1) larger scale application test of hairy vetch was conducted in comparison with Chinese milk vetch on the uniform paddy field of 1000 m^2. This field was divided into 16 block and 4 replications of 4 different cover crop trials were designed. Cover crop design were; (1) hairy vetch, (2) hairy vetch and oat, (3) Chinese milk vetch, (4) no treatment as control. This experiment was started in 1992 and continued. Data from 1992 to 1994 was reported in this paper. 2) Changing the seeding ratio from standard seeding to increased into four times was examined using a field of 500 m^2, and arranged with split-split-plot test with 4 replications. This test start from October 25, 1993 and weed and crop yield were measured in May 20. 3) Changing the seeding date from October to February was tested using the same field with tree replications. This test was started from 1992 and ended in 1994. 4) Effect of soil water contents on the growth of hairy vetch was tested in the lysimeter. This lysimeter was designed to know the suitable water ratio for crops, and it is possible to change the water level by stair step system of over flowing. Each block was about 10 m^2, and there were four stages with two replications. Soil type of above four tests was [sandy loam, paddy field converted to upland condition.] 5) The combination of leguminous cover crops and barley were examined on the slope land experimental field of Shikoku National Agricultural Experiment Station on O-asa mountain. The slope angle of this field was 9 degree. Seeding date was November 6, 1993, and crop and weed yield were measured on May 30. The total area of this field was about 800 m^2. Soil type of this field is light volcanic ash soil.

Direct Application Test of Hairy Vetch to Farmer's Field

Direct application and exhibition trial of hairy vetch were done using the field of cooperative farmers by the courtesy of each district counselors for farmers (in Japan, there are counselors for farmers in each county. These counselors are public service officers belong to the Ministry of Agriculture.) Six different farming systems were chosen. 1) Paddy field in Man'nou town. The owner is Mr. Tsune-kane. The area is about 800 m^2. This paddy field is in a slightly mountainous area, and slightly dry condition. This field was just stop rice production. 2) Paddy field in Marugame city. The owner is Mr. Hirata. The area is about 1000 m^2. This field is close to road and in humid condition. This field was abandoned two years ago. 3) Paddy field in Zentsuji city. The owner is Mr. Kawada. The area is about 900 m^2. This field was abandoned for 4 or 5 years and they used Chinese milk vetch as green cover crop, but suffered from serious weed infection. 4) Grassland in Tyu-nan town. The owner is Mr. Morichika. The area is about 600 m^2. This grassland in in a slope land, and used as exhibition. 5) Orchard for Kaki, Japanese persimmon in Kounan town, Oka village. The owner is Mr. Oka. The area used for cover crop trial is about 50,000 m^2 in first year, 1992, and extended to 10,000 m^2 in the following year and continue until now. Oka family is a pioneer in this orchard area, and produces sweet type of Kaki of supreme quality. 6) Pear Orchard in Toyohama town. The owner is Mr. Oohiro. The area used for the trial for hairy vetch is about 80000 m^2. He continued cover crop trial after 1993.

Isolation and identification of Allelochmicals

Nuclear magnetic resonance (NMR) spectra for 1H and 13C were recorded by using a JNM a-600 (JEOL, Tokyo) spectrometer. Infra-red (IR) spectra were obtained using a Spectrum GX-AutoIMAGE system (PerkinElmer, Boston, MA, USA). High performance liquid chromatography (HPLC, 626 pump with 996 photodiode array detector; Waters, Milford, MA, USA) equipped with an ODS column (Inertsil ODS-3, 250 × 4.6 mm i.d.; GL-Sciences, Tokyo, Japan) eluted with water at a flow rate of 1.0 ml min^{-1} with detection at UV 210 nm was used for the isolation and quantification of cyanamide.

Plant materials

For isolation of plant growth inhibitors, hairy vetch, which had been cultivated in our institute's experimental field (Tsukuba, Japan) from October 2000 to May 2001, was used. Neither fertilizer nor pesticide had been applied in the field at least for 3 years. For quantification of cyanamide in the seedlings of hairy vetch, seven seeds were placed on vermiculite (Fukushima Vermi, Fukushima, Japan) filled in a pot and covered with the vermiculite. The germinated seeds were subjected to HPLC analysis. The pot was incubated in an illuminated growth chamber (Eyelatron FLI-301NH; EYELA, Tokyo, Japan) for 9 d at 25°C with 25000 lx.

RESULTS AND DISCUSSIONS

Screenings of Allelopathic Cover Crops

The results of screening of candidates for allelopathic cover crops from leguminous and gramineous species by Plant Box Method were shown in Table 1 and Table 2, respectively. In this table, radicle percentage means the percentages of the root radicle by length of the young lettuce plants present in the root zone of each donor plant, based on the calculation of radicle length within the root zone controlled by donor plants. As shown in Table 1, leguminous cover crops such as velvet bean, hairy vetch, yellow sweet clover and white sweet clover have strong allelopathic inhibitory activities. Of these legumes, velvetbean, *Crotaralia, Canavalia, Cajanus, Cicer, Vigna* and *Glycine* are summer cover crops, and most of others are winter cover crops. *Melilotus* and *Pueraria* are perennial crops. Then it is important to know the characteristic of each cover crop to use for the farmland.

Gramineous species, such as oat, wheat, millet (*Setaria*), rye also showed strong inhibitory activity, but most of Compositae family such as *Helianthus* showed only medium inhibitory activity by this method (Table 2).

Extraction of Inhibitory Chemicals from Cover Crops

To know the existence of allelochemicals in plants, we checked the extraction of chemicals for some principal cover crops. Table 3 shows the inhibitory activities of water and methanol extracts of cover crops. Hairy vetch and velvetbean showed the strongest inhibitory activity in both water and methanol extract. We have already published the results about the allelochemicals extracted from velvetbean (Fujii et al. 1990, Fujii 1994). Velvetbean contains an extraordinary high amount of L-3, 4-dihydroxyphenylalanine and the inhibitory activity of this compound is rather high in acidic and non-oxidative conditions. However in the case of hairy vetch, there is a possibility of inclusion of still more inhibitory allelochemicals than velvetbean. The results of solvent extraction are consistent with the results from Plant Box test for root exudates in these experiments.

Screening of Cover Crops for Weed Control in the Experimental Fields

To assess the activity of these cover crops, field experiments was started using the small scale field in our Experimental Station. Spring sown cover crops were not promising as shown in Table 4. In Japanese weather condition, we have a rainy season in June, and soon after the beginning of this season, we suffer from serious infection and vigorous growth of weeds. Some cover crops such as *Helianthus, Celosia* and *Panicum* showed relatively strong inhibitory activity to weeds, and *Mucuna*, *Vigna* and *Cassia* followed to them. Competition for light and nutrients must be the most important factors in the fields, and all of these plants have vigorous growth rate and huge leaves. Most of these cover crops were reported as allelopathic, and have relatively strong inhibitory activity in the Plant Box test (Table 1 and 2). For example, there are many reports on the allelopathy of *Helianthus*, and *Celosia* (Rice, 1984). However the field with more than 20 % of remaining of weed looks like weedy and disagreeable to the farmers and public. In this situation, spring-seeded cover crops in these conditions were not successful in Japanese condition.

The results from the trial for fall seeding are shown in Table 5 and 6. If cover crops are sown in fall, they tend to grow slow but steadily in winter to make enough biomass in spring and could eliminate the vigorous weeds. Table 5 shows a part of result of fall-seeded cover crops. As the primary selection, we had no replications in this test. It is obvious that dry weight of each cover crop is the most important to reduce the growth of weeds. It is true for rye, oat, wheat, woolly pod vetch and Italian ryegrass. Because of the overwhelming canopy of these crops, no weed can survive. But if compared the relationship between dry weight and weed suppression activity, *Brassica*, *Vicia*, and *Medicago* have not enough biomass but show better weed suppression than estimated form their biomass and allelopathy may play a role in these cases.

Table 6 shows the result of more precise study with four replications. Without weeding, hairy vetch, oat, barley, rye, wheat showed strong inhibitory activity of weeds, but Chinese milk vetch, which is a traditional green manure in Japan and China, showed little weed suppression.

In conclusion, spring-seeded cover crops are not promising in Japanese conditions, but fall-seeded cover crops such as hairy vetch, rye, oat, wheat, and barley have enough inhibitory activity for weed from spring to early summer. Their inhibitions are almost the same as that of traditional way of weeding, such as rice straw mulch and herbicide application.

Application of Hairy Vetch to Abandoned Paddy Field in the Experimental Station

The results from large space application of hairy vetch, Chinese milk vetch and mixed planting of vetch and oats were shown in Table 7 and Table 8. These data were taken from 1992 to 1994, and in each year four replications were taken. From these data, it was obvious that hairy vetch almost completely inhibits the growth of weeds in spring. On the other hand, Chinese milk vetch, traditionally used in Japanese paddy field as green manure, could inhibit the weed biomass up to 80 %, but to let 20 % of weeds to grow will make this field weedy next year, and will be abandoned in two or three years without weeding. These results coincide with the observation of farmers that continuous using of Chinese milk vetch will cause a serious infection of weeds.

Addition of oat to hairy vetch was aimed to increase the weed suppression ability. In both years, addition of oat decreases the population of weeds, to nearly perfect inhibition of weed until fall (Table 8). Mixed planting of vetch and oat has companionship and both yields increased per acre increased. However, if no care was taken, the outlook of field from spring to summer of mixed cover field was not beautiful because of the remaining stems of oat. On the other hand, hairy vetch kept the stand height of maximum 50 cm, and the outlook of this field was uniform and flat, and free from weeds. Hairy vetch died itself when maximum temperature reaches to 30 C. In our experimental field, hairy vetch made a straw like mulch without any work and this mulch protect the field for weed invasion after the death of mother plant. Then we concluded that using hairy vetch alone is better to recommend to the farmers because of the simplicity of sowing and reducing the labor.

Some Japanese abandoned paddy fields are suffering from water logging. Then, resistance of hairy vetch to water logging in paddy field was measured by using water logging lysimeter. As shown in Table 9, hairy vetch is resistant to heavy water logging. Water content of 40 % means the heavy water logging, and vetch showed no growth retardation from these conditions. But, in this lysimeter, continuous supply of water is possible, so if heavy water logging condition and no movement of water in the field might disturb the growth of vetches.

The combination of leguminous cover crops and barley were examined on the slope land experimental field of Shikoku National Agricultural Experiment Station. The aim of this experiment was to use barley as a cover crop in slope land. Barley, especially known as 'Hadaka-mugi', a naked barley, is the traditional cultivar and suitable to Shikoku and South East area of Japan. The results are shown in Table 10. Hairy vetch slightly reduces the growth of barley, but the weed inhibitory activity of hairy vetch and combination of vetch and barley were the best. The combination of red clover and barley followed to these.

Table 11 and 12 show the results of optimum seeding rate. Standard seeding rate was enough for the weed control, and increasing the seed volume made no difference for biomass and weed suppression. The cost per 1000 m^2 (Japanese standard unit of farming) is about 2500 yen, 20 dollars on the basis of Japanese market price.

Table 13 shows the optimum seeding date for weed suppression in Japan. Late seeding lead to grow more weeds and it was concluded that early seeding no later than the 1st week of November is recommended.

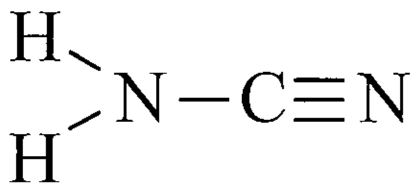
Direct Application and Exhibition of Hairy Vetch to Farmer's Field

Direct application and exhibition trial of hairy vetch were done using the field of cooperative farmers and these results are summarized from Table 14 to Table 17. In most cases, hairy vetch suppress weed enough, and the impression of farmers were very good. Before this trial, there was no custom to use hairy vetch as cover crops to control the weeds. Many farmers came to our Experimental Station or exhibition field of cooperative farmers, and most of them started to try hairy vetch. Now, in the middle of Japan, the use of hairy vetch under Kaki orchard are spreading to 600 ha, and still expanding.

Isolation of Allelochemical from Hairy Vetch

The crude extract of fresh leaves and stems of hairy vetch showed inhibitory activity on hypocotyl and radicle elongations of lettuce. The crude extract from 0.1 g FW of hairy vetch inhibited 50% of hypocotyl elongation of lettuce. This plant growth inhibitory activity was used to guide isolation during all the fractionation procedures. The amounts of samples used for the bioassay were calculated on the basis of the fresh weight of the extracted hairy vetch. The fraction showing the strongest activity was further fractionated chromatographically, finally giving a major plant growth inhibitory compound.

The IR and ^{1}H NMR spectra that this compound was cyanamide (Fig. 1). This was confirmed by comparing all these spectra with those of authentic cyanamide.



Cyanamide

Figure 1. Cyanamide in Hairy vetch.

Quantitative analysis

It was confirmed that the hairy vetch used for the isolation of a plant growth inhibitor contained 130 ug of cyanamide per gram of fresh plant. The crude extract and authentic cyanamide were compared with regard to their ability to inhibit the growth of lettuce hypocotyls on the basis of their cyanamide concentration. In the whole regions of concentrations examined, the growth inhibition of the crude extract on the lettuce hypocotyls was well explained by the presence of cyanamide contained in the extract. The growth inhibition on lettuce radicle, however, was not well coincident, especially in the region of lower concentration. At higher concentration, the crude extract's ability to inhibit radicle growth was well explained by the presence of cyanamide. Other lesser-contributing phytotoxic compounds in the crude extract could contribute to the total inhibitory activity on elongation of lettuce radicle, together with cyanamide. These results indicate that cyanamide is a major possible allelochemical in hairy vetch.

The content of cyanamide in a hairy vetch amounted to 0.13 ± 0.04 ug (± SD) per seed. To clarify whether cyanamide is biosynthesized in vivo or not, hairy vetch was grown without nutrients in an illuminated growth chamber for 9 d, and changes in cyanamide content in each plant were determined. Cyanamide was contained mainly in the shoot part, but was also found in small amounts in the endosperm covered with the seed coat and in the root part. The total amount of cyanamide amounted to 5.1 ± 2.8 ug (± SD) per whole seedling, indicating the presence of an approx. 40 times larger amount of cyanamide than that in the non-germinated seed.

The present report is the first to describe the existence of natural cyanamide. Cyanamide has been produced industrially and utilized for drugs and agrochemicals, but is considered to be absent as a natural product Maier-Greiner et al. 1991). At the early stage of this study, it was not certain whether the cyanamide isolated was from a natural source or not, since unexpected contamination by artificial cyanamide might be possible in the field. However it was confirmed that cyanamide is obviously biosynthesized in hairy vetch. L-Cyanoalanine, known as a neurotoxic compound, has been reported to occur in the seeds of common vetch, *V.* sativa, and other *Vicia* spp. (Odriozola et al. 1990). *Vicia* spp. would be able to biosynthesize cyanides such as cyanamide and L-beta-cyanoalanine.

A disease of cattle that graze in a hairy vetch pasture was first described in the United States of America (Teasdale et al. 1991). This disease appears in cattle fed on a vetch-dominant meadow, and is characterized by dermatitis, conjunctivitis, diarrhea, and so forth. Similar syndromes have been also reported in Australia and Argentina (Teasdale et al. 1993). Morbidity ranges from 1% to 30%, and mortality reaches 50%. Panciera et al. (1966) experimentally caused this vetch-associated disease in an adult cow by feeding hairy vetch. They insisted that contaminations by insects, molds, or other seasonally prevalent factors would not be causative, since the disease occurred in all seasons. However, none of the specific toxic substances causing the vetch-associated disease has been identified. Cyanogenic glycosides and L-beta-cyanoalanine had been suspected as causal compounds, but they induced quite different symptoms than those reported in the vetch-associated disease. In the present report the presence of cyanamide in hairy vetch was revealed. Cyanamide is a toxic compound that is both irritating and caustic; it causes dermatitis on moist skin and its LD_{50} value in rats is 125 mg kg^{-1}. These symptoms caused by cyanamide are identical with those of vetch-associated disease on cattle. In the feeding experiment (Panciera eta al., 1992), a cow (approx. 432 kg) fed hairy vetch (9 kg FW d^{-1}) died after 24 d. The total amount of hairy vetch fed was estimated to 200 kg FW, in which 26 g of cyanamide would be contained if the vetch used contained the same amount of cyanamide as in this experiment (130 ug g^{-1} F.W.). The lethal dose in this case is estimated to be 60 mg kg^{-1}, which is in good agreement with the value in rats. Thus, it would be possible that cyanamide is a causal compound of intoxication of cattle due to ingestion of hairy vetch.

Hairy vetch has many merits other than weed control in the field. 1) Nitrogen fixation to reduce chemical fertilizer. 2) Organic materials to reduce chemical fertilizer or soil conditioner. 3) Prevent soil erosion by surface cover. 4) Deep root system promotes the soil porosity. 5) Thick plant cover ameliorates the micro climates and reduce maximum temperature and increase minimum temperature. 6) Induce carnivorous ladybug to reduce the population harmful insects.

After these serious of experiments, it was concluded that hairy vetch is the most promising cover crop for the control of weeds in abandoned fields, grassland and orchard in the central and southern part of Japan. The value of hairy vetch as fodder and cover crop is well-known for farmers, but its real value was unknown before this finding of cyanamide as bioactive chemical. As cyanamide is well-known synthetic chemical important as nitrogen fertilizer with herbicidal, fungi static and insecticidal activity, it has never been isolated from the nature. There might be natural chemists who isolated cyanamide before us, but it is too simple to be identified as organic chemical. We could not identify without noticing minor contamination of dicyandiamide, which is a dimmer of cyanamide and have more information in NMR and MS spectrum. We can explain the value and disadvantage of hairy vetch by the concentration of cyanamide. We hope that nature friendly agriculture will be promoted by introducing hairy vetch as natural fertilizer and weed controller for vegetable, fruit, wheat, barley, and maize and rice production in addition to its original usage as forage.

REFERENCES

Fujii Y. Shibuya, T. (1991): A new bioassay for allelopathy with agar medium □. Assessment of allelopathy from litter leacheate by sandwich method. Weed Res. Japan 36 (suppl.), 150-151 [In Japanese].

Fujii, Y. Shibuya, T. (1991): A new bioassay for allelopathy with agar medium II. Mixed culture of allelopathic candidates with acceptor plants in agar medium. Weed Res. Japan 36 (suppl.), 152-153 [In Japanese].

Fujii, Y. Shibuya, T. and Yasuda, T. (1990): Method for screening allelopathic activities by using the logistic function (Richards' function) fitted to lettuce seed germination and growth curves. Weed Res. Japan 35, 353-361 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Yasuda, T. (1990): Survey of Japanese weeds and crops for the detection of water-extractable allelopathic chemicals using Richards' function fitted to lettuce germination test. Weed Res. Japan 35, 362-370 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Usami, Y. (1991): Allelopathic effect of Mucuna pruriens on the appearance of weeds. Weed Res. Japan 36, 43-49 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Yasuda, T. (1991): L-3, 4-Dihydroxyphenylalanine as an allelochemical candidate from Mucuna pruriens (L.) DC. var. utilis. Agr. Biol. Chem. 55,617-618.

Fujii, Y. Shibuya, T. and Yasuda, T. (1991): Discrimination of allelopathy of tomato plant by stairstep experiment and rotary greenhouse experiment. Jap. J. Soil Sci. Plant Nutri. 62, 150-155 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Yasuda, T. (1991): Discrimination of allelopathy of velvetbean (*Mucuna pruriens*) with stairstep experiment and rotary greenhouse experiments. Jap. J. Soil Sci. Plant Nutri. 62, 258-264 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Yasuda, T. (1991): Discrimination of allelopathy of upland rice, taro, and oat by substitutive experiment and its modified experiments. Jap. J. Soil Sci. Plant Nutri. 62, 357-362 [In Japanese with English summary].

Fujii, Y. Shibuya, T. and Yasuda, T. (1991): Intercropping of velvetbean (*Mucuna pruriens*) by substitutive experiments: Suggestion of companion plants with corn and kidney bean. Jap. J. Soil Sci. Plant Nutri. 62, 363-370 [In Japanese with English summary].

Fujii, Y. Screening and future exploitation of allelopathic plants as alternative herbicides with special reference to hairy vetch. J. Crop Production 4, 257–275 (2001)

Gershenzon, J. Plant defenses: Surface protectants and secondary metabolites. in Plant Physiology. 2nd ed. 347–376 (Taiz L, Zeiger E (eds), Sinauer Associates, Sunderland, MA, 1998)

Harborne, J.B., & Baxter, H. in Phytochemical Dictionary: a handbook of bioactive compounds from plants. p 66 (Taylor & Francis, London, 1993)

Hoffman, M.L., Regnier, E. E., & Cardina, J. Weed and corn (Zea mays) responses to a hairy vetch (Vicia villosa) cover crop. Weed Technol. 7,594–599 (1993)

Maier-Greiner, UH et al. Isolation and properties of a nitrile hydratase from the soil fungus Myrothecium verrucaria that is highly specific for the fertilizer cyanamide and cloning of its gene. Proc. Nat. Acad. Sci. 88, 4260–4264 (1991)

Odriozola, E, Paloma, E., Lopez, T., & Campero, C. An outbreak of Vicia villosa (hairy vetch) poisoning in grazing Aberdeen Angus bulls in Argentina. Vet. Hum. Toxicol. 33, 278–280 (1990)

Panciera, R. J., Johnson, B. S., & Osburn, B. I. A disease of cattle grazing hairy vetch pasture. J Am. Vet. Med. Assoc. 148, 804–808 (1966)

Panciera, R. J., Mosier, D. A., & Ritchey, J. W. Hairy vetch (Vicia villosa Roth) poisoning in cattle: update and experimental induction of disease. J. Vet. Diagn. Invest. 4, 318–325 (1992)

Peet, R. L., & Gardner, J. J. Poisoning of cattle by hairy or woolly-pod vetch, Vicia villosa subspecies dasycarpa. Aust. Vet. J. 63, 381–382 (1986)

Teasdale, J. R. Contribution of cover crops to weed management in sustainable agricultural systems. J Prod Agric 9, 475–479 (1993)

Teasdale, J. R., & Daughtry, C. S. T. Weed suppression by live and desiccated hairy vetch (Vicia villosa). Weed Sci 41, 207–212 (1993)

Teasdale, J. R,, Beste, C. E., & Potts, W. E (1991) Response of weeds to tillage and cover crop residue. Weed Sci 39,195–199

White, R. H., Worsham, A. D., & Blum, U. Allelopathic potential of legume debris and aqueous extracts. Weed Sci 37, 674–679 (1989)

Allelopathic Potential of Roots of *Parthenium hysterophorus*

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Abstract: The present investigation was undertaken to find out the interference potential of root residues of *Parthenium hysterophorus* that are found plenty in areas of its infestation after the removal of the above ground parts. For this purpose, bioassay studies were planned with root residue extracts and their effect was studied on the growth and development of two crop plants, namely, mustard and turnip. A significant reduction in seedling length and weight was observed in the test crops treated with root extracts. Further, growth studies were also performed in soils amended with root extracts or root residues. Here also, growth retardatory effect was observed on the test species. In order to find out the reasons for inhibitions, studies were further conducted to test the presence of water-soluble phenolics in the various treatments. An appreciable amount of phenolics was present in the *P. hysterophorus* roots. The amount of phenolics correlated well with the observed growth reductions in the test plants. It is concluded that roots of *P. hysterophorus* might exert strong allelopathic influence on other plants by releasing allelochemicals in soil.

Keywords: Amended soils, *Brassica campestris*, *Brassica rapa*, growth performance, phenolics, phytotoxicity, root residues

INTRODUCTION

Parthenium hysterophorus L. (Family Asteraceae) is an aggressive exotic weed that has naturalized almost every part of India. The weed possesses a very high invasive potential and quickly forms dense stands upon invasion. It can be seen growing luxuriantly in gardens, vacant areas, wastelands and agricultural fields. The quick encroachment of an area by the weed can be attributed to several factors such as fast growth rate, high reproductive potential, and possession of adaptive strategies. Moreover, the seeds of the weed remain viable for a long time and can thrive well even under adverse environmental conditions (Williams and Groves 1980). The presence of the weed in large and dense colonies affects biodiversity, crop production, animal husbandry and even human health (Adkins and Sowerby 1996; Kohli and Rani 1994; Navie et al. 1996). The extensive growth pattern of the weed and its adverse effect on the other plants is suggestive of some strong interference potential.

The allelopathic effect of the weed towards crops and other vegetation is well known and is exhibited through the release of a number of allelochemicals like phenolics and sesquiterpene lactone parthenin (Kanchan 1975; Kanchan and Jayachandra 1980; Kohli and Batish 1994; Mersie and Singh 1987). Recently, Batish et al. (2002) have shown that the aboveground residues of *P. hysterophorus* affect growth of radish and chickpea by releasing phenolics, which in turn change the soil chemistry leading to more severe effect. However, most of the studies demonstrate the allelopathic effect of the above ground parts, yet none deals with the role of root residues, which remain in the soil and undergo decomposition especially after the mechanical removal of the above ground parts. It is, therefore, hypothesized that root residues may impart considerable toxicity to crops and play a vital role in the successful establishment of the weed. Studies of Kanchan and Jayachandra (1979) have shown that root exudates of *P. hysterophorus* contain inhibitors, which adversely affect the growth of wheat. However, nothing is known about the phytotoxicity of root residues towards other plants. The present study was, therefore, undertaken to assess the phytotoxic nature of root residues and quantify of the amount of phytotoxic chemicals released by them.

MATERIALS AND METHODS

For the present study, root residues of ragweed parthenium (*P. hysterophorus* L.) were collected from an infested area on campus of Panjab University, Chandigarh, India. For bioefficacy or growth studies, soil (0-10 cm profile) was collected from an area free of *P. hysterophorus*. It was air-dried, and sieved through a 2 mm mesh. Seeds of mustard (*Brassica campestris* L.) and turnip (*Brassica rapa* L.) were procured from Punjab Agricultural University, Ludhiana, India.

In order to determine the phytotoxic nature of root residues of *P. hysterophorus,* bioassay studies were conducted with its root residue extract, and soil amended with root residue extracts or root residues. To prepare aqueous extracts 4 g of root residues were dipped in 100 ml of distilled water for 16 h at 20°C. These were filtered through a double layer of muslin cloth followed by Whatman no. 1 filter paper so as to get 4% aqueous extracts. These were further diluted with distilled water so as to get 2 % solution and were kept in refrigerator at 4°C till further use. Effect of different concentrations of residue extracts was studied on the seedling growth and dry weight of mustard and turnip in a laboratory bioassay. For this, 25 seeds each of mustard and turnip were placed in a 6" petri dish lined with two layers of Whatman no. 1 filter circle moistened with 10 ml of residue extracts. Treatment in a similar manner with distilled water served as control. For each treatment 5 replicates were maintained in a completely randomized design. After 7 days, the length and dry weight of the seedlings were determined.

In order to prepare amended soils, 5 and 10 g of residues were added to 250 g of control soil so as to get 2 and 4 % residue amended soils. Likewise, 100 mL of 2 and 4% root residue extracts were added to the 250 g soils so as to get 2 and 4 % residue extract amended soils. These residue and residue extract amended soils were used for growth studies. 250 g each of respective root residue or its extract-amended soil was taken in 6" diameter petri dishes. Ten seeds each of mustard and turnip were sown in these amended soils. Likewise, the seed were also sown in the unamended soils to serve as control. Five replicates were maintained for each treatment. All the petri dishes were placed in a growth chamber at 23±2°C, 75±1 % relative humidity and 16 / 8 light / dark photoperiod. Each petri dish was sprayed daily with 25 mL of distilled water. After 7 days, length and dry weight of ten seedlings from each treatment were measured.

Amount of total water-soluble phenolics in the root residue extracts, and residue and extract amended soils was estimated following the method of Swain and Hillis (1959) using Folin-ciocalteu reagent. The concentration was read at 700 nm against standard of ferulic acid. For each treatment 5 replications were kept.

All the experiments were repeated and means of results of both the experiment are presented. The data for both bioefficacy studies and growth experiments were analyzed by one-way analysis of variance and the means were separated by Duncan's multiple range test. Besides, the correlation coefficient values between amount of phenolics and the parameter were also determined.

RESULTS AND DISCUSSSION

It is clear from the results that root residues of *P. hysterophorus* are inhibitory towards both test crops – mustard and turnip. Root residue extracts significantly reduced the seedling growth as well as seedling biomass of both test crops. With the treatment of 4% root residue extracts seedling length was reduced by 47 and 65%, respectively, in mustard and turnip (Table 1). Likewise, seedling biomass was also appreciably reduced. Further, the decrease in seedling growth was also observed when the test crops were sown in soils amended with root extracts or root residues (Table 1). In general, seedling length was reduced more than dry weight. Nearly 30% reduction in seedling growth of mustard and turnip was observed when grown in soil amended with 2 % root residue extracts. Likewise, growth inhibitory effect was also observed when test crops were sown in soils amended with root residues. In this case more effect was observed in mustard (Table 1).

Table. 1 Effect of root residue extracts, and soil amended with the root residues and their extracts on the growth of *B. campestris* and *B. rapa*.

Treatment	Concentration (%)	*B. campestris* SL	*B. campestris* SW	*B. rapa* *SL*	*B. rapa* *SW*
Root residue extracts	2	73.0^{a}	86.7^{a}	66.8^{c}	75.7^{a}
	4	52.2^{c}	77.2^{b}	34.8^{e}	59.6^{c}
Root extract amended soil	2	65.5^{b}	69.0^{c}	71.9^{b}	71.9^{a}
	4	56.3^{c}	56.4^{e}	58.7^{d}	65.4^{b}
Root residues amended soil	2	61.4^{b}	76.4^{b}	80.6^{a}	77.7^{a}
	4	35.1^{d}	61.2^{d}	55.8^{d}	58.7^{c}

Data presented as with respect to control
SL: seedling length; SW: seedling dry weight
Different alphabets in a column represent significance at 0.05 % level.

Based on overall bioassay studies, it can be concluded that root residues release some inhibitors in soils, which adversely affect the growth of other plants. In other words, root residues also contribute towards the phytotoxic / allelopathic nature of the weed. Several studies have indicated that aggressive weeds suppress growth of other plants through their root exudates or residues (Rice 1984). Recently, Kiemnec and McInnis (2002) reported that root extracts prepared from hoary cress (*Cardaria draba*) reduce germination and root growth of plants such as wheat, alfalfa, crested wheatgrass and bluebunch wheatgrass. They attributed these inhibitions to the release of three glucosinolates compounds in the soil. In the present study, the inhibitory substances might be accumulating in bioactive concentrations to bring about the observed growth reductions. Since *P. hysterophorus* is known to release several phenolics (Batish et al. 2002; Kanchan and Jayachandra 1980; Kohli and Batish 1994), their presence was tested in root residues too. Appreciable amount of phenolics was present not only in root extracts but also in the residue amended soils (Table 2).

Table 2. Amount of phenolics present in different treatments of root residues.

Treatment	Concentration	Amount of Phenolics
Root residue extracts (μg mL^{-1})	2	49.5 ± 3.45
	4	172.7 ± 4.17
Unamended control soil (mg kg^{-1})	-	5.60 ± 0.90
Root extract amended soil (mg kg^{-1})	2	17.1 ± 0.80
	4	18.9 ± 1.60
Root residues amended soil (mg $100g^{-1}$)	2	25.3 ± 4.30
	4	28.0 ± 1.50

The amount of phenolics was, however, less in amended soils than in extracts. Nevertheless, the accumulation of phenolics in soil exerts an appreciable phytotoxicity towards other plants. In the present study, the amount of phenolics was found to be strongly correlated with the observed growth inhibitions in both test crops (Table 3).

Table 3. Values of correlation (r) between amount of phenolics and phytotoxicity.

Treatment	*B. campestris*		*B. rapa*	
	SL	SW	SL	SW
Root residue extracts	-0.998	-0.997	-0.999	-0.996
Root extract amended soil	-0.997	-0.987	-0.981	-0.991
Root residues amended soil	-0.996	-0.994	-0.996	-0.999

Phenolics are the most potent inhibitors among the water-soluble allelochemicals that are implicated in allelopathy and are widespread in plant species (Blum et al. 1999; Mizutani 1999). A variety of phenolic acid such as ferulic, caffeic, syringic, gallic, vanillic, and *p*-hydroxybenzoic acid are known to be present in *P. hysterophorus* (Kanchan and Jayachandra 1980) besides sesquiterpene lactone parthenin. The lesser amount of phenolics in the soil as observed in the present study can be due to the fact that upon entering the soil, these phenolics undergo a number of transformations and their bioactive concentration changes dramatically (Blum et al. 1999; Chou 1999). Moreover, studies of Batish et al. (2002) have shown that phenolics also interfere with the nutrients and other physico-chemical properties of soil and thus change soil chemistry. In the changed edaphic conditions, the inhibitory effect on test species is further increased. So phenolics not only have a direct impact on growth reductions of test species but also do so indirectly through changes in soil chemistry. Even burning of residues of above ground parts affects soil conditions and thus availability of nutrients (Singh et al. 2002). All these factors coupled with allelopathy due to root residues as revealed in the present study further alleviate the allelopathic potential of the weed. Thus from the present study, it could be concluded that root residues of *P. hysterophorus* release water-soluble phenolics which affect the growth and development of other plants. Root mediated release of allelochemicals and their impact on competing species may also help in the successful establishment of *P. hysterophorus* and formation of its large monocultures.

LITERATURE CITED

Adkins S.W. and M.S. Sowerby 1996. Allelopathic potential of the weed *Parthenium hysterophorus* L. in Australia. Plant Protection Quarterly, 11:20-23.

Batish, D.R., et al. 2002. Phytotoxic effect of *Parthenium* residues on the selected soil properties and growth of chickpea and radish. Weed Biology and Management, 2:73-78.

Blum U., et al. 1999. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. an experimental model. Critical Reviews in Plant Science, 18:673-693.

Chou C.H. 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. Critical Reviews in Plant Science, 18:609-636.

Kanchan S.D. 1975. Growth inhibitors from *Parthenium hysterophorus* Linn. Current Science, 44:358-359.

Kanchan S.D. and Jayachandra. 1979. Allelopathic effects of *Parthenium hysterophorus* L. I. Exudation of inhibitors through roots. Plant and Soil, 53:27-35.

Kanchan S.D. and Jayachandra. 1980. Allelopathic effects of *Parthenium hysterophorus* L. IV. Identification of inhibitors. Plant and Soil, 55:67-75.

Kiemnec, G.L. and M.L. McInnis. 2002. Hoary cress (*Cardaria draba*) root extract reduces germination and root growth of five plant species. Weed Technology, 16:231-234.

Kohli R.K. and D. Rani. 1994. *Parthenium hysterophorus* – a review. Research Bulletin Science, Panjab University, 44:105-149.

Kohli R.K. and D.R. Batish. 1994. Exhibition of allelopathy by *Parthenium hysterophorus* L. in agroecosystems. Tropical Ecology, 35:295-307.

Mersie W. and M. Singh. 1987. Allelopathic effect of parthenium (*Parthenium hysterophorus* L.) extract and residue on some agronomic crops and weeds. Journal of Chemical Ecology, 13:1739-1747.

Mizutani J. 1999. Selected allelochemicals. Critical Reviews in Plant Sciences, 18:653-671.

Navie S.C., et al. 1996. The biology of Australian weeds 27. *Parthenium hysterophorus* L. Plant Protection Quarterly, 11:76-88.

Rice, E.L. 1984. Allelopathy. 2nd edition. Academic Press, Orlando, Florida.

Singh, H.P., et al. 2002. Assessment of allelopathic properties of *Parthenium hysterophorus* residues. Agriculture, Ecosystems and Environment, Accepted, In Press.

Swain T. and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of constituents. Journal of the Science of Food and Agriculture, 10:63-68.

Williams J.D. and R.H. Groves. 1980. The influence of temperature and photoperiod on growth and development of *Parthenium hysterophorus* L. Weed Research, 20:47-52.

Allelopathic Effects of Five Weed Species on Seed Germination and Seedling Growth of Rice

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Abstract: An investigation was done at the Bangladesh Agricultural University, Mymensingh from September to December 2001 to study the allelopathic effects of five weed species, namely, *Amaranthus spinosus*, *Polygonum hydropiper, Monochoria hastate, Sphenoclea zeylanica* and *Commelina benghalensis* on seed germination, growth of shoot and root length of rice (cv. BRRI Dhan28). The relative effects of different plant parts of a particular weed, e.g., leaf, stem, root and whole plant, and different concentrations, 2.5% and 5.0%, were also investigated. All the weeds, except *P. hydropiper,* were found detrimental to rice seed germination. The highest reduction of seed germination was due to *S. zeylanica* and lowest reduction (>1%) was due to *P. hydropiper*. Five percent stem extract of *S. zeylanica* reduced more than 98% seed germination of rice. The ranking of the species was *A. spinosus* ≈ *S. zeylanica* > *M. hastata* ≈ *C. benghalensis* > *P. hydropiper*. Shoot and root lengths of rice seedling were also reduced due to allelopathic effects of the weeds. The ranking of the species in respect of their effects on shoot length and root length was different. The highest reduction of shoot length (63%) and root length (<80%) was due to *A. spinosus* and lowest (35% and 52%, respectively) due to *P. hydropiper*. Among the plant parts, leaf and stem extracts were more detrimental than root extract. Higher concentration of weed extract was more toxic than lower concentration.

Key words: allelopathy, rice, weed

INTRODUCTION

Allelopathy denotes the toxic effects of chemicals produced by one plant on another. Many weeds exert chemical stress in some crops by their phytotoxic root exudates and other plant leachates, which are released to the soil. Due to these phytotoxic substances the growth of plants in the proximity is adversely affected. Allelopathic substances are most commonly found plant extracts, plant residues in the soil, live plant exudates, and as volatile gases liberated from leaves and rhizomes (Keeley 1987). Allelopathic potential may also vary in different parts of an individual weed species (Bansal and Singh 1986; Rajangum *et al.* 1997).

A number of weeds grow in rice fields, which compete with the crop and reduce crop yield. In addition to competition, many weeds pose threat to crops due to allelopathy. It is, therefore, important to know which weeds have the potential for allelopathy so that those species can be removed from the fields before contributing to crop suppression. Sometimes weed straw are left in the crop field with no consideration of future effects. The straw of allelopathic weeds may again hamper the seedling growth of the next crop. With these ends in view, a study was conducted to evaluate the allelopathic influences of selected five weeds on the seed germination, growth of shoot and root lengths of rice.

MATERIALS AND METHODS

The experiment was conducted at the Agronomy Laboratory, Bangladesh Agricultural University (BAU), Mymensingh from September to December 2001. Five common weed species, namely, *Amaranthus spinosus* (Spiny pig weed), *Polygonum hydropiper* (Smart weed), *Monochoria hastata* (Monochoria weed), *Sphenoclea zeylanica* (Sphenoclea weed) and *Commelina benghalensis* (Spider wort), were selected as test materials. The weeds were collected from the Agronomy Field Laboratory, BAU. They were washed, air-dried and then oven-dried at 80^0C for 72 h. Two hundred grams of oven-dried whole plant, leaf, stem and root materials of each of the five weeds were taken and ground with a grinding machine. Two and a half g of the powdery sample of each weed part was mixed with 100 mL of distilled water to make 2.5% solution of the weed part. Similarly, mixing 5 g of powdery mass with 100 mL of distilled water made 5% aqueous extracts. The effect of weed extracts on the germination of rice seed (BRRI Dhan28) was tested under the laboratory conditions of BAU (temperature = 28^0C, RH = 89% and diffused sunlight passed through glass-fitted window). The growing medium was sterilized germinating paper in petri dishes. Rice seeds at the rate of 20 seeds petri dish^{-1} were kept for germination. Twenty mL aqueous extract of different treatments were put in each petri dish with three replications. Only distilled water was used as control. Observation was done regularly after two days from seed setting and was continued for 6 days to record the seed germination. Root and shoot lengths of rice seedlings under different treatments were measured after 14 days of seed setting. Percent reduction in seed germination, root and shoot lengths, due to allelopathic effects were calculated in comparison to control. The collected data on different parameters of rice were statistically analyzed and the mean differences were adjudged using DMRT.

RESULTS AND DISCUSSION

Effects on seed germination

Rice seed germination was significantly reduced due to allelopathic effects of most of the test weeds under study. The highest mean reduction was due to *S. zeylanica* (36.5% reduction), identically followed by *A. spinosus* (34.6% reduction) and the lowest reduction (>1%) was noted *P. hydropiper* (Table 1). Similar inhibitory effect on rice growth was noted due to *A. spinosus* (Rahman *et al.* 1996; Gaffer *et al.* 1996) and due to *S. zeylanica* (Olofsdotter, 1998; Premasthira and Zungsontiporn, 1996). *C. benghalensis* was found comparatively less allelopathic to rice by other authors but the species produced significant reduction of seed germination in this study. However, severe inhibitory effects of the species were noted in wheat by Uppar *et al.* (1993). Prosad and Srivastava (1991) noted that the aqueous extract of the species reduced seed germination of groundnut by 54%. The weed *M. hastata* reduced the seed germination by 20% in this study. Similar retarding effects of root and shoot extracts of the species on rice seed germination was observed by Sobhana *et al.* (1990).

When the effects of different plant parts of the weeds were compared, leaf and stem extracts reduced the seed germination more than other plant parts (Table 1).

Table 1. Effect of aqueous extract of different plant parts of five weed species on seed germination (%) of rice seeds.

Weed species	Plant part				Mean
	Leaf	Stem	Root	Whole plant	
A. spinosus	55.0g	38.4h	80.9cde	74.2ef	62.1C
P. hydropiper	92.5abc	94.2ab	97.5a	98.4a	95.7A
M. hastata	89.2bcde	60.0g	93.4abc	78.4de	78.9B
S. zeylanica	30.9hi	22.5i	96.7ab	90.9abc	60.2C
C. benghalensis	65.0fg	88.4abcd	93.4abc	89.2abcd	84.0B
No weed	96.7ab	96.7ab	96.7ab	96.7ab	
Mean	70.7C	66.7C	93.1A	87.9B	

Figures in the column and row for means having different capital letters differ significantly. Figures in interaction between weed species and plant parts having different small letters differ significantly.

In a study in India, Ambika and Suma (1999) observed that the concentration of allelochemicals was the greatest in leaves of *A. spinosus* and the lowest in roots. Roy (1995) observed that the dried mass of stem of weeds like *P. hydropiper, A. spinosus, C. album, C. rotundus* and *I. cylindrica* were more detrimental than leaf and root. Therefore, the allelopathic effects of weeds were species specific and depended on source and concentration. Degree of toxicity in different plant parts of a weed is variable and the trend is not similar in all weeds (Kim et al. 1999). The concentration of different weed extracts significantly affected the seed germination of rice. A concentration of 5% was more inhibitory that a concentration of 2.5%. Significant interaction of weed species, plant parts and concentration was also observed. In this study the highest reduction (98.4%) of rice seed germination occurred when stem extract of *S. zeylanica* with 5% concentration was used (Table 4).

Effects on shoot length

A significant effect of weed species on shoot length of rice was observed due to weed allelopathy. The highest reduction (79.0%) of rice shoot was found in *A. spinosus* which, was identically followed by *M. hastata* and *S. zeylanica,* and the lowest reduction was in *P. hydropiper* (35.5%) (Table 2). Qashem (1993) observed that the aqueous extract of *A. spinosus* reduced the coleoptile length, root length and shoot dry matter weight of maize and barley. Datta and Bandyopadhaya (1981) also noted similar inhibitory effect of the weed on vegetative and reproductive phase of wheat and mustard. The ranking of the weeds with respect to reduction of shoot length was *A. spinosus* > *M. hastata* > *S. zeylanica* > *C. benghalensis* > *P. hydropiper.*

Among different plant parts, stem and leaf extracts were more detrimental to rice shoot than root extract (Table 5). Although no significant difference in effect of stem and leaf on rice shoot was noted in this study significant differences were observed in other studies (Premasthira and Zungsontiporn 1996; Oudhia *et al.* 1999). Bansal and Singh (1986) found that stem extract of *Avena fatua* was more inhibitory than leaf extract. However, no difference in effect of plant parts of *L. camera* (Oudhia and Tripathi 1999) and *B. lacera* (Oudhia *et al.* 1998) was noted in promoting seed germination and seedling growth of rice. Concentration had similar effect on shoot length as was on seed germination. On the average, about 20% more shoot length was reduced due to increase the concentration from 2.5% to 5% (Table 4). Similar concentration dependent effect of leaf extracts of different weed species on seed germination and seedling growth of rice was noted by Bhatt *et al.* (2001).

Table 2. Effect of aqueous extract of different plant parts of five weed species on the shoot length (cm) of rice seedlings.

Weed species	Plant parts				Mean
	Leaf	Stem	Root	Whole plant	
A. spinosus	2.78cdefg[2]	1.52h	1.42h	2.43defgh	2.04C
P. hydropiper	2.97bcdef	4.11b	3.66bcd	3.86bc	3.65B
M. hastata	1.78fgh	1.38h	3.30bcde	2.13efgh	2.15C
S. zeylanica	1.82fgh	1.84fgh	3.01bcdef	2.51defgh	2.30C
C. benghalensis	1.58gh	2.46defgh	3.41bcd	2.54defgh	2.50C
No weed	5.46a	5.46a	5.46a	5.46a	
Mean	2.73B	2.79B	3.38A	3.15AB	

[1]Figures in the column and row for means having different capital letters differ significantly. [2]Figures in interaction between weed species and plant parts having different small letters differ significantly.

Effects on root length

The effect of different weed species on the root length of rice was similar to that on shoot length. The highest reduction (83.8%) of rice root length was due to the allelopathic effect of *M. hastate,* which was identically followed by *A. spinosus* and *C. benghalensis* (Table 3). The ranking of weed species in respect of reduction of rice root was *M. hastata* > *A. spinosus* ≈ *C. benghalensis* > *S. zeylanica* > *P. hydropiper.*

Different plant parts produced differential effect on rice root length. The highest reduction was due to the application of leaf extract of weed species. Reduction of root length of maize and barley due to treatment with extracts of different plant parts of weed species *C. album* and *A. spinosus* was also reported by Qasem (1993). Concentration had also positive effect on reduction of rice root as was on shoot length.

Table 3. Effect of aqueous extract of different plant parts of five weed species on the root length (cm) of rice seedlings.

Weed species	Plant part				Mean
	Leaf	Stem	Root	Whole plant	
A. spinosus	1.39fg[2]	2.51ef	2.05ef	1.18fg	**1.78CD**
P. hydropiper	3.25cdef	6.37b	4.99bc	2.74def	**4.34B**
M. hastata	0.97fg	0.39g	2.58efg	1.78fg	**1.43D**
S. zeylanica	1.97efg	1.40fg	4.22cde	2.32efg	**2.48C**
C. benghalensis	0.96fg	2.16efg	4.71bcd	1.68fg	**2.38CD**
No weed	8.81a	8.81a	8.81a	8.81a	
Mean	**2.89B**	**3.61B**	**4.56A**	**3.08B**	

[1]Figures in the column and row for means having different capital letters differ significantly.
[2]Figures in interaction between weed species and plant parts having different small letters differ significantly.

Table 4. Interaction effect of plant parts of weeds and concentration on the seed germination, shoot length and root length of rice seedling.

Plant part	Seed germination (%)		Shoot length (cm)		Root length (cm)	
	Conc. 2.5%	Conc. 5%	Conc. 2.5%	Conc. 5%	Conc. 2.5%	Conc. 5%
A. spinosus						
Leaf	92.7abcd	18.4i	2.16	3.40	2.55	0.22
Stem	65.0fg	11.7i	1.69	1.34	2.55	2.48
Root	78.4cdefg	83.4abcde	1.66	1.17	3.80	0.31
Whole plant	89.7abcd	61.7g	2.91	1.95	1.38	0.98
P. hydropiper						
Leaf	92.7abcd	93.4abcd	3.34	2.59	4.02	2.48
Stem	92.7abcd	96.7abc	4.98	3.24	6.41	6.32
Root	100.0a	95.0abcd	3.92	3.40	6.08	3.91
Whole plant	100.0a	96.7abc	3.90	3.82	2.98	2.50
M. hastata						
Leaf	92.7abcd	76.7defg	2.01	1.54	1.33	0.61
Stem	80.0bcdef	40.0h	1.66	1.09	0.52	0.26
Root	95.0abcd	92.7abcd	3.78	2.82	3.61	1.54
Whole plant	88.4abcd	68.4efg	2.70	1.55	2.70	0.87
S. zeylanica						
Leaf	43.4h	18.4i	2.66	0.99	2.98	0.96
Stem	43.4h	1.65i	2.27	1.42	2.17	0.63
Root	98.4ab	95.0abcd	3.69	2.33	5.19	3.25
Whole plant	93.8abcd	88.4abcd	3.09	1.93	2.98	1.66
C. benghalensis						
Leaf	61.7g	68.4efg	2.00	1.17	1.12	0.80
Stem	95.0abcd	81.7abcdef	3.02	1.89	3.67	0.64
Root	93.4abcd	93.4abcd	3.73	3.09	4.97	4.46
Whole plant	92.7abcd	86.7abcd	3.27	1.80	3.00	0.38
No weed	96.7abc	96.7abc	5.46	5.46	8.81	8.81
Mean	**86.1A**	**73.1B**	**3.34A**	**2.68B**	**4.14A**	**2.94B**
Significance	P > 0.01	P > 0.01	NS[2]	NS	NS	NS

[1]Figures in the row for means having different capital letters differ significantly. Figures in interaction between weed species, plant parts and concentration having different small letters differ significantly[2]. NS = not significant

It can be concluded that the weed extract of all the weed species selected in this study had inhibitory effect on the seed germination and seedling growth of rice. The effect on seed germination was not similar on root length and shoot length. The ranking of the species in respect of inhibitory effect on seed germination was - *A. spinosus* ≈ *S. zeylanica* > *M. hastata* ≈ *C. benghalensis* > *P. hydropiper;* the ranking of weed species in respect of effect on shoot length of rice was *A. spinosus* > *M. hastata* > *S. zeylanica* > *C. benghalensis* > *P. hydropiper;* and the ranking in respect of effects on root length was *M. hastata* > *A. spinosus* ≈ *C. benghalensis* > *S. zeylanica* > *P. hydropiper.* Among the plant parts of the test weed species, leaf and stem extract were more detrimental to rice seedling than root. Higher concentration, 5% exhibited greater inhibitory effect than lower concentration (2.5%). Therefore, care should be taken so that no such weed species grow in rice field especially during early stage of rice growth. Allowing straw of these weeds to decompose in the rice field should also avoided to save the crop from toxic effect of these allelopathic weeds.

LITERATURE CITED

Ambika, S.P. and S.S. Suma. 1999. Influence of pollutants on the allelopathic potential of A. spinosus. Allelopathic J. 6(1):49-50.

Bansal, G.L. and Singh, C.M. 1986. Allelopathic effects of different plant parts of grassy weeds of wheat (*Triticum aestivum* L.) on the germination and growth of rice (*Oryza sativa*). Indian J. Weed Sci. 18(2):108-110.

Bhatt, B.P.; Tomar, J.M.S. and Misra, L.K. 2001. Allelopathic effects of weed on germination of legumes and cereal crops of North eastern Himalayas Division. Allelopathy J. 7(1):143-146.

Datta, S.C. and Bandyopadhaya, A.K. 1981. Allelopathic influence of three weeds on two crop plants. Proc. Asian Pacific Weed Sci. Soc. Conf. 1:392-400.

Gaffer, M.A.; Reza, M.S. and Rahman, M.M. 1996. Allelopathic effects of several plant species in controlling weeds in jute. Progress. Agric. 6(1):69-76.

Keeley, P.E. 1987. Interference and interaction of purple (*Cyperus rotundus*) and yellow nutsedge (*Cyperus esculentus*) with crops. Weed Tech. 1(1):74-81.

Kim, S.Y. de, Robles, R.P.; Kim, K.U.; Lee, S.C. and Shin, D.H. 1993. Allelopathic effects of sorghum extract and residues on selected crops and weeds. Korean J. Weed Sci. 14(1):40-41.

Olofsdotter, M. 1999. Allelopathy - a future component of weed management. Weeds, DJF Rapport, Markbrug, 9:101-112.

Oudhia, P. and Tripathi, R.S. 1999. Allelopathic effect of *Lantara camara* L. on rice. Agril. Sci. Digest, 19(1):423-45.

Oudhia, P.; Kolhe, S.S. and Tripathi, R.S. 1998. Allelopathic effects of Blumea lacera L. on rice and common kharif weeds. Oryza, 35(2):175-177.

Premasthira, C.U. and Zungsontiporn, S. 1996. Allelopathic effects of extract substances from goose weed (*Sphenoclea zeylanica* Geartn) on rice seedlings. Weed Res. Japan, 41(2):79-83.

Prosad, K. and Srivastava, V.C. 1991. Telotoxic effects of some weeds on germination and initial growth of groundnut. Indian J. Agril. Sci. 61(7):493-494.

Qasem, J.R. 1993. Allelopathic effects of some common weeds on growth of barley and maize. Applied Science 20(2):5-28.

Rahman, M.M.; Rahman, M.M.; Roy, B. and Ahmed, S. 1996. Allelopathic effects of weeds on germination and growth of rice and jute seeds. Progress. Agric. 7(1):69-72.

Rajangum, M. 1997. Allelopathic effects of *Heliotropium indicum* on paddy var. IR 20 and Ponmani. J. Eco. Environ. Monito., 7(3):207-209.

Roy, K.C. 1995. Allelopathic effects of five selected weed species on corn (cv. Barnali) seed germination, plant height and biomass weight. M.Sc. (Ag) in Agronomy thesis, Bangladesh Agril. University, Mymensingh.

Sobhana, S.; George, S. and Sheela, K.R. 1990. Preliminary studies on allelopathic effects of weeds on rice seed germination. Oryza, 27(1):94-95.

Upper, D.S.; Nalini, A.S.; Hiremath, S.M. and Kamater, M.Y. 1995. Current Res., University of Agriculture and Science, Bangalore, 22(3-5):47-48

Phytotoxic Interference of *Ageratum conyzoides*: Role of Residues

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Abstract: *Ageratum conyzoides* L. (Family Asteraceae) is an annual aggressive weed that has invaded agricultural fields in various parts of Southeast Asia including India. A study was undertaken to determine the effect of its decaying residues that are plenty on the soil after removal of the plant. The bioassay studies were undertaken with radish and mustard grown in soil amended with residues and residue extracts. Seedling growth in terms of length and dry weight accumulation was drastically reduced in response to residue extracts or when grown in amended soils. The observed effect was concentration dependent. The reasons for the decrease in growth of test plants were explored and it was found that the residues of *A. conyzoides* are rich in water-soluble allelochemicals – the phenolics. Even the soils amended with *A. conyzoides* residues or residue extracts were found to be rich in phenolics. A strong correlation was observed between phytotoxicity and the amount of phenolics. Based on the study, it could be concluded that residues of *A. conyzoides* release phytotoxic phenolics in the soil, which interfere with the growth of crop plants.

Keywords: allelopathy, amended soils, *Brassica campestris*, growth performance, phenolics, *Raphanus sativus*

INTRODUCTION

Several aggressive weeds exhibit the phenomenon of allelopathy as a mechanism of interference, which provides them competitive advantage over other plants. The phenomenon of allelopathy occurs through the release of chemicals into the environment, which are known as allelochemicals (Whittaker and Feeny 1971). Allelochemicals may be present in almost every plant part such as leaves, roots, flowers, buds, bark, and even pollen grains (Rice 1984; Singh et al. 2001). Both living and dead parts may contribute allelochemicals. However, studies have shown that decaying residues are more inhibitory than fresh parts. The reasons for this may be attributed to the changes in composition and quality of allelochemicals during the decomposition (An et al. 2000). It is therefore essential to study phytotoxicity of decaying residues on succeeding crops and to correlate it with the amount of inhibitory chemicals

A. conyzoides L. (Family Asteraceae), commonly known as billy goat weed, is an exotic aromatic weed that has spread to various parts of Southeast Asia including India. In India, it is very common in the hilly tracts of the northern regions. It is predominantly a weed of cultivated areas, but also spreads to pastures, rangelands, and other open areas due to its high invasive potential. Soon after invasion, it has a capacity to form dense stands. The weed is highly adaptive and thrives well on a variety of habitats. The monoculture of the weed probably results from its extensive growth due to stolon formation, competitive nature and possible allelopathic interference. Kong et al. (1999, 2002) have established that volatile essential oils composed of several mono- and sesquiterpenes, and precocenes and their derivatives impart allelopathic property to this weed. However, little is known about the role of non-volatile allelochemicals such as water-soluble phenolics, which are commonly implicated in allelopathy (Rice 1984). The extensive growth of *A. conyzoides* and formation of new plants on stoloniferous stem is indicative of its strong interference potential. In agricultural fields prior to the planting the next crop, the weed plants are either manually removed or cut mechanically with swords or shrub masters and the residues are left to decay. It is hypothesized that the residues of the weed left in the field may release water-soluble chemicals either through leachation or microbial decay. The accumulation of these chemicals may adversely affect the growth and development of crop species.

This study was done to determine (a) the phytotoxicity of *A. conyzoides* residues left on soil towards commonly growing winter crops, *i.e.*, mustard and radish, and (b) amount of water soluble allelochemicals present in residues.

MATERIALS AND METHODS

For the present study, soil and residues of billy goat weed (*A. conyzoides* L.) were collected from the *A. conyzoides* infested agricultural fields at on the outskirts of Chandigarh, India. For bioefficacy or growth studies, seeds of mustard (*Brassica campestris* L. var. Pusa Bold) and radish (*Raphanus sativus* L. var. Pusa Chetki) were procured from the Indian Agricultural Research Institute, New Delhi. For growth studies, soil was collected from an area devoid of *A. conyzoides*.

To find out whether residues of *A. conyzoides* contain any water-soluble phytotoxin, 2 g residues of *A. conyzoides* were dipped in 100 ml of distilled water for 18 h at 22°C and filtered through a muslin cloth followed by Whatman no. 1 filter paper. These were further diluted so as to get 0.5 and 1 % solutions and kept in refrigerator at 4°C till further use. The effect of different concentrations of residue extracts was studied on the seedling growth and biomass of radish and mustard in a laboratory bioassay. For this, 20 seeds each of radish and mustard were placed in 15 cm petri dishes lined with a Whatman no. 1 filter circle moistened with 7 ml of different concentrations of residue extracts or distilled water to serve as control. Four replicates were maintained for each treatment. After a week the seedling length and dry weight of the seedlings were determined.

Under field conditions residues of *A. conyzoides* fall on the soil and gradually mix with it. These may undergo decomposition and release water-soluble phytotoxins. Keeping this in mind, two growth experiments were planned. In the first experiment, 0.5, 1, and 2 g of residues were mixed into 100 g of control soil. In the second experiment, 40 ml of residue extracts were mixed with 100 g of soil. These were sprayed with 100 ml of distilled water and used for growth studies.

Two hundred fifty grams each of respective residue amended or residue extract amended or unamended (to serve as control) soil was taken in 15 cm diameter petri dishes for growth experiments. Fifteen seeds each of radish and mustard were sown in these amended soils. Three replicates were kept for each treatment. The entire set up was maintained in a growth chamber at 23±2°C, 75±3 % relative humidity and 16 / 8 light / dark photoperiod. Each petri dish was sprayed daily with 30 ml of distilled water. After a week, length and dry weight of ten seedlings from each treatment were measured.

Amount of total water-soluble phenolics in the aqueous residue extracts, and residue and extract amended soils were estimated using Folin-ciocalteu reagent following the method of Swain and Hillis (1959). Their amount was determined spectrophotometrically at 700 nm against standard of ferulic acid. For each estimation 4 replicates were maintained.

All the experiments were repeated and means of results of both experiments are presented. The data for both bioefficacy studies and growth experiments were analyzed by one-way analysis of variance and the means were separated at $P<0.05$ and $P<0.01$. Besides, the correlation coefficient values between concentration and the parameter were also determined.

RESULTS AND DISCUSSION

It is clear from the results that the extracts prepared from decaying residues of *A. conyzoides* significantly reduced growth (measured in terms of length and dry weight) of both test species – radish and mustard (Table 1). A steady decrease in growth of both test species was seen with increasing concentration of extracts exhibiting a strong reciprocal correlation. With the treatment of 2% extracts, seedling length of radish and mustard was reduced by nearly 53 and 59%, respectively (Table 1).

Table 1. Effect of aqueous residue extracts of *A. conyzoides* on seedling length and dry weight of radish and mustard. Data presented are with respect to control.

Concentration	Seedling Length		Seedling Dry Weight	
(%, w/v)	Radish	Mustard	Radish	Mustard
0.5	89.6*	90.3*	82.3*	74.8*
1.0	57.8**	61.5**	69.0**	72.8*
2.0	46.8**	40.6**	53.3**	64.2*
R	-0.894	-0.961	-0.990	-0.988

* and ** represent significance from control at 0.05 and 0.01 % level
r represents value of correlation coefficient.

After conducting laboratory bioassay with residue extracts, further experiments were performed in the soil, since the soil is the medium where allelochemicals upon release accumulate in bioactive concentrations and bring about growth retardatory effects on other plants. The use of soil as a medium of growth makes bioassay studies more realistic, ecologically important, and close to natural conditions. Moreover, any change (sorption and chemical transformation, etc.) in bioactive concentration of allelochemicals upon entering the soil can be monitored. Thus, in the present study, too, the growth experiments were performed in the soil and for this purpose the soil was amended with either residues or residue extracts.

In case of growth studies conducted in soils amended with residues of *A. conyzoides,* reduction in growth of both the test crops was seen (Table 2). Both seedling length and dry weight were reduced significantly. At 2 % concentration seedling length was reduced by nearly 50 and 56 % in radish and mustard, respectively. A strong correlation was also observed between amount of residue and the parameter.

Table 2. Growth performance of radish and mustard in soil amended with residues of *A. conyzoides*.

Residue Amended Soil (%)	Seedling Length (cm)		Seedling Dry Weight (mg)	
	Radish	Mustard	Radish	Mustard
0 (Control)	17.1	18.2	11.3	11.7
0.5	14.2*	15.1*	11.0	9.0*
1.0	12.1*	11.5**	9.0*	7.7**
2.0	8.5**	8.0**	5.6**	6.4**
r	-0.993	-0.985	-0.980	-0.940

* and ** represent significance from control at 0.05% and 0.01 % level
r represents value of correlation coefficient.

Likewise, inhibitory effect was also observed when soil was amended with residue extracts. With the amendment of 2% extract in the soil nearly 50 and 65% decrease in mustard seedling length and dry weight was observed, whereas in radish, it was comparatively less (Table 3). The extent of reduction increased with increasing concentration and, therefore, a positive strong correlation was observed between concentration of residue extract and the reduction in parameter (Table 3).

Table 3. Percent decrease in seedling length and dry weight of radish and mustard in soil amended with residue extracts of *A. conyzoides*.

Residue Extract Amended Soil (%)	Seedling Length		Seedling Dry Weight	
	Radish	Mustard	Radish	Mustard
0 (Control)	-	-	-	-
0.5	6.2*	16.4*	14.8*	21.6**
1.0	27.6**	26.2**	22.3**	37.9**
2.0	42.3**	49.8**	35.1**	64.6**
R	+0.975	+0.996	+0.978	+0.993

* and ** represent significance from control at 0.05% and 0.01 % level
r represents value of correlation coefficient.

Reduction in growth of both test species in soil amended either with residues or their extracts indicate the release of some inhibitory chemicals by the residues in soil, which adversely affect their growth. How these chemicals come into the soil and in what concentrations these accumulate in it depends upon several biotic and abiotic factors (Blum et al. 1999). Rice (1984) has suggested that leachation, microbial degradation, root exudation and volatilization (specifically in aromatic plants) are responsible for the release of allelochemicals from donor plants into the environment. However, leachation is the most convenient method of escape of allelochemicals into the environment (Guenzi and McCalla 1962; Guenzi et al. 1967; Patrick 1971). In the present study also the release of phytotoxins could be predominantly attributed to leachation as the extracts were prepared in water and added to soil directly. In case of residue amended soil watering prior to sowing of seeds could possibly aid in the leaching mechanism. Most commonly released chemicals during leachation are water-soluble phenolics, which play a significant role in allelopathy (Appel 1993). In the present study, too, appreciable amount of water-soluble allelochemicals was estimated in the various treatments (Table 4).

Table 4. Amount of water-soluble phenolics in the residue extracts and soil amended with residues and extracts of *A. conyzoides*.

Treatment	Amount of Phenolics
Residue extract (μg ml^{-1})	151.8
Unamended control soil (μg g^{-1})	7.4
Residue amended soil (μg g^{-1})	23.1
Extract amended soil (μg g^{-1})	36.0

As regards the concentration of allelochemicals in the soil, several factors like metabolic stage of the plant, age, and prevailing environmental conditions have a direct impact on it. In the present study maximum amount of phenolics was found in the extracts followed by soil amended with extracts and lastly residue amended soils (Table 4). Comparatively lesser amount of phenolics in the amended soils could be attributed to several reasons. There may be chemical transformation (Okumura et al. 1999), utilization by microbes (Blum et al. 1999) or sorption by soil organic matter and clay minerals (Huang et al. 1977; Makino et al. 1996). In spite of lesser amount of phenolics in soil, considerable phytotoxicity has been observed towards both the test plants. The reasons for this could be attributed to the changes in the composition and quality of phenolics in soil with time (Blum et al. 1999). However, no attempt has been made in to determine the composition and dynamics of allelochemicals in the present investigation.

Thus, from the above study it is clear that residues of *A. conyzoides* exhibit considerable toxicity towards both radish and mustard owing to the release of water-soluble phenolics in soil.

LITERATURE CITED

An, M. et al. 2000. Phytotoxicity of *Vulpia* residues: IV. Dynamics of allelochemicals during decomposition of *Vulpia* residues and their corresponding phytotoxicity. Journal of Chemical Ecology, 26:2603-2617.

Appel, H.M. 1993. Phenolics in ecological interactions: the importance of oxidation. Journal of Chemical Ecology, 19:1521–1552.

Blum, U., et al. 1999. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs. an experimental model. Critical Reviews in Plant Sciences, 18:673-693.

Guenzi W.D., et al. 1962. Inhibition of germination and seedling development by crop residues. Soil Science Society of America Proceedings, 26:456-458.

Guenzi, W.D., et al. 1967. Presence and persistence of phytotoxic substances in wheat, oat, corn, and sorghum residues. Agronomy Journal, 59:163-165.

Huang, P.M., et al. 1977. Retention of phenolic acids by non-crystalline hydroxy-almunium and –iron compounds and clay minerals of soil. Soil Science, 123:213-219.

Kong, C., et al. 1999: Allelopathic potential and chemical constituents of volatile oil from *Ageratum conyzoides*, Journal of Chemical Ecology, 25:2347-2356.

Kong, C., et al. 2002: Allelopathic potential and chemical constituents of volatile oil from *Ageratum conyzoides* under stress. Journal of Chemical Ecology, 28:1173-1182.

Makino, T., et al. 1996. Influence of soil chemical properties on adsorption and oxidation of phenolic acids in soil suspension. Soil Science and Plant Nutrition, 42:867-879.

Okumura, M., et al. 1999. Use of ^{14}C-labelled alfalfa saponins for monitoring their fate in soil. Journal of Chemical Ecology, 25:2575-2583.

Patrick, Z.A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Science, 111:13-18.

Rice, E.L. 1984. Allelopathy. 2nd edition. Academic Press, Orlando, Florida.

Singh, H.P., et al. 2001. Allelopathy in agroecosystems. Journal of Crop Production, 4:1-42.

Swain T., and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of constituents. Journal of the Science of Food and Agriculture, 10:63-68.

Whittaker, R.H. and P.P. Feeny. 1971. Allelochemics: chemical interaction between species. Science, 171:757-770.

Weed Suppression by Rat-Tail Fescue (*Festuca myuros*) in Peach Orchard

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Abstract: Rat-tail fescue (*Festuca myuros* L.) is a winter annual grass that originated in the Mediterranean region and widely distributed in Japan. To control weeds and avoid soil erosion in deciduous fruit orchards such as peach and Japanese persimmon orchards and vineyards, we are trying to use the fescue as a ground cover plant. For practical use of the fescue, it is indispensable to clarify the effect on the weed emergence and biomass production in the orchards. Seeds of the fescue were sown at the ratio of 8.5 kg/10 a on the floor of a peach orchard in September 2000 and any weed control was not done after seeding. In the control plot, conventional weed management, i.e., mowing was done three times a year. The seasonal changes of emerging species and their coverage at eight 1 m by 1 m quadrats in each plot were recorded every two weeks and the biomass of each species was measured at five 50 by 50 cm quadrats in each plot in July and November 2001. The effects of water extract of the fescue (30 g L^{-1}) on the germination of 12-weed species and seedling root growth of ten weed species were tested. The weed biomass in July was 59.8 g m^{-2} in the fescue plot and 160.8 g m^{-2} in the control plot, and 66.1 g m^{-2} in the fescue plot and 136.0 g m^{-2} in the control plot in November. Some perennial weeds such as *Artemisia princeps* Pamp. and *Taraxacum officinale* Weber was not effectively suppressed by the fescue. The germination of *Plantago asiatica* L., *Aster subulatus* Michx., *Senecio vulgaris* L., *T. officinale* and *Alopecurus aequalis* Sobol. var. *amurensis* (Komar.) Ohwi, and the seedling root growth of six species were inhibited by the extract. The fescue suppressed weeds by both covering the floor and partially allelopathy.

Key words: Allelopathy, competition, deciduous fruit orchards, ground cover grass, sod culture system, *Vulpia myuros*

INTRODUCTION

Weeding practices in orchards and vineyards are laborious and expensive. To save on labor costs ecological weed management strategies are necessary for fruit production. The use of ground cover plants in orchards is one of the management systems and it reduces soil management problems.

Rat-tail fescue (*Festuca myuros* L.; synonym, *Vulpia myuros* (L.) Gmel.) is a winter annual grass originated in the Mediterranean region and widely distributed in Japan. To control weeds and avoid soil erosion, this fescue is effectively used in citrus gardens in Ehime, southwest Japan (Ishii et al. in press). We are trying to use the fescue as a ground cover plant in deciduous fruit orchards such as peach, Japanese persimmon or kaki orchards and vineyards in Kyoto.

For practical use of the fescue in deciduous fruit orchards, it is indispensable to clarify the effect on weed emergence and biomass production in orchards. In the study, we clarified such effects by both field and laboratory tests and discussed the possibility of the practical use of the fescue in deciduous fruit orchards.

MATERIALS AND METHODS

Field tests on the effects on weed emergence and biomass production

Field tests were made at the University farm of Kyoto Prefectural University in Seika, Kyoto. Seeds of the fescue were sown at the ratio of 8.5 kg/10 a in September 19, 2000 on the floor of a 19.4 by 18.4 m peach orchard, where mowing was done before seeding, and any weed control was not practiced in the fescue plot after seeding. In the control plot adjacent to the fescue plot, conventional weed management, i.e., mowing was done three times a year. Other cultural practices in the orchard were made as the same way as in this region.

The seasonal changes of emerging species and their coverage at eight 1 m by 1 m quadrats randomly placed in each plot were recorded every two weeks from April 5, 2001 to December 27, 2001. The coverage was assessed according to Penfound and Howard (1940).

The aerial parts of the weeds were harvested on each five 50 cm by 50 cm quadrats in July 22, 2001 and November 21, 2001, respectively. They were separated to each weed species and were dried at 80°C for at least 48 h. Then dry matter weight of each species was measured.

Laboratory tests on the effects of water extract of rat-tail fescue on weed germination and seedling growth

The aerial parts of the fescue were harvested on June 25, 2001, when the fescue died. The harvested fescue was cut into 5 cm length pieces and put in a flask with distilled water at the ratio of 30 g L^{-1}. The flask was agitated on a reciprocating shaker for 24 h at room temperature of 25°C as White et al. (1989). For germination test, 12 weed species, *Rumex crispus* L. subsp. *japonicus* (Houtt.) Kitam., *Stellaria media* (L.) Villars, *Capsella bursa-pastoris* (L.) Medik., *Torilis japonica* (Houtt.) DC., *Veronica arvensis* L., *Plantago asiatica* L., *Artemisia princeps* Pamp., *Aster subulatus* Michx., *Conyza sumatrensis* (Retz.) Walker, *Senecio vulgaris* L., *Taraxacum officinale* Weber, *Alopecurus aequalis* Sobol. var. *amurensis* (Komar.) Ohwi, were used. Their seeds were collected in the orchard and prior to the germination tests, seed dormancy was broken by scarrification. Fifty seeds of each weed species were put on two layer filter paper in a 9 cm diameter petri dish with 6 mL extract and for *R. crispus* subsp. *japonicus* and *T. japonica* with 9 mL extract. In the control, the same volume of distilled water was used. The petri dishes were kept in a growth chamber of constant 25°C under 16 h light and 8 h dark condition. Number of germinating seeds was counted every day for 10 days. Three replications were made.

For seedling growth test, 10 species, *R. crispus* subsp. *japonicus*, *S. media*, *T. japonica*, *Lamium amplexicaule* L., *V. arvensis*, *P. asiatica*, *A. subulatus*, *S. vulgaris*, *T. officinale* and *A. aequalis* var. *amurensis* were used. Their seeds were collected in the orchard and seed dormancy was broken by stratification. The seeds of each weed species were put on wet filter paper in petri dishes in a growth chamber of constant 25°C. The seedlings of each weed species for the seedling growth test were incubated for one day after germination at constant 25°C under 16 h light and 8 h dark condition and 15 seedlings of each weed were put on the two layer filter paper to which 6 mL extract was added in a petri dish. In the control, the same volume of distilled water was added. Petri dishes were kept in a growth chamber of constant 25 C under 16 h light and 8 h dark condition. After one week, root length of each seedling was measured. Two replications were made.

RESULTS AND DISCUSSION

Field tests on the effects on weed emergence and biomass production

Rat-tail fescue successfully emerged and established the population on the floor of the peach orchard in October 2000. It headed in early May and lodged in late May 2001. The lodging fescue covered the almost all the floor of the fescue plots in the orchard and died in June.

Main weed species emerging in the orchard were *Equisetum arvense* L., *Persicaria longiseta* (De Bruyn) Kitag., *S. media* and/or *S. neglecta* Weihe, *Oxalis corniculata* L., *T. officinale*, *Commelina communis* L., *A. aequallis* var. *amurensis*, and *Digitaria ciliaris* (Retz.) Koeler.There were no differences in the emerging weed species between both plots.

Coverage of each weed species was conspicuously lower in the fescue plots than in the control plots, because the fescue covered almost all the fescue plots in the orchard floor (Fig. 1). Weed species, especially annual ones seemed not to grow by the cover of the fescue. The relative percentage of perennial weeds was higher in the fescue plots than in the control. Annual weeds such as *P. longiseta*, *Amaranthus* spp. and *D. ciliaris* were less competitive against the fescue, but perennial weeds such as *R. crispus* subsp. *japonicus*, *A. princeps* and *T. officinale* were superior competitors against the fescue and were not effectively suppressed by the fescue. These species regrew quickly after mowing because they had much reserve in their underground parts. It seems to cause the species to be superior competitors against the fescue.

The biomass of the fescue increased gradually and reached 539.4 g m^{-2} on July 22, 2001. The total biomass of weed other than the fescue in July was 59.8 g m^{-2} in the fescue plot and 160.8 g m^{-2} in the control plot, and 66.1 gm^{-2} in the fescue plot and 136.0 gm^{-2} in the control plot in November. The biomass production of weed species, especially annual ones were suppressed effectively by the fescue (Fig. 2).

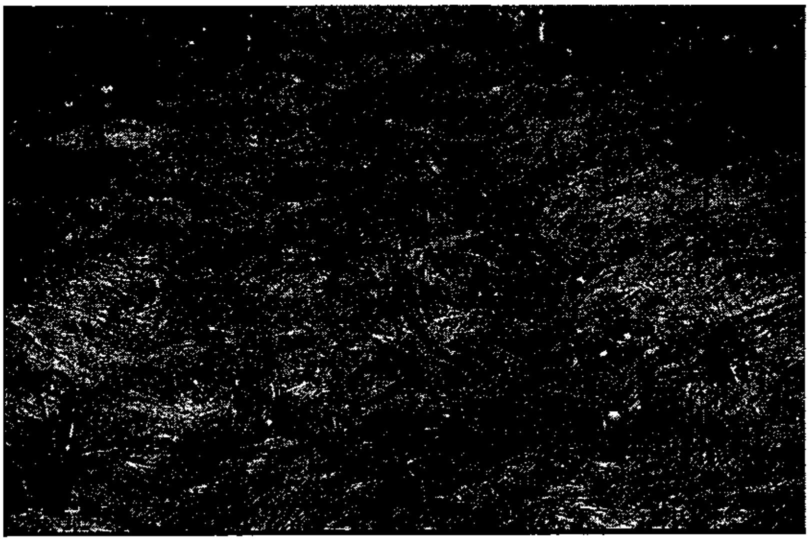

Figure 1. Peach orchard floor covered with rat-tail fescue.

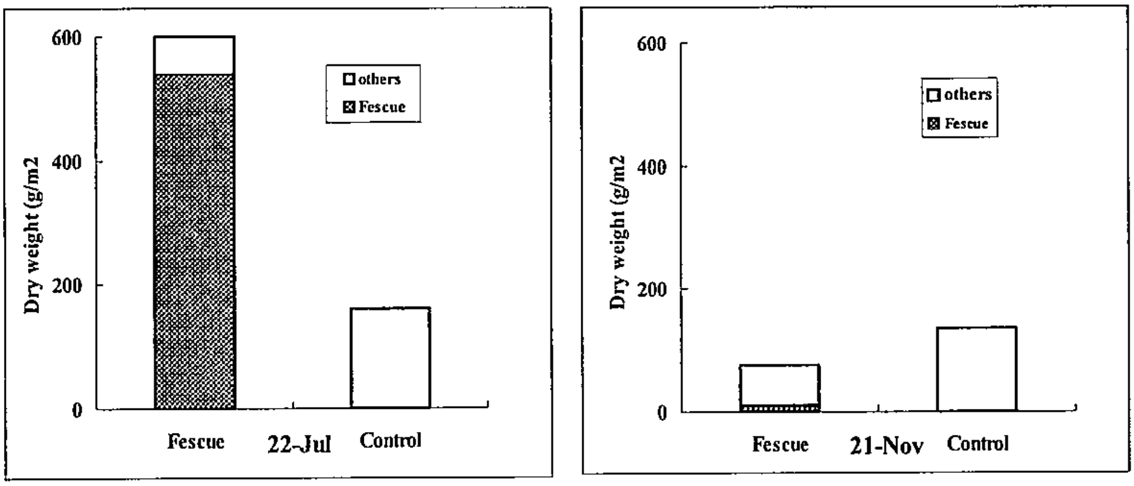

Figure 2. Biomass in the orchard on June 22 and November 21.

Laboratory tests on the effects of water extract of rat-tail fescue on weed germination and seedling growth

Germination response of the weed species to the water extract of the fescue varied among 12 species tested. The water extract of the fescue significantly inhibited the germination of *A. subulatus* (46%), *T. officinale* (39%), *A. aequalis* var. *amurensis* (34%), *P. asiatica* (30%) and *S. vulgaris* (10%) as shown in Figure 3 and no effects on *R. crispus* subsp. *japonicus*, *S. media*, *C. bursa-pastoris*, *T. japonica*, *V. arvensis*, *A. princeps* and *C. sumatrensis*.

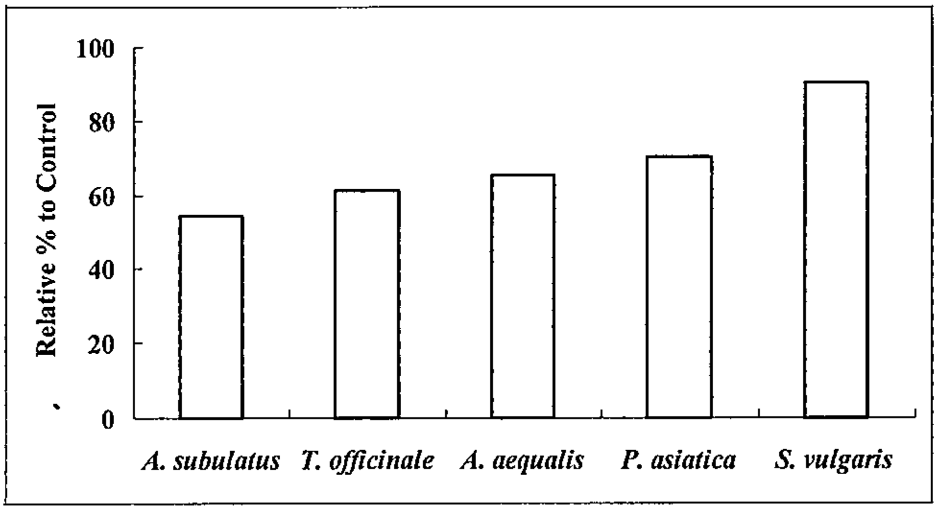

Figure 3. Inhibition of the germination of five weeds by water extract of the fescue.

The root growth of the 10 weed species responded differently to the water extract of the fescue. The water extract significantly inhibited the seedling root growth of *S. vulgaris* (57%), *A. subulatus* (33%), *P. asiatica* (30%), *V. arvensis* (25%), *S. media* (18%) and *R. crispus* subsp. *japonicus* (13%), on the other hand, those of *L. amplexicaule* (8%) and *A. aequalis* var. *amurensis* (18%) were promoted (Fig. 4). There were no differences in the root growth of *T. japonica* and *T. officinale* between the extract treatment and the control.

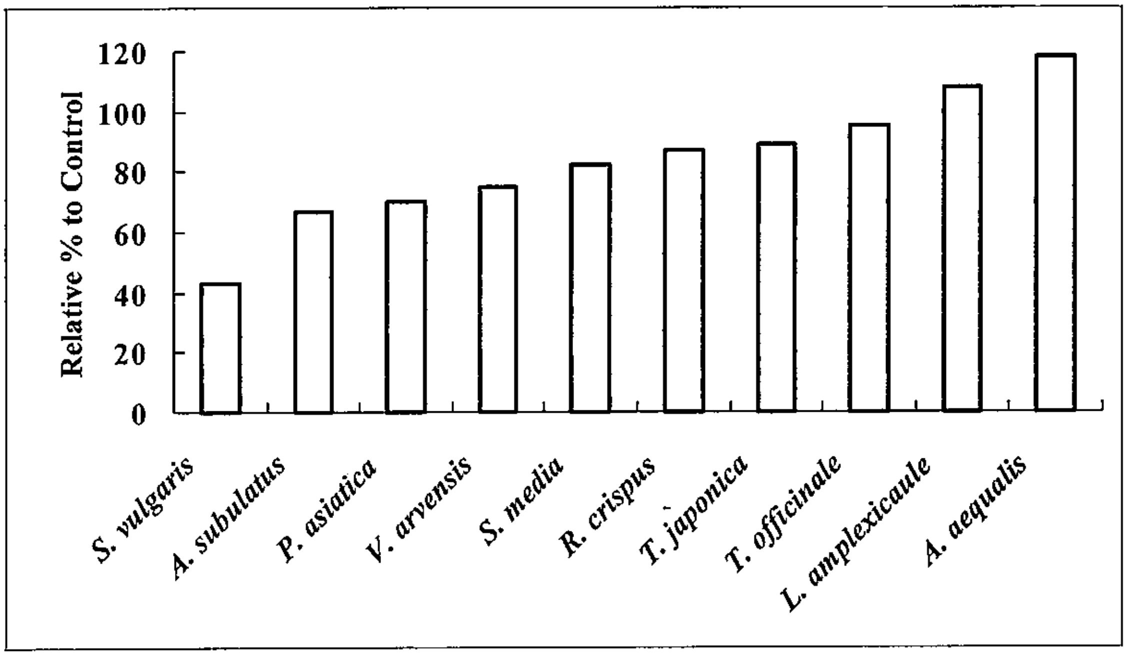

Figure 4. Root growth response of ten weeds to water extract of the fescue.

As shown in our previous experiments (Tominaga and Uezu 1995; Tominaga and Watanabe 1997), allelopathic effects of a species apparently differ among receptor species. The fescue effectively suppressed annual weeds both by covering the floor and partially allelopathy and a spot treatment of herbicide is effective for the control of perennial weeds. Ishii et al. (in press) reported that the fescue stimulated arbuscular mycorrhizal fungal activity and inter-specific competition for nutrients and water between fruit trees and the fescue may be scarcely found when a network system of arbuscular mycorrhizal hyphae between these plants is formed in their rhizospheres. The fescue also supplies organic matter to the soil in orchards. The fescue is useful for sod culture system in orchards.

LITERATURE CITED

Ishii, T. et al. in press. Construction of sustainable citriculture by vesicular-arbuscular mycorrhizal fungi: Introduction of new soil management. Proceedings of the International Society of Citriculture.

Ishii, T. et al. in press. Network establishment of arbuscular mycorrhizal hyphae in the rhizospheres between citrus rootstocks and *Paspalum notatum* or *Vulpia myuros*. Plant and Soil.

Penfound, W. T. and J. A. Howard. 1940. A phytosociological study of an evergreen oak forest in the vicinity of New Orleans, Louisiana. American Midl. Nat. 23: 165-174.

Tominaga and Uezu 1995. Weed suppression by buckwheat. Current Advances in Buckwheat Research, 2: 693-697.

Tominaga and Watanabe 1997. Weed growth suppression by cogongrass (*Imperata cylindrica*) leaves. Journal of Weed Science and Technology, 42:289-293.

White et al. 1989. Allelopathic potential of legume debris and aqueous extracts. Weed Science, 37: 674-679.

Absorption, Translocation and Metabolism of L-3,4-Dihydroxyphenylalanine (L-DOPA) in *Echinochloa crus-galli* L. and *Lactuca sativa* L. at Germination Stage

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Abstract: L-3,4-Dihydroxyphenylalanine (L-DOPA) is known as one of the most potent allelochemicals. Our previous study showed that the phytotoxicity of this phenolic nonprotein amino acid was different among the plant species (Hachinohe *et al.*, 2002). In order to clarify the mechanism of selective action, absorption, translocation and metabolism of ^{14}C-L-DOPA were compared between barnyard grass (*Echinochloa crus-galli* L. ; tolerant) and lettuce (*Lactuca sativa* L. cv. Greatlakes 366 ; susceptible) at the germination stage. Absorption of L-DOPA from roots increased continuously for 5 days in both species, however, total absorbed radioactivity was higher in barnyard grass than in lettuce. Translocation of ^{14}C from root to shoot was higher in lettuce (60.0 % ; 5DAT) than in barnyard grass (20.0 % ; 5DAT). Metabolism of L-DOPA occurred in both species, and several metabolites were detected. Metabolic activity was much greater in barnyard grass than in lettuce. Therefore selectivity between barnyard grass and lettuce is mainly due to metabolic activity of L-DOPA. Involvement of the greater tolerance of barnyard grass roots to L-DOPA is also suggested.

Key words: absorption, barnyard grass, inhibition of root elongation, L-DOPA, lettuce, metabolism, translocation

INTRODUCTION

Some leguminous plants are known to accumulate one of the most potent allelochemicals L-3,4-dihydroxyphenylalanine (L-DOPA). Mucuna (*Mucuna pruriens* L.) is reported to contain greater amount of L-DOPA, exudes it from the root into soil, and causes the growth inhibition of other species (Fujii *et al.* 1991). L-DOPA is also effective against Parkinson's disease (Dougan *et al.* 1975). Therefore the cost-effective methods for production of L-DOPA by plants cell culture have been investigated (Obata-Sasamito et al. 1983; Huizing et al. 1985; Chattopadhyay et al. 1994).

There have been several investigations of phytotoxicic effect of L-DOPA. Plants showed different responses to the chemical, and root elongation was more sensitive than that of shoot in susceptible species. (Fujii et al. 1992; Nakajima et al. 1999; Hachinohe *et al.* 2002). Nakajima et al. (1999) found drastic accumulation of phenylalnine in L-DOPA-treated roots and suggested that their accumulation was due to the detoxific metabolism of L-DOPA, since phenylalanine and tyrosine were non-toxic to plant growth. In L-DOPA-treated perennial rye grass (*Lolium perenne* L.), its metabolism to dopamine and induction of antioxidative enzyme activity were observed (Nishihara *et al.* 2002). However, the mechanisms of phytotoxic action and selectivity of L-DOPA still remain obscure. In order to obtain more information on the mode of action of L-DOPA, its absorption, translocation and metabolism in tolerant, barnyard grass (*Echinochloa crus-galli* L.), and susceptible species, lettuce (*Lactuca sativa* L.), were compared at their germination stage.

MATERIALS AND METHODS

Plant materials

The seeds of barnyard grass (*Echinochloa crus-galli* L.) and lettuce (*Lactuca sativa* L. cv. Greatlakes 366) were sown on an aluminum mesh tray covered with cheese cloth, and set on a plastic box (310 mm × 220 mm × 35 mm) containing water. They were germinated for 1 to 3 days in a growth chamber under 25°C/20°C (day-night, 12 h each, 80-100 $\mu Em^{-2}s^{-1}$). The germinated uniform plants were selected for further experiments.

Effect of L-DOPA on the root elongation of plants

Ten plants of each species were planted in a plastic box (60 mm × 60 mm × 100 mm) containing L-DOPA, tyrosine, phenylalanine, dopamine, caffeic acid or ferulic acid in 200 mL of 0.5% agar culture medium. They were kept in a growth chamber at the same condition as above and the lengths of root and shoot were measured 5 days after treatment. The experiment was conducted with five replications.

Absorption and translocation of L-DOPA

Germinated seeds were planted on agar culture medium (1000 mL) containing 10^{-4}M L-DOPA and ^{14}C- L-DOPA (specific activity of 9.48MBq mg^{-1}) with radioactivity of 186 kBq, and grown under the same condition as above for 5 days. Radioactivity was determined at 1, 2, 3 and 5 days by a liquid scintillation spectrometer (LSS) after combustion with a sample oxidizer. The experiment was conducted with three replications.

Metabolism of L-DOPA

Germinated seeds were planted on the medium (1000 mL) containing 10^{-4}M L-DOPA and ^{14}C- L-DOPA with radioactivity of 833 kBq. At 1, 3 and 5 days after treatment, roots of the plants were harvested and extracted twice with methanol / 0.1N hydrochloric acid (1/1, v/v) (Siddhuraju and Becker 2000; Janicsac et al. 1999). The radioactivities in extracts and residues were determined by LSS. The extracts were evaporated at 40°C and the residues were dissolved in a small amount of the same solution. They were spotted on a thin layer chromatography (TLC) plate, silicagel 60 F245 (Merck) or RP-18 (Merck), and developed with toluene / ethylacetate / formic acid (5 / 4 / 1, v/v/v) or methanol / water (1 / 5, v/v), respectively (Janicsac et al. 1999; Baranowska and Kozlowska 1995). The radioactive spots on the TLC plates were detected by autoradiography and 0.2% ninhydrin in acetone, and their radioactivities were determined by LSS. The experiment was conducted with two replications.

RESULTS AND DISCUSSION

L-DOPA effect on the root elongation of plants

In the previous study, we examined the phytotoxic effect of L-DOPA in 32 species including weeds and crops. Plants showed various responses to L-DOPA, and root elongation was suppressed strongly compared with that of shoot in susceptible species (Hachinohe et al. 2002).

Figure 1 shows the relative root elongation of barnyard grass (tolerant) and lettuce (susceptible) under 10^{-4}M L-DOPA. There was significant difference in the tolerance to L-DOPA between both species and distinct suppression in lettuce was observed 2 days after treatment. Based on GR_{50} value determined at 5 days, barnyard grass was 77-fold more tolerant than lettuce. In shoot elongation, no remarkable suppression was found in both species (data not shown).

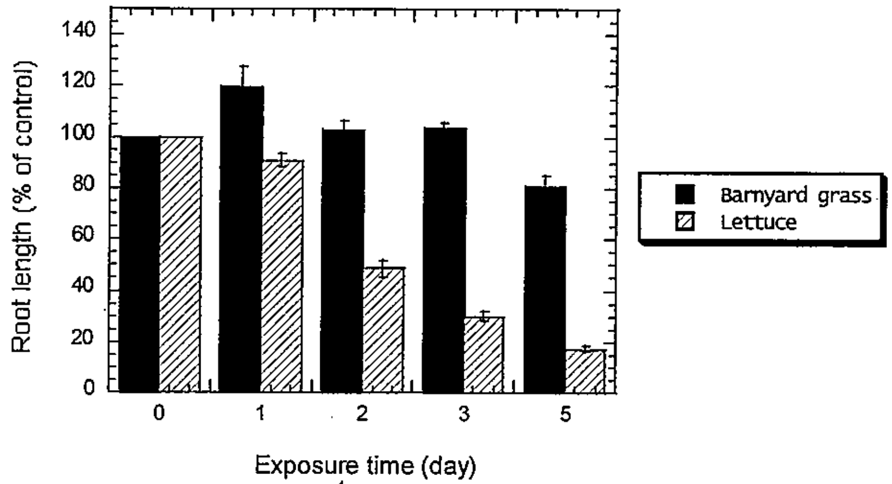

Figure 1. Effects of L-DOPA (10^{-4}M) on root elongations of barnyard grass and lettuce at the germination stage.

Absorption and translocation of L-DOPA

Absorptions of ^{14}C- L-DOPA from 10^{-4}M L-DOPA containing medium by roots of both species increased continuously for 5 days (data not shown). However absorption of ^{14}C-L-DOPA was greater in barnyard grass than in lettuce throughout 5 days (data not shown). The concentration of ^{14}C in root was also higher in barnyard grass than in lettuce (Fig. 2). Lettuce translocated 48.5% and 60% of total absorbed ^{14}C to shoots at 3 and 5 days, respectively. In barnyard grass, only 22.3% and 20.0% of ^{14}C were translocated at 3 and 5 days, respectively. These results indicate that barnyard grass accumulates greater amount of ^{14}C derived from ^{14}C-L-DOPA in roots. Therefore absorption and translocation of might not be involved in the tolerance of barnyard grass to L-DOPA.

Metabolism of L-DOPA

Phytotoxic activity of the putative L-DOPA metabolites were examined. Little inhibition of lettuce root elongation was observed with phenylalanine, tyrosine and dopamine

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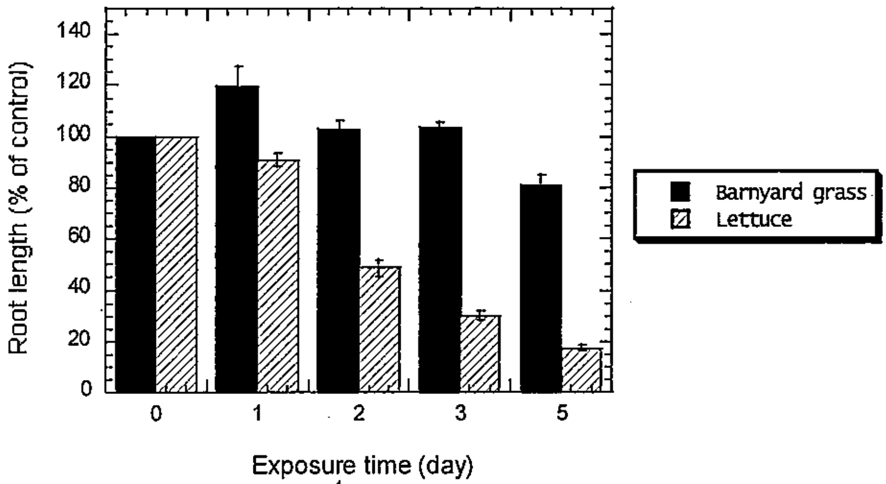

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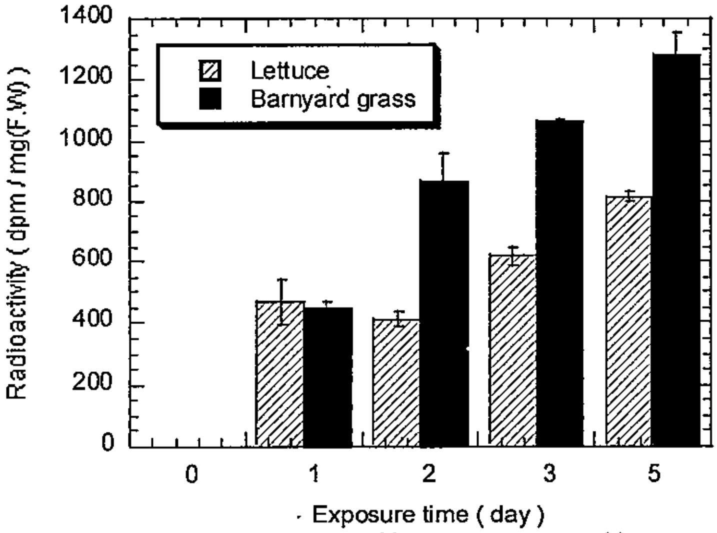


Figure 2. Time courses of concentrations of ^{14}C derived from ^{14}C- L-DOPA in barnyard grass and lettuce at the germination stage.

treatment (Fig. 3). In contrast, caffeic acid and ferulic acid suppressed the root elongation as strong as L-DOPA. Percentage distribution and concentration of ^{14}C- L-DOPA in roots differed obviously between barnyard grass and lettuce (Fig. 4, A). The value of percentage distribution of L-DOPA decreased in barnyard grass with the exposure time, but increased in lettuce. This result suggests that barnyard grass has much greater metabolic activity of L-DOPA than lettuce. Although barnyard grass possesses greater metabolic activity, L-DOPA concentration in the roots is still higher than lettuce due to greater absorption. Continuous increase of L-DOPA in the root of lettuce was found (Fig. 4, B), however, caffeic acid and ferulic acid did not accumulate. There was no significant difference in concentrations of the metabolites between the species (data not shown).

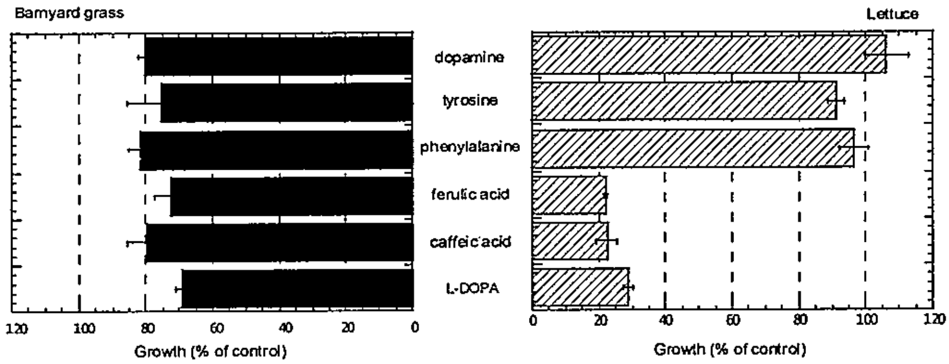
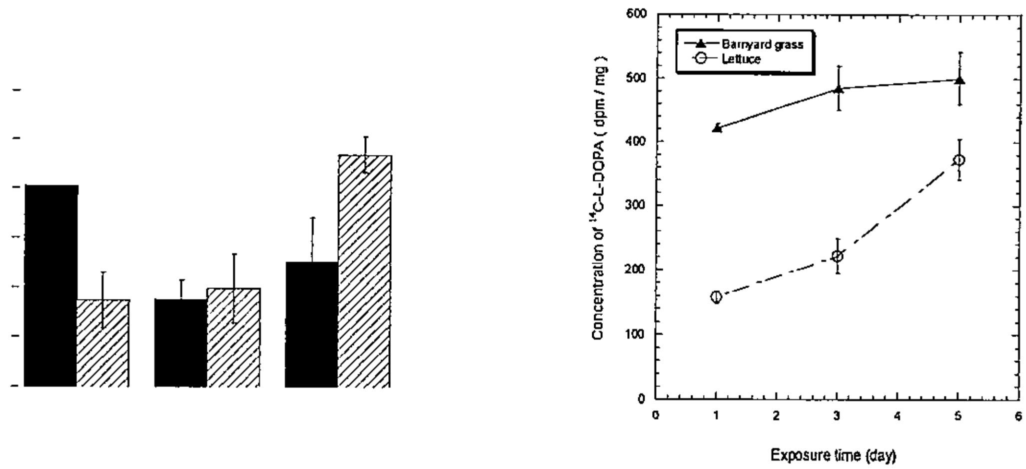


Figure 3. Effect of the putative L-DOPA metabolites (10^{-4}M) on root elongation of barnyard grass and lettuce 5 days after treatment.

Based on these results, L-DOPA itself might be the active form to suppress the root elongation of plants. However, root elongation in barnyard grass was less inhibited under the higher concentration of L-DOPA compared lettuce. Barnyard grass is considered to possess the mechanism to tolerate L-DOPA. Therefore the selectivity between barnyard grass and lettuce might be due to the metabolic activity of L-DOPA and ability to tolerate the chemical's action.



(A) (B)

Figure 4. Percentage distribution (A) and concentration (B) of ^{14}C- L-DOPA in the root of barnyard grass and lettuce.

LITERATURE CITED

Baranowska, I. and M. Kozlowska. 1995. TLC separation and derivation spectrophotometry of amino acid. Talanta. 42:1553-1557.

Chattopadhyay, S. et al. 1994. Production of L-DOPA from cell suspension culture of *Mucuna pruriens f. pruriens*. Plant Cell Rep. 13.519-522.

Dougan, D.D. et al. 1975. Effect of L-DOPA metabolites at dopamine recepter suggest a basis for 'on-off' in Parkinson's disease. Nature. 254,70-72.

Fujii, Y. et al. 1991. L-3.4-dihydroxyphenylalanine as an allelochamicals candidate from *Mucuna pruriens* (L). DV. var. utile. Agric. Biol. Chem. 55.617-618.

Fujii, Y. et al. 1992. Allelopathy of velvetbean : Its discrimination and identification of L-DOPA as a candidate of allelopathic substances. JARQ. 25.238-247.

Hachinohe, M. et al. 2002. Pytotoxic effect, absorption, translocation and metabolism of L-DOPA in plants at the germination stage. Third world congress on allelopathy. 204.

Huizing, H.J. et al. 1985. Production of L-DOPA by cell suspension cultures of Mucuna pruriens L. Initiation and maintenance of cell suspension cultures of Mucuna pruriens and identification of L-DOPA. Plant Cell Tissue Organ Culture. 4.61-73.

Janicsac, G et al. 1999. Comparative studies of the rosmarinic and caffeic acid contents of Lamiaceae species. Biochem. Systems Ecol. 27:733-738.

Nakajima, N. et al. 1999. Cheracteristics of growth inhibitory effect of L-3,4-Dihydroxyphenylalanine (L-DOPA) on Cucumber seedlings. J. Weed. Sci. Tech. 44.132-138 (in Japanese).

Nishihara, E. et al. 2002. Effect of plant interaction by L-DOPA treatment during germination period. J. Weed. Sci. Tech. 46.136-137 (in Japanese).

Obata-Sasamito, H. et al. 1983. Effect of culture conditions on DOPA accumulation in a callus culture of Stizolobium hassjoo. J. Med. Plant. Res. 49.120-123.

Siddhuraju, P. and K. Becker. 2000. Rapid reversed-phase high performance liquid chromatographicmethod for the quantification of L-Dopa (L-3,4-dihydroxyphenylalanine), non-methylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. Food Chem. 73.389-394.

Momilactone B May Play an Important Role in Rice (*Oryza sativa*) Allelopathy

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Abstract: Rice seedlings inhibited the growth of hypocotyls and roots of cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) seedlings when the cress and lettuce were grown with rice seedlings. The putative compound causing the inhibitory effect of rice seedlings was isolated from their culture solution, and the chemical structure of the inhibitor was determined from spectral data as momilactone B. Three-day-old rice seedlings were transferred to hydroponics and the level of momilactone B released into the environment from the seedlings was determined. At 15 days after transfer to hydroponics, the level of momilactone B in the culture solution was 1.8 nmol per seedling, and its endogenous level was 0.32 and 0.63 nmol per root and shoot, respectively, suggesting that the rice seedlings probably release momilactone B into the culture solution very actively. This release may occur from the roots, because only rice roots were immersed in the culture solution. Momilactone B inhibited the growth of cress and lettuce seedlings at concentrations greater than 3 and 30 nmol mL^{-1}, respectively. The doses required for 50% inhibition on roots and hypocotyls of cress were 36 and 41 nmol mL^{-1}, respectively, and on those of lettuce were 56 and 79 nmol mL^{-1}, respectively. These results suggest that the rice seedlings may produce and release momilactone B into the environment, and momilactone B may act as an allelochemical by inhibiting growth of neighboring plants. Thus, momilactone B may play an important role in rice allelopathy.

Key words: donor-receiver bioassay, growth inhibitor, *Lactuca sativa*, *Lepidium sativum*, root exudate

INTRODUCTION

As the use of chemicals increases throughout the world, agricultural weed control alternatives to the present man-made herbicide dominated programs are now being given wide considerations because of the concerns about natural environment as well as human health. Controlling weeds through allelopathy is one strategy to reduce man-made herbicide dependency (Putnam 1988; Rizvi et al. 1992; Seigler 1996; Narwal 1999; Duke et al. 2000).

Rice has been extensively studied with respect to its allelopathic potential for development of rice cultivars with proven allelopathic characteristics as part of a strategy to weed control (Olofsdotter et al. 1995; Mattice et al. 1998; Olofsdotter et al. 1999). A large field experiment was conducted in USA to evaluate the allelopathic potential of 17 000 rice accessions and growth of *Heteranthera limosa* and/or *Ammannia coccinea* was inhibited by 557 of these (Dilday et al. 1994; 1998). Similar attempts were conducted in other countries, and many rice accessions were found to possess allelopathic activity (Narwal 1999). It is obscure, however, whether these inhibitions are caused only by allelopathic interference because plant-to-plant interference is a complex combination of competitive interference for resources and allelopathic chemical reactions. Moreover, competition and allelopathy cannot be separated under field condition (Fuerst and Putnam 1983; Leather and Einhellig 1998; Olofsdotter et al. 1999).

Aqueous extracts of rice plants inhibited the growth of several plant species (Tamak et al. 1994; Kawaguchi et al. 1997) and aqueous extracts of decomposing rice residues inhibited root growth of lettuce seedlings (Chou and Lin 1976). Several phenolic compounds, such as *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid, were found in aqueous extracts of rice residues and straw (Kuwatsuka and Shindo 1973; Chou and Lin 1976; Chou and Chiou 1979). It is not clear, however, whether these compounds are released from living rice plants and act as allelochemicals by inhibiting growth of neighboring plants.

To date, few studies have identified allelochemicals released from living rice to the environment. In this study, a growth inhibitor was isolated from rice root exudates and the biological activity, as well as the release size of the inhibitor into environment from the rice seedlings was determined.

MATERIALS AND METHODS

Donor-receiver bioassay

Seeds of rice (*Oryza sativa* L. cv. Koshihikari) were surface sterilized and allowed to germinate at 25°C with a 12-h photoperiod. Light was provided from above with a white fluorescent tube (irradiance, 2.9 W m^{-2} at plant level; FL40SBR, National, Tokyo). After 4 days, uniform rice seedlings were transferred, in groups of six, to 5.5-cm petri dishes each containing a sheet of filter paper (No. 2; Toyo Ltd) moistened with 3.5 ml of 1 mM MES buffer (pH 6.0), and grown further for 3 days as described by Kato-Noguchi et al. (2002). Then, 10 cress (*Lepidium sativum* L.) or lettuce (*Lactuca sativa* L.) seeds were arranged on the filter paper in the Petri dishes and allowed to germinate and grow with the rice seedlings under conditions as above. After 3 days, the lengths of the hypocotyls and roots of the cress and lettuce seedlings were measured. Control seedlings were incubated without rice seedlings.

Rice hydroponics and quantification of monilactone B

Three-day old seedlings, in groups of 100, were transferred onto a sheet of plastic mesh that was floated on distilled water in container, and grown at 25°C with a 12-h photoperiod. After incubation of rice seedlings for 15 days, the water in the container was collected for isolation and quantification of a growth inhibitor. Rice seedlings were also harvested for quantification of the growth inhibitor. The quantification of the inhibitor, and cress and lettuce bioassay were carried out as described in Kato-Noguchi et al. (2002).

RESULTS AND DISCUSSION

Allelopathic potential of rice seedlings

The growth of hypocotyls and roots of cress and lettuce seedlings was markedly inhibited by the presence of rice seedlings (Fig. 1). Growth of rice seedlings was not significantly reduced by the presence of cress and lettuce seedlings (data not shown). The length of hypocotyls and roots of the cress seedlings was 48% and 46% that of control seedlings, respectively, and the length of hypocotyls and roots of the lettuce seedlings was 63% and 57% that of control seedlings, respectively. The cress and lettuce seedlings may grow with the rice seedlings without competition for nutrients, because no nutrients were added in the bioassay. Light is also unnecessary in the developmental stages of these seedlings, since early developmental seedlings mostly withdraw nutrients from the reserve of their seeds (Fuerst and Putnam 1983). Thus, the rice seedlings inhibited the growth of the neighboring plants and the inhibitory effect may not be due to the competitive interference, suggesting that rice seedlings may produce growth inhibiting substances and release into the neighboring environment.

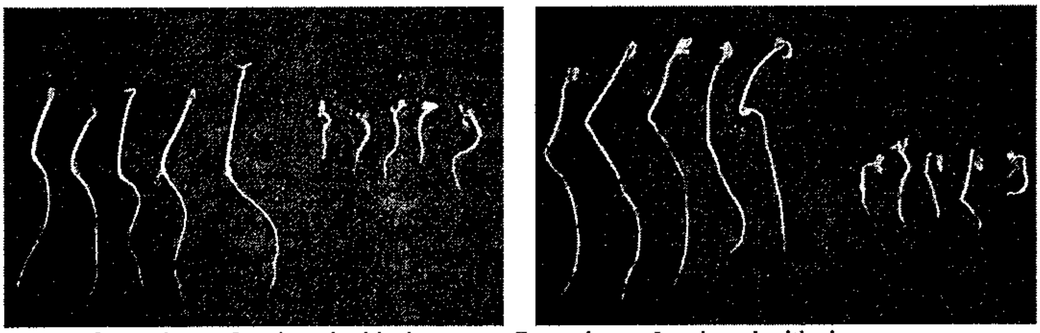


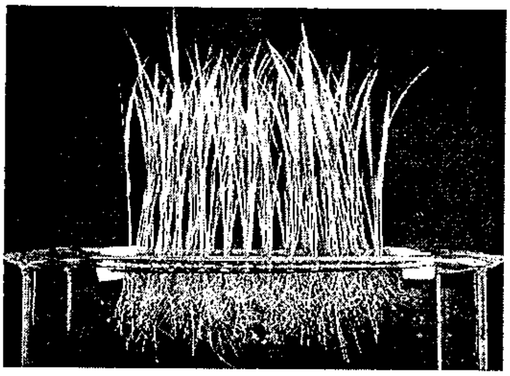
Figure 1. Effects of rice seedlings on the growth of hypocotyls and roots of cress and lettuce seedlings. Cress and lettuce seeds were allowed to germinate and grow with 7-day-old rice seedlings at 25°C and 12-h photoperiod. After 3 days, hypocotyl and root length of their seedlings were determined. Control bioassays did not contain rice seedlings.

Identification of allelochemical

The putative compound causing the inhibitory effect of rice seedlings was isolated from their culture solution (Fig. 2), and the chemical structure of the inhibitor was determined from its high-resolution MS, and ^{1}H- and ^{13}C-NMR spectral data as momilactone B (Fig. 3). Momilactone B was originally isolated from rice husks as a growth inhibitor (Kato et al. 1973) and later found in rice leaves and straw as a phytoalexin (Cartwright et al. 1977; 1981; Kodama et al. 1988; Lee et al. 1999). However, this compound has not been reported to be released from rice plants to the environment.

Momilactone A was also found in rice leaves and straw and its function as a phytoalexin has been extensively studied (Nojiri et al. 1996; Araki and Kurahashi 1999; Takahashi et al. 1999; Tamogami and Kodama 2000). Although biological activity of momilactone

B was much greater than that of momilactone A (Takahashi et al. 1976), the function of momilactone B is obscure.



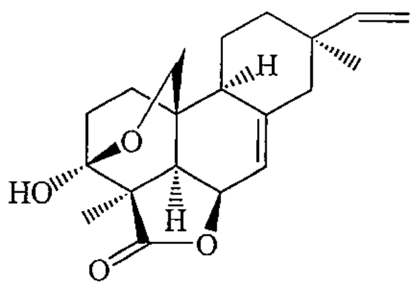


Figure 3. Momilactone B.

Figure 2. Rice hydroponics

Rice seedlings were hydroponically grown and a growth inhibitor, momilactone B, was isolated from the culture solution.

Biological activity

Momilactone B inhibited the root and hypocotyl growth of cress at concentrations greater than 3 nmol mL^{-1}, and those of lettuce at concentrations greater than 30 nmol mL^{-1}. The inhibition was increased with increasing concentrations of momilactone B. The doses required for 50% inhibition on roots and hypocotyls of cress, interpolated from the concentration-response curves, were 36 and 41 nmol mL^{-1}, respectively, and on those of lettuce were 56 and 79 nmol mL^{-1}, respectively.

Release level of momilactone B from rice seedlings

Three-day-old rice seedlings were transferred to hydroponics and the level of momilactone B released into the environment from the seedlings was determined. At 15 days after transfer to hydroponics, the level of momilactone B in the culture solution was 1.8 nmol per seedling and its endogenous level was 0.63 and 0.32 nmol per shoot and root, respectively (Fig. 4). The level in the culture solution was much greater than those in shoots and roots. These results suggest that the rice seedlings probably released momilactone B very actively into the culture solution because of its high level in the culture solution compared with the levels in the seedlings.

However, there is a possibility that momilactone B diffuses from rice husks to the culture solution during incubation because momilactone B was originally found in husks of rice seeds (Kato et al. 1973; Kato et al. 1977). One hundred and fifty mg of momilactone B were isolated from 200 kg of rice husks (Kato et al. 1973; Takahashi et al. 1976), which indicates that one husk (1.2 mg) contains 0.9 ng (2.7 pmol) of momilactone B. This value is negligible in comparison with the level of momilactone B in the culture solution. Thus, momilactone B is not supplied only from rice husks, which indicates that momilactone B may be produced and released from the seedlings into the culture solutions. In addition, since only the rice roots were immersed in the culture solution (Fig. 2), the rice seedlings can release momilactone B from their roots.

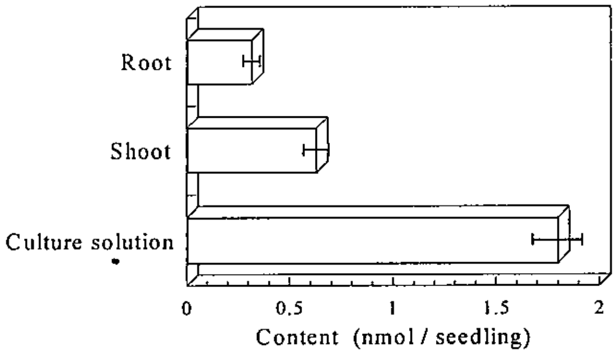


Figure 4. The content of momilactone B in culture solution and in shoots and roots of rice seedlings. One hundred rice seedlings were hydroponically grown with distilled water for 15 days. Then, the content of momilactone B in the culture solutions and in shoots and roots of rice seedlings were determined on per seedling basis. Means $\pm$ SE from three independent experiments with three assay for each determination are shown.

Allelopathy of rice

In the present research, momilactone B was found in roots and shoots of the rice seedlings and in their culture solution. These results and effectiveness of momilactone B on growth inhibition suggest that the rice seedlings may produce and release momilactone B into the environment, and momilactone B may act as an allelochemical by inhibiting growth of neighboring plants. Thus, momilactone B may play an important role in rice allelopathy.

Momilactone B had been found in rice leaves and shown to participate in defense of rice against pathogens (Cartwright et al. 1977; Kodama et al. 1988). Thus, momilactone B may have an important role in the defense system of rice not only against pathogens but also against the competition by neighboring plants for resources such as nutrients, light and water. Much research on rice allelopathy has been directed toward searching for its allelochemicals (Olofsdotter et al. 1995; Mattice et al. 1998; Olofsdotter et al. 1999). This is, however, the first research that found a potent growth inhibitor momilactone B is that released from living rice to the environment.

LITERATURE CITED

Araki, Y. and Kurahashi, Y. 1999. Enhancement of phytoalexin synthesis during rice blast infection of leaves by pre-treatment with carpropamid. J Pestic Sci 24: 369-374.

Cartwright, D. et al. 1977. Chemical activation of host defence mechanisms as a basis for crop protection. Nature 267: 511-513.

Cartwright, D.W. et al. 1981. Isolation and characterization of two phytoalexins from rice as momilactones A and B. Phytochemistry 20: 535-537.

Chou, C.-H. and Lin, H.-J. 1976. Autointoxication mechanism of *Oryza sativa*. I. Phytotoxic effects of decomposing rice residues in soil. J Chem Ecol 2: 353-367.

Chou, C.-H. and Chiou, S.-J. 1979. Autointoxication mechanism of *Oryza sativa*. II. Effects of culture treatments on the chemical nature of paddy soil and on rice productivity. J Chem Ecol 5: 839-859.

Dilday, R.H. et al. 1994. Identification of allelopathy in the USDA-ARS rice germplasm collection. Aust J Exp Agr 34: 907-910.

Dilday, R.H. et al. 1998. Allelopathic activity in rice for controlling major aquatic weeds. Pages 7-26 in M. Olofsdotter, ed. Allelopathy in Rice. International Rice Research Institute, Manila.

Duke, S.O. et al. 2000. Natural products as sources of herbicides: current status and future trends. Weed Res 10: 99-111.

Fuerst, E.P. and Putnam, A.R. 1983. Separating the competitive and allelopathic components of interference: Theoretical principles. J Chem Ecol 8: 937-944.

Kato, T. et al. 1973. Momilactones, growth inhibitors from rice, *Oryza sativa* L. Tetrahedron Lett 39: 3861-3864.

Kato, T. et al. 1977. Growth and germination inhibitors in rice husks. Phytochemistry 16: 45-48.

Kato-Noguchi, H. et al. 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. Physiol Plant 115: 401-406.

Kawaguchi, S. et al. 1997. Effects of aqueous extract of rice plants (*Oryza sativa* L.) on seed germination and radicle elongation of *Monochoria vaginalis* var. *plantaginea*. Plant Grow Regul 23: 183-189.

Kodama, O. et al. 1988. Induction of phytoalexins with heavy metal ions in rice leaves. J Pestic Sci 13: 615-617.

Kuwatsuka, S. and Shindo, H. 1973. Behavior of phenolic substances in the decaying process of plants. I. Identification and quantitative determination of phenolic acids in rice straw and its decayed product by gas chromatography. Soil Sci. Plant Nutr 19: 219-227.

Leather, G.R. and Einhelling, F.A. 1998. Bioassay of naturally occurring allelochemicals for phytotoxicity. J Chem Ecol 14: 1821-1828.

Lee, C.W. et al. 1999. Momilactones A and B in rice straw harvested at different growth stages. Biosci Biotechnol Biochem 63: 1318-1320.

Mattice, J. et al. 1998. Searching for allelochemicals in rice that control ducksalad. Pages 81-98 in M. Olofsdotter, ed. Allelopathy in Rice. International Rice Research Institute, Manila.

Narwal, S.S. 1999. Allelopathy in weed management. Pages 203-254 in: S.S. Narwal, ed. Allelopathy Update, Volume 2, Basic and Applied Aspects. Science Publishers Inc., Enfield, New Hampshire.

Nojiri, et al. 1996. Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. Plant Physiol 110: 387-392.

Olofsdotter, M. et al. 1995. Allelopathic potential in rice (*Oryza sativa* L.) germplasm. Ann Appl Biol 127: 543-560.

Olofsdotter, M. et al. 1999. Weed-suppressing rice cultivars – does allelopathy play a role? Weed Res 39: 441-454.

Putnam, A.R. 1988. Allelochemicals from plants as herbicides. Weed Tech 2: 510-518.

Rizvi, S.J.H. and Rizvi, V. 1992. Allelopathy: Basic and Applied Aspects. Chapman and Hall. London.

Seigler, D.S. 1996. Chemistry and mechanisms of allelopathic interactions. Agron J 88: 876-885.

Takahashi, A. et al. 1999. Lesion mimic mutants of rice with alterations in early signaling events of defense. Plant J 17: 535-545.

Tamak, J.C. et al. 1994. Effect of aqueous extracts of rice stubbles and straw and stubbles on the germination and seedling growth of wheat, oat, berseem and lentil. Crop Res 8: 180-185.

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.

stages. Biosci Biotechnol Biochem 63: 1318-1320.

Mattice, J. et al. 1998. Searching for allelochemicals in rice that control ducksalad. Pages 81-98 in M. Olofsdotter, ed. Allelopathy in Rice. International Rice Research Institute, Manila.

Narwal, S.S. 1999. Allelopathy in weed management. Pages 203-254 in: S.S. Narwal, ed. Allelopathy Update, Volume 2, Basic and Applied Aspects. Science Publishers Inc., Enfield, New Hampshire.

Nojiri, et al. 1996. Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. Plant Physiol 110: 387-392.

Olofsdotter, M. et al. 1995. Allelopathic potential in rice (*Oryza sativa* L.) germplasm. Ann Appl Biol 127: 543-560.

Olofsdotter, M. et al. 1999. Weed-suppressing rice cultivars -- does allelopathy play a role? Weed Res 39: 441-454.

Putnam, A.R. 1988. Allelochemicals from plants as herbicides. Weed Tech 2: 510-518.

Rizvi, S.J.H. and Rizvi, V. 1992. Allelopathy: Basic and Applied Aspects. Chapman and Hall. London.

Seigler, D.S. 1996. Chemistry and mechanisms of allelopathic interactions. Agron J 88: 876-885.

Takahashi, A. et al. 1999. Lesion mimic mutants of rice with alterations in early signaling events of defense. Plant J 17: 535-545.

Tamak, J.C. et al. 1994. Effect of aqueous extracts of rice stubbles and straw and stubbles on the germination and seedling growth of wheat, oat, berseem and lentil. Crop Res 8: 180-185.

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.

Herbicide Resistance

Managing A Biotype of Wild Radish (*Raphanus raphanistrum*) Resistant to Diflufenican and Triazines

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Abstract: A long-term rotation experiment confirmed that when challenged by a dense population of wild radish (*Raphanus raphanistrum* L.) that has evolved multiple resistances to diflufenican and triazines, the ability to control the population at the outset is crucial to prevent enrichment of the wild radish seedbank. A single break year of very little seed set in 2000 was found to have a dramatic impact on the enrichment process. This was achieved by planting a wheat crop, followed by the application of bromoxynil + MCPA. Planting lupins or triazine-tolerant canola only exacerbated the problem because no suitable alternative herbicide could be used to control the population. Mowing the population in a single pasture year resulted in regrowth of the wild radish, which subsequently produced seeds. In 2001, when the pasture *Cadiz serradella* was planted to allow seed set management of wild radish in spring, green or brown manuring of Cadiz was found to be more effective than mowing to prevent wild radish seed set. This long-term experiment will continue over the next three years and the wild radish seed bank will be monitored following the implementation of more control options. The final objective is to identify the best strategies in managing this population. In addition to this long-term experiment, an herbicide screening trial confirmed that picolinafen, a lupin herbicide with the same mode of action as diflufenican, failed to control the population. Diflufenican on its own or mixtures of diflufenican with simazine or metribuzin also failed to give effective control. However, the herbicide mixtures chlorsulfuron + MCPA, diflufenican + bromoxynil, diflufenican + MCPA, and picolinafen + MCPA, which are all common mixtures for wild radish control in cereals, gave effective control. The effectiveness of diflufenican + bromoxynil is interesting and could be explained in terms of bromoxynil having a different binding behaviour from the triazines at the binding protein D1 in photosystem II.

Key words: diflufenican, herbicide resistance, *Raphanus raphanistrum*, triazines

INTRODUCTION

Wild radish (*Raphanus raphanistrum* L.), an intractable broadleaf weed of Australian agriculture, is rapidly evolving resistance to several herbicide groups in Western Australia (Cheam et al. 2000). Its resistance to ALS-inhibiting herbicides (Group B herbicides) is already widespread (Hashem et al. 2001; Walsh et al. 2001). The most significant recent discovery is a biotype that has evolved multiple resistances to diflufenican (a Group F herbicide) and triazines (Group C herbicides). This development of multiple resistances, if left unchecked, can become a serious problem because of the importance of wild radish in Australian and world agriculture. In Australia alone, such a development means the loss of diflufenican and triazines for wild radish control in lupins and the loss of triazines for wild radish control in triazine-tolerant (TT) canola. With the loss of diflufenican and triazines, growing lupins or TT canola where wild radish is a problem may not be possible using current varieties. No suitable alternative herbicides with a different mode of action are currently available to replace this loss in both crops. In anticipation of these problems, long and short term experiments have been initiated to learn quickly how to control and manage this first case of wild radish that has evolved multiple resistance to diflufenican and triazines.

MATERIALS AND METHODS

Long-term experiment

A long-term experiment was started in 2000 on the original site with confirmed wild radish resistance to diflufenican and triazines. The aim of this experiment is to develop suitable rotation systems to quickly run down the wild radish seed bank to enable sustainable cropping to continue. The experimental design was a randomised complete block with four replicates of five rotation treatments; each treatment within a 6 m by 25 m plot. The cropping programs and management practices in the first two years of the experiment are shown in Table 1.

Table 1. Rotation treatments imposed in 2000 and 2001.

Year	Rotation					
	A	B	C	D	E	F
2000	TT Canola (Atrazine 2 L pre-em., 2 L post-em.)	Wheat (Buctril MA 1.4 L)	Lupin (Simazine 2 L pre-em., Brodal 100 mL + Lexone 100 g)	Lupin (Simazine 2 L pre-em., Brodal 200 mL)	Lupin (Brodal only 200 mL)	Cadiz serradella (mowed)
2001	Cadiz serradella (Green manuring followed by glyphosate 3 L to kill survivors)	Cadiz serradella (Mowing once only)	Cadiz serradella (Hay freezing with glyphosate 3 L and followed by mowing of survivors)	Cadiz serradella (Hay freezing with glyphosate 3 L)	Cadiz serradella (Green manuring with cultivation)	Cadiz serradella (Mowing twice)

The active ingredient concentrations of the herbicides used were as follows: atrazine (500 g L^{-1}), Buctril MA® (bromoxynil 200 g L^{-1} + MCPA iso-octyl ester 200 g L^{-1}), simazine (500 g L^{-1}), Brodal® (diflufenican 500 g L^{-1}), Lexone® (metribuzin 750 g kg^{-1}) and glyphosate (450 g L^{-1}).

The experiment was diverted to a pasture phase using *Cadiz serradella* in the 2001 season. This was to allow the use of non-selective herbicides and management practices, such as green manuring and slashing, to control the seed production of wild radish in spring. Forty soil cores per plot were obtained at the start of the 2001 season in early May to determine the wild radish seed bank level. The amount of seed in the soil is a good criterion to establish the efficacy of the 2000 treatments. Each soil core measured 4.5 cm in diameter and 10 cm deep. After the soil sampling, the top halves of all plots received a shallow cultivation to stimulate wild radish germination. The bottom halves were left undisturbed. Cadiz pasture was seeded in mid-June 2001. Wild radish emergence was assessed in all plots 10 weeks after pasture seeding. A final assessment of wild radish regeneration and seed production was done at the end of the 2001 season to determine the effectiveness of the spring management treatments.

Short-term experiment

A short-term field experiment with the aim of assessing the impact of alternative herbicides on the population was established in the 2002 season in a non-cropping situation. Some of the key herbicides investigated included mixtures like chlorsulfuron + MCPA, diflufenican + simazine, diflufenican + metribuzin, diflufenican + bromoxynil, diflufenican + MCPA and picolinafen + MCPA. Herbicides on their own included diflufenican, atrazine and picolinafen. Four of the treatments are herbicides for wild radish control in cereals only, not in lupins. The rest are herbicides for use in lupins (Table 3).

A known susceptible wild radish population was included for comparison. Seeds from the resistant and susceptible populations were sown to a depth of 2 cm in 1 m^2 plots. One hundred seeds per population were sown separately onto each plot. All herbicide treatments were applied when the wild radish seedlings were at the three-to four-leaf stage of development. The rates used are shown in Table 3. Five weeks after treatment, plant survival was recorded. Survival was expressed as a percentage of the number of established plants in each plot prior to treatment.

RESULTS AND DISCUSSION

Long-term experiment

The wild radish seed bank level determined at the start of the 2001 season ranged from 1,290 seeds m^{-2} in rotation B to 13,480 seeds m^{-2} in rotation A (Fig. 1). As expected, it was the efficacy of the wild radish control in 2000 that determined the seed bank density in 2001. The lowest density corresponded with the best 2000 treatment (Rotation B), while the highest density corresponded with the worst 2000 treatment (Rotation A). In 2000, Rotation B was under a wheat phase sprayed with Buctril MA (bromoxynil + MCPA), which resulted in excellent control of wild radish with very low seed input at the end of the season. In contrast, with Rotation A, wild radish survival was high in the 2000 TT canola because of the failure of atrazine to control the triazine-resistant wild radish. This resulted in a high seed rain in Rotation A. The relatively high seed bank, ranging from 5,210 to 8,120 seeds m^{-2} in Rotations C, D, and E, is expected because diflufenican on its own or in combination with metribuzin gave unsatisfactory control of wild radish in 2000 because of its multiple resistance to diflufenican and the triazines. The 2000 slashing treatment in Rotation F which resulted in a lower seed bank than Rotations A, C, D and E, was considered a satisfactory treatment despite the regrowth of wild radish, which subsequently produced seeds to recharge the seed bank.

Based on the wild radish emergence data obtained in 2001, the amount of seed loss from the seed bank due to emergence ranged from 0.8 to 4.7 %. Cultivation did not result in higher wild radish emergence than the undisturbed treatment. Cultivation could have contributed to further drying of the soil due to a delay in rainfall after cultivation.

Figures below each rotation treatment indicate the soil seed reserves of wild radish (seeds m^{-2} to 10 cm depth) in the respective treatment at the start of 2001 season. Vertical bars indicate ± SE of the means. Results of the 2001 spring treatments are shown in Table 2.

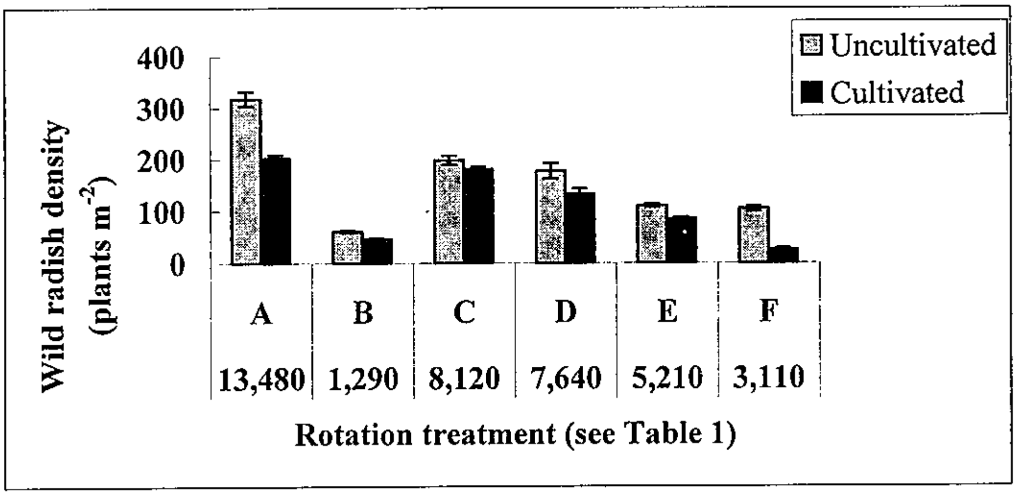

Figure 1. Density of wild radish by the end of winter 2001.

Table 2. Number of reproductive wild radish plants and seed production per plant in 2001.

Treatment	Spring programme	Reproductive wild radish (plants m^{-2})		Wild radish seed production (seeds $plant^{-1}$)	
		Uncultivated	Cultivated	Uncultivated	Cultivated
B	Mowed once	12	10	136	225
F	Mowed twice	19	11	132	264
A	Green manuring, glyphosate	0	0	0	0
C	Glyphosate, mowing	0	0	0	0
D	Glyphosate	0	0	0	0
E	Green manuring	0	0	0	0

Except the mowing treatments (B and F), all treatments gave total kill of wild radish. The late spring rainfall sustained the survival of regrowths after mowing. The survivors managed to produce seeds at the end of the season. Greater seed production per plant was noted in cultivated plots because of the larger wild radish plants that emerged earlier than plants in uncultivated plots. Therefore, overall, green or brown manuring of Cadiz is a more effective method than mowing to prevent wild radish seed set. Further monitoring of this long-term experiment is in progress and more treatments will be imposed over the next few years to run down the wild radish seed bank. Since wild radish has a long-term seed longevity, it is hoped that after six years or so, sustainable and efficient strategies for managing the diflufenican and triazine multiple resistant population can be identified.

Short-term experiment

The herbicide screening results (Table 3) confirmed that the commonly recommended herbicides for wild radish control in lupins are no longer effective on the diflufenican and triazine multiple resistant wild radish. Cross-resistance to picolinafen is not surprising since both diflufenican and picolinafen have the same mode of action, targeting the enzyme phytoene desaturase. Therefore, any resistance management strategy involving diflufenican-resistant wild radish should not be based on switching to picolinafen in lupins. As expected, atrazine did not control the population. However, the population can still be controlled with herbicides in the wheat phase, as seen in the screening results (Table 3).

Table 3. Survival of resistant and susceptible populations of wild radish 5 weeks after treatment with various herbicides at the recommended rate.

Herbicide and rate (g a.i. ha^{-1})	Survival (%)	
	Resistant	Susceptible
Lupin herbicides		
Diflufenican (100 g)	55.1	0
Diflufenican (50 g) + Simazine (500 g)	47.9	0
Diflufenican (50 g) + Metribuzin (75 g)	22	0
Picolinafen (37.5 g)	60.2	0
Atrazine (1.0 kg) + 1 % Ulvapron	100	0
Cereal herbicides		
Chlorsulfuron (3.75 g) + MCPA LVE 50% (250 g)	7	3
Diflufenican (12.5g) + Bromoxynil (125 g)	0	0
Diflufenican (12.5g) + MCPA ester (125 g)	74.1 (4.8)*	65.9 (5.8)*
Picolinafen (12.5 g) + MCPA ester (125 g)	81.3 (2.9)*	63.9 (2.9)*

* Figures within brackets are fresh weight per plant expressed as a percentage of the unsprayed control.

Since the population is still susceptible to the ALS inhibitors and phenoxy herbicides, based on preliminary tests in the glasshouse, the common mixture chlorsulfuron + MCPA still gave excellent control, resulting in complete kill at 10 weeks after application despite the initial small number of survivors at 5 weeks afer spraying.

The effectiveness of diflufenican + bromoxynil is interesting. Since diflufenican and bromoxynil are classified under Group F and Group C, respectively, based on the present Australian herbicide classification system, one would be tempted to think that the mixture is unlikely to be effective on the population. However, under the international Herbicide Resistance Action Committee (HRAC) classification system, bromoxynil is placed under Group C3 and the triazines and metribuzin under C1 (Schmidt 2002). Unlike the triazines, bromoxynil has a different binding behaviour at the binding protein D1 in photosystem II. This protein is also called the herbicide or Q_B binding protein (Pfister et al. 1981; Kyle 1985). Trebst (1987) grouped herbicides that bind in the Q_B niche into two families based on their interaction with amino acids at this site: the triazine/urea family which shows a strong interaction with Ser 264 and the phenol family that interacts strongly with His 215. Bromoxynil, being a nitrile, belongs to the phenol family. According to Trebst (1991), mutations in triazine resistance lead to an increased sensitivity to phenol-type herbicides. This, together with the fact that bromoxynil has an additional mode of action involving membrane disruption (Schmidt 2002), probably accounts for the effectiveness of the bromoxynil + diflufenican mixture.

Another significant result was the effectiveness of the mixtures diflufenican + MCPA and picolinafen + MCPA, despite the resistance of the population to one of the components in the mixture. This was clearly evident in the suppression of the wild radish biomess rather than plant mortality. Therefore, once a herbicide is no longer effective on a population due to resistance, mixing it with an appropriate herbicide having a different mode of action may result in the control of the resistant population, as shown in this study. All knowledge gained to date has been extended and demonstrated to farmers.

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LITERATURE CITED

Cheam, A.H. et al. 2000. Triazine and diflufenican resistance in wild radish (*Raphanus raphanistrum* L.). Proceedings 3rd International Weed Congress, Foz do Iguassu, Brazil, pp.143.

Hashem, A. et al. 2001. Resistance of wild radish (*Raphanus raphanistrum*) to acetolactate synythase inhibiting herbicides in the Western Australian Wheatbelt. Weed Technology, 15: 68 – 74.

Kyle, D. J. 1985. The 32 000 Dalton Q_B protein of photosystem II. Photochemistry and Photobiology, 41:107 – 116.

Pfister, K. et al. 1981. Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes. Proceedings of the National Academy of Sciences USA, 78: 981 –985.

Schmidt, R. R. et al. 2002. HRAC classification of herbicides according to mode of action. Herbicide Resistance Action Committee. http://plantprotection.org/HRAC/MOA.html

Trebst, A. 1987. The three-dimensional structure of the herbicide binding niche on the reaction centre polypeptides of photosystem II. Zeitschrift fur Naturforschung, 42 C: 742 –750.

Trebst, A. 1991. The molecular basis of resistance of photosystem II herbicides. In J. C. Caseley, G. W. Cussans and R. K. Atkin, eds. Herbicide Resistance in Weeds and Crops. Oxford: Butterworth – Heinemann. Pp. 145 –164.

Walsh, M. J. et al. 2001. High frequency of wild radish (*Raphanus raphanistrum* L.) resistance to the ALS-inhibiting herbicides across the Western Australian Wheatbelt. Weed Technology, 15:199 –203.

Effect of Bentazon Plus MCPA (Basagran M60*) on *Monochoria vaginalis* Resistant and Susceptible to Sulfonylurea Herbicides in Rice (*Oryza sativa*)

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Abstract: *Monochoria vaginalis* is one of the troublesome resistant weeds in Korean rice. In greenhouse studies, GR_{50} ratios between resistant and susceptible biotypes of *M. vaginalis* were 31, 38, 3172 and 7 to bensulfuron-methyl, cyclosulfamuron, imazosulfuron and pyrazosulfuron-ethyl, respectively, when applied at 2-leaf stages, indicating cross-resistance to the ALS-inhibiting herbicides used in this study. ALS inhibitors combined with annual herbicides did not control the resistant biotypes of *M. vaginalis* satisfactorily. *In vitro* ALS assay showed that the ratio of I_{50} values between resistant and susceptible biotypes to bensulfuron-methyl, cyclosulfamuron, imazosulfuron and pyrazosulfuron-ethyl were 35, 13, 183 and 31, respectively, when applied at 2-leaf stages. In field studies, bentazon plus MCPA at 1200+180 g a.i. ha^{-1} or higher gave good to excellent control of *M. vaginalis* resistant and susceptible to sulfonylurea herbicides but the recommended rate of application must be doubled to control it with bentazon solo. Also, bentazon plus MCPA showed faster activity than bentazon solo or bentazon plus cyhalofop-butyl in controlling *M. vaginalis*. This study indicates that bentazon plus MCPA is a promising formulation to rescue the resistant weed problem in Korean rice.

Key words: acetolactate synthase (ALS), bentazon plus MCPA, herbicide resistance, *Monochoria vaginalis* (MOOVA), rice, sulfonylurea herbicides, weed control.

INTRODUCTION

Bentazon plus MCPA is a liquid formulation in a well balanced combination of two proven herbicidal active ingredients for post-emergence control of the most important broadleaved weeds and sedges in rice, developed and produced by BASF AG. Bentazon is a contact herbicide with exceptional selectivity in rice and blocks the photosynthetic electron transport in susceptible plants. MCPA is a hormone-type compound with systemic action (BASF 1995).

The sulfonylurea herbicides inhibit the enzyme acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched-chain amino acids valine, leucine and isoleucine (Brown 1990). Once translocated to the site of action, the sulfonylureas inhibit ALS activity, causing death of meristematic cells, resulting in plant death.

In Korea, sulfonylurea herbicides, combined with soil-applied annual herbicides, have been consecutively used in rice fields since 1990. Such repeated use of the sulfonylureas resulted in the development of resistant weeds such as *M. vaginalis*(Kwon et al. 2000), *Monochoria korsakowii* (Park et al. 1999), *Rotala indica* (Kwon et al. 2001), *Lindernia dubia* (Park et al. 2000), *Cyperus difformis* (Kuk et al. 2002), and *Scirpus juncoides* (Kuk et al. 2002). The resistant biotype of *M. vaginalis* was first reported in 2000 in Korea (Kwon et al. 2000). In most cases, resistance to ALS-inhibiting herbicides is due to an insensitive site of action caused by point mutation in the ALS gene (Saari et al. 1994).

M. vaginalis resistant to sulfonylurea herbicides can grow as vigorously as the susceptible one. When competed with rice for season-long, resistant biotypes of *M. vaginalis* caused severe yield loss of 70% and 44% in seeded- and transplanted-rice, respectively (Kwon et al. 2002). The problem with resistant weeds in rice would greatly be increased with time unless there are alternative chemicals or rotations of herbicide use with different modes of action.

The objectives of this study were to evaluate the response of *M. vaginalis* resistant to sulfonylurea herbicides and to suggest an alternative herbicide to solve the resistant problem with *M. vaginalis*, once failed to control, in seeded- and transplanted-rice in Korea.

MATERIALS AND METHODS

Plant materials

Seeds of suspected SU-resistant *M. vaginalis* biotypes were collected from a field of Jeonnam Agricultural Research and Extension Service in Korea, where sulfonylurea herbicides were applied for the past 8 years consecutively and had failed to control *M. vaginalis* in 2001. The susceptible biotype of *M. vaginalis* seeds was collected from a field where sulfonylurea herbicides have never been used for the past three years. The seeds of both biotypes were stored at 4°C for one month to break dormancy.

Response of *M. vaginalis* to sulfonylurea herbicides

Seeds of *M. vaginalis* were sown in plastic pots (280 cm^2) filled with clay loam soil. Then, the pots were flooded 3-cm deep and arranged in a completely randomized design in a greenhouse at 30/25°C and 14/10 h in day/night. At 2-leaf stages of *M. vaginalis*, sulfonylurea herbicides were applied at various application rates. The recommended rates of bensulfuron-methyl, cyclosulfamuron, imazosulfuron, and pyrazosulfuron-ethyl were 51, 60, 75, and 21 g a.i. ha^{-1}, respectively. The responses of *M. vaginalis* were evaluated at 20 days after treatment by measuring shoot dry weight.

In vitro ALS assay

Enzyme extraction and assay were done based on the method of Ray (1984) with some modifications. Four g of leaf tissues harvested from the seedlings of 6- to 7-leaf stages was frozen in liquid nitrogen and ground in 10 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate (TPP), 10 μM flavin adenine dinucleotide, 0.5% polyvinylpyrrolidone, and 10%(v/v) glycerol. The homogenate was filtered through four layers of Miracloth, then centrifuged at 20,000 g for 20 min at 4°C. ALS in the supernatant was precipitated by 45% ammonium sulfate. The crude extract was placed on ice for 30 min and centrifuged at 20,000 g for 20 min at 4°C. The pellet was suspended in 1 mL elution buffer. The extract was desalted on Sephadex G-25 column (PD-10) equilibrated with elution buffer [70 mM potassium phosphate buffer (pH 7.5) containing 80 mM sodium pyruvate, 0.5 mM $MgCl_2$, and 0.5 mM TPP], and the crude enzyme preparation was collected in 2.5 mL tube. Two hundred μl of the enzyme extract and 10 μL of herbicide solution were added to 790 μL of assay buffer [70 mM potassium phosphate buffer (pH 7.5) containing 80 mM sodium pyruvate, 0.5 mM $MgCl_2$, and 0.5 mM TPP] and the solution was incubated at 37°C for 1 h. The final concentrations of each herbicide in the assay solutions were 0, 0.001, 0.01, 0.1, 1.0, 10, 100, and 1,000 μM. At the end of the reaction time, 50 μl of 6 N H_2SO_4 was added to 500 μL of each solution and the solutions were incubated at 60°C for 30 min to convert acetolactate to acetoin (pink color). To evaluate the color change due to ALS activity, 50 μl of 2 N NaOH was added to 500 μL of the remaining supernatant, instead of H_2SO_4, and a reference absorbance was measured according to the following procedure. Acetoin was quantified by a modified colorimetric assay (Westerfeld 1945). The color was developed by adding 0.5 ml of 0.5% (w/v) creatine and 0.5 ml of 5% (w/v) α-naphthol prepared in 2.5 N NaOH, then heated at 60°C for 15 min. After cooling, the absorbance was measured by a spectrophotometer at 530 nm. Total ptotein content in the crude ALS extracts was determined by the method of Bradford (1976). Data were analyzed by using non-linear regressions, using SAS program. I_{50} values were calculated from the regression equations.

Field experiments

Experiments were conducted in 2002 to investigate the effect of Bentazon plus MCPA to control *M. vaginalis* resistant and susceptible to sulfonylureas (SUs) and control *Sagittaria trifolia* in seeded- and transplanted-rice.

In water-seeded rice at Jeonnam Agricultural Research and Extension Service, pyrazosulfuron-ethyl plus molinate at 21+1500 g a.i. ha^{-1} was applied at 10 days after seeding but failed to control *M. vaginalis*. In the field, a trial of randomized block design with 3 replicates was laid out in the plot size of 15 m^2 (3 by 5 m). At 65 days after seeding, bentazon solo at 960, 1920 and 3840 g a.i. ha^{-1}, and bentazon plus MCPA at 1200+180, 1800+270 and 2400+360 g a.i. ha^{-1} were sprayed over the top. The recommended rates of application for bentazon and bentazon plus MCPA are 1920 and 1200+180 g a.i. ha^{-1}, respectively. Visual evaluations on efficacy and crop injury were made at 7 and 14 days after application.

In transplanted rice, two trials were conducted in Kyonggi and Chungnam, Korea in 2002. Randomized block designs with 3 replicates were laid out in the plot size of 15 m^2. Rice was transplanted on May 17 and 21, 2002 and herbicide applications were made at 35 and 48 days after rice transplanting in Kyonggi and Chungnam, respectively. Efficacy and crop injury were visually evaluated at 10, 20 and 30 days after application.

Table 1. Weed species, location and growth stage at the time of treatment in the trials.

Location	Weed	
	Species	Growth stage
JARES[a]	*Monochoria vaginalis*	8-main leaf, 40 cm high
Chungnam	*Monochoria vaginalis*	4-main leaf, 20 cm high
Kyonggi	*Monochoria vaginalis*	2-main leaf, 15 cm high
	Bidens tritiparca	15 cm high
	Sagittaria trifolia	3-4 early leaf, 10 cm high

[a]Jeonnam Agricultural Research and Extension Service.

RESULTS AND DISCUSSION

Response of *M. vaginalis* to sulfonylurea herbicides

The susceptible *M. vaginalis* was very sensitive to the herbicides used in the test and was completely controlled at one-fourth of the recommended rates (data not shown). This could be possible because it was tested in pots in a controlled environment. The GR_{50} values of the resistant and susceptible *M. vaginalis* when applied at 2 leaf stages with bensulfuron-methyl, cyclosulfamuron, imazosulfuron and pyrazosulfuron-ethyl are shown in Table 2. The response of *M. vaginalis* between resistant and susceptible biotypes to the herbicides used was great. Resistance levels of *M. vaginalis* to bensulfuron-methyl, cyclosulfamuron, imazosulfuron and pyrazosulfuron-ethyl were 31, 38, 3172 and 7 times, respectively, indicating that *M. vagianlis* showed cross-resistance to the ALS-inhibiting herbicides being used for weed control in rice, although there is a big difference in the level of resistance among the herbicides tested. Hwang et al. (2001) reported a similar pattern with bensulfuron-methyl and pyrazosulfuron-ethyl when ALS inhibitors were applied at 10 days after seeding in a greenhouse. Such a big difference in the response between resistant and susceptible biotypes of *M. vaginalis* would really be a problem in rice fields practically.

When the ALS-inhibiting herbicides combined with annual herbicides that have different modes of action were applied at 2-leaf stages of *M. vaginalis*, the susceptible biotypes were completely killed, except for the combination of pyrazosulfuron with molinate, which reduced shoot dry weight 85% (Table 3). But the shoot dry weight of the resistant biotypes was reduced only by 22 to 56% depending on the combinations.

This result suggest that control of *M. vaginalis* resistant to sulfonylurea herbicides might not be possible with butachlor, molinate and pretilachlor when applied at 2-leaf stages. It is not sure whether *M. vaginalis* resistant to sulfonylurea herbicides is to some

Table 2. Response of *M. vaginalis* is resisteant ® and susceptible (S) to ALS-inhibiting herbicides.

Biotypes	GR_{50}[a] (g a.i. ha^{-1})			
	Bensulfuron-methyl	Cyclosulfamuron	Imazosulfuron	Pyrazosulfuron-ethyl
R	56	67	1586	3.67
S	1.83	1.77	0.5	0.49
R/S ratio	31	38	3172	7

[a]GR_{50} values were herbicide concentrations that reduced shoot dry weight by 50%, calculated from the regression equations.

resistant to sulfonylurea herbicides did not show a mutiple resistance to the herbicides such as butachlor, simazine and thiobencarb. Further trials in terms of application timings and rates are necessary to evaluate the efficacy of herbicides having different modes of actions from sulfonylurea herbicides.

***In vitro* ALS activity**

The specific activity of in vitro ALS extracted from the shoot tissue of *M. vaginalis* was similar in both resistant and susceptible biotypes in terms of acetoin production (data not shown). *In vitro* ALS activity of resistant biotype was 35, 13, 183 and 31 times higher than that of susceptible biotype to bensulfuron-methyl, cyclosulfamuron, imazosulfuron, and pyrazosulfuron-ethyl, respectively (Table 4). It was confirmed that the ALS of susceptible biotype was sensitive but that of resistant one was very insensitive to all sulfonylurea herbicides tested.

Table 3. Effect of sulfonylurea mixtures on susceptible (S) and resistant ® biotypes of *M. vaginalis* when applied 2 leaf stages.

Treaments	Rate applied (g a.i, ha^{-1})	Shoot dry weight (%) [a]	
		S	R
Bensulfuron-methyl +butachlor	51+750	100	56
Cyclosulfamuron +molinate	60+2100	100	49
Imazosulfuron +pretilachlor	75+300	100	52
Pyrazosulfuron-ethyl +molinate	21+1500	85	22

[a]Reduction of shoot dry weight in percentage based on the untreated check.

Table 4. In vitro inhibition of ALS activity of M. vaginalis resistant (R) and susceptible (S) to sulfonylurea.

Biotype	I_{50} value (uM)[a]			
	Bensulfuron-methyl	Cyclosulfuron	Imazosulfuron	Pyrazosulfuron-ethyl
R	39.06	0.035	5.294	12.87
S	1.11	0.0027	0.059	0.42
R/S[b]	65	13	183	31

[a]Herbicide concentrations that reduced ALS activity by 50% calculated from the regression equations.
[b]The ratio calculated from I_{50} value of R-type divided by that of S-type.

Field studies

Control of resistant M. vaginalis in water-seeded rice. After failure of *M. vaginalis* control by the treatment of pyrazosulfuron plus molinate at 10 days after seeding, a spray application of bentazon solo or bentazon plus MCPA was made at 65 days after seeding to control *M. vaginalis* resistant to a sulfonylurea herbicide pyrazosulfuron-ethyl. The application timing was later than the recommended in this trial. At 14 days after application, bentazon at 3840 and bentazon plus MCPAat 1200+180 or higher controlled the resistant biotype more than 90%, but bentazon at the recommended rate (1920 g a.i. ha^{-1}) did not control it satisfactorily (Fig.1). The result at 7 days after application was the same as that at 14 days after application (data not shown). This result indicated that even at later stages of growth, bentazon plus MCPA can control *M. vaginalis* resistant to sulfonylurea herbicides in water-seeded rice. A similar result was obtained in a field study that found *M. vaginalis* resistant to ALS-inhibiting herbicides well controlled by the treatment of bentazon combined with 2,4-D after failure of control by the treatment of pyrazosulfuron-ethyl plus molinate (Kwon et al. 2000). No phytotoxic symptom was observed by any treatment in the study.

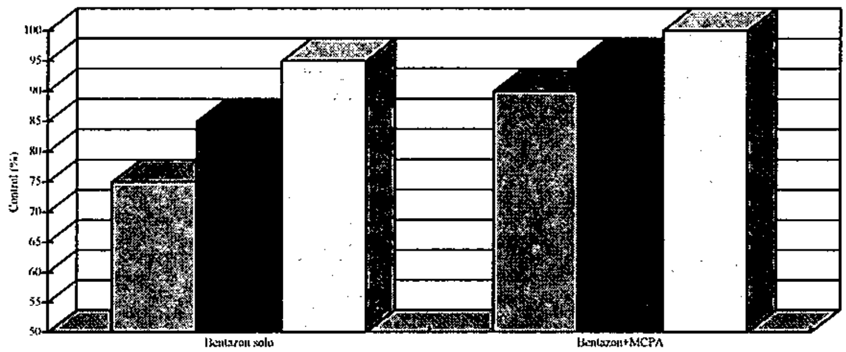

Figure 1. Control of M. vaginalis resistant to sulfonylurea herbicides by applying bentazon solo and bentazon plus MCPA in water-seeded rice. (Bentazon was applied at 960, 1920 and 3840 g a.i. ha^{-1}; bentazon plus MCPA was applied at 1200+180, 1800+270 and 2400+360 g a.i. ha^{-1}).

Control of susceptible M. vaginalis, S. trifolia and *Bidens tripartita in transplanted rice.* Excellent control of susceptible *M. vaginalis* was obtained by the treatment of bentazon plus MCPA, regardless of the application rates (Table 5). In addition, bentazon plus MCPA showed faster acting characters than bentazon solo or bentazon plus cyhalopfop-butyl which was not satisfactory in controlling *M. vaginalis.* In controlling *B. tripartita,* bentazon plus MCPA at 1200+180 g a.i. ha^{-1} or higher gave satisfactory control but bentazon solo or bentazon plus cyhalofop-butyl failed to control it in this trial. All treatments controlled *S. trifolia,* the most difficult-to-control weed in rice, 80 to 90% at 30 days after application. There was a slight crop injury with the treatment of bentazon plus MCPA observed at 10 days after application but it was recovered soon (data not shown).

Table 5. Effect of Bentazon solo and Bentazon+MCPA on *M. vaginalis, S. trifolia* and *Bidens tritiparca* in transplanted rice (Kyonggi).

Herbicide	Rate applied	Weed control (%)								
		Monochoria vaginalis			*Sagittaria trifolia*			*Bidens tripartita*		
	(g a.i./ha)	10 DAA[a]	20 DAA	30 DAA	10 DAA	20 DAA	30 DAA	10 DAA	20 DAA	30 DAA
Bentazon solo	2400	40	75	90	40	60	90	20	40	55
Bentazon+Cyhalofop-butyl	1650+300	30	70	85	30	50	80	10	20	20
Bentazon+MCPA	800+120	64	90	98	30	50	80	48	70	60
Bentazon+MCPA	1200+180	70	100	100	50	70	85	50	90	90
Bentazon+MCPA	2400+360	85	100	100	50	70	90	60	95	95

[a]DAA indicates Days After Application.

Another trial conducted in transplanted rice of Chungnam showed that bentazon plus MCPA at the recommended rate controlled *M. vaginalis* susceptible to sulfonylurea herbicides excellently and fast (Fig. 2). On the other hand, the activity of bentazon solo and bentazon plus cyhalofop-butyl at the recommended rate was slow and low, compared with that of bentazon plus MCPA. No crop injury was observer in any treatment in this location.

Results of this study indicate that there is a big difference in the response between resistant and susceptible biotypes of *M. vaginalis* to ALS- inhibiting rice herbicides. Such a difference would not make it possible to practically control the resistant *M. vaginalis* with sulfonylurea herbicides. Therefore, it is essential to use herbicides with different modes of action or to develop post-emergence herbicides to rescue the problem. In this study, bentazon plus MCPA when applied post-emergent performed excellently to control *M. vaginalis* resistant and susceptible to sulfonylurea herbicides. Also, it showed faster activity than bentazon solo or bentazon plus cyhalofop-butyl in controlling *M. vaginalis*, regardless of sensitivity to ALS-inhibiting herbicides.

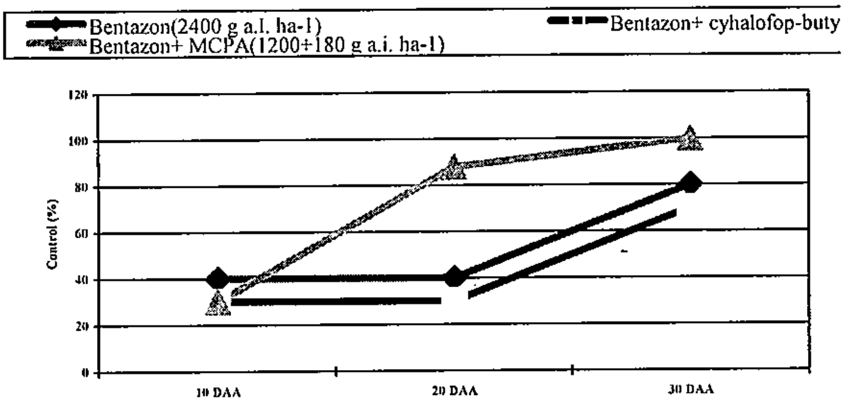

Figure 2. Control of M. vaginalis susceptible to sulfonylurea herbicides by applying bentazon solo, bentazon plus MCPA and bentazon plus cyhalofop-butyl in transplanted rice (Chungnam).

LITERATURE CITED

BASF. 1995. Basagran M60 – A liquid formulation for post-emergence weed control in rice. 12 pages.

Bradfors, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

Brown, H.M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides.Pestic. Sci. 29:263-281.

Hwang, I.T., K.H. Lee, S.H. Park, B.H. Lee, K.S. Hong, S.S. Han, and K.Y. Cho. 2001. Resistance to acetolactate synthase(ALS) inhibitors in a biotype of *Monochoria vaginalis* discovered in Korea. Proc. Of the 18th Asian-Pacific Weed Sci. Soc. Conf. 18:465-470.

Kuk, Y.I., O.D. Kwon, I.B. Im. 2002. Sulfonylurea herbicide-resistant Scirpus juncoides Robx. In Korean rice culture. Korea J. of Weed Sci. 22:296-305.

Kuk, Y.I., K.H. Kim, O.D. Kwon, D.J. Lee, N.R. Burgos, S.C. Lee, and J.O. Guh. 2002. Cross-resistance pattern and alternative herbicides for *Cyperus difformis* resistant to sulfonylurea herbicides in Korea. Pest Management Sci.(sumbitted).

Kwon, O.D., S.J. Koo, J.S. Kim, D.J. Lee, H.J. Lee, T.S. Park, Y.I. Kuk and J.O. Guh. 2000. Herbicide response and control of sulfonylurea-resistant biotype of *Monochoria vaginalis* in paddy fields in Chonnam province, Korea. Korea J. of Weed Sci. 20:46-52.

Kwon, O.D., Y.I. Kuk, H.I. Jung, D.J. Lee and J.O. Guh. 2001. Control of resistant *Rotala indica* biotypes to sunfonylurea herbicides. Korea J. of Weed Sci. 21(Suppl.):33-38.

Kwon, O.D., I.K. Kuk, D.J. Lee, H.R. Shin, I.J. Park, E.B. Kim, and J.O. Guh. 2002. Growth abd yield of rice as affected by competitive period of resistant *Monochoria vaginalis* biotype to sulfonylurea herbicides. Korean J. of Weed Sci. 22:147-153.

Park, T.S., C.S. Kim, J.P. Park, Y.K. Oh, and K.U. Kim. 1999. Resistance biotype of *Monochoria korsakowii* against sulfonylurea herbicides in the reclaimed paddy fields in Korea. Proc. Of the 17th Asian-Pacific Weed Sci. Soc. Conf. 17: 252-254.

Park, T.S., C.S. Kim, B.C. Moon, I.Y. Lee, J.E. Park, and K.U. Kim. 2000. Occurrence and control of sulfonylurea-resistant biotype *Lindernia dubia* in paddy fields in southern province of Korea. Korea J. of Weed Sci. 20:73-75.

Ray, T.B. 1984. Site of action of chlorsulfuron: Inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75:827-831.

Saari, L.L., J.C. Cotterman and D.C. Thill. 1994. Resistance to acetolactate synthase inhibiting herbicides. Pages 83-139 in *Herbicide Resistance in Plants*: Biology and Biochemistry (edite by Prowles, S.B. and J.A.M. Holtum), Lewis publishing, Boca Raton, FL.

Westerfeld, W.W. 1945. A colorimetric determination of blood acetoin. J. of Biol. Chem. 161:495-502.

Paraquat-Resistant *Crassocephalum crepidioides* in Sri Lanka

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Abstract: Experiments were conducted to investigate the occurrence of paraquat-resistant *Crassocephalum crepidioides* (Benth.), a major weed of tea growing areas in the central province of Sri Lanka. Mature seeds collected from suspected resistant (R-biotype) and susceptible (S-biotype) populations of the weed from three different districts (two R biotypes and one S-biotype per district) were used in the experiments. The germination percent of seeds from R and S-biotypes was not significantly different. In tray experiments, paraquat (1.1'-dimethyl-4, 4' bypiridilium ion) was applied at dosages ranging from 0.05 to 3.2 kg a.i. ha^{-1} to the weed seedlings at 2/4 leaf stage. The results indicated that the six R-biotypes used in the study have developed resistance to paraquat with Resistant Index (RI) varying from 2.6 to 6.2.

Key words: *Crassocephalum crepidioides*, herbicide resistance, paraquat, Sri Lanka

INTRODUCTION

Among the agricultural crops that play a major role in Sri Lankan economy, the tea (*Camelia sinensis* (L) O. Kuntz) sector occupies the highest position in terms of export earnings. Annual tea production in Sri Lanka is 306 million kg from 180,000 ha of land, and the export earnings amount to Sri Lankan Rs million 53,133 (Central Bank Report, 2000; 1 Rs = approx 96 US $). However, the average yield in Sri Lanka stands low (Marambe et al. 2002), and of the many factors, weeds have been recognized as of major importance. Several researchers have estimated the yield losses due to weed competition in Sri Lankan tea to be 5 to 9% in mature clonal tea and 5 to 15% in mature seedling tea (Waidyanatha 1966). Kulasegaram (1980) and Luxmie (1993) reported that the cost for weed control in tea contributes to about 10 to 14% of the total cost of all field operations, which is attributed to high cost of labour and chemicals.

Among the many weeds found in tea fields of different agroecological regions of Sri Lanka, *Crassocephalum crepidioides* (Benth.) is a noxious weed. The weed has mostly spread in mid and up country regions. *C. crepidioides* can be easily removed by hand pulling, however, but the root systems come out with a large root bole leading to soil erosion, more manual labour is needed for hand pulling thus, increasing cost of production. Scraping is not recommended, too, due to its detrimental effect on the soil. Therefore, the most appropriate method of weed control is by the use of herbicides, and paraquat (1.1'-dimethyl-4,4'bypiridilium ion) was the most common herbicide used to control *C. crepidioides* and other weeds in tea plantations in Sri Lanka since late 1950's.

In the recent past, there have been complaints from tea planters that *C. crepidioides* is no more controlled effectively by the herbicide paraquat. Dense populations of the weed that cannot be controlled by paraquat has been recorded in various tea estates in different tea growing areas, which have been subjected to continuous application of the herbicide for more than a decade, at a frequency of 6 to 10 times a year. Continuous use of herbicides has imposed selection pressure for increased resistance within species that were formally susceptible (Holt 1996 Valverde et al. 2000), thus making the task of weed control more difficult. There are 22 weeds that have developed resistance to paraquat (www.weedscience.com, 2002) and of those paraquat-resistant weeds, *C. crepidioides* has previously reported in Malaysia. Development of resistance in *Erigeron sumatrensis* to paraquat was recently reported in Sri Lanka (Marambe et al. 2002).

The present study was conducted to investigate the occurrence of paraquat-resistant *C. crepidioides* in different tea growing districts in Sri Lanka.

MATERIALS AND METHODS

Pot and field experiments were carried out in the mid country wet zone of Sri Lanka (450 m amsl; 26-28°C, 65% RH). Pot experiments were carried out in the plant house of the Faculty of Agriculture of the University of Peradeniya, Sri Lanka. Mature seeds of *C. crepidioides* were collected from nine tea different plantations covering three districts, namely, Kandy, Matale and Nuwera Eliya, to include three sites per district. In each district, seed samples were collected from two sites based on the experiences of planters who claimed that the weed has not been controlled by paraquat (R-biotypes; R1 and R2), and from one site with no such experience (S-biotype). In all the estates where suspected R-biotypes were found, paraquat has been applied continuously for the past 15 to 20 years at a frequency of 6 to 8 times a year. The estates with S-biotypes either did not have experience of using paraquat in the past, or the herbicide has used for 2 to 3 years and later been shifted to a mix of other herbicides.

The seed germination tests were conducted in Petri dishes under laboratory conditions to investigate whether the R and S biotypes differ in their germination. Fifty seeds from each seed sample collected were placed in separate Petri dishes laid with a moistened filter paper and kept in a germination cabinet at 28°C and a light intensity of 250 lx. The experiment was replicated six times and the number of seeds germinated was counted after 10 days.

Tray experiments were conducted at the plant house of the Faculty of Agriculture, University of Peradeniya, Sri Lanka. The mature seeds of R and S biotypes collected from different tea estates were grown separately in trays (8 x 32 x 42 cm^3) filled with a mixture of the topsoil and sand at 1:1 ratio. Each tray was row seeded with 150 seeds, and the seedlings were thinned to 100 plants per tray at 1 week after germination. Soil was kept moistened continuously throughout the experiment. The paraquat treatments ranging from dosages 0.05-3.2 kg a.i. ha^{-1} (recommended dosage 0.4 kg a.i. ha^{-1}) were imposed on trays with seedlings of R and S biotypes of *C. crepidioides* at 2/4 leaf stage. From the preliminary studies, 2/4 leaf stage was identified as the most susceptible stage of the growth of the weed to paraquat. The herbicide was sprayed using a knapsack sprayer (16 L) equipped with a flat fan nozzle (spray volume 475 L ha^{-1}). A herbicide-free tray was maintained as the control. The experiment was conducted in a completely randomized design with three replicates. Samples were taken at 10 days after treatment, where the remaining plants of each tray were uprooted and the dry weight was measured after oven drying the samples at 80°C until constant weight.

The data were subjected to statistical analysis. The germination percentages of the mature seeds of R and S biotypes collected from different estates were analyzed using ANOVA. The data from tray experiments were analyzed using the non-linear regression using the log-logistic model described by Seefeldt et al. (1995), the ED_{50} values of R and S biotypes were estimated from the dose-response curves and the Resistance Index (RI) was calculated. The computer software package Statistical Analysis System (SAS 1994) was used to analyze the data.

RESULTS AND DISCUSSION

Seed germination of *C. crepidioides*

The percent seed germination of the R and S biotypes of *C. crepidioides* collected from all locations did not show any significant difference (Fig. 1). The maximum germination percentage recorded was 82%.

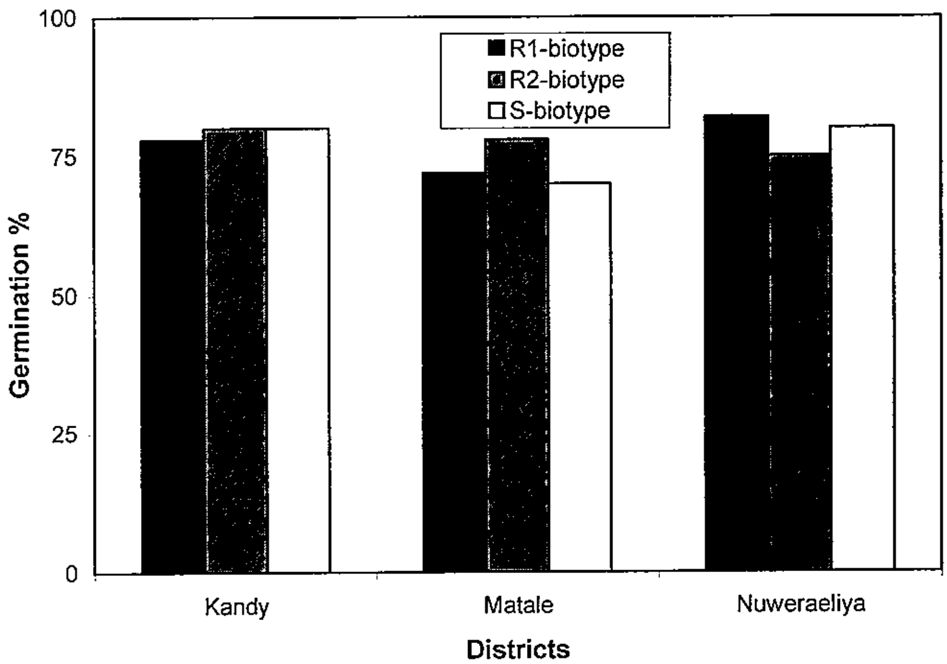

Figure 1. Percent seed germination of R and S biotypes collected from three districts.

Control of *C. crepidioides* by paraquat

The recommended dosage of paraquat (0.4 kg a.i. ha^{-1}) gave a significantly lower control of *C. crepidioides* in all suspected R-biotypes (Fig. 2). The S-biotypes were successfully controlled by the recommended dosage of the chemical. Paraquat at the recommended dosage totally failed to control the mature plants of R-biotypes at the 6/7 leaf stages and flowering stage. In S-biotype, too, the weed at the flowering stage was not effectively controlled by the herbicide. Increasing dosage of the chemical enhanced the phytotoxicity on the weed (Fig. 2).

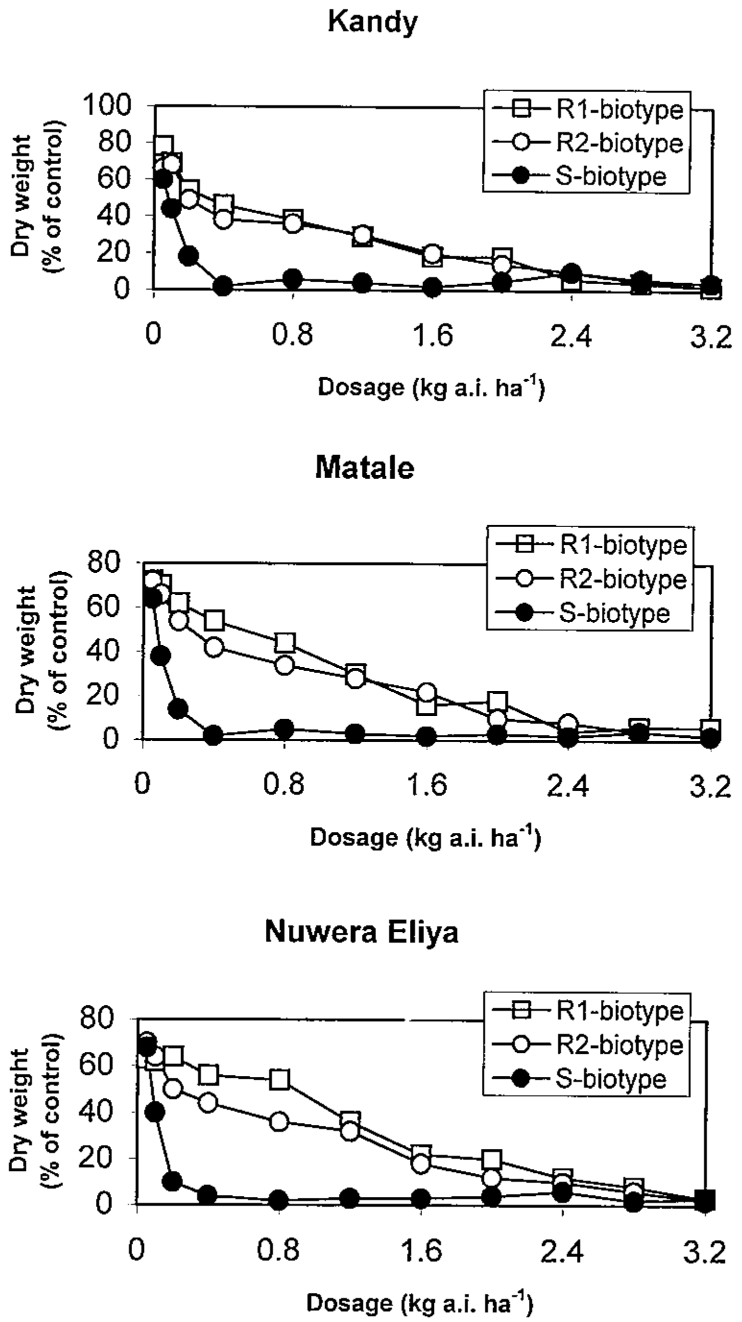

Figure 2. Change in dry weight of *C. crepidioides* seedlings (2/4 leaf stage) treated with different dosages of paraquat.

Resistant index of R-biotypes

The Resistant Index (RI) of the R-biotypes collected from different sites is shown in Table 1. The results indicated that the R-biotypes used in this study have developed resistance ranging from 2.6 to 6.2-fold to paraquat.

Table 1. Resistant Index (RI) of different R-biotypes of *C. crepidioides* collected from three districts of Sri Lanka.

District (biotype)	RI
Kandy (R1)	3.8
Kandy (R2)	2.6
Matale (R1)	4.2
Matale (R2)	4.5
Nuwera Eliya (R1)	6.2
Nuwera Eliya (R2)	5.4

Repeated use of a herbicide could result in development of resistance in weeds (Valverde et al 2000). The R-biotypes used in the present study were selected from sites that have used paraquat for more than 15 years at a frequency ranging from 4-8 times a year. The results clearly indicated that *C. crepidioides* has developed resistance to the herbicide paraquat. The S-biotypes used in the present study has not been exposed to paraquat continuously, and hence susceptible to the herbicide. Paraquat-resistant *C. crepidioides* has previously being reported only from Malaysia in 1990 (www.weedscience.com, 2002). Marambe et al. (2002) reported that *E. sumatrensis*, another noxious weed found in upcountry tea fields of Sri Lanka, has developed resistance to paraquat. Glyphosate, a systemic, non-selective herbicide is currently used by many planters in Sri Lanka to control paraquat-resistant *C. crepidioides* in non-crop lands and in pruned tea fields.

LITERATURE CITED

Central Bank Report 2000. Annual Report, Central Bank of Sri Lanka.

Holt, J.S. 1996. Ecological fitness of herbicide-resistant weeds. Proc. Second Int. Weed Control Congress, 387-392.,

Kulasegaram, S. 1980. Technical developments in tea production. Tea Quarterly, 49: 157-183.

Luxmie, M. 1993. Studies in biology and management of *Crassocephalum crepidioides* (Benth.), and *Erigeron sumatrensis* (Retz.) in high grown tea. M.Phil. Thesis, PGIA, University of Peradeniya, Sri Lanka

Marambe, B. et al. 2002. Occurrence of paraquat-resistant *Erigeron sumatrensis* (Retz.) in upcountry tea lands of Sri Lanka. J. Plant Diseases and Protection, 18: 973-978.

SAS 1994. SAS/STAT User's Guide, Ver. 6.0, 4th Edition. SAS Institute Inc., USA.

Seefeldt, S.S. et al. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technology, 9: 218-227.

Valverde, B.E. et al. 2000. Prevention and management of herbicide-resistant weeds in rice: Experiences from central America with *Echinochloa colona*. Grafos, S.A., Costa Rica.

Waidyanatha, U.P. De S. 1966. Investigation with paraquat (Gramoxone) as a herbicide for weed control in low grown tea. Tea Quarterly, 39: 11-18.

Control of Herbicide-Resistant *Phalaris minor* in Rice-Wheat Cropping System

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Abstract: The investigation was conducted during the *Rabi* seasons of 1998-99 and 1999-2000 at the Indian Agricultural Research Institute, New Delhi to determine the suitability of new selective herbicide flufenacet in wheat crop infested with herbicide-resistant *Phalaris. minor*. The investigation consisted of field and pot experiments. In the field experiment, the effects of different doses of flufenacet, viz., 180, 240 and 300 g ha^{-1}, its time of application, viz., pre-emergence and two weeks after sowing, and its lowest dose in combination with isoproturon, metribuzin and 2,4-D on behavior of *P. minor* and wheat growth and yield were studied. In the pot experiment cross-resistance to flufenacet among six biotypes of *P. minor* was investigated. Insignificant difference was observed between times of application. Increasing dose of flufenacet from 180 g ha^{-1} to 300 g ha^{-1} significantly decreased dry matter and resistant population of *P. minor* at 30 days after sowing. Combination of 180 g ha^{-1} of flufenacet with metribuzin and 2,4-D recorded broad-spectrum control of weeds. Slight phytotoxicity was observed in wheat field that was vanished by the time of harvesting. Highest dry matter accumulation in wheat crop was recorded 240 g ha^{-1} flufenacet and 2,4-D and metribuzin in combination with lowest dose of flufenacet. Number, height and, consequently, dry matter in all biotypes of *P. minor* notably decreased with increasing dose of flufenacet beyond 60 g ha^{-1}.

Key words: control, flufenacet, herbicide resistance, *Phalaris minor*, wheat

INTRODUCTION

In recent decades the predominant weed control method in many parts of the world has been the use of effective and reliable chemical herbicide. Although herbicides are major factors contributing to world crop production, their intensive use is held responsible for environmental pollution, shift in weed flora and evolution of resistant weed biotypes, which jeopardize herbicide utility, availability and longevity and impose the threat to productivity of world agriculture (Duary and Yaduraju 1999). The increase in the number of new herbicide-resistant weeds has remained relatively constant since 1978. A total of 64 weed species have developed resistance to triazine herbicide. Acetolactate synthase (ALS) and acetyl coenzyme A carboxylase (ACCase) inhibitor-resistant weeds will cause great problems in the future. (Heap1997). Indian farmers have enjoyed the benefits of rice-wheat cropping system since long ago but many problems have risen in the system in the recent past, which has lowered the profitability of the system. One of the reasons is the poor control of *Phalaris minor* in wheat crop. The poor control is due to genetic resistance of some *P. minor* biotypes to isoproturon. The herbicide resistant biotypes have been reported from many parts of Punjab, Haryana and U.P. (Wallia *et al.* 1997). Isoproturon-resistant *P. minor* infesting wheat fields in north West India is of significant economic importance. The lack of alternative herbicides to control this weed with multiple herbicide resistance makes this the most challenging resistance problems

(Heap 1997). New herbicides such as clodinofop, fenoxaprop, sulfosulfuron and tralkoxydim are being tested for the control of herbicide resistant *P. minor* (Brar and Walia 1997). Lately, flufenacent (foe 5043) has also been reported to be very effective against *P. minor*. Flufenacet, with application possibilities on many crops around the world, represents a major new herbicide. Flufenacet belongs to the oxiacitamide group of herbicides (Forester *et al.* 1997).

MATERIALS AND METHODS

The experiments were conducted during the *Rabi* seasons (November – April) of 1998-99 and 1999-2000 at the farm and net house of the Division of Agronomy, IARI, to evaluate flufenacet for the control of herbicide-resistant *P. minor*. The main field experiment was laid out in a randomized complete block design with 12 treatments. Treatments included three different doses of flufenacet, i.e., 180, 240 and 300 g ha^{-1}, applied as pre and post emergence (2 weeks after sowing or WAS) in addition to lower dose of flufenacet followed by Isoproturon (0.5 kg ha^{-1} 4 WAS), Metribuzin (150 g ha^{-1} 4 WAS), 2,4-D (0.4 kg ha^{-1} 5 WAS). Following treatments were also included in the experiment: Isoproturon alone 0.75 kg ha^{-1} 4 WAS, hand weeding 2, 4 and 6 WAS, unweeded control. Sowing was done by tractor-drawn seed drill calibrated for the recommended seed rate of 100 kg ha^{-1} and adjusted for the spacing of 22.5 cm between rows. The first irrigation was given 21days after sowing at crown root initiation (CRI). The other 4 irrigations were given at critical stages of growth.

Observations recorded

Weed population counts and weed sample for dry matter accumulation were taken to assess the effect of various treatments on weed growth at 30, 60 and 100 days after sowing. An area of 0.25 m^2 was selected randomly by throwing a quadrat. Species-wise weed population count was made from the quadrat area in both years. However, the dry weight was recorded by summing up as grass and broad-leaved weeds. Collected weeds were first dried in the sun and then in an electric oven at 70°C for 48 h and dry weight was recorded and expressed as g m^{-2}. Observations on the crop were taken at 30, 60 and 100 DAS and at the time of harvest in both years of study.

Cross resistance in isoproturon-resistant biotypes of *P. minor*

To study the possibility of cross resistance to flufenacet among biotypes of *P. minor*, a pot experiment was laid out using six biotypes of Phalaris collected from different areas in 1999. Young plants were harvested 30 days after germination to record dry weight and height of plants for finding out the existence of cross resistance among collected biotypes. 0, 60, 120, 240, 300 g ha^{-1} applied at 2 WAS.

Randomized complete block design was employed to analyze collected data. Each treatment was replicated 5 times. Considering dry matter production in control pots equal to 100 percent, regression equations were established between doses of flufenacet and scaled dry matter production for all biotypes of *P. minor* using Curve fit computer software.

Biotype	Place of collection	Susceptibility
B12	Asand	Resistant
B25	Kurukshetra	Resistant
B34	Karnal	Resistant
B57	Kithal	Sensitive
B59	Kithal	Sensitive
B95	Pusa	Sensitive

RESULTS AND DISCUSSION

P. minor population and density

Number of *P. minor* per m^2 at different stages of growth is shown in Table 1. Interaction between years and treatments was not significant but general mean of experiment in first year was lower than in second year. It was observed that increasing dose of flufenacet from 180 g ha^{-1} to 300 g ha^{-1} significantly decreased population of *P. minor* at 30 DAS in both years. This was mainly due to the fact that flufenacet is basically a grass killer. Insignificant difference was observed between times of application of the herbicide. This may be due to mechanism of action of flufenacet as it interferes in growth and development in early stage of growth. Such result can be considered as an advantage of this chemical because it provides a wider window of application time. Combination of 180 gha^{-1} of flufenacet with metribuzin and 2,4-D recorded the best control. It decreased *Phalaris* population down to about 85% compared to hand weeded plot. Difference between the doses of flufenacet disappeared for both times of application at 100 DAS.

Table 1. Density of *Phalaris minor* (No. m^{-2}) at different stages of growth.

Treatments (kg ha^{-1})	30 DAS		60 DAS		100 DAS	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	425.2 b	492.0 b	90.6 c	110.7 cd	200.0 a-d	186.7 bc
Flufenacet 240 PE	310.5 c	350.7 c	29.2 e	42.7 e	100.0 c-f	122.8 c-e
Flufenacet 300 PE	197.3 d	244.0 d	14.8 g	22.7 g	82.7 ef	111.0 de
Flufenacet 180 2WAS	436.0 b	517.2 b	93.2 c	113.3 c	146.7 b-e	173.2 b-d
Flufenacet 240 2WAS	310.7 c	350.8 c	65.2 d	85.3 d	90.7 c-f	108.0 c-e
Flufenacet 300 2WAS	206.7 d	242.7 d	22.8 ef	37.2 e	88.0 d-f	106.8 c-e
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	450.8 b	530.8 b	100.0 cd	100.0 cd	166.7 a-d	190.2 bc
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	89.3 e	109.3 e	22:8 ef	38.8 ef	88.0 d-f	106.7 c-e
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	93.2 e	113.3 e	20.Q f	30.8 f	84.0 d-f	104.0 c-e
Isoproturon 750 4WAS	613.2 a	692.0 a	204 .0 b	238.7 b	262.8 ab	302.4 ab
Two hand weedings (4 and 6WAS)	20.0 f	30.8 f	25.2 ef	43.0 e	136.9 f	66.8 e
Unweeded control	666.8 a	753.2 a	312.0 a	352.0 a	358.8 a	422.8 a

The higher the dose of flufenacet the lower was the dry mater accumulation by *P. minor*. Time of application did not affect *Phalaris* dry matter production at 30 DAS. Combinations of isoproturon with flufenacet and isoproturon alone had no effect on *Phalaris* in respect to dry matter accumulation, while combinations of metribuzin and 2,4-D with flufenacet regardless of time of application significantly decreased dry matter at 30 DAS. Unsuccessful control of grassy weeds by isoproturon alone confirmed that *P. minor* biotypes present in the field were really isoproturon-resistant. Unweeded control and isoproturon alone recorded higher dry matter in all stages. Difference between treatments was significant at 60 DAS in first year but the general trend was the same as 30 DAS. Difference between 180 g ha^{-1} and 300 g ha^{-1} remained significant even at 100 DAS.

Table 2. Total dry matter production (gm^{-2}) of *P. minor*.

Treatment (g ha^{-1})	30 DAS		60 DAS		100 DAS	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	3.8 a-c	4.3 a-c	7.6 a-d	8.3 a-c	72.1 b-d	82.3 bc
Flufenacet 240 PE	2.9 d	3.9 bd	2.4 ef	2.9 de	63.2 b-e	70.3 bd
Flufenacet 300 PE	0.8 d	1.0 d	1.6 f	2.0 e	40.9 ef	45.8 de
Flufenacet 180 2WAS	2.5 a-c	3.0 cd	10.2 a-c	12.1 ab	68.4 b-e	76.0 bd
Flufenacet 240 2WAS	1.6 cd	1.8 cd	4.8 b-e	5.2 a-d	44.7 d-f	49.6 ce
Flufenacet 300 2WAS	0.8 d	1.0 d	2.1 ef	2.4 de	40.9 e-f	45.6 ce
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	6.2 ab	6.8 ab	3.7 b-f	4.3 be	73.5 b-d	81.4 bc
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	0.6 d	0.8 d	2.0 ef	2.3 ce	36.0 e-f	40.0 c-e
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	0.7 d	0.9 d	1.8 f	2.1 ce	32.1 f	37.2 c-e
Isoproturon 750 4WAS	7.2 a	8.2 a	20.2 a	24.9 a	104.8 ab	124.0 ab
Two hand weedings (4 and 6WAS)	0.0 e	0.0 e	0.4 g	0.4 e	23.2 f	27.6 e
Unweeded control	6.1 a	7.0 a	26.1 a	29.2 ab	168.2 a	197.6 a

*fb. : Followed by ** Any two means having a common letter are not significantly different at 5% level of significance

Broad-leaved weed population

Mean range comparison on number of broad-leaved weeds, mostly *Chenopodium album, Melilotus indica* and *Cannabis sativ,* is illustrated in Table 3. Similar result was obtained in both years. The population of broad-leaved weeds in the first year remained significantly higher than the second year. Since 2,4-D was not applied at 30 DAS the results obtained from these plots at 30 DAS did not reflect their effects but later at 60 DAS and at harvest this treatment significantly reduced the number of broad-leaved weeds. Metribuzin applied at 4 WAS had similar effect as 2,4-D had on broad-leaved weeds at 30 and 60 DAS. Combination of 2,4-D with flufenacet reduced weed population even higher than hand weeding treatment (Table 3). Such a result was due to the fact that flufenacet affected grassy weeds and the others, i.e., metrobiuzine/2,4-D decreased broad-leaved weed growth.

Table 3. Population of broad-leaved weeds (No.m^{-2}) at different stages of growth.

Treatment (g ha^{-1})	30 DAS		60 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	59.7 a	53.1 a	133.0 a	92.4 a	78.2 ab	70.0 a
Flufenacet 240 PE	47.5 a	37.2 a	101.2 a	81.2 a	78.0 ab	63.2 ab
Flufenacet 300 PE	84.0 a	58.1 a	166.3 a	150.0 a	57.0 a-c	41.2 a-c
Flufenacet 180 2WAS	65.3 a	43.5 a	189.9 a	172.1 a	40.3 a-c	29.6 a-d
Flufenacet 240 2WAS	56.0 a	74.4 a	253.5 a	202.8 a	69.1 a-c	56.0 a-c
Flufenacet 300 2WAS	56.1 a	49.6 a	152.0 a	105.2 a	88.0 a	80.0 a
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	56.6 a	38.8 a	63.5 a	56.0 ab	34.2 a-c	32.0 a-c
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	72.0 a	50.8 a	25.2 bc	22.8 cd	3.0 d	5.0 d
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	60.0 a	48.0 a	29.4 b	25.6 bc	4.0 d	4.5 d
Isoproturon 750 4WAS	81.2 a	73.2 a	60.0 a	50.0 a	38.1 a-c	61.3 ab
Two hand weedings (4 and 6WAS)	5.1 b	5.0 b	13.3 bc	10.1 cd	21.1 cd	10.0 cd
Unweeded control	57.3 a	46.2 a	112.0 a	89.6 a	278.2 a	19.6 a-d

Wheat height

The height of wheat plants at different stages of growth is presented in Table 4. Height of wheat crop was significantly higher in the first year than in the second year but no interaction was found significant between years and treatments. The difference in plant height between hand weeding and other treatments was significant, except unweeded control and isoproturon alone at 30 DAS. Significant differences were observed between different doses of flufenacet in the early stages of growth. Treatments that received higher level of flufenacet showed lesser height. No significant difference was found between times of application of flufenacet in respect to plant height at all stages.

Table 4. Wheat plant height (cm) at different stages of growth.

Treatment (g ha^{-1})	30 DAS		60 DAS		100 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	16.6 b	15.7b	46.3bc	43.6 bc	72.3 cd	68.9cd	92.9	92.0
Flufenacet 240 PE	14.0 d	13.2d	40.7d-f	38.6 de	76.9a-c	72.6a-c	93.4	91.3
Flufenacet 300 PE	13.2 e	12.7 e	37.0 ef	35.2 e	71.3 cd	68.5 cd	91.3	91.0
Flufenacet 180 2WAS	15.7 c	14.8 c	46.7 bc	43.4 bc	73.9b-d	69.5b-d	93.8	92.5
Flufenacet 240 2WAS	14.3 d	13.6 d	43.3c-e	41.0 cd	69.7 de	66.3 de	91.4	92.1
Flufenacet 300 2WAS	14.0 d	13.4 d	35.3 ef	33.2 ef	72.1 cd	69.2 cd	92.6	91.6
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	16.0bc	15.3bc	40.5d-f	38.0 de	64.9 e	62.6 e	93.1	92.0
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	16.5 b	15.4 b	43.3c-e	40.0 d	77.1a-c	71.7a-c	91.8	91.3
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	16.1bc	15.4bc	42.6c-e	40.7 d	72.6 cd	69.5 cd	92.6	92.1
Isoproturon 750 4WAS	19.0 a	18.1 a	49.7 bc	46.7 b	72.3a-c	69.0 cd	92.7	91.8
Two hand weedings (4 and 6WAS)	19.2 a	18.1 a	56.0 a	52.9 a	79.7a-c	75.2a-c	93.1	92.1
Unweeded control	19.1 a	18.3 a	53.3 ab	50.8 a	82.2 ab	78.9 ab	92.4	93.1

Wheat dry matter production

The data pertaining to dry matter production (DM) of wheat presented in Table 5 clearly show the competitive effects of *P. minor* as well as the phytotoxic effect of flufenacet at the highest dose on wheat biomass. At all stages in both years, hand weeded plots showed maximum dry matter production.

Although two hand weedings, unweeded control and isoproturon alone did not significantly differ with 180 g ha^{-1} flufenacet and its combination with other herbicides except combination with 2,4-D in second year, they held maximum dry matter at 30 DAS. Minimum dry matter was obtained in plots treated with 300 g ha^{-1} flufenacet. Observation at 60 DAS showed almost a similar trend as at 30 DAS but dry matter was reduced with post-emergence application of 180 g ha^{-1} flufenacet, its combination with other herbicides and isoproturon alone as compared to hand weeding treatment. Mean comparison of treatments at 100 DAS revealed that dry matter was at its highest level in plots treated with pre-emergence application of 240 g ha^{-1} flufenacet and in hand weeded one. Unlike plant height, difference in dry matter between highest dose of flufenacet and other treatments was observed even at harvest. Besides hand weeded plots, the higher dry matter accumulation was observed on 240 g ha^{-1} flufenacet and also combination of the lowest dose of flufenacet with 2,4-D and metribuzin. It was obvious that the lowest dose (180 g ha^{-1}) of flufenacet was not enough to cause a reasonable level of control, while highest dose of flufenacet was quiet excessive.

Table 5. Dry matter production by wheat (gm^{-2}) at different stages of growth.

Treatment (g ha^{-1})	30 DAS		60 DAS		100 DAS		Harvest	
	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000	1998-1999	1999-2000
Flufenacet 180 PE	28.0 ab	26.4 ab	293.6ab	277.0ab	510.8de	486.6de	735.0bd	693.4b-d
Flufenacet 240 PE	21.8b-d	20.6 bc	223.6 bc	209.8 c-e	779.2 ab	735.0 ab	753.6 a-d	718.4 a-c
Flufenacet 300 PE	16.4 d	15.8 cd	134.4 e	127.8 e	610.4 b-e	586.2 b-e	678.6 e	647.0 e
Flufenacet 180 2WAS	24.4 a-c	22.8 bc	223.0 bc	207.8 c-e	545.6 c-e	514.0 c-e	733.2b-d	685.2 c-e
Flufenacet 240 2WAS	19.8 cd	18.8 cd	199.0 cd	188.4 de	569.2 c-e	539.6 c-e	740.4 a-d	706.4 b-d
Flufenacet 300 2WAS	16.4 d	15.6 d	154.8 de	145.0 e	486.8 de	467.8 de	725.8 c-e	683.4 c-e
Flufenacet (180 2WAS) *fb.Isoproturon (500 4WAS)	24.7 a-c	23.2 a-c	190.4 cd	179.4 de	612.0 b-e	589.6 b-e	733.6b-d	696.8 b-d
Flufenacet (180 2WAS) *fb. Metribuzin (150 4WAS)	25.8 a-c	24.0 a-c	193.6 cd	178.2 de	688.0 a-d	649.0 a-d	786.6 ab	740.2 a-b
Flufenacet (180 2WAS) *fb. 2,4-D (400 5WAS)	23.6 a-d	22.6b-d	189.9 cd	181.8 de	666.8 a-e	637.2 a-e	776.6 a-c	740.2 a-b
Isoproturon 750 4WAS	29.8 ab	28.2 ab	248.6 bc	234.2 cd	462.0 e	439.8 e	743.0 a-d	710.6 b-d
Two hand weedings (4 and 6WAS)	30.8 a	29.0 a	346.0 a	325.0 a	820.0 a	787.2 a	789.6 a	753.0 a
Unweeded control	30.0 a	28.8 a	278.0 ab	264.8 bc	596.0 b-e	554.2 b-e	728.8 cd	672.6 c-d

A Population of Sprangletop (*Leptochloa chinensis)* is Resistant to Fenoxaprop

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Abstract: This study reports the resistance to fenoxaprop-p-ethyl evolved in a BLC1 population of sprangletop (*Leptochloa chinensis* L. Ness.*)* in a rice field in Thailand. Following eight applications of fenoxaprop-p-ethyl, a farmer reported that in the herbicide experiments were conducted separately. Firstly, a glasshouse experiment using Syngenta Quick-test revealed that BLC1 population survived at 600 g ai ha^{-1} of fenoxaprop-p-ethyl and plants looked unaffected. Secondly, BLC1 population was treated with fenoxaprop-p-ethyl and the other ACCase-inhibiting herbicides (quizalofop-p-tefuryl, cyhalifop-butyl and clefoxydim) under field condition. BLC1 exhibited a high resistance level to fenoxaprop-p-ethyl and low level of resistance to the other cases ACCase-inhibiting herbicides. Thirdly, seeds of susceptible SLC1 and resistant BLC1 populations. A low level of resistance to the other ACCase-inhibiting herbicides indicates a need for alternative herbicides to control this population. Future research will identify mechanisms of resistance in this population.

Key words: sprangletop, *Letochloa chinensis* (L.) Ness, fenoxaprop resistance

INTRODUCTION

Sprangletop (*Leptochloa chinensis* (L.) Ness) is a major grass weed in wet-seeded rice in Thailand. It can be controlled by pre- and post-emergence herbicides. Fenoxaprop-p-ethyl, an ACCase inhibiting herbicide, has been widely used since the last 15 years. It is an effective post-emergence herbicide for controlling grass weeds in rice cultivation. In 2002, a farmer reported that a population of sprangletop (*Leptochloa chinensis* (L.) Nees) in Minburi District of Bangkok could not be controlled with fenoxaprop-p-ethyl. This population had been exposed to 10 applications of fexonaprop but this herbicide failed. Infestation was dense and rice was reduced by more than 70%. Resistance to ACCase-inhibiting herbicide did occur after three herbicide applications (Tardif et al. 1993). Hence, this population may develop resistance to fenoxaprop-p-ethyl.

Recently, resistance to fexonaprop-p-ethyl was documented in some grass species such as *Avena fatua* (Friesen et al. 2000), *Digitaria ischaemum* (Derr 2002), *Echinocloa colona* (Riches et al. 1996 cited in Kim et al. 2000). However, resistance to fexonaprop-p-ethyl has not been reported in *Leptochloa* spp. The study was aimed to determine whether this sprangletop population was resistant to fenoxaprop-p-ethyl and other ACCase-inhibiting herbicides.

MATERIALS AND METHODS

Plant materials

A resistant population BLC1 was collected from a paddy field in Minburi District, Bangkok in 2002. The history of herbicide application in this field is shown in Table 1. A susceptible populaion SLC1 was collected from an area with no history of application in Supanburi Province.

Table 1. History of herbicide application to the field prior to the resistant population BLC1 was collected.

Year of Application	Herbicides	No. of Season
1997	two applications of fenoxaprop-p-ethyl	2
1998	two applications of fenoxaprop-p-ethyl	2
1999	two applications of fenoxaprop-p-ethyl	2
2000	two applications of fenoxaprop-p-ethyl	2
2001	one application of butachlor/propanil and one application of cyhalofop-butyl	2
2002	two applications of fenoxaprop-p-ethyl	1

Fenoxaprop-p-ethyl was the most frequently used in BLC1 population (Table1). Initially, sprangletop was successfully controlled with fenoxaprop at the rates of 37.5-75 g ai ha^{-1}. After eight applications of fenoxaprop, the farmer could no longer control this population. In 2002, BLC1 field was treated twice with RICESTAR® (fenoxaprop-p-ethyl+safener, ethyl 5, 5-dephenyl-2-isoxazoline 3-carboxylate). Firstly, RICESTAR® at 25 g ai ha^{-1} (a recommended rate for killing the susceptible) was sprayed at 12 days after sowing rice (DAS). Seven days after application, BLC1 plants were normal.m Then RICESTAR® at 35 g ai ha^{-1} was secondly sprayed at 19 DAS. Again, no phytotoxic symptom was found in sprangletop plants, whereas the herbicide did cause moderate to severe damage to rice plants.

Glasshouse experiment

The survival plants from BLC1 field were confirmed resistant by Syngenta quick-test (Boutsalis 2001). The experiment was conducted in the glasshouse during May-June 2002 at the Department of Agriculture, Bangkok, Thailand. Survival plants at 26 days old were randomly collected from the infested field. Shoots and roots were cut as described in Syngenta Quick-Test. Five cuttings were transplanted into 15-cm long were sprayed with fenoxaprop-p-ethyl (Whip) at 0, 75, 150, 300 and 600 g ai ha^{-1}, respectively. For each herbicide rate, six replicates pots were used. At 21 days after application, survival plants were cut at soil level and dried at 70° C for 48 h prior to dry weight measurement.

Field Experiment

After rice harvest in August 2002, BLC1 field was densely covered with sprangletop plants germinated from seeds. Thirty days after germination, plants were sprayed with three APP herbicides (fenoxaprop-p-ethyl at 150, 300 and 1500 g ai ha^{-1}, quizalofop-p-tefuryl at 30 and 150 g ai ha^{-1}, cyhalofop-butyl at 300 an d1500 g ai ha^{-1}) and one Cyclohexenadiones (CHD) herbicide (clefoxydim at 100 g ai ha^{-1}).

Treatments were arranged in complete block design with four replicates. Plot size was 1m x 1m. Two weeks after treatment, visual estimates of plant injury were recorded using a scale of 0 to 10 (0 = no injury, 1-3 = slightly toxic, 4-6 = moderately toxic, 7-9 = severely toxic, 10 = completely killed). Stem and panicle length of 10 plants were measured in each replicate. Data were analyzed by ANOVA and $LSD_{0.05}$ was used for mean comparison.

Laboratory experiment

Four hundred seeds of each resistant BLC1 and susceptible SLC1 were sown separately on 50 mL 0.6% (w/v) cooled agar contained in 400-mL plastic container (with 11 cm diameter). Various concentrations (0, 0.06, 0.12, 0.48, 0.96, and 1.2 mg ai L^{-1}) of fenoxaprop-p-ethyl (Ricestar, 69 g ai L^{-1}) were added to agar. At each herbicide concentration, four replicate containers were used. After sowing seeds, the lids were closed to maintain the moisture. Containers were kept in a growth room at a light density of 50 $\mu Em^{-2} s^{-1}$ during a 12 h photoperiod, at 27°C. Ten days after treatment, the number of seedlings with shoots extended above celeoptile were counted as survival. The results were expressed as a percentage of the control. LD_{50} value of both resistant and susceptible populations was calculated from the graph data.

RESULTS AND DISCUSSIONS

Glasshouse Experiment

All plants from the BLC1 field survived at 75-600 g ai ha^{-1} of fenoxaprop-p-ethyl (Fig. 1A). In addition, shoot dry weight was not significantly decreased when exposed to high rates of the herbicide (Fig. 1B). No phytotoxic symptom was observed on leaves of the survival plants.

In Thailand, fenoxaprop-p-ethyl at 75-150 g ai ha^{-1} is recommended at 15-20 days after sowing rice. The majority of *L. chinensis* is three-to-four-leaf stage with one to two tillers. Rice and *L. chinensis* could be readily killed with 4-8 times the recommended dosage of fenoxaprop-p-ethyl. Although there was no susceptible weed in this experiment, the highest rate of 600 g ai ha^{-1} (8 times the recommended dosage) indicated that a certain level of herbicide resistance was developed in this population.

To date, there are a few post-emergence herbicides for the control of *L. chinensis* that are available in the market, i.e., quizalofop-p-tefuryl, cyhalofop-butyl and clefoxydim. All of them are ACCase-inhibiting herbicides. There are other herbicides with different modes of action such as bispyribac-sodium and quinclorac. However, they give a poor control of *L. chinensis*. So, all the four ACCase-inhibiting herbicides were used in a follow-up experiment.

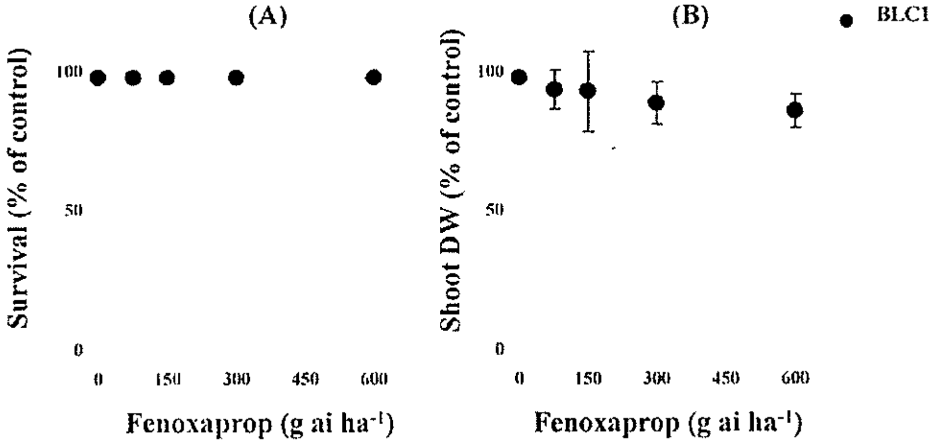

Figure 1. Survival and shoot dry weight of putative resistant sprangletop BLC (*L. chinensis)* population at 21 days after herbicide application. Points are the average of six replicates ± standard errors.

Field Experiment

Resistance to fenoxaprop-p-ethyl and other ACCase-inhibiting herbicides in BLC1 population was determined in the field. The results indicated that BLC1 was highly resistant to fenoxaprop-p-ethyl. BLC1 poplation could not be killed by 1,500 g ai ha^{-1} fenoxaprop-p-ethyl (20 times of recommended rate). Slight phytotoxicity was observed on the old leaves. Stem elongation was significantly inhibited when compared to the untreated plants. However, the panicle length was not affected (Table 2).

In addition, BLC1 showed a low level of resistance to the other APP herbicides, quizalop-p-tefuryl and cyhalofop-butyl. At 5-fold of the recommended rate of both APP herbicides, a moderate phytotoxicity appeared on the exposed leaves (Table 2). However, the newly emerged leaves were normal. Stem elongation was slightly decreased by CHD herbicide, clefoxydim while panicle length was not affected by the herbicide.

Laboratory Experiment

When seeds were geminated in agar containing fenoxaprop-p-ethyl, the herbicide readily controlled the susceptible population SLC1. The concentration pf 0.48 mg ai L^{-1} killed all of this population (Fig. 2A). In contrast, BLC1 population was not killed even at the highest concentration of 1.2 mg ai L^{-1}. The LD_{50} (concentration of herbicide required to kill 50% of a population) was 0.08 mg ai L^{-1} for the susceptible SLC1 compared to >1.2 mg ai L^{-1} for the resistant BLC1. The resistant BLC1 exhibited .15-fold resistance to fenoxaprop-p-ethyl. Similar result was observed in total dry weight of both populations. Total dry weight of BLC1 seedlings was reduced when exposed to high concentration of herbicide (Fig. 2B). However, the highest concentration did not give 50% total dry weight of BLC1 while that of the susceptible was completely reduced (Fig. 2B). GR_{50} of the resistant BLC1 and the susceptible SLC1 were .1.2 and 0.15 mg ai L^{-1} respectively.

Table 2. Effects of post-emergence herbicides on phytotoxicity, stem length and panicle length of *Leptochloa chinensis* BLC1 14 days after herbicide treatments.

Herbicides	Trade Name	Rate (g ai ha^{-1})	Times of recommended rate	Phytotoxicity[a] 14 DAA	Stem length (cm)	Panicle length (cm)
Fenoxaprop-p-ethyl	Whip® 7.5%EW	150	2	0	88.3a	40.4a
Fenoxaprop-p-ethyl	Whip® 7.5%EW	300	4	1	78.9ab	39.5a
Fenoxaprop-p-ethyl	Whip® 7.5%EW	1500	20	2	75.7b	36.6a
Quizalofop-p-tefuryl	Farmer® 4% EC	30	1	3	73.5bc	35.9a
Quizalofop-p-tefuryl	Farmer® 4% EC	150	5	6	49.7d	22.8bc
Cyhalofop-butyl	Grandstand 1 0%EC	300	1	2	63.8c	26.9b
Cyhalofop-butyl	Grandstand 1 0%EC	1500	5	5	48.4d	18.2c
Clefoxydim	Tetris® 7.5%EC	100	1	1	83.8b	37.3a
Untreated check		-	-	0	96.3a	38.1a
$LSD_{0.05}$					8.03	5.80

[a]Phytotoxicity level: 0 = no injury, 1-3 = slightly toxic, 4-6 = moderately toxic, 7-9 = severely toxic, 10 = completely killed

An agar germination bioassay could detect fenoxaprop-resistance in jungle rice (*Echinochloa colona*) (Kim et al. 2000). They reported that the largest difference between the susceptible and the resistant populations was made by fenoxaprop at 0.24 mg ai L^{-1}. In contrast, a concentration of 0.15 mg ai L^{-1} could be used to discriminate between susceptible and resistant populations of sprangletop. It may due to seeds of sprangletop are much smaller than that of jungle rice. Hence, less concentration is required to inhibit growth and germination of sprangletop.

Resistance to herbicides in rice production was found in 30 weed species (17 broadleaved weeds, 8 grass weeds and 5 species of Cyperaceae) (Vaverde and Itoh 2001). Of most importance, 7 species of *Echinochloa* evolved resistance to several thiobencarb. To date, herbicide resistance has not been reported in sprangletop (*L. chinensis*).

Here we documented the resistance to fenoxaprop-p-ethyl evolved in a population of sprangletop in a rice field in Thailand. As this population was also slightly resistant to the other ACCase-inhibiting, alternative herbicides with different modes of action are needed. Future research will elucidate the mwchanism of herbicide resistance in BLC1 sprangletop population.

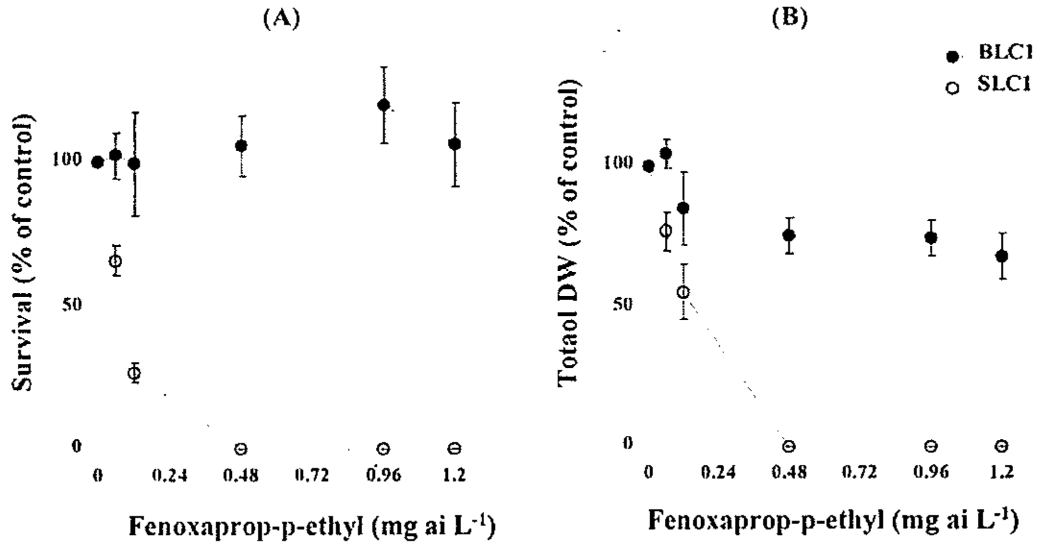

Figure 2. Survival (A0 and total dry weight (B) of resistant BLV (•) and susceptible SLC (o) sprangletop populations when grown in 0.6% agar containing with various concentrations of fenoxaprop-p-ethyl (0, 0.06, 0.12, 0.48, 0.96 and 1.2 mg a.i. L^{-1}). Data represented in % of untreated control. Points are the average± standard erroe of our replicates exposed to high concentration of herbicide (Fig. 2B). However the highest concentration did not give 50% total dry weight of BLC1 while that of the susceptible was completely reduced (Fig. 2B). GR_{50} of the resistant BLC1 and the susceptible SLC1 were >1.2 and 0.15 mg a.i. L^{-1}, respectively.

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LITERATURE CITED

Boutsalis, P. 2001. Syngenta-test: a rapid whole-plant test for herbicide resistance. Weed Technology 15:257-263.

Derr, J.F. 2002. Detection of fenoxaprop-resistant smooth crabgrass (*Digitaria ischaemum*) in turf. Weed Technology 16:396-400.

Friesen, L.F., T.L. Jones, R.C.V. Acker and I.N. Morisson. 2000. Identification of *Avena fatua* populations resistant to imazamethabenz, flamprop, and fenoxaprop-P. Weed Science 48:532-540.

Kim, D., C.R. Riches, andB.E. Valverde. 2000. Rapid detection of propanil and fenoxaprop resistance in *Echinochloa colona*. Weed Science 48: 695-700.

Tardif, F.J., J.A.M. Holtum and S.B. Powles. 1993. Occurrence of a hebicide-resistant acetyl-coenzyme A carboxylase mutant in annual ryegrass (*Lolium rigidum*) selected by sethoxydim. Planta 190: 176-181.

Valverde, B.E. and K. Itoh. 2001. World rice and herbicide resistance. *In* Herbicide Resistance and World Grains. S.B. Powles and D.L. Shanner, Eds., pages 195-249. CRC Press LLC,Boca Raton, FL.

Seedling Emergence and Growth of Glyphosate-Resistant and Susceptible Biotypes of Goosegrass in Relation to Fertilizer Use

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Abstract: Greenhouse studies were conducted to investigate the effects of nitrogen (N), phosphorus (P), potassium (K) and chicken manure on seedling emergence and growth of glyphosate-resistant (R) and -susceptible (S) *Eleusine indica* (L.) Gaertn. biotypes. Generally, the emergence of both R and S seedlings declined as N levels increased regardless of P and/or K levels. At high N rates (120 kg ha^{-1}), little or no emergence was observed in both biotypes. Chicken manure applied at 6,000 kg ha^{-1} stimulated the emergence of R and S biotypes by approximately 60% and 270% of the control, respectively. There was no significant difference between the R and S biotypes in total biomass at N-deficient (0 kg ha^{-1}) and at moderate N rates (60 kg ha^{-1}) regardless of P and/or K levels. However, the R biotype apportioned greater dry matter into the roots than the S biotype at the N-deficient rate. The R biotype exhibited lower total biomass than the S biotype on exposure to high rates of N (120 kg ha^{-1}) regardless of P and/or K rates. Although both biotypes did not exhibit significant difference in total biomass when chicken manure was applied at 3,000 and 6,000 kg ha^{-1}, the R biotype partitioned more biomass into inflorescences relative to the S biotype.

Key words: chicken manure, *Eleusine indica*, fertilizer, glyphosate, nutrient stress.

INTRODUCTION

Eleusine indica (L.) Gaertn., a tufted annual grass, commonly known as goosegrass, presents one of the most serious weed problems in the cultivation of vegetables, orchards, oil palm and rubber plantations as well as in wasteland and along the roadsides of Peninsular Malaysia (Holm et al. 1977). Glyphosate, a postemergence, systemic and non-selective herbicide, has been used very successfully to control perennial and annual weeds such as *E. indica.*

The first appearance of glyphosate resistance was recorded in 1996 in *Lolium rigidum* Gaud (Powles et al. 1998). Recently, Teng and Teo (1999) and Lim and Ngim (2000) reported glyphosate-resistant *E. indica* in orchards, vegetable farms, nurseries, and young oil palm plantations in several regions throughout Peninsular Malaysia. On-site field trial results have demonstrated that the resistant biotype of *E. indica* was able to withstand glyphosate application rates of more than four times the recommended dosage of 1.08 kg ae ha^{-1} in Bidor, Perak (Teng and Teo 1999). Lim and Ngim (2000) reported that glyphosate applied at 5.76 kg salt ha^{-1} provided only about 25% control of the R biotype in Teluk Intan, Perak. The level of resistance in the R biotype in Teluk Intan was found to be 8- to 12-fold higher than the susceptible biotype.

Weed species can vary in their response and sensitivity to various forms of nitrogen (N). For example, witchweed infestations are affected by the form and concentration of N in the fertilizers used. Urea and, to some extent, ammonium salts were demonstrated to reduce witchgrass infestations by inhibiting germination and radicle elongation (Mumera and Below 1993; Pesch and Pieterse 1982). Teyker et al. (1991) reported no effect on shoot dry weight of corn when N was applied either as nitrate or the ammonium salt plus an experimental nitrification inhibitor (EAS). In contrast, EAS applied with ammonium salt not only caused chlorosis and crinkling in redroot pigweed leaves, but also reduced pigweed shoot dry weight by 75% (Teyker et al. 1991).

To date, there is little published information on the effects of different nutrient sources such as N, phosphorous (P), potassium (K) and chicken manure on the glyphosate-resistant (R) and susceptible (S) biotypes of *E. indica*. This study was initiated to examine seedling emergence and growth of the R and S biotypes under different nutrient regimes. A better understanding of the differences in seedling emergence responses and growth of the R and S biotypes under various nutrient levels is very valuable for improving weed management of the R biotype.

MATERIALS AND METHODS

Seed source

E. indica seeds were collected from the Bidor district in Perak, Malaysia where glyphosate-resistant *E. indica* had been identified (Teng and Teo 1999). Putative glyphosate-resistant seeds were collected from a soursop farm at Batu 12, Bidor, where treatment with glyphosate had been carried out at least 10 times a year for five consecutive years, while the putative susceptible population was collected from along roadside (2 km apart). Each population consisted of 30 plants. Inflorescences from each plant were placed in envelopes individually.

Screening for the R and S biotypes

Seeds of plants from putative resistant and susceptible populations were germinated in an incubator with 12 h photoperiod, a light intensity of 50 $\mu E\ m^{-2}\ sec^{-1}$ and alternate temperature of $35/20^0C$ day/night. After two weeks, seedlings were individually transplanted into 94-cell measuring 28 cm- by 56-cm flats containing commercial potting mix soil and were grown in the greenhouse at 29 ± 4^0C, with a 12-h photoperiod and light intensity of 800 $\mu E\ m^{-2}\ sec^{-1}$. Plants were watered twice daily. Three weeks after transplanting, seedlings of each plant type were screened for resistance to glyphosate by spraying the seedlings at the recommended rate of 1.08 kg ae ha^{-1} in order to confirm resistance or susceptibility.

Resistant (R) plants that survived glyphosate treatment and untreated susceptible (S) plants were grown in isolation to facilitate self-pollination. Each plant was grown in a 12.5-cm polybag filled with commercial potting mix soil and placed under an iron framed box covered with transparent plastic on the four sides and a nylon mesh material at the top. The R and S biotypes were grown in separate greenhouse bays to prevent cross-pollination. The R and S biotype seeds collected from this first generation were used in the subsequent studies.

Seedling emergence study

Twenty scarified seeds of the R and S biotypes of *E. indica* were sown in 12-cm diameter-pots containing sterilized and washed sand on the soil surface in a greenhouse maintained at 29±4°C, a 12-h photoperiod and light intensity of 800 $\mu E\ m^{-2}\ sec^{-1}$. The pots were sealed to prevent drainage. N was applied (as urea - 46% N) at rates equivalent to 0, 60 and 120 kg ha^{-1}, P (as superphosphate - 46% P) at 0, 75 and 150 kg ha^{-1} and K (as muriate of potash - 60% K) at 0 and 70 kg ha^{-1}. Chicken manure was applied at rates equivalent to 3,000 and 6,000 kg ha^{-1}.

From the start of the experiment, the soil was kept at 90% field capacity and this watering regime was continued for 11 weeks. A preliminary study using the tetrazolium test had shown that both biotype seeds were 100% viable. Seedling emergence was counted weekly for 11 weeks. Seedlings were considered emerged if the shoots appeared on the soil surface. Seedlings that had emerged were gently removed after each count.

Plant growth study

Scarified seeds of the R and S biotypes were germinated in 96-cell measuring 28 cm- by 56-cm flats containing commercial potting mix soil (Right Grow®). They were placed in a greenhouse (29±4°C, with a 12-h photoperiod and light intensity of 800 $\mu E\ m^{-2}\ sec^{-1}$) and watered twice daily.

After two weeks, uniform R and S seedlings were transplanted into individual 12-cm diameter pots containing 800 g sterilized and washed sand and placed in the greenhouse. Holes at the bottom of the pots were sealed to prevent leaching of nutrients. N was applied (as urea - 46% N) at rates equivalent to 0, 60 and 120 kg ha^{-1}, P (as superphosphate - 46% P) at 0, 75 and 150 kg ha^{-1} and K (as muriate of potash - 60% K) at 0 and 70 kg ha^{-1}. Chicken manure was applied at rates equivalent to 3,000 and 6,000 kg ha^{-1}.

The soil surface was watered to 90% field capacity twice daily and the regime was continued for four weeks. The number of tillers and spikes for each plant were counted at one-week intervals for one month. The shoots, vegetative structures, inflorescences and root dry weights were determined at the end of 4 weeks. At four weeks, shoots were cut to soil level and dried at 55°C for one week in an oven. Roots were separated after removing the soil by gently running water over them and dried before weighing.

Statistical analysis

All greenhouse experiments were designed as randomized complete block with three replications. Percentage data in the seedling emergence study was transformed to arcsine value. All data were subjected to analysis of variance and means were compared by the LSD test at the 5% level.

RESULTS AND DISCUSSION

Effect of fertilizer type on seedling emergence

Table 1 shows the main effects of N, P, K and chicken manure on seedling emergence of the R and S biotypes of *E. indica*. Percentage emergence of both biotypes declined with increasing levels of N with the highest N rate (120 kg ha^{-1}) showing the highest decline. This result suggests that high N rates in NH^{+} form has a suppressive effect on the emergence of both biotypes, which is in agreement with a previous study (Pesch and Pieterse 1982) on witchgrass. The effects of P on both biotypes are rather different. Seedling emergence of the R biotype decreased when the level of P was increased from 0 to 150 kg ha^{-1} with P at 75 kg ha^{-1} causing the highest decline. However, P had no significant effect on seedling emergence of the S biotype. The main effect of K on emergence for both biotypes was also different. It was observed that 70 kg ha^{-1} K appeared to cause increased emergence of the S biotype, while no significant effect was observed for the R biotype.

Table 1. Main effects of nitrogen (N), phosphorus (P), potassium (K) and chicken manure on seedling emergence of the resistant (R) and susceptible (S) biotypes of goosegrass.

	Emergence (%)	
Main effect (kg ha^{-1})	R	S
N = 0	15.1 a	15.3 a
N = 60	9.7 a	17.3 a
N = 120	0 b	2.4 b
P = 0	17.3 a	17.0 a
P = 75	4.2 b	10.7 a
P = 150	3.3 b	7.4 a
K = 0	7.4 a	6.3 a
K = 70	8.0 a	17.8 b
Chicken manure (kg ha^{-1})		
$N_oP_oK_o$	39 a	15 a
3,000	38 a	20 a
6,000	63 b	53 b

[#] N_o, P_o, K_o are 0, 0 and 0 kg ha^{-1} respectively (control).
Means within the same column at the chicken manure treatment with the same letter are not significantly different at the 5% level by the LSD test.

The percentage emergence of both biotypes treated with 3,000 kg ha^{-1} chicken manure did not differ significantly from the control at $N_oP_oK_o$. However, when the application rate of chicken manure was increased to 6,000 kg ha^{-1}, the percentage emergence of the R and S biotypes was stimulated by approximately 60% and 270%, respectively.

Effect of fertilizer type on plant growth

Table 2 shows the main effects of N and chicken manure on the numbers of tillers, spikes, total biomass, shoot/root ratio, percentage vegetative structure, inflorescence and root of glyphosate-resistant (R) and susceptible biotypes (S) of goosegrass. Increased number of tillers and spikes were observed in the R and S biotypes with increasing N levels. At moderate N (60 kg ha^{-1}) and high N rates (120 kg ha^{-1}), the R biotype produced more spikes and apportioned greater dry matter to the inflorescences than the S biotype. At the same nutrient regime, the S biotype partitioned more dry matter to vegetative structure than the R biotype.

Both biotypes exhibited increase in total biomass when the level of N was increased from 0 to 60 kg ha^{-1}. No significant difference in total biomass was observed between the two biotypes when N was deficient (0 kg ha^{-1}), irrespective of the P and K rates. However, the R biotype allocated more to root growth and lower shoot/root ratio than the S biotype at these nutrient regimes. This result implies that with N deficiency, the R biotype may have an advantage over the S biotype in nutrient acquisition.

At moderate rate of N (60 kg ha^{-1}), the total biomass of the S biotype did not differ from the R biotype. The shoot/root ratio, however, was similar for the R and S biotypes, suggesting that both biotypes had similar efficiency in nutrient absorption at the moderate N level. There was no significant difference in total biomass of the S biotype as the N rate increased from 60 to 120 kg ha^{-1}, but the total biomass of the R biotype was shown to be marginally reduced. Total biomass of the S biotype was higher than the R biotype at the highest N rate (120 kg ha^{-1}) regardless of the P and K levels. This observation suggests that the S biotype may be more competitive as than the R biotype in fertile soils. Interaction between biotype x P or K was not significant in total dry weight, shoot/root ratio, number of spikes and tillers, percentage inflorescence, and vegetative structures.

Chicken manure applied at 3,000 kg ha^{-1} and 6,000 kg ha^{-1} did not exhibit any significant difference in total biomass, shoot/root ratio and percentage vegetative structure of both biotypes. However, the proportion of dry matter apportioned to inflorescence growth in the R biotype was higher than that of the S biotype at both rates of chicken manure. Percentage apportioned for root growth of both biotypes at these rates was higher than the high and moderate rates of N, suggesting that both biotypes were under nutrient stress. At 3,000 kg ha^{-1} chicken manure, however, the S biotype may be more competitive than the R biotype in nutrient absorption as shown by the higher allocation to root growth.

Overall, the growth response of both biotypes to N, P and K obtained in this study suggests that coexistence of the R and S biotypes most likely takes place in habitats with moderate N. In habitat with low N, the R biotype may have an advantage over the S biotype. On the other hand, in fertile habitats high in N, P and K content, the S biotype may have an advantage over the R biotype. Nevertheless, more study is needed to compare the growth of the R and S biotypes under competitive conditions by using a set of replacement series experiments to asses the relative fitness of both biotypes under different nutritional regimes.

Table 2. Main effects of N and chicken manure on total dry weights, numbers of tillers and spikes, percentage of vegetative structure, inflorescences and root of glyphosate-resistant (R) and susceptible (S) biotypes of goosegrass.

Main effect (kg ha^{-1})	Total dry weight (g plant)$^{-1}$ R	Total dry weight (g plant)$^{-1}$ S
N = 0	1.4 a	1.4 a
N = 60	5.4 b	6.1 b
N = 120	4.8 c	6.3 b *
	No. tiller plant^{-1} R	**No. tiller plant^{-1} S**
N = 0	4.7 a	3.4 a
N = 60	7.6 b	6.8 b
N = 120	9.6 b	7.3 b
	No. spikes plant^{-1}	
N = 0	5.2 a	3.4 a
N = 60	23.4 b *	7.7 b
N = 120	19.9 b *	5.7 b
	Vegetative structure (%)	
N = 0	48.4 a	66.9 a *
N = 60	49.9 a	59.4 b *
N = 120	56.2 b	68.7 a *
	Inflorescence (%)	
N = 0	10.6 a	10.6 a
N = 60	25.9 b *	14.7 b
N = 120	18.2 c *	8.1 c
	Root (%)	
N = 0	41.0 a *	21.8 a
N = 60	24.1 b	25.9 a
N = 120	25.6 b	23.3 a
	Shoot: root	
N = 0	1.6 a	3.9 a *
N = 60	3.3 b	3.1 b
N = 120	3.0 b	3.5 b
Chicken manure (kg ha^{-1})	**Inflorescence (%)**	
3000	15.3 a *	7.7 a
6000	17.5 a *	9.6 a
	Root (%)	
3000	34.4 a	42.4 a *
6000	36.1 a	38.4 a

Means within the same column at each growth parameter with the same letter are not significantly different at the 5% level by the LSD test. * R and S are significantly different at the 5% level.

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LITERATURE CITED

Holm et al. 1977. The World's Worst Weeds: Distribution and Biology, Pages 47-53. Honolulu, The University Press of Hawaii.

Lim, J. L. and Ngim J. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L.) Gaertn.) in Malaysia. Pest Management Science, 56:336-339.

Mumera, L.M. and Below, F.E. 1993. Roles of nitrogen in resistance to *Striga* paratism in maize. Crop Science, 33:758-763.

Pesch, C. and Pieterse, A.H. 1982. Inhibition of germination in *Striga* by means of urea. Experientia, 38:559-560.

Powles et al. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. Weed Science, 46:604-07.

Teng, Y. T and Teo, K. C. 1999. Weed control and management of resistant goosegrass (*Eleusine indica*) in Malaysia. Proceedings of 17th Asian-Pacific Weed Science Society Conference I(B), Pages 753-758. Bangkok.

Teyker et al. 1991. Maize and pigweed response to nitrogen supply and form. Plant soil, 135:287-292.

Inheritance of Glyphosate Resistance in *Eleusine indica* (L.) Gaertn.

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Abstract: Reciprocal crosses were made between a resistant (R) population of *Eleusine indica*, which has been treated repeatedly with glyphosate, and a known susceptible (S) population. The hybridity was confirmed by isozyme analyses and the resulting F_1 hybrids were selfed to produce F_2 populations. Both the reciprocal F_1 hybrids displayed uniform intermediate levels of resistance to that of the parental types with no indication of maternal or paternal inheritance. A segregation ratio of 1:2:1 (S:I:R) was observed in the F_2 generation following glyphosate application, suggesting that glyphosate resistance is most likely inherited as a single, nuclear and incompletely dominant gene.

Key words: *Eleusine indica,* glyphosate, herbicide resistance, inheritance

INTRODUCTION

Herbicide resistance appears as a result of selection of pre-existing and continually occurring rare mutant genotypes that allow the evolution of populations under the pressure of the selecting herbicide. Resistance hereby refers to the capacity of a weed to withstand a herbicide used at its normal rate within populations of susceptible biotypes of weeds. The evolution of resistance can also occur naturally in populations at very low frequencies through recurrent mutation (Jasieniuk et al. 1996) prior to the application of herbicides. Resistant weeds will predominate in a population only when they have a selective advantage. Repeated use of the same herbicide or herbicides with a common mode of action generally provides the necessary selection pressure to select and increase the frequencies of resistant individuals in the population. Herbicide-resistant weeds were first confirmed in the late 1960s. Since then, about 261 resistant biotypes from 157 species (95 dicots and 62 monocots) in over 210,000 fields have been reported (Heap 2002).

Eleusine indica (L.) Gaertn. is a serious agronomic problem in orchards, immature oil palm plantations and vegetable farms (Holm et al. 1977). Until recently, glyphosate has been used successfully to control this weed. Late in 1997, the existence of a resistant biotype of *E. indica* was reported by Lim and Ngim (2000). These resistant biotypes identified in Teluk Intan, Malaysia showed an 8- to 12 –fold resistance to glyphosate compared to the susceptible biotypes. An understanding of the mode of inheritance of resistance will aid in predicting how readily the resistant gene in *E. indica* can spread and influence the rate of evolution of resistance.

The mode of inheritance varies with the plant species as well as with the herbicide type. In most instances where resistance is encoded by a nuclear gene, it is expressed as either a dominant (Jasieniuk et al. 1996) or incompletely dominant trait (Lorraine-Colwill et al. 2001), although trifluralin resistance has been reported to be controlled by a recessive gene (Zeng and Baird 1997). The only known example of maternal inheritance of herbicide resistance was the target-site triazine (Darmency 1994). Resistance to other herbicides most commonly results from an alteration in a single nuclear gene (Jasieniuk et al. 1996) except resistance to chlorotoluron in *Alopecurus myosuroides* which was reported to be controlled by two additive genes.

In a recent genetic analysis of glyphosate resistance in *Lolium rigidum*, the inheritance was inferred to be controlled by a single, incompletely dominant nuclear-encoded gene (Lorraine-Colwill et al. 2001). The resistance mechanism in this ryegrass population is a non-target site resistance mechanism that is manifested as limited glyphosate translocation. On the other hand, an altered target site confers the resistance in *E. indica*. In order to improve our understanding of the glyphosate resistance, we studied the inheritance of glyphosate resistance in *E. indica* using reciprocal F_1 and F_2 populations.

MATERIALS AND METHODS

Glyphosate-resistant (R) and –susceptible (S) parental plants used in the crosses were derived from seeds collected in Bidor (Perak, Malaysia) and Lenggeng (Negeri Sembilan, Malaysia), respectively. To ensure homozygosity for resistance in the Bidor and susceptibility in the Lenggeng populations, single seeds from each population were selected and selfed for two generations. The parental lines and all subsequent generations were selfed by enclosing inflorescences within glassine bags prior to anther dehiscence.

Seeds were germinated in petri dishes with moist filter paper under controlled conditions (12 h, 35°C, 50 $\mu Em^{-2}s^{-1}$ light period; 12 h, 20°C, dark period) in an incubator. After 2 weeks, the seedlings were transplanted into potting mix (Right Grow®) and thereafter maintained in a greenhouse. A total of 15 reciprocal crosses were made between the R and S biotypes by routine clipping and contact methods (Fehr 1980). Pollination was done by direct contact between the parental inflorescence in the early morning. It was left inside the glassine bag for four days until the pollination period was over. After four days, the glassine bag was removed and one month later, seeds were harvested, cleaned, dried and stored at room temperature.

The mixture of hybrids and "selfed" seeds produced from each of the reciprocal crosses was germinated and a total of 302 F_1 (184 seedlings from S♀ X R♂ and 118 seedlings from R♀ X S♂) seedlings were screened for hybridity at the three-leaf stage by conducting isozyme electrophoresis and ACP staining (Wickneswari and Norwati 1991). The acid phosphatase (ACP) locus is polymorphic in *E. indica* populations and is represented by two alleles (a "fast-band" allele and a "slow-band" allele) (Werth et al. 1994). The individual Lenggeng S biotype is a homozygous "fast-band" whilst the Bidor R biotype is a homozygous "slow-band". The F_1 hybrids will yield three bands due to the dimeric structure of the ACP enzyme (De Cherisey et al. 1985). All the identified F_1 hybrids were allowed to grow to maturity and their inflorescence was bagged with glassine paper to ensure self-pollination. The F_2 seeds from each of these F_1 plants were harvested as individual seed lots.

Glyphosate dose-response experiments were conducted on the S, R and F_1 plants. The confirmed hybrid F_1 from each cross was transplanted to polybags containing potting soil with one tiller plant per bag. Glyphosate was applied to the tiller plant at rates ranging from 0 to 1728 g a.e. ha^{-1}. For each herbicide dosage, 16 replicates of the S, R and hybrid F_1 (maternal S and R origin) populations were used. The F_1 dose-response experiment was a randomized complete block design and the replicates were rearranged weekly to ensure uniformity of growth. Twenty-one days after treatment, shoots were clipped at the soil level and weighed. The fresh shoot weight was expressed as the mean of 16 plants for each biotype.

The fresh weight was subjected to log-logistic analysis (Seefeldt et al. 1995). The dose response curves were calculated using the non-linear regression analysis procedure of SigmaPlot 2000 (Jandel Scientific, 2591 Kerner Blvd., San Rafael, CA 94901). Resistance ratio was determined from the ED_{50} values (ED_{50} R/ED_{50} S). The mathematical expression relating the response y to the dose x in the log-logistic analysis is given below:

$$y = C+(D-C)/(1+(x/ED_{50})^b)$$

where C = lower limit (corresponds to mean response at very high doses)
D = upper limit (corresponds to the mean response of the control)
b = slope (describes the slope of the curve around the ED_{50} value)
ED_{50} = effective dose, causing 50% reduction

The F_2 populations were screened in two steps. Firstly, the plants at the three-leaf stage were treated at 540, 789 and 868 g a.e. ha^{-1}, respectively. Based on the visual observations of the plants, two appropriate discriminatory dosages of glyphosate that can identify intermediate (I) plants were used. For each herbicide rate, a total of three replicates of the F_2 populations from both maternal S and R origin were used. The observed segregation ratios of S, I and R phenotypes were tested for goodness-of-fit to the expected Mendelian ratios using the chi-square test.

RESULTS AND DISCUSSION

F_1 dose-response curves

Isozyme analysis shows that 43 out of 184 S♀XR♂ and 41 out of 118 R♀XS♂ seedlings tested were hybrid progenies. The F_1 hybrids were evaluated for their glyphosate response by conducting a dose-response experiment. The response of F_1 hybrids obtained from reciprocal crosses of S and R parents was intermediate to those of the parents (Fig. 1). The ED_{50} of the susceptible population was calculated to be 250 g a.e. ha^{-1}, whilst the ED_{50} for the resistant population was 1685 g a.e. ha^{-1} (Table 1). Resistant *E. indica* therefore requires a 6.74-fold higher rate for 50% mortality compared to the susceptible population. The ED_{50} values for the combined F_1 (S♀ X R♂) or F_1 (R♀ X S♂) families were 856 and 913 g a.e. ha^{-1}, respectively. Both crosses resulted in F_1 progenies with an approximately 3.5-fold increase in resistance relative to the susceptible biotype.

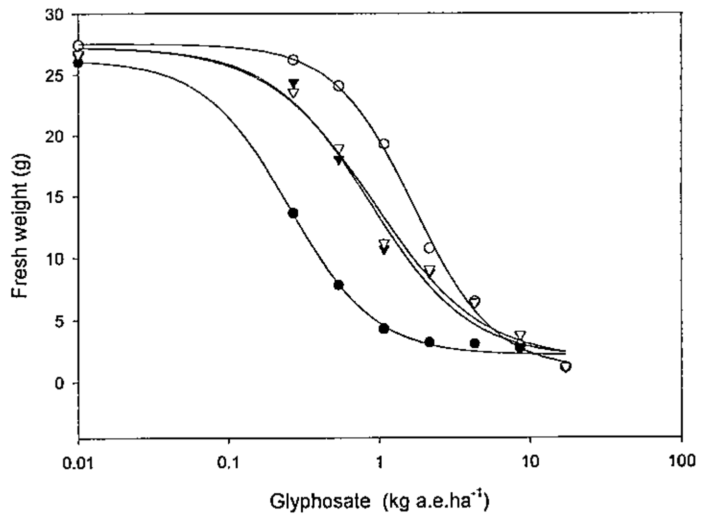

Figure 1. Dose-response of the susceptible (•), F_1 maternal S (▼), F_1 maternal R ( ), and resistant (○) populations of *E. indica* to glyphosate using the log-logistic model. Lines are the response curves predicted from nonlinear regression. Symbols represent mean fresh weight of 16 replicates.

If the dose response curves are closely parallel to the susceptible, a recessive gene would control the resistant plant. Conversely, a dominant gene will control the resistant plants if the dose response curves are closely parallel to the resistant curve. In these experiments, the dose response curves of the reciprocal F_1 plants were located in between the susceptible and resistant curves. Therefore, glyphosate resistance in *E. indica* most probably exhibits incomplete dominance. As both the reciprocal F_1 plants exhibited similar intermediate response to glyphosate, the glyphosate resistance in *E.indica* is most likely inherited as a nuclear trait.

Table 1. ED_{50} values of *E. indica* populations calculated by log-logistic analysis of the full dose-response.

Population	ED_{50} (g a.e. ha^{-1})	Resistance ratio
Susceptible (parent)	250 ± 21.6	
Resistant (parent)	1685 ± 107.9	6.74
F_1 (S♀ X R♂)	856 ± 189.8	3.4
F_1 (R♀ X S♂)	913 ± 188.2	3.7

Resistance ratio is the ED_{50} values of the resistant or F_1 populations divided by the ED_{50} value of the susceptible population. ED_{50} values are glyphosate dose ± standard error

Segregation of the resistance trait in F_2 populations

Seeds from six self-pollinated F_1 parents were collected to generate six F_2 families. Treatment of the F_2 seedlings with 789 g a.e. ha^{-1} (Table 2) and 868 g a.e. ha^{-1} (data not shown) provided the best discrimination between biotype responses. At these dosages, S, I and R biotypes could be identified among the F_2 progenies; the S biotype was entirely necrotic and obviously dead; the I biotype recovered from herbicide treatment but was stunted with leaf necrosis, similar to the F_1 parents and the R biotype exhibited no injury symptoms. Chi-square tests showed no significant difference for deviation from the expected Mendelian ratios in the F_2 populations (Table 2). This implies that a single and incompletely dominant gene controls glyphosate resistance in *E. indica*. Glyphosate resistance in *L. rigidum* was also reported to be controlled by a single incompletely dominant gene where the I phenotype and 1:1 (S:R) ratio in the backcross generations was observed (Lorraine-Colwill et al. 2001).

Table 2. Chi-square analysis for segregation in F_2 families for glyphosate resistance (789 g a.e. ha^{-1} treatment).

Family	Phenotype				χ^2 (1:2:1)	Probability
	S	I	R	Total		
F_2 (S♀ X R♂)						
1	80	157	70	307	0.811	0.667
2	69	160	81	310	1.252	0.535
3	84	149	81	314	0.873	0.646
F_2 (R♀ X S♂)						
1	72	164	80	316	0.861	0.650
2	83	147	76	306	0.791	0.673
3	75	164	78	317	0.438	0.803
Observed	463	941	466	1870	0.087	0.958
Expected	467.75	935.50	467.75			

The findings of this study allow us to designate the genotype of the F_1 hybrids based on the first alphabet of glyphosate as g^+/g^+ (susceptible), g^+/g^- (intermediate) and g^-/g^- (resistant). The symbol of 'g' denotes the glyphosate herbicide response phenotype locus. At the molecular level, incomplete dominance is generally caused by a quantitative effect of the number of "doses" of a wild-type allele; two doses (g^+/g^+) produce the most functional transcript and therefore the most functional product that can form bonding with glyphosate ; one dose (g^+/g^-) produces less transcript and product, whereas zero dose (g^-/g^-) has no functional transcript or product that can be bonded to glyphosate (Griffiths et al. 2000).

E. indica plants tested so far were either homozygous R or homozygous S. The heterozygosity for resistance to glyphosate has not been observed in the field population. The production of a heterozygous population by intercrossing is not normally expected due to the autogamous nature of reproduction in *E. indica* (Werth et al. 1994). The use of glyphosate removes S plants and most of the surviving progenies are homozygous R. The resistance trait would become the predominant allele due to the highly self-compatible behavior of *E. indica*. Thus an increased proportion of R plants in the population might occur with intensive use of glyphosate, especially if R plants are currently widely distributed in the natural population.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Darmency, H. 1994. Genetics of herbicide resistance in weeds and crops. Pages 263-297 in S.B. Powles and J.A.M. Holtum, eds. Herbicide resistance in plants : biology and biochemistry. Lewis Publishers, Boca Raton, Florida.

De Cherisey, H. et al. 1985. Inheritance of some marker genes in *Setaria italica* (L.) P. Beauv. Theoretical Applied Genetics, 71: 57-60.

Fehr, W.R. 1980. Artificial hybridization and self-pollination. Pages 105-132 in W.R. Fehr and H.H. Hadley, eds. Hybridization of Crop Plants. Madison, Wisconsin, USA.

Griffiths, A.J.F. et al. 2000. Gene interaction. Pages 106-140. An introduction to genetic analysis. W.H. Freeman and Company, New York.

Heap, I. 2002. International survey of herbicide-resistant weeds. Annual Report Internet, http://www.weedscience.com.html September 2002.

Holm, L.G. et al. 1977. *Eleusine indica* (L.) Gaertn. Pages 47-53. The world's worst weeds. Distribution and biology. The University Press of Hawaii, Honolulu.

Jasieniuk, M. et al. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Science, 44: 176-193.

Lim, J.L. and Ngim, J. 2000. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn.) in Malaysia. Pest Management Science, 56: 336-339.

Lorraine-Colwill, D.F. et al. 2001. Inheritance of evolved glyphosate resistance in *Lolium rigidum* (Gaud.). Theoretical Applied Genetics, 102: 545-550.

Seefeldt, S.S. et al. 1995. Log-logistic analysis of herbicide dose-response relationship. Weed Technology, 9: 218-227.

Werth, C.R. et al. 1994. Isozymes of *Eleusine* (Gramineae) and the origin of finger millet. American Journal of Botany, 81: 1186-1197.

Wickneswari, R. and Norwati, M. 1991 Techniques for starch gel electrophoresis of enzymes from acacias. Pages 88-100 in L.T. Carron and K.M. Aken, eds. Proceedings : Breeding Technologies for Tropical Acacias. ACIAR Proceedings No 37.

Zeng L. and Baird, W.V. 1997. Genetic basis of dinitroaniline herbicide resistance in a highly resistant biotype of goosegrass (*Eleusine indica*). Journal of Heredity, 88: 427-432.

Efficiency of Sulfosulfuron Against Isoproturon Resistant and Susceptible Biotypes of Little Seed Canarygrass (*Phalaris minor*)

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Abstract: Pot culture studies were conducted during rabi 2000-01 at CCSHAU, Regional Research Station Uchani to evaluate the efficiency of sulfosulfuron against isoproturon-resistant and susceptible biotypes of *Phalaris minor*. Seeds of isoproturon resistant *P. minor* were collected from 11 different sites in Haryana and Punjab. The isoproturon susceptible seeds were taken from the Uchani research station field. Sulfosulfuron was applied at four doses 6.25, 12.5, 25 and 50 g a.i. ha^{-1}. Sulfosulfuron was applied at 2-3 leaf stage of the *P. minor*. The study revealed that sulfosulfuron caused complete mortality of both resistant and susceptible biotype even at 6.25 g a.i. ha^{-1}, whereas the recommended dose of sulfosulfuron is 25 g ai ha^{-1}.

Key words: biotypes, resistant, sulfosulfuron, susceptible

INTRODUCTION

Wheat is an important winter cereal crop in India. Wheat fields are generally infested with annual grasses and broadleaf weeds. With the introduction of high yielding dwarf varieties of wheat, the ecological conditions of croplands have undergone a significant change leading to the intensification of grassy weeds, which are difficult to control by traditional methods. Little seed canarygrass (*Phalaris minor* Retz.) is the most abundant weed infesting wheat crop in the Northern part of India. The management of this weed has become pivotal to sustain the productivity of wheat. Isoproturon became a major input in wheat production since 1978 (Gill *et al.* 1978) and was able to stabilize the production of wheat by shielding at least a quarter of loss in the potential yield of wheat (Malik and Singh 1993). With indiscriminate and continuous use of isoproturon the *Phalaris minor* has already developed resistance against the herbicide in the rice-wheat cropping zone and the new generation herbicides sulfosulfuron, clodinofop and fenoxaprop are being used by farmers to tackle this menace. These new herbicides were applied on a considerable area for the first time in Rabi 1999. The factors, which were responsible for the development of resistance in *P. minor* against isoproturon, if repeated with these new chemistries, may once again cause resistance in *P. minor* against these new generation herbicides. Thus, the present investigation was done to eliminate any such possibility of resistance in this wheat bowl of India.

MATERIALS AND METHODS

Earthen pots were filled with 4.5 kg soil prepared by mixing soil and well rotten farmyard manure in the ratio of 6:1 by volume after passing it through 2 mm sieve. Twenty canarygrass seeds were planted in each pot, which were thinned to 10 seedlings per pot after germination. Pots were watered as needed to maintain adequate moisture. Sulfosulfuron was evaluated in the dose range of 6.25 to 50 g a.i. ha^{-1}.

The application of herbicide was done at 2 to 3 leaf stage of the weed using a knapsack spray with flat fan nozzle. The treatments were replicated thrice. Percent mortality was recorded at 10, 20, 40 and 60 days after treatment on 0 to 100 scale (0 for no control and 100 for complete control). The experiment was terminated after three months.

RESULTS AND DISCUSSION

Data recorded on percent mortality by the two biotypes of *P. minor* revealed that sulfosulfuron, when applied at a dose range of 6.25 – 50 g ai ha^{-1} caused complete mortality of both biotypes. Sulfosulfuron caused up to 50 percent mortality 20 days and after application and complete mortality at 40 DAA. There was no difference with respect to mortality between resistant and susceptible biotypes. Similar control with sulfosulfuron has also been reported by Malik and Yadav (1997).

Table 1.Visual Percent Biomass Reduction (percent mortality).

Sr.		10 DAT				20 DAT				40 DAT				60 DAT			
No.	**Entries**	**0.25X**	**0.5X**	**X**	**2X**	**0.25X**	**0.5X**	**X**	**2X**	**0.25X**	**0.5X**	**X**	**2X**	**0.25X**	**0.5X**	**X**	**2X**
1	RRS UCHANI	10	10	20	25	20	25	40	55	85	95	98	98	86	96	100	100
2	SSN RAMBA	15	15	25	30	25	25	40	50	90	95	98	98	92	96	100	100
3	CFP RAMBA	10	10	25	25	20	25	45	45	95	96	98	98	96	98	100	100
4	SSN G.GARH	10	15	15	30	20	30	40	45	95	96	97	98	96	98	100	100
5	CFP G.GARH	10	10	25	30	15	20	45	45	96	94	98	98	98	96	100	100
6	KHARWAN YNR	15	15	30	30	20	25	35	40	95	94	97	98	96	95	100	100
7	JAGADHARI	10	10	20	25	20	25	40	50	94	96	96	98	96	98	100	100
8	GAGSINA KNL	15	10	25	25	25	20	40	50	95	96	98	98	96	98	100	100
9	PANSARA YNR	10	20	20	25	20	25	35	45	92	96	98	98	95	98	100	100
10	PANJOLI PB	15	15	30	35	20	20	45	40	90	94	97	98	92	95	100	100
11	COOM PB	15	15	20	30	20	25	45	50	90	94	97	98	96	95	100	100
12	SAGGA	15	15	25	25	20	25	45	50	95	96	98	98	96	98	100	100

LITERATURE CITED

Malik, R. K. and A. yadav. 1997. Potency of alternate herbicides against isoproturon resistant littleseed canarygrass. *Proc. 16th APWSS Conf. Integrated Weed Management Towards Sustainable Agriculture,* Kaula Lumpur, Malaysia, 8-12 September, 208-210.

Malik, R. K. and S. Singh. 1993. Evolving strategies for herbicide use in wheat. Resistance and integrated weed management. In: *Proc. Int. Symp. Indian Soc. of Weed Sci., Hisar, Nov. 18-20* , 1: 225-238.

Gill, H.S., U.S. Walia and L.S. Brar. 1978. Control of *Phalaris minor* Retz. And wild oats in wheat with new herbicides. *Pesticides* 12: 55-56

Glyphosate Tolerance of *Dicliptera chinensis*: Dose-Response and EPSPS Activity

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Abstract: *Dicliptera chinensis* Juss. is an indigenou Acanthaceae of Taiwan and East Asia. In the last two decades, this plant has become a dominant weed in some of the orchards in central Taiwan. Many farmers complained that glyphosate failed to effectively control this weed. ED_{50} (visual rating) of *D. chinensis*, *Biden pilosa* var. *minor*, *Scoparia dulcis*. *Ageratum houstonianum* and *Amaranthus viridis* were 2.90, 0.51, 0.49, 0.34, and 0.15, respectively. Dose-responses were same for *D. chinensis* collected from areas of different herbicide-using history, suggested that the observed tolerance was not related to screening pressure of glyphosate. Pattern on absorption and translocation of glyphosate between *D. chinensis* and *A. houstonianum* were similar. Analysis of metabolic products showed that glyphosate in *D. chinensis* was not degraded in the study period. *D. chinensis* had higher EPSPS activity than *A. houstonianum*. Glyphosate treatment resulted in increase of EPSPS mRNA and protein level of *D. chinensis*. The increase was apparent at about 8 h of glyphosate treatment, and peaked at about 16 h. Our studies strongly suggested that the higher endogenous EPSPS activity and the induced formation of novel EPSPS in *D. chinensis* were the major factors contributed to its tolerance against glyphosate.

Key Words: *Dicliptera chinensis,* dose response, gene expression, glyphosate, resistance, 5- enolpyruvylshikimate -3-phosphate synthase

INTRODUCTION

Glyphosate (N-(phosphonomythyl) glycine) is a non-selective, broad-spectrum herbicide that is very effective against both grasses and broadleaf weeds. The mode of action of glyphosate in plants is inhibition of the EPSP synthase reaction (Amrhein et al., 1980). 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EPSPS, EC 2.5.1.19) is an enzyme in the shikimate pathway that leads to phenylalanine and from here tryptophan and tyrosine.

Tolerance to glyphosate has been reported for several bacteria and plant cells (Bradshaw et al. 1997). Glyphosate-tolerant petunia, tobacco, tomato, maize and *Corydalis sempervirens* were obtained from cell cultures after stepwise selection (Steinrücken 1986; Dyer et al. 1988; Forlani et al. 1992; Hollander-Czytko et al. 1992). Tolerance of their selected plants is related to over-expression of EPSPS due to gene amplification. In the field, repeated application of glyphosate also resulted in resistant ryegrass (*Lolium rigidum*) (Powles et al. 1998) and goosegrass (*Eleusine indica*) (Tran et al. 1999). Nature resistant biotype was previously reported on field bindweed (*Convolvulus arvensis* L.) (DeGennaro and Weller 1984).

Dicliptera chinensis Juss. is a naturally occurring glyphosate-resistant annual weed (Yuan et al. 2001). In the last two decades, this plant has become a dominant weed in some of the lowland orchards in central Taiwan, as a result of chemical weed control. In this paper, we present the results of our studies on *D. chinensis* related to dose-response and mechanisms of tolerance to glyphosate.

MATERIALS AND METHODS

Chemicals

Commercially formulated glyphosate, 41% solution were used for greenhouse and field tests. Chemicals of analytical grade were used in all other studies. ^{14}C-glyphosate was obtained from Amersham. A ^{32}P-EPSPS full length cDNA probe was prepared from the 1.9 kb PCR DNA product, labeled with ^{32}P-dCTP.

Dose-response tests

Seeds of *D. chinensis*, *B. pilosa* var. *minor*, *S. dulcis*, *A. houstonianum* and *A. viridis* were from field. Pot-raised seedlings were treated overhead with glyphosate at 0.04 to 6.6 kg ai ha^{-1}. The plant dry weight and control rating (%) were obtained. ED_{50} were caculated using the log-logistic model (Seefeldt et al. 1995).

Glyphosate uptake and translocation experiments

Seedlings were raised with Hoagland's solution in growth chamber. Five weeks old plants were treated with ^{14}C-label glyphosate. Glyphosate applied was equivalent to 0.5 kg ae ha-1. Labeled glyphosate solution consisted of 20% ^{14}C-glyphosate. Treated plants were sampled at 6, 12, 24, 48, 168 h after treatment and sectioned into treated leaves, shoots below treated leaves, shoots above treated leaves, stems and roots. External ^{14}C was removed by washing treated leaves for 1 min in 10 mL of distilled water. Aliquots of all plant homogenates were measured by liquid scintillation spectrometry. Glyphosate-sensitive *A. houstonianum* was used as a control plants.

EPSPS activity assay

The methods for EPSPS enzyme extraction and activity assay were modified from Shyr et al. (1992), Dyer et al. (1988) and Westwood and Weller (1997). Two to five g of fresh young leaves of *D. chinensis* and *A. houstonianum* seedlings were floated on 0.2 to 8 mM glyphosate solutions with 0.2 % Tween 20 or on deionized water with 0.2 % Tween 20 only. Samples were homogenized in extraction buffer and centrifuged at 2, 4, 8, 12, 18 and 24 h after treatment. Ten to 50 μL of the leaf extracts were added to the incubation mixtures. The supernatant was added to assay buffer. Phosphenolpyruvate remaining after the EPSPS reaction was measured. EPSPS specific activity was μmoles of PEP consumed mg protein per min. I_{50} was obtained using the log-logistic model.

Cloning of EPSPS cDNA of *D. chinensis*

Total RNA of *D. chinensis* young leaves was extracted with Trizol Reagent. The primers were designed from a tomato EPSPS gene sequence. A 0.8 kb fragment was obtained by RT-PCR method, and the nucleotide sequence was determined. DNA sequence comparisons using the NCBI GenBank BLAST programs confirmed that the 0.8 kb fragment contains part of the EPSPS gene. PCR RACE was performed in a thermal cycler. A 1.9 kb fragment was obtained. The complete nucleotide sequence was determined and confirmed, as described above.

Northern blots

Twenty μg of total RNA for each sample was loaded in a formaldehyde/agarose gel (1.2 %) and transferred to Hybond-N membrane. The hybridization probe was ^{32}P-EPSPS full-length cDNA of *D. chinensis*. The transcript level was quantified densitometrically analyzed by autoradiographs, using imaging software.

Immunoblotting

The coding region for the putative mature *D. chinensis* EPSPS enzyme was deduced from the cDNA sequence (Val^{29} to Thr^{293}). The amplified product was digested and ligated into the vector pEX-2T to make a GST-EPSPS fusion gene. The N-terminal amino acid sequence of the purified EPSPS was determined. Then the EPSPS protein was injected intravenously into rabbit to produce a polyclonal against EPSPS. Antibodies directed against the *D. chinensis* EPSPS at 1: 3000 dilutions in TBST. The protein levels were densitometrically analyzed using imaging software.

RESULTS AND DISCUSSION

Dose-response tests

Greenhouse tests showed that ED_{50} (visual rating) of *D. chinensis*, *Biden pilosa* var. *minor*, *Scoparia dulcis*. *Ageratum houstonianum* and *Amaranthus viridis* were 2.90, 0.51, 0.49, 0.34, and 0.15, respectively (Table 1). The ED_{50} for *D. chinensis* was 2-7 times higher than that of other species. We found that responses to glyphosate were similar for *D. chinensis* collected from areas with different herbicide-use history in Taiwan (Yuan et al., 2001). This suggested that the resistance of *D. chinensis* to glyphosate is not derived from selection by repeated use of this herbicide.

Table 1. Comparison of five weeds to foliar application of glyphosate.

Species	Plant stage at application		ED_{50} (kg a.i. ha^{-1})	
	Height(cm)	No. of leaves	Visual rating[1)]	Dry weight
Ageratum houstonianum	13	32	0.15 d	0.13 c
Amaranthus viridis	15	16	0.34 c	0.37 b
Bidens pilosa var.*minor*	18	16	0.51 b	0.48 b
Dicliptera chinensis	14	28	2.90 a	1.00 a
Scoparia dulcis	10	28	0.49 b	0.14 c

1) Visual basis was rated at 21 days after application on a scale of 0=no injury and 10=dead.

Absorption and distribution of ^{14}C glyphosate in *D. chinensis*

Table 2 shows total recoveries of applied radioactivity through the first 168 hours after treatment. Ratio activity recovered from different parts of treated plant showed that applied glyphosate was well translocated in both species. Level of glyphosate increased from 0.2 to 3.5 % in roots of *D. chinensis*. Glyphosate was absorbed more rapidly by leaf of *D. chinensis* than *A. houstonianum* and much more glyphosate (86.3 %) was translocated in root tissues of *D. chinensis*. Our data suggested that tolerance of *D. chinensis*, against glyphosate was not related to absorption and translocation of the applied herbicide.

EPEPS activity and gene cloning

The I_{50} of EPSPS from *D. chinensis* and *A. houstonianum* were approximately 6.8 and 0.8 mM glyphosate, respectively (Fig 1). EPSPS of *D. chinensis* was less sensitive to glyphosate than that of *A. houstonianum*. Two cDNA clones of EPSPS from a glyphosate-resistant *D. chinensis* were obtained by RT-PCR using a pair of primers designed from tomato EPSPS sequence. The complete sequences of the two EPSPS cDNA were determined and information sented to (NCBI GeneBank Accession number: AF371965, AF371966). Both cDNAs (EPSPS-1 and EPSPS-2) had an open reading frame encoding a mature enzyme of 446 amino acids plus a transit peptide of 70 amino acids. The two cDNAs had identical 5' and 3' ends and differed by only 26 base pairs.

Table 2. Distribution of radio-activity from *Dicliptera chinensis* and *Ageratum houstonianum* treated* with ^{14}C-glyphosate.

Treated Plant	Harvest Time (h)	Rinse of treated leaves	% of total ^{14}C recovered					Total dpm X 10^2 recovered
			Treated leaves	Upper leaves	Lower leaves	Stem	Roots	
D. chinensis	6	98.7	0.4	0.2	0.2	0.2	0.2	201
	12	98.7	0.4	0.2	0.2	0.2	0.3	206
	24	96.9	0.7	0.4	0.4	0.5	1.0	132
	48	95.2	0.8	0.6	0.6	0.8	2.1	84
	168	92.5	3.0	1.9	0.8	0.3	3.5	79
A. housto-nianum	6	98.0	0.2	0.2	0.2	0.3	0.3	234
	12	98.4	1.0	0.2	0.1	0.2	0.1	119
	24	98.6	0.9	0.1	0.1	0.1	0.3	157
	48	97.5	1.3	0.4	0.2	0.3	0.2	127
	168	88.5	6.8	1.8	0.5	1.3	1.2	124

- Glyphosate solution, 20% labeled, was applied on young leaf next to the apex. Upper and lower leaves are relative to the treated leaves.

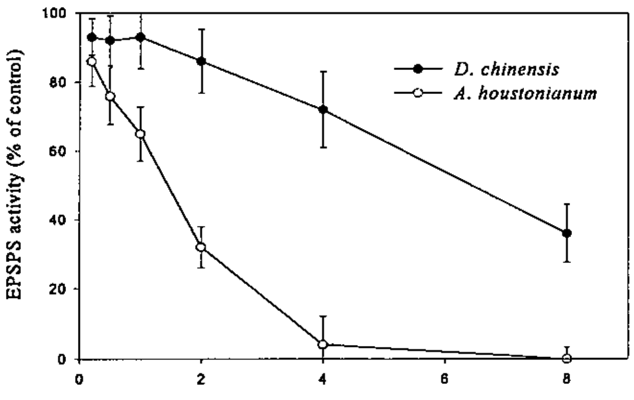

Fig. 1. Dose-response of EPSPS activity to glyphosate. Young leaves of *Diclipera chinensis* and *Ageratum houstonianum* were floated on 0.2-8 mM glyphosate solution and 0.2% Tween-20. Control leaves were floated on 0.2% Tween-20. EPSPS activity was determined at 12.5 h after glyphosate treatment as μmoles of PEP consumed per mg protein per minute. The error bars denotes standard deviation (n=3).

Effect of glyphosate on EPSPS at the mRNA and protein level

Glyphosate is known to inhibit the enzymatic activity of EPSPS, we checked to determine if glyphosate treatment would alter the level of EPSPS mRNA and protein.

The total RNA was isolated from leaves at 2, 4, 8, 12, 18, and 24 h. There was no significant difference in EPSPS mRNA expression level in control leaves exposed to buffer only (Fig. 2). Glyphosate treatment induced a significant and steady increase of EPSPS mRNA in *D. chinensis* from 8 to 18 h, then declined at 24 h. Induction was higher with 2 mM glyhosate than with 0.5 mM glyphosate (Fig. 2). In comparison, glyphosate caused a faster, shorter and weaker induction of EPSPS in *A. houstonianum* (Fig. 2). Protein extracted from the young leaves of *D. chinensis* and *A. houstonianum* were analyzed by immunoblotting using a polyclonal antiserum to *D. chinensis* EPSPS (Fig. 3). In *A. houstonianum,* glyphosate caused a reduction of the mature EPSPS protein level (Fig. 3). Consistent with the report that glyphosate also inhibits the translocation of EPSPS into the plastids and the concomitant cleavage of its transit peptide (dell-Cioppa and Kishore 1988), the pre-EPSPS level increased (70 %) at 4.5 h (Fig. 3). In contrast, in *D. chinensis* the level of mature EPSPS protein showed significant increase with time after 2 mM glyphosate treatment. The induction was apparent at 8.5 h and increased at 16.5 h (Fig. 3).

Our results showed that *D. chinensis* has triple novel mechanisms in its tolerance to glyphosate. First, an inherently more resistant EPSPS, based on its I_{50}, in *D. chinensis.* Second, its EPSPS had higher enzymatic activity even before glyphosate treatment. This was most likely due to higher specific enzyme activity, since the EPSPS mRNA and protein level were comparable in the two plants. Third, EPSPS was induced in response to glyphosate treatment. Both the steady state mRNA and protein level increased after glyphosate treatment. The induction explained why the EPSPS enzymatic activity increased after glyphosate treatment. Increases in specific RNA levels without gene amplification also have been reported in glyphosate-tolerant *C.*

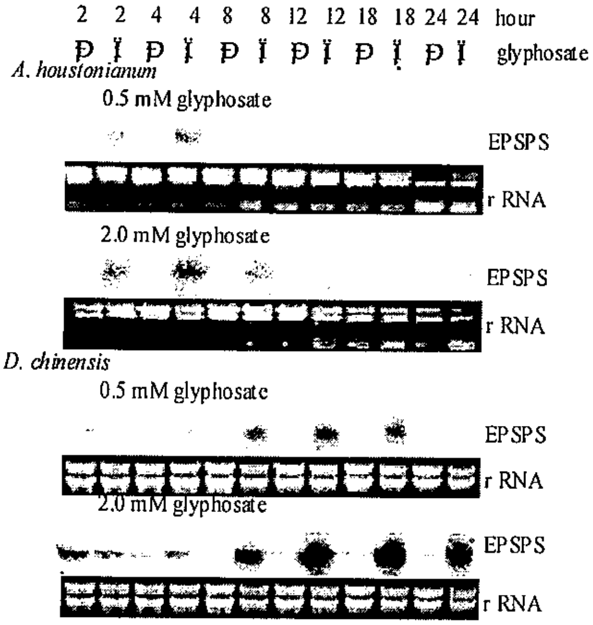

Fig. 2. Effect of glyphosate on EPSPS RNA level in *Diclipera chinensis* and *Ageratum houstonianum* young leaves. The leaves were treated with or without 0.5mM or 2.0 mM glyphosate for the indicated hours. Total RNA was extracted and analyzed by northern blot using a ^{32}P-labeled *D. chinenesis* EPSPS cDNA probe.

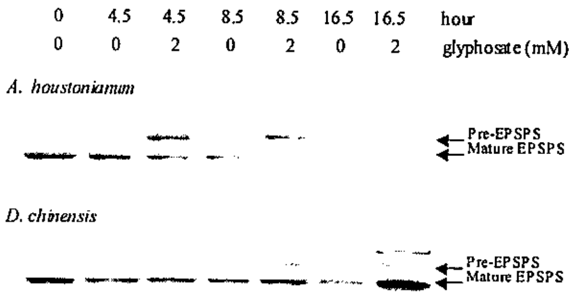

Fig. 3. Effect of glyphosate on EPSPS protein level in *Diclipera chinensis* and *Ageratum houstonianum* young leaves. The leaves were treated with or without 2.0 mM glyphosate for the indicated hours. Proteins were extracted, separated by SDS-PAGE and transferred to a nitrocellulose membrane. The western blot was probed with antiserum raised against *D. chinensis* EPSPS.

sempervirens and paraquat-resistant *Conyza bonariensis* (Hollander-Czytko, 1988; Ye and Gressel, 2000). In addition, our results showed that the enzymatic activity of EPSPS in different tissues did not correlate with the mRNA level, suggesting a further level of regulation at the posttranscriptional step.

LITERATURE CITED

Amrhein, N., B. et al. 1980. The site of the inhibition of the shikimate pathway by glyphosate. Plant Physiol. 65:830-834.

Bradshaw, L.D., S. et al. 1997. Perspectives on glyphosate resistance. Weed Technology 11: 189-198.

deGennaro, F.P. and S.C. Weller. 1984. Differential susceptibility of field bindweed (*Convolvulus arvensis*) biotype to glyphosate. Weed Sci. 32: 472-476.

della-Cioppa, G. and G.M. Kishore.1988. Import of a precursor protein into chloroplast is inhibited by the herbicide glyphosate. The EMBO Journal. 7: 1299-1305.

Dyer, W.E., S. et al. 1988. Glyphosate tolerance in tobacco (*Nicotiana tabacum* L.). Plant Physiol. 88: 661-666.

Forlani, G., E. et al. 1992. A glyphosate-resistant 5-enol-pyruvyl-shikimate-3-phosphate synthase confers tolerance to a maize cell line. Plant Sci. 85: 9-15.

Herbicide Physiology

Light Effect on Induction of 1-Aminocyclopropane-1-carboxylic Acid Synthase Activity in Quinclorac- or 2,4-D-Treated Maize Seedlings

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Abstract: Light involvement in quinclorac (3,7-dichloro-8-quinolinecarboxylic acid)-induced injuries and enhancement of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity in intact maize (*Zea mays* L. cv. Honey Bantam) seedlings were investigated. Root-treatment of intact seedlings with quinclorac significantly reduced the water and chlorophyll contents in the leaves under light, whereas no significant decrease was detected in the dark. In contrast, root-applied 2,4-D had no significant effect on chlorophyll content in the leaves under both light and dark conditions. Quinclorac (50 or 100 μM)-treated seedlings produced approximately two-fold higher levels of ethylene in the light than in the dark. For determining ACC synthase activity, the seedlings were treated with quinclorac (50 μM) for 12 h, and then transferred to light or kept in darkness. Quinclorac significantly enhanced ACC synthase activity in the shoot 6 h after exposure to light, while no significant activation was observed in the dark. In contrast, in 2,4-D-treated seedlings, a significant increase of the enzyme activity compared to untreated control was detected during 12 h dark treatment. These results suggested that light, ethylene biosynthesis and unknown light-regulated factor(s) are involved in the herbicidal action of quinclorac in intact maize seedlings and the mechanism of quinclorac-induced ACC synthase induction in maize is different from that induced by 2,4-D.

Key words: ACC synthase, auxinic herbicide, chlorophyll content, ethylene production, 2,4-D, water content

INTRODUCTION

Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) controls major grasses and broad-leaved weeds in direct-seeded and transplanted rice fields (Menck et al. 1985; Wuerzer and Berghaus 1985). Quinclorac is classified as auxin type herbicide, based on morphological responses of treated plants (Berghaus and Wuerzer 1987). In grass species, typical quinclorac symptoms are the appearance of necrotic spots in the developing leaves and subsequent chlorosis and necrosis of whole leaves. There have been many reports concerning the mode of action of quinclorac (Grossmann and Kwiatkowski 1995; Koo et al. 1996; Grossmann and Scheltrup 1997; 1998). Grossmann and Scheltrup (1997) reported that quinclorac stimulated ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity and endogenous levels of ACC in a concentration-dependent manner in barnyard grass. Furthermore, quinclorac elevated β-cyanoalanine synthase activity and cyanide accumulation, and concluded that cyanide, derived from quinclorac-stimulated ethylene biosynthesis, was the causative factor of leaf chlorosis in several grass species (Grossmann and

Kwiatkowski 1995). In addition, quinmerac (7-chloro-3- methylquinoline-8-carboxylic acid), which belongs to the same chemical family as quinclorac, stimulated ACC synthase activity, ethylene formation, and levels of ACC and abscisic acid (ABA) in sensitive dicotyledonous weed, cleavers (*Galium aparine* L.) (Grossmann and Scheltrup 1997; 1998). From these results, the involvement of an accumulation of ABA, the synthesis of which is enhanced by ethylene, has been suggested in the actions of quinmerac. We previously reported the enhancement of quinclorac action under illumination in maize leaf disks by demonstrating a strong correlation between light intensity and reduction of total chlorophyll content or level of ethylene production (Sunohara and Matsumoto 1997). This was the first report suggesting the involvement of light and/or light-related factors in the herbicidal action of quinclorac. Recently, Grossman *et al.* (2001) reported that quinmerac and quinclorac stimulated the generation of one of reactive oxygen species (ROS) H_2O_2, and hypothesized that the H_2O_2 contributed to the induction of cell death in *Galium* leaves. Although light is required for the generation of ROS in plants, the precise role of light in the actions of quinclorac is still unclear. The present study was conducted to examine the involvement of light in the phytotoxic action of root-applied quinclorac on intact maize plants, and to examine the influence of light on quinclorac-enhanced ACC synthase activity.

MATERIALS AND METHODS

Plant Materials

Seeds of maize (*Zea mays* L. cv. Honey Bantam) were washed with tap water, and soaked in water at room temperature for 3-6 h. Seeds were sown on paper towels moistened with distilled water in plastic trays. The trays were covered with aluminum foil and incubated at 30□ for 3 days in darkness. The dark-grown seedlings were transplanted to plastic trays containing Kasugai's nutrient solution and grown in a growth chamber at 25/20□, with 12 h of light (30-60 μmol m^{-2} s^{-1}) a day. Seedlings at approximately the second-leaf stage were used for experiments.

Water content

Roots of intact maize seedlings were immersed in a 0, 10, 50 or 100 μM quinclorac or 2,4-D solution containing 0.1% (v/v) dimethyl sulfoxide (DMSO) at 30□ for 12 h in darkness. Roots of untreated control seedlings were immersed in distilled water containing 0.1% DMSO. After the herbicide treatment, the roots were washed with distilled water and transplanted to a herbicide-free nutrient solution under continuous light (250-280 μmol $m^{-2}s^{-1}$) or darkness for 48 h. The first leaves of the seedlings were excised 48 h after treatment, then their fresh weights (FWs) were measured. The leaves were dried at 80□ for 48 h for dry weights (DWs) measurement. Water content (%) was calculated by the equation, (FW-DW)100/FW.

Chlorophyll content and ethylene production

Maize roots were treated with a 0, 10, 50 or 100 μM quinclorac or 2,4-D solution containing 0.1% DMSO at 30□ for 12 h under dark. After treatment, roots were washed with distilled water. Two seedlings were transferred into a 100 ml tall beaker containing a herbicide-free nutrient solution. The beakers were covered with another 100 ml tall beaker and their joint portions were sealed with six layers of Parafilm® and four layers of Sellotape®. The beakers were incubated at 30□ in continuous light or darkness. One milliliter of the gas phase was sampled with a syringe 0, 12, 24, 36 and 48 h after the sealing and ethylene content was analyzed by gas chromatograph (GC: Shimadzu GC-7A) (Sunohara et al. 2002). For chlorophyll determination, the first leaves of the seedlings were excised at 48 h, soaked in DMSO (10 ml g^{-1} FW) in a glass tube. The tubes were tightly sealed and incubated at 30□ for 24 h under dark. The concentrations of the extracted pigments (total chlorophyll, chlorophylls a and b) were calculated based on the absorbance values at 664, 648 and 470 nm using the method described by Chappelle and Kim (1992).

ACC synthase activity

Roots of maize seedlings were immersed for 12 h in the 0 or 50 μM quinclorac or 2,4-D solution containing 0.1% DMSO under dark. After the treatment, they were washed with distilled water and transplanted to the herbicide-free nutrient solution under light or dark conditions. Shoots of the seedlings were sampled at 0, 3, 6 and 12 h after application and stored at -80□ until assayed. ACC synthase activity was determined according to the procedures of Tsai *et al.* (1988) with minor modifications. The sampled seedlings were homogenized with 100 mM potassium phosphate buffer (1 ml g^{-1} FW), pH 8.0, containing 4 mM dithiothreitol (DTT), 0.4 μM pyridoxal phosphate and 1 mM EDTA in a mortar on ice with sea sand. The homogenate was centrifuged at 20,000 g for 20 min. The supernatant was then desalted on a Sephadex G-25 column (Pharmacia) pre-equilibrated with 20 mM potassium phosphate buffer, pH 8.0, containing 3 μM pyridoxal phosphate and 0.4 mM DTT. Four hundred microliters of the crude extract was transferred into a 5 ml vial and incubated at 30□ for 5 min with 100 μl of 500 μM *S*-adenosylmethionine (SAM) solution and 500 μl of 100 mM potassium phosphate buffer (pH 8.0) containing 4 mM pyridoxal phosphate. Three hundred microliters of 10 mM $HgCl_2$ solution was added to terminate the reaction. The vials were sealed and 0.2 ml of an ice-cold mixture of 5% NaOCl: saturated NaOH (2:1 v/v) was injected by a syringe. Vials were thoroughly shaken and incubated for 10 min at 30□. The ethylene content of the vial was analyzed by GC. One unit represented the activity that produced 1 nmol ACC h^{-1}. ACC synthase activity was evaluated in units per ml of the crude extract.

Statistical analyses

The relationship between quinclorac concentration and water content was analyzed by linear regression analysis. The difference in chlorophyll content or ethylene production between non-treated maize seedlings and quinclorac- or 2,4-D-treated seedlings was analyzed by Tukey's HSD test. The effects of quinclorac or 2,4-D on chlorophyll content and ethylene production were also analyzed by Kruskal-Wallis test. Linear regression analysis was performed to determine the relationship between ethylene production and chlorophyll content in maize seedlings. The difference in ACC synthase activity between non-treated control and 50 μM quinclorac- or 2,4-D-treated seedlings at the same time points after treatment was analyzed by *t*-test. The difference is considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Effect of light on quinclorac-induced phytotoxicity in maize seedlings

The water content of the first leaves of quinclorac-treated maize seedlings (Fig.1) decreased with increasing quinclorac concentration under light (p<0.01), but not in the dark (p=0.14). Root-applied quinclorac (50 or μ100 M) also significantly (p<0.05) reduced chlorophyll content of the first leaves under illumination, but not in the dark (Fig. 2a). In contrast, root-applied 2,4-D had no effect on chlorophyll content in the leaves under light and dark conditions (Fig. 2b). These results indicated that light was involved in the herbicidal action of quinclorac, such as chlorosis, reduction of water content in maize leaves.

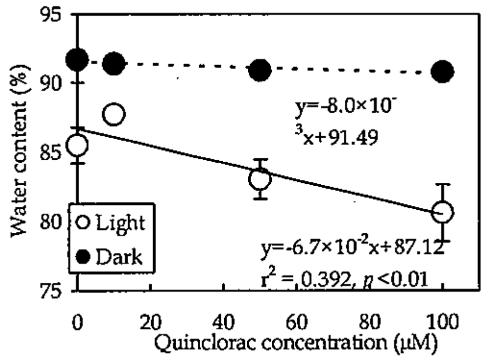

Figure 1. Relationship between quinclorac concentration and water content in the first leaf of quinclorac-treated maize seedlings 48 hr after treatment under light or dark conditions. Vertical bars indicate ±1 SE (n=3) of the means.

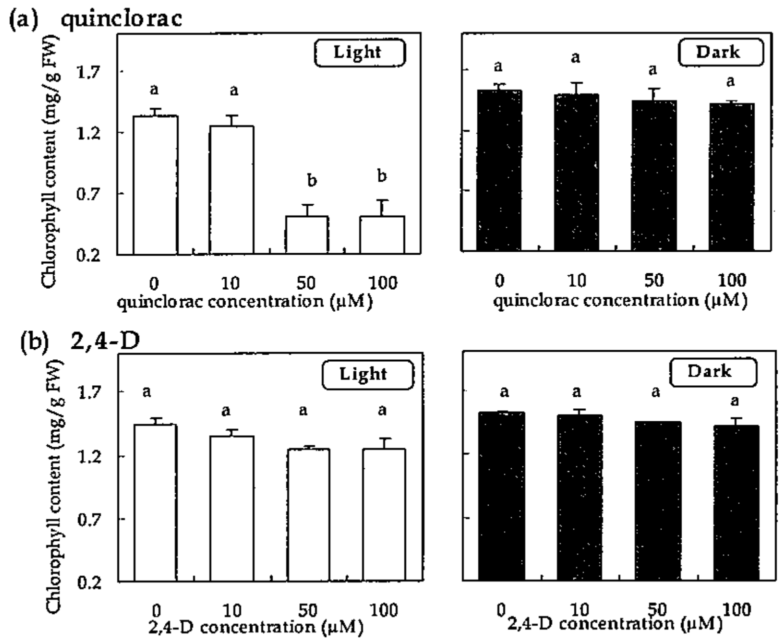

Figure 2. Effect of light on chlorophyll content in maize first leaf 48 h after root application of quinclorac or 2,4-D. Vertical bars represent ±1 SE (n=3) of the means. Means with different letters, a and b, differ significantly (p<0.05).

Effect of light on quinclorac-induced ethylene production

Ethylene production increased under illumination and reached the maximum at 24 h in 50 or 100 μM quinclorac-treated plants (Fig. 3a). Although ethylene induction was detected in 50 or 100 μM quinclorac-treated seedlings in the dark, the amount was approximately half of that under light conditions. In contrast, 2,4-D induced greater and comparable amounts of ethylene under light and dark conditions (Fig. 3b). Our previous studies (Sunohara and Matsumoto 1997; Sunohara et al. 1999) demonstrated the marked induction of ethylene production in quinclorac-treated maize leaf disks under illumination. In the study (Sunohara and Matsumoto 1997), quinclorac (50 μM)-treated leaf disks produced 35-fold increase in ethylene under light than dark conditions. Although the amount of ethylene in the present study was considerably small compared with that in leaf disks, light enhancement of quinclorac-induced ethylene production was confirmed with the intact seedlings. In this study, quinclorac was applied to roots of maize seedlings so that the amount of quinclorac translocated to leaves affects the ethylene production. These results suggest that quinclorac-induced ethylene produced under light mainly evolved from leaves.

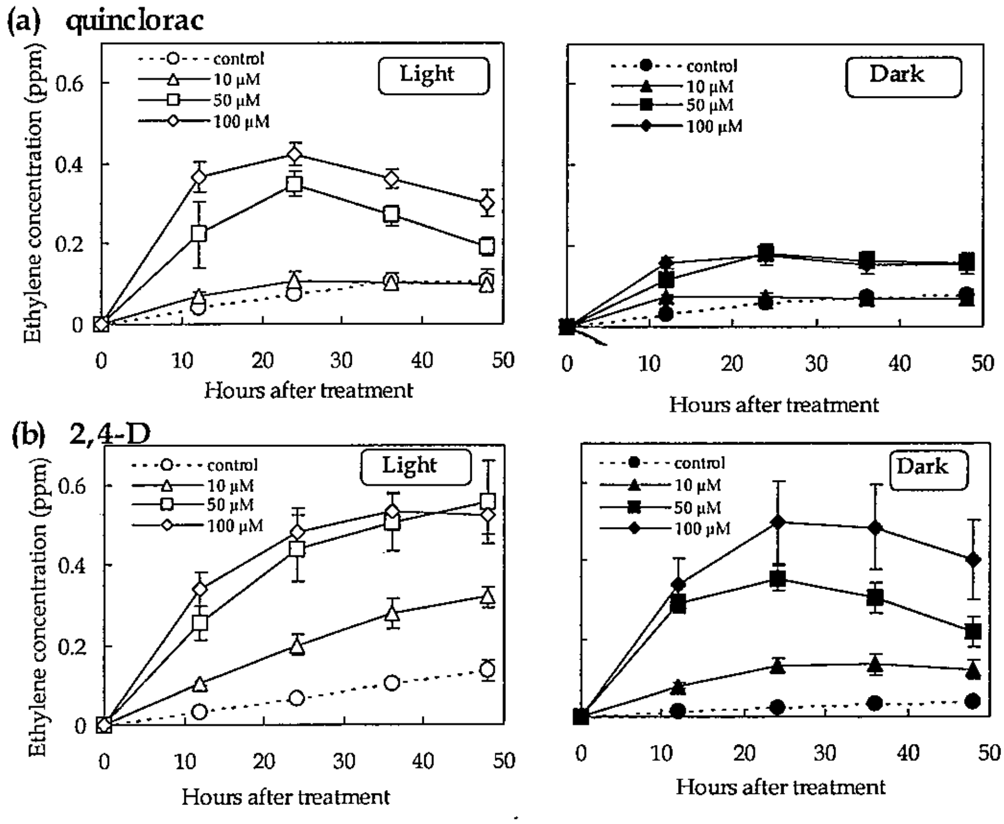

Figure 3. Time course of ethylene production in maize seedlings after quinclorac or 2,4-D treatment under light or dark conditions. Vertical bars represent ±1 SE (n=3) of the means.

Linear regression analysis showed that 84.3% of the variation in the chlorophyll content in maize leaves after root application of quinclorac was explained by quinclorac-induced ethylene production (Fig. 4). The percentage was much higher than that by 2,4-D-induced ethylene production (49.4%). These results show that quinclorac-induced ethylene biosynthesis is related to the herbicide-induced chlorosis. However, the quantity of ethylene after root application of 2,4-D (50 or 100 μM) under light and dark conditions were similar to quinclorac (50 or 100 μM) in the light (Figs. 3a and 3b), while no significant decrease in chlorophyll content was detected after 2,4-D application (Fig. 2b). These results imply that chlorosis in maize leaves after quinclorac-treatment cannot be explained only by the action of ethylene. Quinclorac-induced ethylene seems to be related to herbicidal action. However, additional unknown factor that leads to the enhancement of ethylene biosynthesis and its action seems to be strengthened in the interaction with ethylene might be mutually involved in the quinclorac-induced water loss and chlorosis in maize leaves.

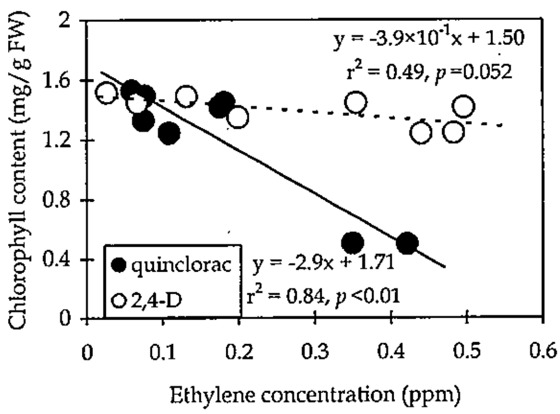

Figure 4. Relationship between quinclorac- or 2,4-D-induced ethylene production and chlorophyll content in maize seedlings. Ethylene concentration was measured 24 h after root application of 0, 10, 50 and 100 μM quinclorac or 2,4-D. Chlorophyll content was measured 48 h after root application of 0, 10, 50 and 100 μM quinclorac or 2,4-D.

Effect of light on quinclorac-induced ACC synthase activity

Root-applied quinclorac significantly ($p<0.05$) enhanced ACC synthase activity in maize shoots from 6 h after light exposure, while no significant activation of the enzyme was observed in the dark (Fig. 5a). In this experiment, a close relationship between quinclorac-induced ethylene production and the herbicide-enhanced ACC synthase activity was observed (Figs. 3a and 5a). Root-applied quinclorac did not considerably increase the conversion of ACC to ethylene (ACC oxidase) in the leaves under light and dark conditions (data not shown). Thus, we conjecture that quinclorac-induced ethylene synthesis is mainly due to the activation of ACC synthase. Six h of light exposure is needed for significant activation of ACC synthase in quinclorac-treated maize seedlings. Therefore, light may act as an inducer of ACC synthase activity following quinclorac application in the plants. In 2,4-D-treated seedlings, a significant ($p<0.05$) increase of ACC synthase activity was detected at 0 h (i.e., the end point of herbicide treatment) and thereafter the activity increased under both light and dark conditions (Fig. 5b). These results, therefore, indicated that 2,4-D caused activation of ACC synthase during the herbicide pre-treatment in the dark.

ACC synthase is encoded by a multi-gene family, and many of the isogenes in the gene family are differentially expressed in response to different signals (Kende 1993). So far various types of ACC synthase genes have been characterized (Nakajima et al. 1990; Yip et al. 1992; Yoon et al. 1997; Tuomainen et al. 1997). The gene expression in response to auxin is often rapid, detectable within 30 min of IAA treatment (Yip et al. 1992; Yoon et al. 1997), that is usually much faster than that induced by wounding (Mori and Tatsuki 1998). In the studies concerning ozone-regulated ACC synthase genes, the mRNA hybridizing to a probe for ACC synthase (*LE-ACS2*) showed a very weak induction 1 h after the onset of ozone exposure and peaked at 2 h (Tuomainen et al. 1997). Therefore, it is possible that the increase of ACC synthase activity following the application of quinclorac is due to the expression of an ACC synthase gene different from that induced by auxins, such as 2,4-D, but it is stimulated by an unknown light-regulated factor. These results support our previous study (Sunohara and Matsumoto 1997), suggesting that quinclorac does not act by entirely the same mechanism as 2,4-D and other auxins in maize.

All the results presented above showed that light is involved in herbicidal injuries of root-applied quinclorac in intact maize seedlings. Light also has to do with quinclorac-enhanced ethylene production and activation of ACC synthase. Quinclorac-induced chlorosis and loss of water in maize leaves in the light may be due to enhancement of ethylene biosynthesis pathway and unknown light-regulated factor. The mechanisms of quinclorac action in the light might be different with those of 2,4-D and possible involvement of relatively slowly induced ACC synthase is suggested.

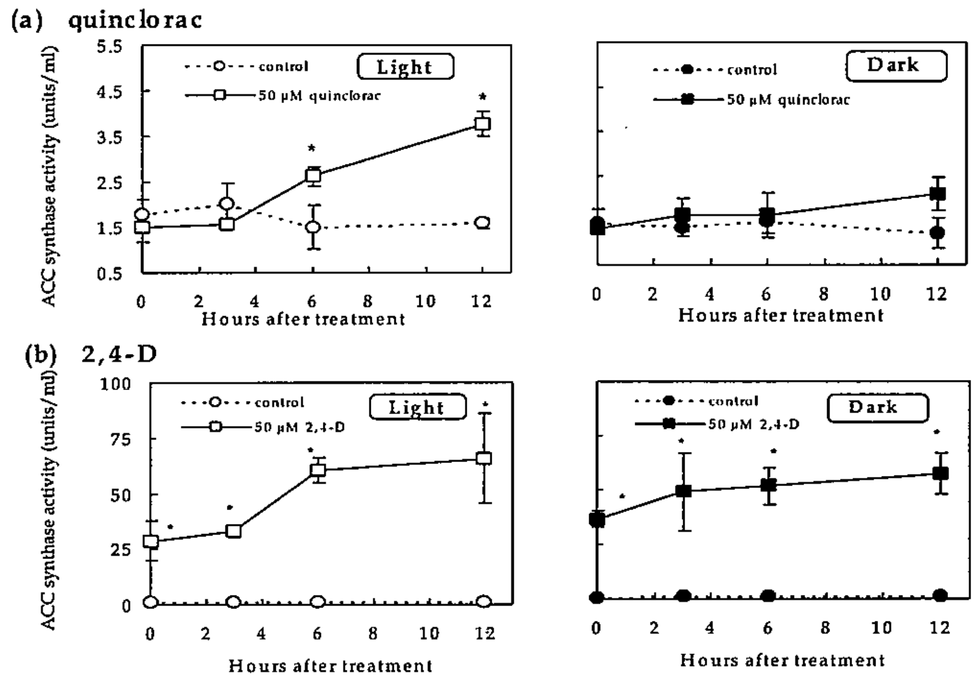

Figure 5. Time course of the changes in ACC synthase activity in maize shoots after root application of quinclorac or 2,4-D under light or dark conditions. Roots of the seedlings were pre-treated with quinclorac or 2,4-D for 12 h in the dark, and then the seedlings were transplanted to a herbicide-free nutrient solution and kept for a further 12 h under light or dark conditions. Vertical bars represent ±1 SE (n=3) of the means. *: $p<0.05$ (quinclorac or 2,4-D treatment vs non-treated control).

ACKNOWLEDGEMENT

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LITERATURE CITED

Berghaus, R. and B. Wuerzer. 1987. The mode of action of the new experimental herbicide quinclorac (BAS 514H). Proc. 11th Asian-Pac. Weed Sci. Soc. Conf., 81-87.

Chappelle, E.W. and M. S. Kim. 1992. Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. Remote Sens. Environ., 39:239-247.

Grossmann, K. and F. Scheltrup. 1997. Selective induction of 1-aminocyclopropane-1-carboxylate acid (ACC) synthase activity is involved in the selectivity of the auxin herbicide quinclorac between barnyard grass and rice. Pestic. Biochem. Physiol., 58:145-153.

Grossmann, K. and F. Scheltrup. 1998. Studies on the mechanism of selectivity of the auxin herbicide quinmerac. Pestic. Sci., 52:111-118.

Grossmann, K. and J. Kwiatkowski. 1995. Evidence for a causative role of cyanide, derived from ethylene biosynthesis, in the herbicidal mode of action of quinclorac in barnyard grass. Pestic. Biochem. Physiol., 51:150 160.

Grossmann, K. et al. 2001. Auxin herbicides induce H_2O_2 overproduction and tissue damage in cleavers (*Galium aparine* L.). J. Exp. Bot., 52:1811-1816.

Kende, H. 1993. Ethylene biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Bio., 44:283-307.

Koo, S.J. et al. 1996. 3,7-dichloroquinolinecarboxylic acid inhibits cell-wall biosynthesis in maize roots. *Plant Physiol.* 112:1383-1389.

Menck, B.H. et al. 1985. BAS 514. H-quinclorac field experience to control *Echinochloa crus-galli* in rice. Proc. 10th Asian-Pac. Weed. Sci Soc. Conf., 107-113.

Mori, H. and M. Tatsuki. 1998. Regulatory mechanism of ethylene biosynthesis. Pages 138-150 in H. Fukuda et al. eds. Signal transduction of plant hormones. Shujunsha, Tokyo. (in Japanese)

Nakajima, N. et al. 1990. Molecular cloning and sequence of a complementary DNA encoding 1-aminocyclopropane-1-carboxylate synthase induced by tissue wounding. Plant Cell Physiol., 31:1021-1029.

Sunohara, Y. and H. Matsumoto. 1997. Comparative physiological effects of quinclorac and auxins, and light involvement in quinclorac-induced chlorosis in maize leaves. Pestic. Biochem. Physiol., 58:125-132.

Sunohara, Y. et al. 1999. Effect of temperature on quinclorac-induced ethylene production from maize leaves. J. Pesticide Sci., 24:375-380.

Sunohara, Y. et al. 2002. Effects of trampling on morphology and ethylene production in asiatic plantain. Weed Sci., 50:479-484.

Tsai, D.S. et al. 1988. Purification of 1-aminocyclopropane-1-carboxylate synthase from etiolated mungbean hypocotyls. Arch. Biochem. Biophys. 264, 632-640.

Tuomainen, J. et al. 1997. Ozone induction of ethylene emission in tomato plants: regulation by differential accumulation of transcripts for the biosynthetic enzymes. Plant J., 12:1151-1162.

Wuerzer, B. and R. Berghaus. 1985. Substituted quinolinecarboxylic acid - new elements in herbicide systems. Proc. 10th Asian-Pac. Weed Sci. Soc. Conf., 177-184.

Yip, W.-K. et al. 1992. Differential accumulation of transcripts for four tomato 1-aminocyclopropane-1-carboxylate synthase homologs under various conditions. Proc. Natl. Acad. Sci. USA, 89:2475-2479.

Yoon, I. S. et al. 1997. *VR-ACS6* is an auxin-inducible 1-aminocyclopropane-1- carboxylate synthase gene in mungbean (*Vigna radiata*). Plant Cell Physiol., 38:217-224.

Characterization of a Gene Encoding 7-keto-8-Aminopelagonic Acid Synthase in *Arabidopsis thaliana*, and its Potential as a Novel Herbicide Target

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Abstract: To investigate a possibility for the 7-keto-8-aminopelargonic acid (KAPA) synthase as a novel potential herbicide target, we isolated *bioF* homologue, *AtKAPAS*, from *Arabidopsis thaliana*. An *AtKAPAS* encoded KAPA synthase of 469 amino acids, and shared extensive amino acid identity bacteria and yeast KAPA synthases, ranging from 28% to 38%, respectively. It contained the domain of predicted aminotransferase class II in the C-terminal region, as well as two putative membrane-spanning domains. The partially purified *AtKAPAS* protein had enzyme activity fit Michaelis-Menten kinetics for pimeloyl CoA, and a K_m for pimeloyl CoA of about 5.4×10^{-7} M, indicating that the *AtKAPAS* protein is a KAPA synthase with substrate specificity for pimeloyl CoA. Biotin auxotrophs transformed with antisense *AtKAPAS* construct, exhibited considerable phenotypic alterations such as growth inhibition, severe growth retardation, yellow-green cotyledon and leaf, and lethal phenotypes due to the levels of the antisense suppression. On the other hand, the auxotrophs were rescued in the presence of biotin. These results suggest, therefore, that the antisense disruption of *AtKAPAS* gene causes lethality in the early stage of plant development. It is proposed that new and better inhibitor design strategies could be focused on the preparation of mimics of the pimeloyl CoA as substrate because of a lower K_m value.

Key words: anti-sense, biotin, herbicide, 7-Keto-8-aminopelargonic acid (KAPA) synthase, pimeloyl CoA, target

INTRODUCTION

Biotin is an essential cofactor for a small number of enzymes involved in CO_2 transfer during carboxylation, decarboxylation, and transcarboxylation reactions that are related to fatty acid and carbohydrate metabolism (Alban et al. 2000). Bacteria, plants, and some fungi make their own biotin directly from chemical intermediates, whereas other organisms such as most fungi and animals must obtain from their surrounding environments. Therefore, studies on the inhibition of the enzymes involved in the biotin pathway will potentially offer an attractive target for herbicide development.

After Eisenberg (1973) and Pai (1975) first investigated the biosynthetic pathway of biotin in *Escherichia coli* and *Bacillus subtilis* through biochemical studies, numbers of researchers have widely investigated the biosynthetic pathway of biotin through biochemical and genetic studies in bacteria (Huang et al. 1995). Also, there have been numerous studies on the related gene for biotin synthesis identified in a variety of other microorganisms (Bult et al. 1996).

Herein, we describe the identification and characterization of *bioF* homologue, *AtKAPAS* from *Arabidopsis thaliana*, as well as the isolation of a third biotin auxotroph of *A. thaliana* disrupted at a different step of biosynthetic pathway, the decarboxylative condensation of L-alanine and pimeloyl CoA into KAPA. Antisense expression of the *AtKAPAS* gene induced the lethality in the transgenic *A. thaliana* plants, suggesting that the *AtKAPAS* gene could be an excellent target for the development of an herbicide.

MATERIALS AND METHODS

Recombination DNA technique

Isolation of plasmid DNA and plant DNA, restriction digestions, modification and ligation of DNA, polymerase chain reaction (PCR), agarose and polyacrylamide gel electrophoresis, transformation and culture of *E. coli* and *Agrobacterium* strains, and DNA gel blot analysis were carried out with some modifications of standard protocols. RNA gel blot (northern) analysis were performed as follows: 10 μg of total RNA were denatured, size-fractionated in 1.2% formaldehyde-agarose gels, and transferred onto nylon membranes (ICN, Costa Mesa, Calif., USA). The filters were hybridized and washed as described by Oh et al. (1996).

cDNA library construction

Total RNA isolated from leaf tissues of *A. thaliana* was used for preparation of poly(A)$^+$RNA. Double-stranded cDNA was synthesized from 5 μg of poly(A)$^+$RNA with the Time Saver cDNA synthesis kit (Pharmacia, Piscataway, N.J., USA), using *Not*I-$(dT)_{18}$ as a primer.

Expression of the AtKAPAS protein in *E. coli*

The primers encompassing the full-length cDNA of *AtKAPAS*, KAPAFB (5' GGCGGATCCTTCGCCCAAATCACAATTC 3') and KAPARH (5' GGCAAGCTT TTCACTGACAATATCAGAAACAA 3'), were synthesized to include *Bam*H1 and *Hind*III restriction site, respectively. Primers of KAPAFB and KAPARH were used in a PCR reaction to amplify the *AtKAPAS*-encoding region. The resulting PCR fragment was digested with *Bam*H1 and *Hind*III, and cloned into pCAL-n vector (Stratagene, La Jolla, CA, USA) to generate construct pCKAPA. *E. coli* containing expression vector pCKAPA was grown to early stationary phase at 37°C in Luria-Bertani (LB) broth media (USB, USA) supplemented with 100 μg· mL^{-1} of ampicillin, and collected by centrifugation at 4,000g for 15 min. The suspension grown at 37°C with shaking at 150 rpm until the A_{600} was attained to ca. 0.7 was incubated with isopropyl-D-thiogalactoside (IPTG) to a final concentration of 1 mM for the induction of the protein. The culture was grown for a further 2 h. The cells were washed with 50 mM-potassium phosphate buffer, pH 7.0, containing 50 mM $MgSO_4$ and 0.4 M NaCl, centrifuged at 4,000 g for 15 min, and kept at -20°C until use. The cell pellets were resuspended and pooled in 30 ml of $CaCl_2$ binding buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 10 mM β-mercaptoethanol, 1.0 mM magnesium acetate, 1.0 mM imidazole, 2 mM $CaCl_2$). Lysozyme was added to the cell suspension to a final concentration of 200 μg mL^{-1} and the mixture was mechanically rotated for 15 min. The sample was sonicated for 30 sec with the microtip at an intermediate setting. Sonicated-sample was cooled on ice for 3 to 5 minutes, and the procedure was repeated three times. After centrifugation at 10,000 g for 5 min, the supernatant was purified through calmodulin affinity chromatography.

Synthesis of substrate

Transesterification was performed as previously described by Wieland & Rueff for the synthesis of acyl-CoA thiol esters. CoA (lithium salt), (50 mg, 65 μmol) was dissolved in 1 ml of degassed 2% (w/v) $NaHCO_3$, and 6-(phenylthiocarbonyl)hexanoic acid (35 mg, 130 μmol) dissolved in 0.5 ml of methanol was added. The pH was adjusted to 8 by addition of 2% (w/v) $NaHCO_3$ and the solution was stirred at room temperature for 1 hr. After the reaction was completed (analyzed by HPLC), the pH was adjusted to 2 by the addition of conc. HCl. The aqueous phase was washed three times with diethyl ether and concentrated under reduced pressure. Pimeloyl CoA was purified by HPLC on a LiChrospher 100 RP-8e 5 μm (Merck) column with 15% (v/v) acetonitrile in 0.25 M-triethylammonium phosphate buffer, pH 5.5, as eluent (2 ml/min). Compound (II) was kept in an acidic solution (pH 2) at -20°C without noticeable degradation.

Enzyme assay

KAPAS activity was determined at 340 nm on a Backman DU series 60 spectrophotometer, thermostatically controlled at 30 °C with the method of Alexeev et al. (1994). A typical assay contained 20 mM potassium phosphate (pH 7.5), 1 mM α-ketoglutarate, 0.25 mM thiamine pyrophosphate, 1 mM NAD^+, 3 mM $MgCl_2$, 0.1 unit of α-ketoglutarate dehydrogenase, and 2 to 10 μg of KAPAS in a total volume of 1 mL. L-Alanine and pimelyl CoA were added to each assay to give the desired final concentration. Data were analyzed using Soft-Pac Module kinetics software. Prior to analysis, enzyme samples were dialyzed for 2 h at 4°C against 20 mM potassium phosphate (pH 7.5) containing 100 μM pyridoxal 5'-phosphate. The KAPAS concentrations in all analyses were 10 μM in 20 mM potassium phosphate (pH 7.5). Reference cuvettes contained all other compounds except KAPAS.

Analysis of T_1 transgenic plants

Transgenic plants were selected by soaking the seeds in a 0.1% solution of Basta herbicide for 30 min (Saito et al. 1992). To elucidate the biotin requirement of the transgenic plants transformed with the 'antisense' construct, the 5,000 transgenic seeds supplemented with Basta solution for 30 min were grown in petri-dish containing agar-solidified MS medium treated with and/or without 5 μM biotin as well as in the pot containing sand treated daily with and/or without 1 mM biotin (Schneider et al. 1989).

RESULTS AND DISCUSSIONS

As a number of enzymes related in the metabolic pathway of plants are essential to growth and development, those can be utilized as potential herbicide targets. We have, thus, performed molecular genetic dissection using reverse genetics of antisense approach to identify *AtKAPAS* gene encoding KAPA synthase in the pathway of biotin biosynthesis (Fig. 1) and to characterize the phenotypic consequences of loss-of-function mutations.

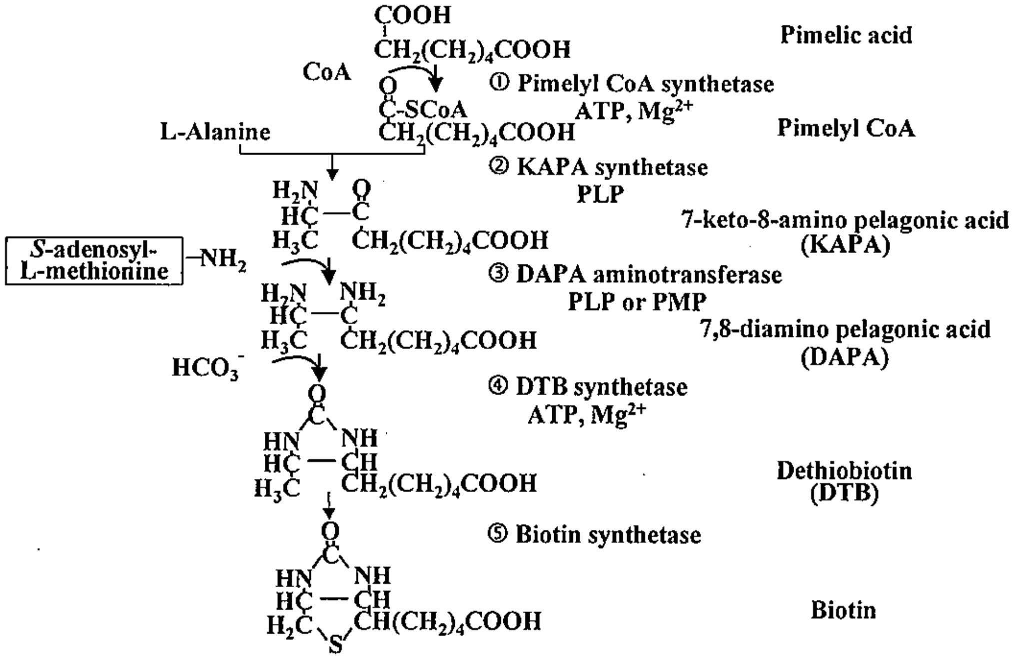

Figure 1. Biotin biosynthetic pathway in microorganisms.

The deduced amino acid sequence of *AtKAPAS* (Fig. 2) exhibits extensive identity with that of bacteria and yeast KAPA synthases, ranging from 28% identity with *E. coli BioF* to 38% identity with *B. sphaericus BioF*. Recently, it is also reported that AtKAPAS shares 35% amino acid identity and 55% amino acid similarity with *Kurthia* sp. (GeneBank accession number BAB39457, Kiyasu et al. 2001). The *AtKAPAS* locus has been assigned to position 8.7 cM on chromosome 5 of the classical genetic map. The loci of *bio1* and *bio2* related to the biosynthetic pathway of biotin described previously maps to 74 cM on chromosome 5 and 67 cM on chromosome 2, respectively. The *AtKAPAS* may be a transmembrane protein because the protein contains the putative membrane spanning regions. Sequence comparisons have been also reported stating that 7,8-diamino- pelargonic acid (DAPA) synthase in *E. coli* is identified as a member of the aminotransferase subclass II, and the overall structure of the protein is very similar to that of KAPA synthase, suggesting that two enzymes might be possibly derived from a common ancestor. Together with the amino acid sequence homology of *AtKAPAS* encoding KAPA synthase and *bio1* encoding DAPA synthase containing aminotransferase subclass III, it is suggested that two enzymes of *A. thaliana* may be evolutionary related, or possibly derived from a common ancestor as those of *E. coli*. On the other hand, *bio1* mutant, the first plant auxotroph in biotin biosynthesis, has been shown to result in embryonic lethality, and in the present study, the antisense repression of *AtKAPAS* gene causes the lethality of *A. thaliana*. It is also reported that antisense suppression of amino acid:glyoxylate aminotransferase containing aminotransferase class V domain results in substantial inhibition of the expression of the target gene so as to kill the plant, or at least inhibit normal plant growth or development (personal communication). The proteins containing the conserved domains of aminotransferase could be, therefore, utilized as novel target enzymes for new and better herbicides inhibiting such conserved domains.

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   1 atggcggatcattcgtgggataaaactgtggaagaagcagtgaatgtgcttgaatccaggcaaattcttcgatctttgaggcccatttgc
     M  A  D  H  S  W  D  K  T  V  E  E  A  V  N  V  L  E  S  R  Q  I  L  R  S  L  R  P  I  C
  91 atgtctaggcaaaacgaagaagaaatagtgaaaagcagagccaatggaggagacgggtacgaggtgttcgacggtttgtgtcaatgggat
     M  S  R  Q  N  E  E  E  I  V  K  S  R  A  N  G  G  D  G  Y  E  V  F  D  G  L  C  Q  W  D
 181 cggacttcagttgaggtgtctgtctcgattcctacatttcagaaatggcttcacgatgaacccagcaacggagaagagatttttagtgga
     R  T  S  V  E  V  S  V  S  I  P  T  F  Q  K  W  L  H  D  E  P  S  N  G  E  E  I  F  S  G
 271 gatgcattagctgagtgtagaaaagggagattcaagaagctgcttttgttctctgggaatgattatttgggtttgagctcacatcctaca
     D  A  L  A  E  C  R  K  G  R  F  K  K  L  L  L  F  S  G  N  D  Y  L  G  L  S  S  H  P  T
 361 atatcaaacgctgctgcaaacgcagtcaaagaatatggtatgggacctaagggttctgctttaatatgtggctataccacttatcatcgt
     I  S  N  A  A  A  N  A  V  K  E  Y  G  M  G  P  K  G  S  A  L  I  C  G  Y  T  T  Y  H  R
 451 ttgcttgagtctagtttggcgcaactgaagaaaaaagaggattgtcttgtttgtcctactgggtttgctgccaatatggctgcaatggtt
     L  L  E  S  S  L  A  Q  L  K  K  K  E  D  C  L  V  C  P  T  G  F  A  A  N  M  A  A  M  V
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     A  I  G  S  V  A  S  L  L  A  A  S  G  K  P  L  K  N  E  K  V  A  I  F  S  D  A  L  N  H
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     A  S  I  I  D  G  V  R  L  A  E  R  Q  G  N  V  E  V  F  V  Y  R  H  C  D  I  S  N  C  K
 721 atgaagaggaaggtcgtggtgactgatagcttatttagtatggacggtgactttgcaccaatggaagagctctctcagcttcggaagaag
     M  K  R  K  V  V  V  T  D  S  L  F  S  M  D  G  D  F  A  P  M  E  E  L  S  Q  L  R  K  K
 811 tatggcttccttctagttattgatgatgctcatggaacatttgtctgtggagaaaacggtggtggcgtggctgaggaatttaactgtgaa
     Y  G  F  L  L  V  I  D  D  A  H  G  T  F  V  C  G  E  N  G  G  G  V  A  E  E  F  N  C  E
 901 gctgatgtagatttatgtgtgggcactttgagtaaggcagcagggtgtcatggcggtttcatagcttgcagcaaaaaatggaagcaactg
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1081 aaggagatatggagaagaaaggcaatatgggagagggtaaaagagttcaaggaattatctggagttgacatctcaagccccattatctca
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1171 cttgttgtagggaatcaagagaaagccctcaaagcgagccggtatctattaaaatcaggcttccatgtaatggcaatacgaccgcccaca
     L  V  V  G  N  Q  E  K  A  L  K  A  S  R  Y  L  L  K  S  G  F  H  V  M  A  I  R  P  P  T
1261 gtgccacccaattcttgcaggctaagggtgacactgagtgcagcacataccacagaagatgtgaagaaactcatcactgcgctttcttct
     V  P  P  N  S  C  R  L  R  V  T  L  S  A  A  H  T  T  E  D  V  K  K  L  I  T  A  L  S  S
1351 tgtttggactttgacaacacagccactcacattccttcctttctatttcccaaattataa 1410
     C  L  D  F  D  N  T  A  T  H  I  P  S  F  L  F  P  K  L  *
```

Figure 2. Nucleotide and deduced amino acid sequence of the putative AtKAPAS.
The practical pl/Mw: 8.27/51312.05
Putative Plasma Membrane Spanning Region:
LVCPTGFAANMAAMVAI & SRGRSFIFSTAIPVPMA

KAPA synthase has a substrate specificity for pimeloyl CoA and L-alanine. For the product of *bioF* gene, KAPA synthase of *E. coli*, the K_m values for L-alanine and pimeloyl CoA are reported to be 0.50 mM and 25 μM, respectively, whereas for that of *B. sphaericus*, the values are reported to be 2.5 mM and 1.5 μM, respectively (Webster et al. 2000). To confirm whether *AtKAPAS* protein might be a KAPA synthase or not, we carried out the kinetic assay for the partially purified protein using synthesized pimeloyl CoA as substrate. The enzyme activity fit Michaelis-Menten kinetics for pimeloyl CoA, and the K_m and V_{max} values for pimeloyl CoA were about 5.4×10^{-7} M and 7.93, respectively (Fig. 3). These results indicate, therefore, that the *AtKAPAS* protein is a KAPA synthase at least with substrate specificity for pimeloyl CoA. The K_m determined for pimeloyl CoA is slightly greater than those for the corresponding *E. coli* and *B. sphaericus* enzymes, indicating that the *Arabidopsis* enzyme has a higher specificity for pimeloyl CoA than to *E. coli* and *B. sphaericus* enzymes. On the other hand, the mechanistic information to design inhibitors of the KAPA synthase and to test the inhibitors for herbicidal activity was absent on the practical source of the plant enzyme as a model so far. Thus, it is plausible that new and better inhibitor design strategies could be focused on the preparation of mimics of the pimeloyl CoA as substrate because of a lower K_m value.

Embryo-defective mutants of *A. thaliana* are valuable to identify the gene with essential functions during plant growth and development. But it is difficult to find the auxotrophs among embryo-defective mutants because they arrest early in the development of the plant. The following proposal with respect to biotin auxotrophs has made it easy to identify the auxotrophs among embryo-defective mutants: 1) the nutrient deficiency cannot be recovered by duplicate genes or maternal sources of biotin, 2) mutant embryos arrest at globular and cotyledon stages because maternal tissues cannot supply enough biotin to support rapid cell division and increased lipid biosynthesis associated with later stage of development, and 3) the auxotrophs can be effectively rescued in culture with the supplement of biotin (Patton et al. 1998). It should, therefore, be possible to complete genetic dissection of the entire biotin pathway in *Arabidopsis* by analyzing the collections of biotin auxotrophs among embryo-defective mutants. Mutant *bio1*, first plant auxotroph for biotin, has shown to result in embryonic lethality, and its embryos remain pale throughout development, typically arrest between germination and cotyledon stage of embryogenesis (Shellhammer and Meinke 1990). The supplement of biotin has been reported to induce the rescue of *bio1* auxotroph. Mutant *bio2* has also shown to result in embryonic lethality and can be rescued with supplemental biotin. In addition, it is suggested that its embryos typically arrest at the globular stage (Patton et al. 1998).

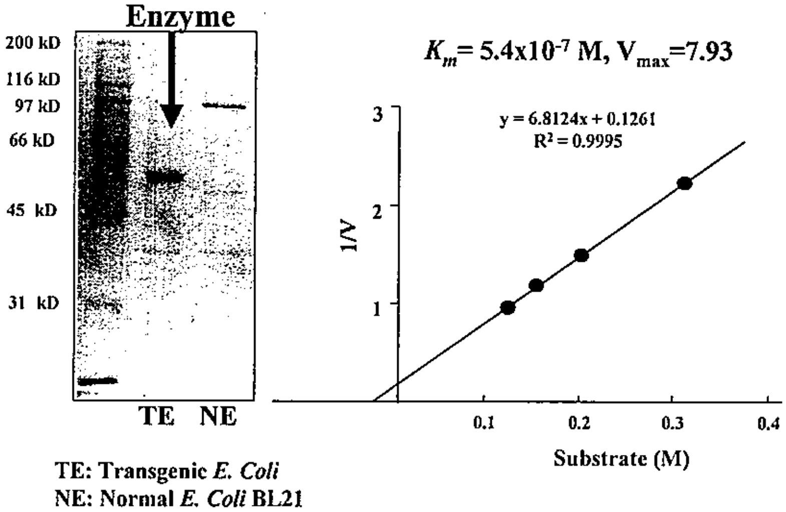

Figure 3. Purification and kinetics of *AtKAPAS*.

Genetic dissection and function on KAPA synthase associated with the pathway of biotin biosynthesis is understood in detail in microorganisms, but is little understood in plants. In the present study, we generated biotin auxotrophs disrupted at a step of biosynthetic pathway, the decarboxylative condensation of L-alanine and pimeloyl CoA into KAPA through direct antisense approach, and elucidated their functions on the phenotypic alteration of mutants supplemented with and/or without biotin. Considerable phenotypic alterations of the biotin auxotrophs for KAPA synthase without biotin supplement were exhibited as growth inhibition, severe growth retardation, yellow-green cotyledon and leaf, and so far as lethal phenotype due to the levels of antisense suppression. On the other hand, transgenic plants rescued in the presence of biotin did not considerably change in phenotypic characters compared to the wild type. In some plants, supplements containing required biotin partially failed to rescue the corresponding mutants, suggesting that it may be due to the partial disruption of the transport and utilization of biotin. These results suggest, therefore, that transgenic plants transformed with the 'antisense' construct might be biotin auxotroph such as *bio1* and *bio2* auxotroph, and antisense disruption of *AtKAPAS* gene cause lethality in the early stage of plant development (Fig. 4). The *AtKAPAS* gene may be an excellent target in the development of a herbicide because of the lethality by antisense disruption of the gene and the biotin requirement of animals from their environments. It is proposed, therefore, that new and better inhibitor design strategies could be focused on the preparation of mimics of the pimeloyl CoA as substrate because of a lower K_m value as well as mimics of the AtKAPAS protein

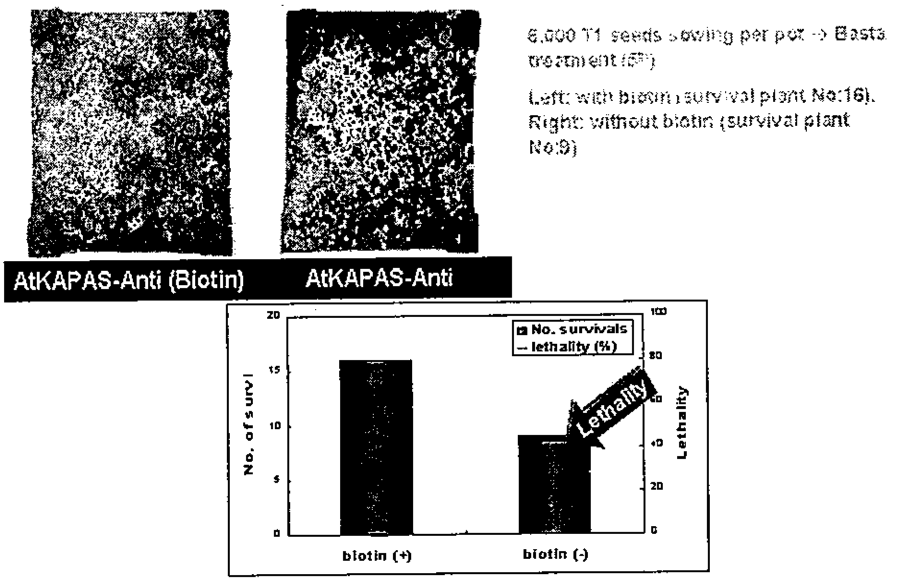

Figure 4. Phenotypic characteristics of *AtKAPAS* transgenic plants.

LITERATURE CITED

Alban C., Job D., Dource R. (2000) Biotin metabolism in Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 17-47.

Eisenberg M.A. (1973) Biotin: biogenesis, transport, and their regulation. Adv. Enzymol. 38: 317-372.

Pai C.H. (1975) Genetics of biotin biosynthesis in *Bacillus subtilis*. J Bacteriol. 121: 1-8.

Huang W., Jia J., Gibson K.J., Taylor W.S., Rendina A.R., Schneider G., Lindqvist Y. (1995) Mechanism of an ATP-dependent carboxylase, dethiobiotin synthetase, based on crystallographic studies of complexes with substrates and a reaction intermediate. Biochemistry 34: 10985-10995.

Bult C.J., White O., Olsen G.J., Zhou L., Fleischmann R.D., Sutton G.G., Blake J.A., FitzGerald L.M., Clayton R.A., Gocayne J., and others (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science 273: 1-58-1073.

Oh S.A., Lee S.Y., Chung I.K., Lee C-H, Nam H.G. (1996) A senescence-associated gene of *Arabidopsis thaliana* is distinctively regulated during natural and artificially induced leaf senescence. Plant Mol. Biol. 30:739-754.

Alexeev D., Bury S.M., Boys C.W.G., Turner M.A, Sawyer L., Ramsey A.J., Baxter

R.L. (1994) Sequence and crystallization of *Escherichia coli* dethiobiotin synthetase, the penultimate enzyme of biotin biosynthesis. J. Mol. Biol. 235: 774-776.

Saito K., Yamazaki M., Anzai H., Yoneyama K., Murakoshi I. (1992) Transgenic herbicide-resistant *Atropa belladonna* using an Ri binary vector and inheritance of the transgenic trait. Plant Cell Rep. 11:219-224.

Schneider T., Dinkins R., Robinson K., Schellha J., Meinke D.W. (1989) An embryo-lethal mutant of *Arabidopsis thaliana* is a biotin auxotroph. Dev. Biol. 131: 161-167.

Kiyasu T., Nagahashi Y., Hoshino T. (2001) Cloning and characterization of biotin biosynthetic genes of *Kurthia* sp. Gene 265:103-113.

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

Patton D.A., Schetter A.L., Franzmann L.H., Nelson K., Ward E.R., Meinke D.W. (1998) An embryo-defective mutant of Arabidopsis disrupted in the final step of biotin synthesis. Plant Physiol. 116: 935-946.

Shellhammer J., Meinke D. (1990) Arrested embryos from the *bio1* auxotroph of *Arabidopsis thaliana* contain reduced levels of biotin. Plant Physiol. 93: 1162-1167.

Rapid Measurement of Herbicide Translocation and the Effect of Climatic Conditions on the Translocation of LGC-42153

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Abstract: The leaf excision method (Koo et al. 2000) was applied to measure translocations of LGC-42153, pyribenzoxim, and glyphosate. Application doses for spot treatment to *Echinochloa formosensis* were 1.0, 2.0, and 0.5 μg plant^{-1} for LGC-42153, pyribenzoxim, and glyphosate, respectively and to *Brassica napus* were 10, 20, and 10 μg plant^{-1}, respectively. Herbicide translocation test using the leaf excision method revealed that LGC-42153 translocated the most quickly in both species, followed by glyphosate and pyribenzoxim. The activities of LGC-42153 against *E. formosensis* and *B. napus* were significantly reduced when the plants were subjected to shaded or low temperature conditions, and the translocation of LGC-42153 was delayed under shaded or low temperature conditions. In conclusion, the leaf excision method was very useful and applicable to compare translocation speed of different herbicides and variations of translocation under different climatic conditions.

Key words: *Echinochloa formosensis*, *Brassica napus*,leaf excision method, LGC-42153, light intensity, pyribenzoxim, translocation, temperature

INTRODUCTION

Absorption and translocation studies of herbicides are important to understand the modes of action of herbicides. The amount and rate of absorption and translocation affect the activities of herbicides, particularly for systemic herbicides. Herbicidal activity is also influenced by environmental factors such as temperature, humidity and light condition (e.g. Kudsk 1990), implying that these factors may affect herbicide absorption and translocation. For conventional studies of herbicide absorption and translocation, radiolabeled compounds are used to monitor radioactivity as an indicator of the herbicide movement in a plant. However, although this method provides quantitative information of translocation, biological implication often cannot be directly linked. Also, radio-chemical availability and equipment can become the limiting factor. In comparison with this method, the leaf excision method (Koo et al. 2000) does not use radio-chemical, and can compare variety of different herbicides in one time. The idea of the method is very simple, based on the concept that a systemic herbicide will kill the whole plant by spot application to a specific tissue of the plant. This method involves spot application of a defined amount of an herbicide on a recently mature leaf, then excision of the applied leaf at various time intervals. If the applied leaf is excised before a lethal amount of herbicide translocates, the plant will survive; however, if the applied leaf is excised after a lethal amount of herbicide translocates, the plant will die. By this simple procedure, it is possible to obtain the time required to obtain certain control value, thereby understand the translocation speed directly linked with biological efficacy.

LGC-42153, a new sulfonylurea herbicide recently developed by LG Life Sciences, Ltd., is a POST herbicide providing broad-spectrum weed control in rice and cereals, and inhibits acetolactate synthase (ALS) (Koo et al. 2003; Hwang et al. 2003). However, its translocation has not yet been studied.

In this study, we applied the leaf excision method to measure herbicide translocation of LGC-42153 in comparison with pyribenzoxim and glyphosate. Additionally, the effects of light and temperature conditions on herbicidal activity of LGC-42153 were investigated in relation with its translocation using the leaf excision method.

MATERIALS AND METHODS

Pot experiments were conducted in a glasshouse at the Agrochemical Research Institute of LG Life Sciences, Ltd., Daejeon, Korea in 2002. For all experiments, 3 plants pot^{-1} of *Echinochloa formosensis* and *Brassica napus* were grown in a pot of 10 cm diameter and 12 cm deep in the glasshouse

Measurement of herbicide translocation

Prior to the translocation study, whole plant assay was conducted to determine the application dose rate for the spot treatment in the leaf excision method (Koo et al. 2000). LGC-42153 (50% WP), pyribenzoxim (15% EC), and glyphosate (62% IPA) were spot treated at a range of dose rates; 0.02 to 20.48 µg, 0.01 to 10.24 µg, and 0.0001 to 10 µg plant^{-1}, respectively. Each herbicide solution of 10 µl at each dose rate was treated to the third leaf of *E. formosensis* at 3-leaf stage and the second leaf of *B. napus* at 2-leaf stage using a micropipette. Tween 20 was added to the solution at 0.1% concentration to guarantee good retention on the leaf. Assessment of fresh biomass was made at two weeks after herbicide treatment.

To measure herbicide translocation using the leaf excision method, 3 plants in a pot were spot treated with LGC-42153 at 1.0 and 10 µg plant^{-1}, pyribenzoxim at 2.0 and 20 µg plant^{-1}, and glyphosate at 0.5 and 20 µg plant^{-1} for *E. formosensis* and *B. napus*, respectively. The dose rates were determined based on the ID_{90} values (Table 1). After treatment, the treated leaf was excised sequentially at different timings; 1, 3, 6, 12, 24, 48, 72, and 96 h.

Table 1. ID_{50} and ID_{90} values of herbicides spot-treated for *E. formosensis* and *B. napus*.

Herbicide	*E. formosensis* (□ plant^{-1})		*B. napus* (□ plant^{-1})	
	ID_{50}	ID_{90}	ID_{50}	ID_{90}
LGC-42153	0.05	0.15	0.18	6.24
Pyribenzoxim	0.03	0.35 .	2.31	10.24
Glyphosate	0.09	0.11	0.98	11.34

*ID_{50}: herbicide dose required to get 50% inhibition, ID_{90}: herbicide dose required to get 90% inhibition

Effects of light and temperature on herbicide translocation

To evaluate the effects of light and temperature on the herbicidal activity and translocation of LGC-42153, whole plant assay and translocation measurement by the leaf excision method were conducted. Plants were initially grown in the glasshouse. At 14 days after sowing, for the whole plant assay, LGC-42153 was applied at 0.02 to 20.48 g a.i. ha^{-1} for *E. formosensis* and 0.0002~20.0 g a.i. ha^{-1} for *B. napus* using a CO_2-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8001E flat fan nozzle adjusted to deliver 300 L ha^{-1} at 300 kPa. For translocation measurement, spot treatment was made as described above. The treated plants were then grown in different light (0% (10~12 MJ m^{-2} sec^{-1}), 50% (4~6 MJ m^{-2} sec^{-1}), and 75% (2~3 MJ m^{-2} sec^{-1}) shading) and temperature conditions (20, 27, and 20/30□). Leaf excision was made at different timings for the rapid translocation measurement.

Statistical analysis

All experiments were conducted in a completely randomized design with at least 3 replications. For the herbicide dose-response experiments, Streibig's (1981) standard dose-response curve was used, while a general logistic curve was used for the herbicide translocation experiments. All data analyses were conducted using Genstat.

RESULTS AND DISCUSSION

Herbicide translocation

For measurement of herbicide translocation, a dose response to each herbicide by spot treatment was obtained. In the case of *E. formosensis*, the ID_{90} value of LGC-42153 was 0.15 μg $plant^{-1}$, while those of pyribenzoxim and glyphosate were 0.35 and 0.11 μg $plant^{-1}$, respectively (Table 1). For *B. napus*, those of LGC-42153, pyribenzoxim and glyphosate were 6.24, 10.24, and 11.34 μg $plant^{-1}$, respectively. To measure herbicide translocation by measuring plant response after leaf excision followed by herbicide spot treatment, the herbicide application dose rate must be sufficient enough to get the asymptotic level of response to the herbicide. Thus, we decided the application dose rates of LGC-42153, pyribenzoxim, and glyphosate to be 1.0, 2.0, and 0.5□ $plant^{-1}$ for *E. formosensis,* while 10, 20, and 10 μg $plant^{-1}$ were decided for *B. napus*, respectively.

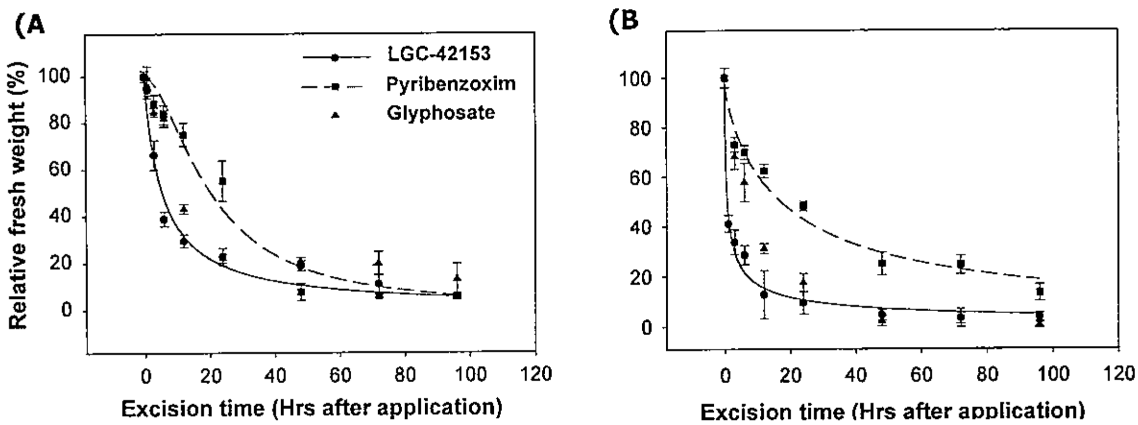

Figure 1. Herbicidal activity of LGC-42153 (●), pyribenzoxim (■), and glyphosate (▲) on *E. formosensis* (A) and *B. napus* (B) when the treated leaf was excised at different timings after spot treatment.

The effect of light condition on herbicide translocation

Figures 2A and 2B clearly show that the herbicidal activities of LGC-42153 on both *E. formosensis* and *B. napus* decreased in lower light conditions. The ID_{50} value for *E. formosensis* was 0.0012 g a.i. ha^{-1} at 0% shading, while it was 0.086 g a.i. ha^{-1} at 75% shading condition. This result agrees with Doran and Andersen's (1976) study of bentazon on *Abutilon theophrasti*. Herbicide translocation measured using the leaf excision method revealed that the decreased herbicidal activity at lower light conditions could be linked to delayed herbicide translocation (Figs. 2C and 2D). The time required to get 50% inhibition of *E. formosensis* and *B. napus* were 2.936 and 0.381 h after spot application at 0% shading condition while it was 8.680 and 8.688 h after application at 75% shading condition. This result indicates that light affects the production and translocation of assimilates, and can influence the movement of systemic herbicides within plants (Devine 1986).

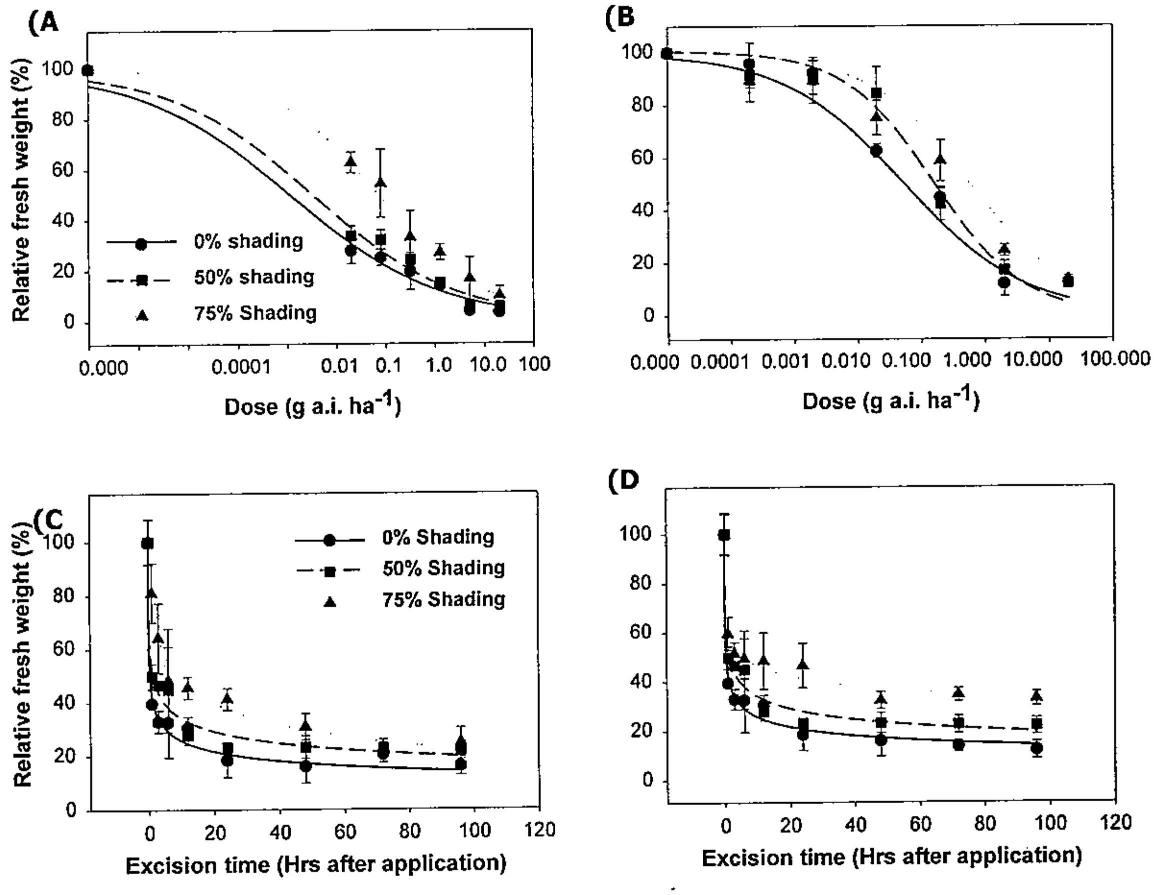

Figure 2. Dose-responses for the relative fresh weight of *E. formosensis* (A) and *B. napus* (B) to LGC-42153 at different light conditions, and herbicidal activities of LGC-42153 on *E. formosensis* (C) and *B. napus* (D) when the treated leaf was excised at different timings after spot treatment.

The effect of temperature on herbicide translocation

The herbicide LGC-42153 was found to be progressively more active on both the tested plants as temperature increased (Figure 3A and 3B). The ID_{50} values for *E. formosensis* and *B. napus* were 0.543 and 0.749 g a.i. ha^{-1} at 20°C, while those for *E. formosensis* were 0.0012 and 0.055 g a.i. ha^{-1} at 20/30 °C, respectively. Herbicide translocation was also influenced by temperature as shown in Figure 3C and 3D, with increased herbicide translocation at higher temperatures. The time required to get 50% inhibition of *E. formosensis* and *B. napus* were 21.115 and 4.559 hours after spot application at 20 °C, while at 20/30 °C those were 2.936 and 0.381 h after application, respectively.

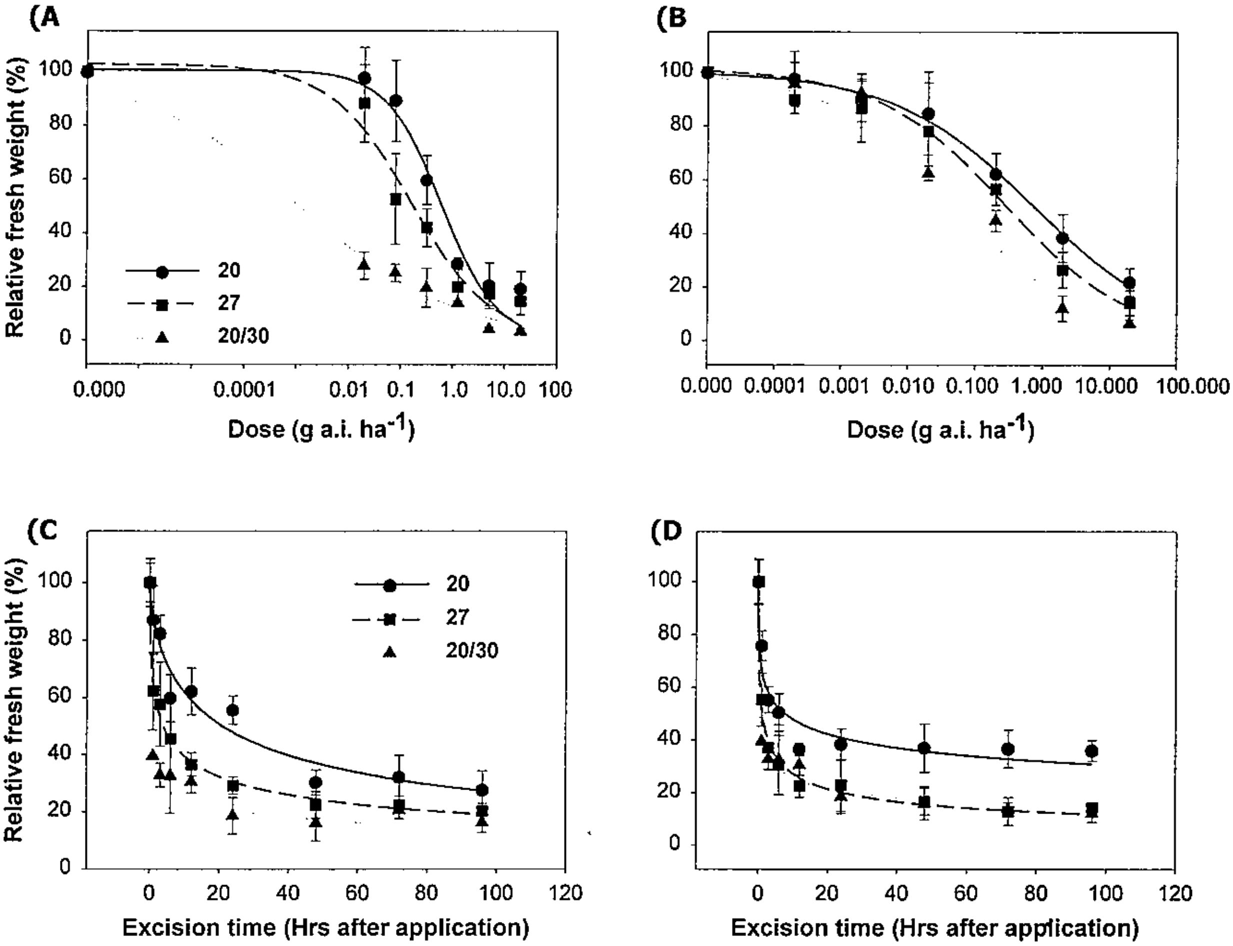

Figure 3. Dose-responses for the relative fresh weight of *E. formosensis* (A) and *B. napus* (B) to LGC-42153 at different temperature conditions, and herbicidal activities of LGC-42153 on *E. formosensis* (C) and *B. napus* (D) when the treated leaf was excised at different timings after spot treatment.

In conclusion, the leaf excision method was very useful and applicable to measure translocations of herbicides including LGC-42153, and measured significant difference in the translocation of LGC-42153 even under different light or temperature conditions. Herbicide translocation measured by the method thus helped to conclude that the reduced herbicidal activities under shaded or low temperature conditions could be linked to delayed translocation. Further study will be conducted to compare this leaf excision method directly with the conventional method using radiolabeled active ingredients.

LITERATURE CITED

Bevitori, R. et al. 2001. Transformation of spinach (*Spinacia oleracea* L.) for glyphosate resistance. The Proc. of the 18th Asian-Pacific Weed Sci. Soc. Conf.. 433-438.

Devin M. D. 1989. Phloem translocation of herbicide. In: reviews of weed science, Vol. 4 (ed. CL Foy). 191-213. Weed Science Society of America., Champain, USA.

Doran D. L. and Andersen R. N. 1976. Effectiveness of bentazon applied at various times of the day. *Weed Science* 24, 567-570.

Hwang, K. H., D. S. Kim, J. N. Lee, and S. J. Koo, 2003. Crop and weed selectivity to a new sulfonylurea herbicide LGC-42153. Proceedings of the 19th Asian-Pacific Weed Science Society Conference, 17-21 March 2003, Manila, Philippines.

Koo, S.J. et al. 2000, A simple bioassay to measure herbicide translocation in plants. Kor. J. Weed Sci. 20(3): 217-224.

Kudsk P. et al. 1990. The influence of temperature, humidity and simulated rain on the performance of thiameturon-methyl. Weed Research 30: 261-269.

Streibig, J.C.1980. Models for curve fitting herbicide dose response data. Acta Agriculturae Scandinavica 30:59-64.

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And
Weed Technology Extension

New Interactive Software for Weed Science Research and Education

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Abstract: The University of Queensland has been involved in the development of a number of interactive software tools for weed science research and education. In one such example, a series of identification keys to the weeds of the Australasian region have been developed using the computer program LucID. Once a specimen has been identified to a particular species, the key then provides multi-media fact sheets (photos, pictures and text descriptions) and links to web sites for further information. In a second example, an interactive simulation model of pesticide application based on the dynamics of spray droplet movement and the architectural development of crop plants has been developed. Models of crop architectures, different boom sprayer configurations and the simulation of individual pesticide droplet movements have been combined. The model also allows for particular nozzle types to simulate pesticide application from different configurations of overhead and dropper boom sprays.

Key words: education, identification, keys, LucID, simulations, software, training

INTRODUCTION

The University of Queensland has been involved in the development of a number of interactive software tools for weed science research and education. In one such example, a series of identification keys to the weeds of the Australasian region have been developed using the computer program LucID. Unlike dichotomous keys, LucID keys allow the user to decide on which order to work through the characters in the key, depending on the specimen being investigated and the user's ability to distinguish between different character states. Hence, if a user has a weed specimen without certain important taxonomic characters present, such as floral structures, they can use the characters that are present to identify the specimen. This is a major advantage over dichotomous keys, wherein progress cannot be made through the key if a certain character is absent. As the user selects character states, those weeds to which these do not apply are rejected, reducing the list of possible weeds. Once a specimen has been identified to a particular species, the keys then provide multi-media fact sheets (with photos, pictures and text descriptions) and links to web sites for further information. The keys come with tutorials, which provide a step-by-step walk-through that the user can interact with.

In a second example, a series of interactive simulation models of weed and crop growth are possible. To create the three-dimensional plant growth (virtual plants), a combination of mathematical equations, computer science and biological expertise are used. Using such systems, it is possible to model the canopy interaction of a crop and a weed or the fate of a pesticide application onto plants and off target locations such as the soil surface. It is also possible to observe insect (for example, a biological control agent) damage on plants at different stages of growth.

Interactive Weed Identification Software

A series of interactive weed identification and information packages are being created for a wide variety of users. Firstly, these identification 'keys' are being used for educating students involved in tertiary level weed science courses. Secondly, local government authorities are adopting them, as a tool to increase the effectiveness of their weed management programs. Finally, they are also being sold commercially to a much wider audience. The series of keys includes some that are very specific to certain regions and land uses, while others are designed to be much more general educational tools. Currently two 'keys' have been completed and another two are about to be released. They are as follows:

- Common Garden Weeds (a general key designed for educational uses)
- Suburban and Environmental Weeds (a key that focuses on the south-east Queensland region)
- Noxious Weeds of Australia (a key to all the state declared species in Australia)
- Crop Weeds of Australia (an educational key covering the main cropping weed species in Australia)

All of these keys are being created using LucID, a powerful yet easy to use software package developed by the Centre for Pest Information Technology and Transfer (CPIT¯ at The University of Queensland. LucID is a multi-media system designed specifically to help users make a correct identification of a biological specimen or to correctly diagnose a particular problem.

LucID

A major feature of LucID is its ease of use for those creating keys, using the 'Builder', and for those working through a key, using the 'Player'. The 'Builder' allows taxonomists or decision support developers to build and modify keys to meet the particular requirements of specific users. The 'Player' allows users to view and investigate these keys, incorporating text, images, video, and or sound to help the user select those taxonomic and diagnostic characteristics which best describe the particular case being investigated. As the user selects character states present on the specimen being identified, those species to which these character states do not apply are rejected, reducing the list of possible species or taxa remaining.

Unlike conventional dichotomous keys, LucID is a matrix key that allows the user to decide in which order to work through the characters in the key, depending on the specimen being investigated and the user's ability to distinguish between different character states (Norton *et al.* 2000). Hence, if a user has a specimen without certain important taxonomic characters present, such as floral structures, he or she can use the characters that are present to identify the specimen or at least narrow the field of candidates. This is a major advantage over dichotomous keys, where if a certain character is absent one cannot progress further through the key. Once a specimen has been identified to a particular taxon or species, the user can then view attached multi-media (i.e., photos, pictures, and text descriptions) or access linked websites for further information or recommendations. Demonstration versions of the *Lucid* Builder and Player are available on the internet (http://www.lucidcentral.com/). Similar identification keys include EUCLID (a eucalypt identification package) and a key to the Families of Australian Flowering Plants.

An example: The 'Noxious Weeds of Australia' key

This CD product is designed to allow easy identification of, and provide information about, all the state declared or noxious weed species in Australia (a group of about 350 weed species). It consists of a LucID 'key' and a large amount of additional content in HTML format. The LucID 'key' uses a relatively basic set of plant characters so that a great deal of botanical knowledge is not required for its use. Many vegetative and seed characters are also included so that identification will be easier if, as is often the case, floral characters are not present on weed specimens. As well as these noxious weeds, many other common weeds that may be confused for these species (i.e., about 300 additional species) are also included in the key. This is so that a user can identify an unknown species, and then determine if it is noxious or otherwise.

All characters present in the key are described, and their different states illustrated and defined. Each species has comprehensive information attached so that once an identification is made it can be checked. This includes photos of different stages of the plant's life cycle, a distribution map, as well as text descriptions including its declaration status in the various states of Australia and links to appropriate government web sites for control recommendations. In many cases photos of plants that are most commonly confused with these noxious weeds have also be included.

A tutorial can be accessed from the front screen that explains how to use the CD. An interactive tutorial is also included within the LucID key, which provides a step by step demonstration of its use. The CD can also be browsed like a web page to view additional resources such as reference lists, links to weed related web sites, and a substantial glossary. A search function is also provided so that finding information about a certain subject or species is simplified.

It is hoped that the CD will be regularly updated and in subsequent versions, it is anticipated that potential weed threats that are not yet present in Australia will also be added to the product along with any newly declared noxious species. All those concerned with the management of weeds in Australia should find this product useful. In particular, it is aimed at state and local weed control officers, primary producers, weed scientists, extension officers, and tertiary and secondary teachers.

Interactive Simulation Models

The growth of plants and their responses to different internal and external stimuli can now be simulated in computers. To do this, scientists at the Centre for Plant Architecture Informatics, the University of Queensland have harnessed technology to measure, in three dimensions, the structural development of weeds and crops. These measurements are transformed into models that are interpreted by software, developed by the Centre's collaborators, at the University of Calgary in Canada, to generate virtual plants: mathematical representations of crop and weed structure that can be displayed on computer screens (Hanan and Room 1997). This approach is revolutionizing the way weed scientists can study plant interactions with one another. Some expensive field trials, which take many months to complete, will be replaced by virtual experiments that provide answers in minutes, allowing the researcher to only undertake key field experiments. As well as simulating crop and weed growth and development, the simulations can incorporate the movement of insects over a plant, and the movement of metabolites, plant growth regulators or systemic pathogens and pesticides within a plant. Simulating insect movement and behavior, for example, can allow scientists to predict the amount of damage a weed will sustain when subjected to certain kinds of biological control agents at different stages of growth and in different environments. It is also possible to simulate how herbicide is sprayed on to weeds, to see where it is deposited on leaves and how much is lost into the environment. Different kinds of application can be simulated under a wide range of environmental conditions

L-Systems

To create plant growth simulations or virtual plants a combination of mathematics, computer science and biological expertise are used. The models simplify reality, but provide an animated representation of field measurements that would normally appear as numbers in tables. To convert these numbers into pictures, a series of 'growth rules' expressed in mathematical formulae, are used. This growth rule notation is called an 'L-System', after its inventor, Lindenmayer (1968).

In an L-system plants are considered to be modular and, at their simplest, can be divided into repeating units consisting of a segment of stem, a leaf, an apical bud and an axillary bud. Each apical bud grows to produce another whole unit, its development can be represented by the growth rule, A to IL[B]A, where A = apical bud, I = internode, L = leaf, B = axillary bud and [] represents the start and end of a branch (Room et al. 1996). Computer software 'interprets' such L-systems graphically. For example, applying the rule A to IL[B]A in a series of 'time steps' generates a series of growth stages which appear as a growing, virtual plant (see http://www.cpai.uq.edu.au/virtualplants.html). For a more realistic image, digital scans of plant tissues can be incorporated into the simulation. The simulation can focus on a whole plant or on a part of a plant. Images may also be viewed from any angle and can be displayed in sequence to give the impression of growth.

An example: Pesticide fate at the canopy interface

In this interactive simulation model, it is possible to apply a pesticide and, using knowledge of the dynamics of spray droplet movement, see the fate both on a simulated plant and onto the soil below (Fig. 1). This is achieved by combining the models of plant architecture, different boom sprayer configurations and the simulation of individual pesticide droplet movements in the atmosphere. The equations used allow for the effects of gravity and air drag, but not air turbulence. The model allows for particular nozzle types to simulate pesticide application from different configurations of overhead and dropper boom sprays. Routines can be run to detect when droplet trajectories intersect plant parts, to calculate the relative amounts of pesticide reaching different plant parts, and to show the amounts of pesticide in visualizations reaching the crop/weed or soil surface (Fig. 2).

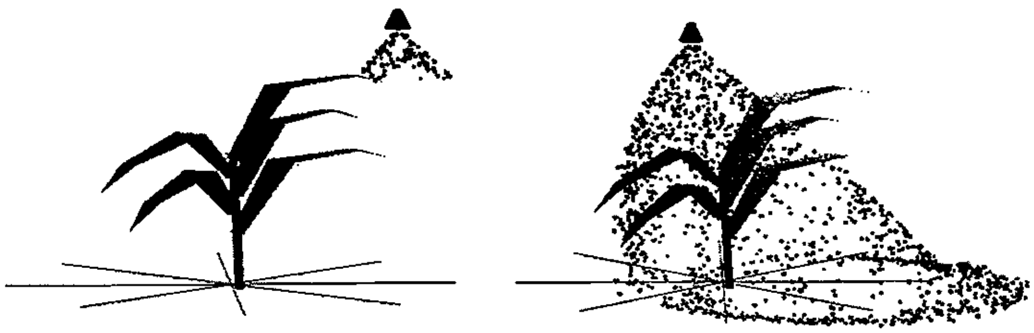

Figure 1. Two stages from a simulation of an overhead spray. Droplets are about 500 times real size and numbers are about 1/10000 of actual.

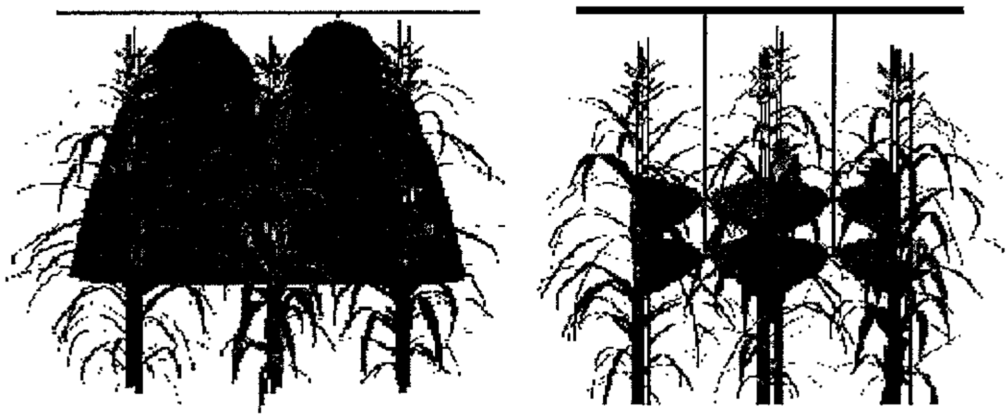

Figure 2. Views along furrows during a simulated pass of a spray boom with overhead nozzles (left) and with droppers having 2 nozzles each side (right).

CONCLUSION

We have developed a number of interactive software tools for weed science research and education. In one such example, a series of identification keys to the weeds have been developed using the computer program LucID. Once a specimen has been identified to a particular species, the key then provides multi-media fact sheets (photos, pictures and text descriptions) and links to web sites for further information. In a second example, an interactive simulation model of pesticide application based on the dynamics of spray droplet movement and the architectural development of crop plants has been developed. We visualize how different dropper arrangements may affect spray deposition. By linking these pesticide deposition models with models of insect behavior, also modeled using L-systems (Hanan *et al.* 2002), valuable insights can be gained into the dose acquisition process.

LITERATURE CITED

Hanan, J.S. and Room, P.M., 1997. Practical Aspects of Virtual Plant Research. In: Michalewicz, M.T.(Ed.), Plants to ecosystems: Advances in Computational Life Sciences. CSIRO Publishing, Melbourne, pp. 28-44.

Lindenmayer, A. 1968. Mathematical models for cellular interaction in development, Parts I and II. Journal of Theoretical Biology 18, 280-315.

Norton, G.A, Patterson, D.J. and Schneider, M. 2000. 'LucID: A multimedia education tool for identification and diagnostics.' CAL-laborate, June 2000, 15-18.

Room, P., Hanan, J. and Prusinkiewicz P. 1996. Virtual Plants: new perspectives for ecologists, pathologists and agricultural scientists. Trends in Plant Science 1(1), 33-38.

Building Capacity to Link Research with Implementation: Empowering Community Weed Action Groups

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Abstract: One of the stumbling blocks in improving or increasing weed management by members of the general public is their feeling of being overwhelmed by weeds and information, as well their own lack of capability. There is often an absence of local coordination in weed control, and lack of ownership of weed problems. This paper will discuss the project *Integration and Coordination of Weed and Pest Control* in Western Australia. The project aims to build the capacity of community groups'-Weed Action Groups - to cut through these and other limiting factors. An important focus of the project is to bridge the gap between researchers and community groups by providing the processes and information needed by the groups to prioritize, plan, act capably on weed control in their local area, and to take on ownership of the issues. The paper draws on the Western Australian experience with Weed Action Groups, their origins, processes and practices to build capacity within community groups to implement action in weed and pest management.

Key words: community empowerment, groups, participatory approach, Weed Action Group, weed management

INTRODUCTION

During a period of extensive community consultation with regard to natural resource management issues in the South Coast region of Western Australia during the mid 1990s, it became apparent that weed and pest control was rated as one of the community's top 10 issues. The community felt that pests and weeds were thwarting much of their effort and that this was a major threat to sustainable agriculture in the region. Many landholders had experienced the loss of tree seedlings to rabbits and witnessed native animals disappear because of predation by foxes and cats. Environmental weeds such as bridal creeper (*Asparagus asparagoides*) were devastating the remaining remnant vegetation in the region. Downgrading or removal of weeds from the declared list[1] was also a source of concern. The perceived a lack of coordination between pest and weed control on public and private land was a major source of community frustration. Unless pests and weeds are tackled in a coordinated way the chances of success are limited.

[1] The Department of Agriculture manages a Declared Plant (and Pest) list, which prescribes various levels of eradication, control or management for plants, or pests, which are perceived to threaten agricultural productivity. The Department has legislative powers under the *Agriculture and Related Resources Protection Act 1976* (ARRPA) to enforce specific action on declared weeds or pests. Many other problem weeds and pests are not on the declared list, and are considered environmental weeds or pests and therefore do not have any legislative powers attached to their control or management by the Department of Agriculture.

In 1997 a pilot project, *"Integration and Coordination of Pest and Weed Control"* was developed and jointly funded by the Department of Agriculture, Western Australia (DAWA) and the Natural Heritage Trust (NHT) to address these concerns over the South Coast region. Since 2000 the pilot approach has been extended to a state -wide basis and is resourced with three part time coordinators, weed scientists and a project manager until July 2003. National interest in the project has extended to the Cooperative Research Centre for Australian Weed Management (Weeds CRC) who are developing a project, *"Building Capable Community Groups"* based on aspects of the NHT project.

MATERIALS AND METHODS

A community empowerment approach

To a degree the project is duplicating the community empowerment approach used by the Australian landcare movement over the past two decades (Campbell 1994; Chamala and Mortiss 1990). Within the landcare movement, these processes have helped shift the dominant extension culture operating in government agricultural and natural resource management departments from technology transfer to ones which also encompass participatory approaches and a community empowerment philosophy (Campbell 1994 and Curnow and Burnside 1995). During the mid to late 1990s, DAWA was shifting in favor of groups over individuals in a move away from the one-to-one servicing of the past. Therefore there was congruence between DAWA's changing approach to extension and the approach being developed for the project.

In March 1998, a workshop was held in the South Coast community of Jerramungup. The workshop, *Weeds Wisdom,* addressed future weed management issues such as: enforcement of weed control, the need for agreed local plans, improved coordination and information, weed control, clear leadership in taking action on weed management, and prioritisation processes to guide community action (Wheeler and Hurst 1998). A clear message from the meeting, which was attended by 90 people, was the strong desire among the participants (mainly farmers) to be involved in organizing and coordinating weed management, particularly in the area of environmental or non-declared weeds. The *Weeds Wisdom* workshop provided the starting point for the main direction of the project, one of supporting community based groups to take ownership of the management of environmental weeds.

This led to the formation of the first Weed Action Group (WAG) in Jerramungup in August 1998. A facilitation process was used to engender community ownership and action in localized weed management - the key statement being: "Today's workshop, *Action on Weeds,* is focussing on the Jerramungup Shire[2] and what this community wants to do to act more effectively on weed control." The facilitation process used in this and additional workshops for WAGs involved the community deciding on key weeds for priority areas, choice of appropriate strategies/actions and developing clear roles and responsibilities for people as well as determining processes to make sure actions planned actually happened. Some of the principles were simplicity of process,

[2] Shire is the term commonly used for a Local Government Area, predominately rural based.

valuing and using both local and expert knowledge, working in partnership, starting with simple achievable steps and working on locally "owned" visions, strategies and actions.

The formula for the first workshop was so successful that it has been used as the basis for most initial meetings looking at setting up WAGs. In addition a wide range of stakeholders[3] in weed control are invited to participate, particularly during the first meeting of a group. Independent facilitators are used at key stages in order to minimize the perception of bias and fear of an outside agenda being imposed.

The idea of local empowerment in planning for action is an approach that has been followed since. The critical point is that no attempt is made to impose any outside agenda. Processes, information and expertise are provided to support the community in deciding its own direction, thereby forming the basis of a true partnership. By basing the project on the formation, and support, of community groups that are committed to taking coordinated action on weed control, the project had a mechanism to ensure any "improvements" continued beyond the formal project life.

Structure and functions of Weed Action Groups - based on using existing structures and harnessing local support

Though the structure of a WAG tends to vary somewhat from shire to shire, a general model, which appears to be adaptable to most situations, is built on the existing landcare structure. The WAG provides shire-wide coordination and support. Sub-catchment groups act as the "drivers" for action, providing representatives for a Shire-based WAG. They have their own local priorities, and are the groups who carry out most on-ground actions. Shire-based WAGs are well structured to harness stakeholder involvement and are the building block of local level coordination. Not all WAGs follow this model, however; some are still at the stage of being very local, or subcatchment based, while others are Shire-based but lacks the underpinning subcatchment structure.

The Shire Council is a key player in the group. If a true partnership is developed with the Shire then the people feel supported and the Council can benefit in terms of collaborative community weed control programs. Support from the Shire can be such things as having councillor/s attend meetings, and covering volunteer workers under Shire insurance. Other support for WAGs includes: having staff take and distribute meeting agenda and minutes, assisting with mailouts and loan of equipment.

The following are some of the functions of a WAG, though more may be added as time goes on: define needs, establish priorities, establish and coordinate actions, raise awareness, develop strategies, and act as a reference group for the Shire.

[3] Stakeholders typically include other farmers/community members and where appropriate members of the following: Landcare groups, Wildflower Society, Local Government, environment groups, local Aboriginal groups, community based natural resource management groups, mining industry and government departments involved in natural resource management or ownership of land (i.e.Main Roads, railways, utilities for power and telecommunications).

Key people

Three key groups of people are recognized as being invaluable for supporting WAGs. The Landcare Coordinator supports communication between the WAG and the sub-catchment groups (who do the actual work on weed control). This fits well with the normal functions of a Landcare Coordinator, only the subject matter (weeds) is different. Landcare Coordinators generally have excellent local networks and knowledge of funding opportunities.

The involvement of the Agriculture Protection Officer[4] (APO) is vital, particularly for the knowledge and expertise on general weed control that most have, and especially for their local knowledge. Over the years the role of APOs has evolved from being very regulatory, focussing largely on declared weeds and animal pests to one of working in conjunction with landholders. It now extends to identifying and, in some cases, providing management advice on environmental weeds. The project can take some of the credit for supporting this evolution since it legitimizes APO involvement in supporting WAGs to work on declared and environmental weeds. However some APOs still hold on to their old style of working being directive rather than participative and keeping a focus only on declared weeds.

The Weed Scientist is able to provide a broad in-depth knowledge of weed ecology and control. Their support is often sought for the difficult issues. Their involvement has been very useful in assisting WAGs prioritize weeds for subsequent action and allows top quality information on weed control to reach the community. It also means that research, which is wanted by the community, is being done on their behalf.

The link between research and the community

The people in WAGs are volunteers, and most have little weed science training. Some, particularly those without a farming background, are opposed to, or uncomfortable, with the use of herbicides. As a result DAWA Weed Scientists have developed extension aids for WAGs, which aim to address these issues, meet their needs and keep the cost to an absolute minimum by seeking government funding or sponsorship. Moore 2002, describes three extension aids developed to assist WAGs in detail.

Weed Action Groups across southern Western Australia were surveyed and 130 introduced species that were causing the greatest concern identified and described in, a pocket sized field book, *"Southern Weeds and their control"* (Moore and Wheeler 2002). It is printed on waterproof paper and uses plain language and has photographs and descriptions of common weeds as well as recommendations for control.

HerbiKey is a computer-based electronic weed identification key covering over 500 weeds with descriptions, photos and management suggestions. It is designed for people with little botanical training and has been used in weed identification workshops. Its users feel confident about keying and visual recognition in the identification process.

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[4] APOs are DAWA employees, based in local communities, with responsibility for administering ARRPA to manage declared plants and animal pests.

An internet mapping program, based on *WeedMapper* (Johnson 2001), is being modified for use in Western Australia. Data will include weeds that various WAGs are targeting and some of the declared weeds. Locations of weeds can be entered via a form or the user can go to a map and locate the position of their weed or determine if it has already been mapped. It is ideal for community groups because they can enter data or view maps of the weed distribution from home.

Other examples of collaboration between WAGs and DAWA weed researchers are:

- the production of information booklets targeting specific weeds across the State (i.e. wild radish (*Raphanus raphanistrum L.)*,
- collated weed information papers specific to the needs of a particular WAG, conducting herbicide trials on weed species (and testing for tolerance of native species),
- assisting groups gain permission for "off label" use of selected herbicides, and
- development and testing of equipment for weed control, which can be used by community groups, e.g., blanket wiper (Rayner and Peirce 2002).

WAGs have also produced their own extension materials, largely information and awareness raising brochures. These brochures typically include photographs of the WAGs " priority weeds" and control and management information.

RESULTS & DISCUSSION

Actions on the ground

Figure 1 below illustrates several examples of WAG activity from the initial South Coast pilot. With the expansion of the project on a statewide basis, an additional 12 WAGs have been supported to date. Activities from some of these groups are very innovative, i.e. in the tropical Kimberley region, local tourist operators are giving discounts to tourists (canoeists) who undertake weed control activities in certain areas.

Changes in awareness and empowerment levels

Attitudes towards agencies, and attitudes of agencies

Many of the groups expressed concern that government agencies such as the Department of Conservation and Management (CALM) and Westrail did not have their weeds under control, so any plan for widespread control would fail. An example of how this can change was seen in Tambellup, where CALM, the Main Roads Department and Westrail all participated in a "Weed Action Week." Westrail's action (apart from donation of chemical to the cause) was to substantially increase weed control on their reserve. A recent meeting of the Tambellup WAG was told of Westrail's concern that unless the adjacent farmers did something about their weeds it wasn't much use Westrail spraying their reserve! This is a far cry from the first meetings of the Tambellup group (and others) which expressed concern about their ability to achieve widespread success with agencies ignoring the problems!

Change of the roles of "experts"

There has been growing awareness among DAWA staff involved with weed actions that they are no longer the "expert authority figure" but a partner, and that active listening skills, willingness to being open to do things differently, and having different priorities are essential here. The best example of this is the change in DAWA's agriculture protection activities away from an exclusive focus on declared weeds. APOs are now supported in their involvement with WAGs, whose focus is not necessarily on declared weeds.

Figure 1. Activities of outh coast weed action groups, 1998-2002 .

Jerramungup. Initiated pamphlet on radish control and demonstration sites for radish and doublegee (*Emex australis*)control. Bremer Bay residents received $4,500 for the fight against Victorian Tea Tree (*Leptospermum laevigatum*) and includes awareness raising, supply of control information, and community involvement in control.

Ravensthorpe. A survey has shown landholders are keen to control onion weed (*Asphodelus fistulosus*) and Afghan thistle (*Solanum hoplopetalum*). The Main Roads Department has since indicated that it will work on these roadside weeds. The group also ran a busy bee at the local sports oval, targetting boxthorn (*Lycium ferocissimum*) and caltrop (*Tribulus terrestris*). Their next activity is a competition to rid the townsite of boxthorn and other target weeds with the support of the townspeople. Secured funding and chemicals from other stakeholders.

Esperance. Held a "Weedarama" at the State Landcare Conference highlighting successful weed control. Held trial of the control of pyp grass (*Ehrharta villosa*) . Conducted roadside survey including eight weeds of significance for Esperance. A caltrop campaign is established on a continuing basis, with the Esperance Shire Council having incorporated the funding for it into their annual budget. The Shire also helps out with some support for other projects, such as the control of Victorian tea tree.

Tambellup. Developed a Strategic Plan for the Shire, which is a highly significant step. Set up a cooperative program with the Shire to control radish and turnip, on roadsides. Initial control of chincherinchee (*Ornithogalum thyrsoides*) along riverbanks, using a Greencorps team. Involvement in the bridal creeper leafhopper (*Zygina* sp.) breeding programme in the local school – reported on TV news.

Denmark/Walpole. Undertook a weed inventory which was used as background for priority setting and action planning. The group also had a display at the Denmark Fair aimed at awareness raising of weeds, promotion and collecting information on weed distribution. Very action-oriented, and has spent lots of time over the year weeding some 25 sites in and around Denmark town. The group was successful in attracting a $4,000 grant for weed control in a significant urban reserve in the Denmark townsite.

Kojonup. Developed local policy, and on ground action, for control of tagasaste (*Chamaecytisus palmensis*), bridal creeper and wild radish on roadsides. Undertaken weed survey. The group has put in an application to the Blackwood Basin Group for a grant for the control of environmental weeds in the Myrtle Benn reserve. The outcome of this application will be known shortly. Developed Shire weed policy/strategy. With the support of the Shire have removed tagasaste from Hillier and Frankland Roads, and revegetated these areas.

Albany. The Bushcarers Group is going from strength to strength. The City Council appointed two bushcare assistants (part time) to remove woody weeds from urban reserves, as part of its commitment to the Environmental Weed Strategy, and in support of local community groups. The Bushcarers group was party to successful applications for funding for control of *Senecio glastifolius* ($25,000) Gorse (*Ulex europeus*) ($125,000) and Blackberry (*Rubus* sp.) ($151,500). They will soon have their own computer and office furniture: office space is being arranged, and members are being trained in GIS systems so that they can map infestations as a basis for the coming campaigns.

Widespread interest in weeds

Farmers and agencies are not the only ones who are interested in weed control. There is a rising awareness of weeds, and the need for action, across the community as a whole. For example over 100 people turned up for a weed pull on Mt Clarence in Albany and in Esperance, the "Scooter Club" (people who have electric wheelchairs), have been catalyzed into doing surveys of weeds as they travel around. These changes have been noted in the short period of time Weed Action Groups have been operating and they give hope that more involvement and participation will continue.

Where to now? - Cultural change now and in the future

WAGs are a partnership between all parties involved. They are the pioneers in a major cultural shift in approaches to weed control. This shift has been recognized in the State Weed Plan (State Weed Plan Steering Group 2001), which is perpetuating and emphasizing this cultural change, because it embodies the partnership approach to weed control. Under the plan local issues are seen as the drivers for planning, priority setting, coordination and action - matching well with the roles and activities of WAGs.

DAWA is strongly supportive of the development of the State Weed Plan and while this change is officially recognized it still needs to properly resourced, institutionalized and operationalized within DAWA so that the momentum of the current State project (due to finish by July 2003) is not lost.

Given the project's relatively short time frame the approach has been to work with those APOs willing to change rather than trying to force change on them. There is still room to change the attitudes of some APOs and those in the community who see the responsibility for controlling weeds as the responsibility of "someone else". Many of the weeds of concern to the community are not declared weeds, but the benefits of having APOs involved with community groups concerned with <u>all</u> weeds are beginning to be recognized.

Moreover the idea of working in a real partnership where the community's knowledge, issues and concerns are seen as equally as valid as those of the experts will take some time to normalize itself as an established part of the culture operating among weed management agencies and specialist researchers. However the positive experience to date with WAGs suggests that this cultural change is taking place and gives hope that new experiences and changes will strengthen the partnership between the community, government and other stakeholders in taking a coordinated approach to weed management in Western Australia.

LITERATURE CITED

Campbell, A. 1994. *Landcare: Communities Shaping the Land and the Future*. Allen & Unwin. St Leonards, Sydney, Australia.

Chamala, S. and P.D. Mortiss.1990. *Working Together for Land Care*. Australian Academic Press, Bowen Hills, Queensland, Australia.

Curnow, M. and D. Burnside. 1995. *Technical Support to Land Conservation Districts 1989-1995 : An Evaluation.* Agriculture Western Australia, Miscellaneous Publication 14/95

Johnson, D. E. 2001. *WeedMapper.* www.weedmapper.org, (Oregon State University)

Moore, J. 2002. *Extension Aids for Community Based Weed Action Groups.* Pages 457-458 in Jacob, H., J. Dodd and J. Moore, eds 2002. Proceedings,13th Australian Weeds Conference, 8th-13th September, 2002, Sheraton Perth Hotel, Perth, Western Australia.

Moore, J and J. Wheeler. 2002. *Southern Weeds and their Control.* Department of Agriculture Western Australia Bulletin No. 4558/02

Rayner, B and J. Peirce.2002. *Control of weeds using an experimental blanket wiper* Page 158-160. in Jacob, H., J. Dodd and J. Moore, eds. 2002. Proceedings,13th Australian Weeds Conference, 8th-13th September, 2002, Sheraton Perth Hotel, Perth, Western Australia.

State Weed Plan Steering Group (2001). A Weed Plan For Western Australia. Bulletin 4490 Department of Agriculture [or see: **www.agric.wa.gov.au/swp**]

Wheeler, S and R. Hurst (1998) *Weeds Wisdom.* Proceedings of a seminar and workshop about future weed management, 11th March, 1998, Jerramungup Entertainment Centre.

Village Level Integration of Integrated Weed Management Strategies in Rice-onion Systems

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Abstract: A participatory on farm trial was conducted in three villages in Nueva Ecija Province, Philippines to integrate IWM strategies to rice-onion cropping system. Ten farmer cooperators from each village participated as IWM adopters. Another set of ten farmers that served as non-IWM adopters were likewise monitored during the season. The IWM strategies tested were cultural weed management practices like rice hull burning and stale seedbed techniques followed by one herbicide and one handweeding, The average reduction in herbicide application was 31% in San Jose to 51% in Bongabon in two years. Bulb yield of onion increased by 9% to 33%, and net income increased by 33 to 48% in the two sites.

In the village level integration activity, farmers were encouraged to integrate good farming practices that will complement other integrated pest management practices. Farmers had unique practices integrated to the IWM strategies example of which are rice hull burning to control weeds and soil borne pathogens This integration activity empowers the farmers to decide on their pest management practices and at the same time understand the science behind the pest management strategies that we are teaching. Farmers have greater appreciation of the alternative IPM strategies as they have the sense of ownership of the technology being used in their farm.

Key words: Village level integration, integrated weed management, participatory approach, rice-onion cropping systems

INTRODUCTION

Weeds remain to be a pressing problem among farmers in their crop production. Yield losses are high, ranging from 40 to 100%, depending on the duration and intensity of crop-weed competition (Brewster, 1994 ; Casimero et al , 2001). The weed problem in onion is complex. Broadleaf weeds and grasses dominate during the early stage of onion growth. After these weeds have been controlled, sedges emerge and largely dominated by *Cyperus rotundus*. *C. rotundus* cannot be controlled by herbicides when it is growing with onion. Farmers spend large amounts of money through handweeding to control this weed.

Weed control in onion is intensive and largely depends on herbicide use and hand weeding. Weed control accounts to 40 to 60% share in the cost of onion production. There is a need therefore to integrate chemical control with cultural methods to provide a longer duration of weed control and reduce the negative impact of prolonged herbicide use. Tests on herbicide usage combined with mechanical and physical methods showed better grass and broadleaf weed control with significant increases in bulb yield. In the

Philippines, the integration of chemical with cultural, mechanical and physical control result in lesser cost of production and increased yields without reducing efficacy of weed control (Baltazar 1996, Baltazar et al. 1999, Casimero et al,. 2001).

Weed management strategies such as stale seedbed technique, rice hull burning and rice straw mulching in onion combined with one herbicide and one handweeding have been shown to reduce herbicide application and lessen the handweeding cost by as much as 50 to 60% (Casimero et al. 2001, Baltazar et al. 2001). However, these technologies have reached a limited number of farmers because of problems with the current extension system. The extensionists who were under the national government were devolved to local government units and have been assigned tasks other than agriculture. This has created a vacuum in the extension of new crop management technologies and failure in bringing it to farmers. Likewise, the traditional approach had been "top down" where farmers were told what to do about their crops without letting them understand and discover the why's and how's these technologies work (Ooi 2001). Researchers develop new technologies on station and transfer them to farmers. This had been unsuccessful and resulted in very little adoption of research-generated technologies that were not acceptable and adapted to the farming systems of farmers.

The Food and Agriculture Organization of the United Nations (FAO-UN) had shifted the paradigm of technology extension from a top down to participatory approach though the Rice IPM project in several countries in Southeast Asia (Ooi 2000). As many of the rice farmers have been trained on Rice IPM, the project built upon this experience of farmers in the implementation of the village level integration activity. This project on village level integration would shorten the time for technologies to reach farmers. The approach starts with a participatory appraisal activity involving the scientists, extension personnel assigned in the village and farmers. In this activity, farmers identify and rank their existing problems related to weeds. Farmers also identify the possible solutions to the problems and the solutions proposed serve as inputs in formulating the integrated weed management (IWM) strategy to be implemented. Because it involves active farmer participation, farmers can readily evaluate and discover the whys and how's of the technologies and select which of these would fit into their current farming practices. It is envisioned that with the direct involvement and participation of farmers in the project, they will discover and understand the scientific basis of these technologies and how these would increase their yields and profits as well as promote the sustainability of their system.

OBJECTIVES

1) To involve active participation of farmers in technology development and adaptation through hands-on evaluation of alternative IWM technologies; 2) To integrate the alternative IWM strategies into the current farming practices of farmers; 3) To gather farmers' feedback on the IWM strategies introduced; 4) To do comparative analysis between alternative IWM technologies and farmers' weed management practices.

MATERIALS AND METHODS

2001 Dry Season

The study was conducted in three villages, Sto. Tomas and Palestina in San Jose and in Bongabon, Nueva Ecija province, Philipines where the dominant cropping pattern is rice in the wet season followed by onion in the dry season. Farmer cooperators were identified based on their willingness to test the IWM technology and compare it to their existing practice. Prior to setting up the on-farm trial, a participatory appraisal was conducted where the farmers were interviewed on their current farming practices. A workshop was held to discuss the current weed problems and find solutions to these problems. The draft of the protocol for project implementation was also done in collaboration with the farmers and the local government units. A briefing was held before the planting season to fully explain to the farmer-cooperators the mechanics of the project. Six farmer cooperators were involved and worked together with the researchers in the implementation of the project. Two adjacent plots measuring 500 m^2 each were set up in the farmers' fields. One plot was used for the implementation of the IWM strategies and the other plot for the farmer's practice.

2002 Dry season

The study was conducted in two villages, Palestina in San Jose and in Lusok, Bongabon, Nueva Ecija. The same process as in 2001 was followed in selecting farmers and briefing about the project. The number of farmer cooperators was increased to ten in each site. Plots measuring 2500 m^2 each were set up in the farmers-cooperators' fields for the implementation of the IWM strategies. Ten other farmers served as non-IWM practitioners or control.

RESULTS AND DISCUSSION

Farmers' Practice vs IWM

The frequency of herbicide application and handweeding was compared between the IWM and non-IWM farmers for two years. In 2001, substantial reduction in herbicide application and handweeding was observed in both sites (Table 1). Herbicide application using IWM was reduced by 90% and handweeding frequency declined by 50% in Bongabon. Handweeding was reduced to 0% with IWM in Palestina (San Jose). In 2002, the frequency of herbicide spraying was reduced by 37.2 % in San Jose and 63.4% in the IWM compared to the non-IWM farmers. The reduced frequency of herbicide spraying is a result of the integration of cultural management practices such as rice hull burning is Palestina and stale seedbed technique in Bongabon by farmers to manage weeds.

Weed Management Strategies and Effects

Pre-plant application of glyphosate and rice straw mulch followed by post-plant herbicide application or handweeding were the common weed management practices of farmers in Sto. Tomas. The alternative technology followed the same system with modifications on the time and dosage of herbicide application. In the IWM technology,

Table 1. Number of hand weeding (HW) and sprayings (S) in onion crops for two years in the village level integration project.

Site	2001				2002			
	FP		IPM		FP		IPM	
	HW	S	HW	S	HW	S	HW	S
Sto. Tomas	0	2	0	2	-	-	-	-
Palestina, San Jose	1	1	0	1	1.3	3.2	1.2	1.2
Bongabon	2-3	3	1-2	1	1.3	4.6	1.2	2.9

glyphosate was applied at 2000 ml/ha while oxyfluorfen was applied at 240 ml ha^{-1}. In the farmers' practice (FP), glyphosate was applied 2000 ml/ha and oxyfluorfen at 365 ml/ha. Farmers applied about 50% more oxyfluorfen than the recommended rate.

The dominant weed species in Sto Tomas were *Cyperus rotundus, Cleome rutidosperma* and *Cynodon dactylon.* Similar weed densities were observed in both the IWM and the farmer's practice plots indicating that it was not necessary to apply oxyfluorfen at a high rate to achieve good weed control (Fig.1).

In Palestina, the common practice was rice hull burning (RHB) combined with early-post and post-plant herbicide application followed by hand weeding. Farmers either overdosed or underdosed their application of herbicides. In the IWM plots, oxyfluorfen (240 ml ha^{-1}) was applied at 2 weeks after transplanting followed by one hand weeding before 39 DAT. In the farmers' practice, oxyfluorfen (260 ml ha^{-1}) was applied at 2 weeks after transplanting followed by hand weeding at 39 DAT. A follow up herbicide application using a mixture of fluazifop-butyl (50 ml ha^{-1}) and oxadiazon (50 ml ha^{-1}) was also done at 60 DAT. Low densities of *C. rotundus* and *Trianthema portulacastrum* were observed in both treatments (Fig.1). One herbicide followed by one handweeding was as effective as the farmers' practice that involved two herbicide applications followed by one handweeding. The follow-up herbicide application done by the farmers at 60 DAT did not help in increasing the level of weed control at mid to late season. The dominant weeds observed in Bongabon were *T. portulacastrum* and *C. rotundus.* Farmers normally prepare their land a few weeks before transplanting the onion seedlings. Herbicide application is done at 2 to 3 weeks after transplanting followed by two to three handweedings until 60 DAT. In the IWM plots, the stale seedbed technique was implemented two months before transplanting. Stale seedbed technique was done by plowing followed by harrowing and then the field is left undisturbed for two to three

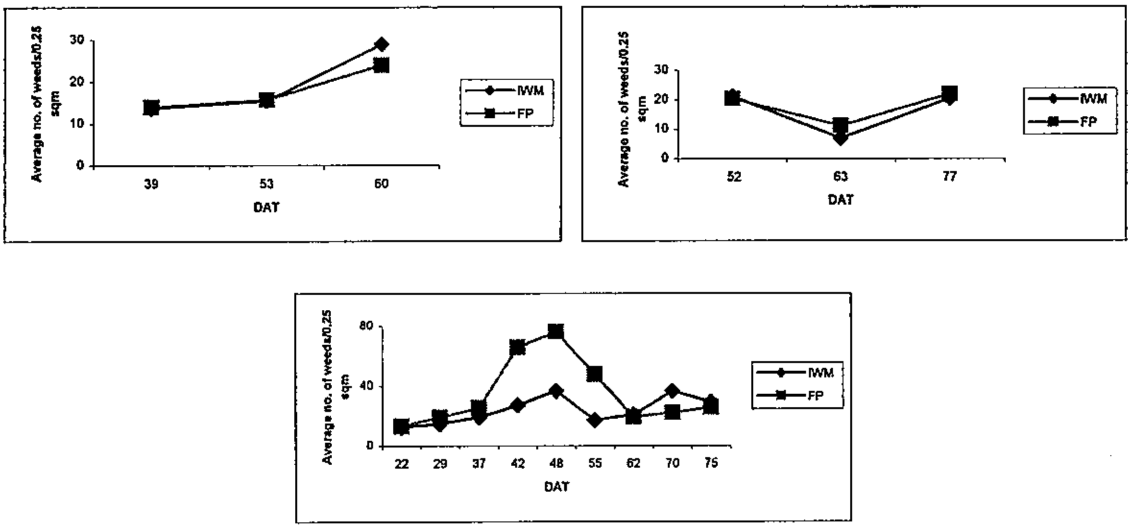

Figure 1. Weed populations at Sto. Tomas (A), Palestina (B), and Bongabon (C) during the 2001 dry season.

weeks to allow the weeds to grow. When the *C. rotundus* plants were at the 6 to 8 leaf stage, the area was again harrowed and the process was repeated until 1 week before transplanting. Oxyfluorfen was applied from 7 to 14 DAT and one handweeding was implemented at 30 to 45 DAT. Inter-row cultivation followed at 53 DAT. Results show lower weed population levels in the IWM plots compared with the farmers' practice plots despite more herbicide applications in the latter (Fig. 1). Moreover, higher rates of herbicides were used in the farmers' practice. This proved that the combination of more strategies is more efficient in maintaining lower weed populations.

In 2002, the dominant weed species observed both on the IWM and non-IWM farmers' fields in both sites were *Cyperus rotundus* and *Trianthema portulacastrum.* Despite the presence of higher weed density in the IWM plots in San Jose, farmers had less herbicide spraying than their non-IWM counterparts. Most of the farmers sprayed herbicide only once either at 10 or 14 DAT using oxyfluorfen or fluazifop-butyl except for one who sprayed twice, once at 10 DAT using oxyfluorfen and at 30 DAT using fluazifop-butyl.

Weed density is much higher in Bongabon than in San Jose as has been observed in earlier weed studies (Figs. 3 and 4). Farmers have a bigger problem on weeds especially *T. portulacastrum*, the early season weed, and *C. rotundus* that occur mid to late in the season. With this weed problem in mind, farmers resorted to more herbicide sprayings. In can be noted however, that IWM farmers reduced their frequency of herbicide spraying by about 60% as a consequence of the stale seedbed technique that they implemented during land preparation. The integration of stale seedbed technique resulted in more than 50% reduction in weed density early in the season until mid season in IPM practicing farmers compared to the non-IWM practitioners. Non-IWM farmers sprayed herbicide two times more than the IWM adopting farmers to control weeds.

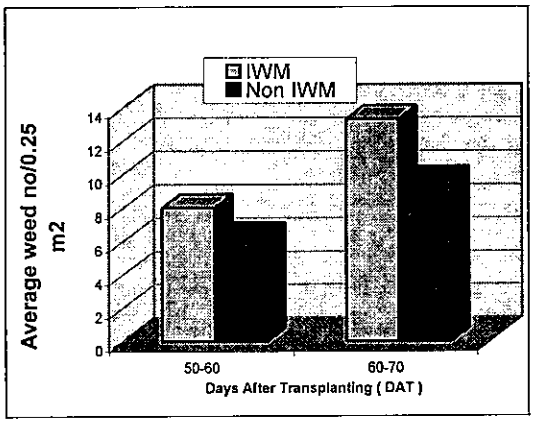

Figure 1. Average weed density/.25m^2 (Brgy. Palestina, San Jose City).

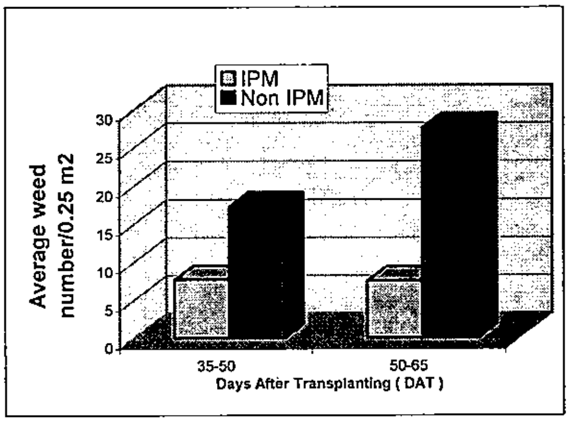

Figure 2. Average weed density/ .25m^2 (Brgy. Lusok, Bongabon).

Onion Yield

The yield obtained from the non-IWM and IWM plots in Sto. Tomas site were similar (Table 2). IWM had 15.3% higher yield than non-IWM in Palestina, while IWM had 8.9% higher yield than FP in Bongabon during the 2001 dry season. In 2002, the average yield obtained by IPM farmers was higher in San Jose and Bongabon than the non-IWM farmers. IWM farmers had a yield advantage of 33.52% in San Jose and 18.26% in Bongabon than the non-IWM farmers.

Cost of Production

In 2001, the production cost incurred in IWM was 7.1% lower than non-IWM in Sto. Tomas site. On the contrary, IWM incurred 10.2% higher production cost than non-IWM in Palestina due to the higher cost of insect control. In Bongabon, IWM also incurred lower production cost than non-IWM (Table 4). The lower costs of IWM than non-IWM in Sto. Tomas and Bongabon sites were due to lower dosage and spray application frequency.

Rice hull has become an expensive investment for farmers adopting rice hull burning to control weeds as well as soil borne pathogens. This resulted in a 27.3% increase in the cost of production of farmers adopting IWM in San Jose (Table 3). Likewise, the integration of stale seedbed technique which makes use of repeated cultivation and spraying of a glyphosate coupled with a more intense insecticide spraying to control leaf miner resulted in a 8.1% increase in the cost of production of onion in Bongabon. Despite these added costs, however, yields were higher and the increase in yield far outweighed the added cost of production.

Net Income

The net income derived from IWM was higher than non-IWM in all sites (Table 4). In 2001, the net income of IWM adopting farmers was 14.7 % higher s in Sto. Tomas, 18.3% in Palestina, and 23.1% in Bongabon than their non-IWM counterpart.

Table 2. Bulb yield (t/ha) obtained in the IWM and non-IWM plots. 2001 and 2002 dry season.

Site	2001		2002		% Difference	
	Non-IWM	IWM	Non-IWM	IWM	2001	2002
StoTomas	3.00	3.0	-	-	0	-
Palestina	40.00	47.26	10.43	15.69	15.3	33.52
Bongabon	5.95	6.29	14.37	17.58	8.9	18.56

Table 3. Production costs (Php ha^{-1}) in onion.

Site	2001		2002		% Difference	
	Non-IWM	IWM	IWM	Non-IWM	2001	2002
StoTomas	25,836.00	23,996.85	-	-	7.1	-
Palestina	84,634.00	94,253.00	38,813	49,410	- 10.2	-27.3
Bongabon	78,956.62	72900.63	53,345	57,685	-7.7	- 8.1

Table 4. Net income derived from onion production. Dry Season 2001.

Site	2001		2002		% Difference	
	Non-IWM	IWM	Non-IWM	IWM	2001	2002
StoTomas	10,164.00	11,912.30	-	-	14.7	-
Palestina	135,366.00	165,692.00	72,776	143,329	18.3	48.57
Bongabon	54,544.90	70,942.40	45,528	70,389	23.1	33.93

Similarly, the IWM adopting farmers in San Jose and Bongabon generated an added net income of 48.57% and 33.93% respectively compared to the income of non-non-IWM farmers (Table 5). This result indicates that although the alternative technology in some cases had higher production cost, the economic benefit was still higher. The increase in net income on both IPM sites is substantial enough to cover up the increase in production cost.

Impact

The IWM strategies are as effective as those of the farmers' practice. Despite the increase in input cost and labor requirement for the IWM methods, the increase in yield and income far outweighed the added cost of production. Results indicate that the farmers have widely adopted rice hull burning and stale seedbed technique as cultural management practices to manage weeds. However, hand weeding is dependent on the density of weeds growing in the field. Farmers may decide to have more than one handweeding if they see that weeds are growing more profusely than they expect.

In the village level integration activity, farmers were taught to decide on their crop management practices. Farmers were encouraged to integrate good farming practices that will complement other pest management practices. Thus, we see that farmers have unique practices integrated to the IWM strategies, examples of which are rice hull burning to control weeds and soil borne pathogens and uprooting of diseased bulbs and sanitation to manage bulb rot. This integration activity empowers the farmers to decide on their pest management practices and at the same time understand the science behind the pest management strategies that we are teaching. Farmers have greater appreciation of the alternative IWM strategies as they have the sense of ownership of the technology being used in their farm.

LITERATURE CITED

Baltazar, AM. 1996. Weeds and weed management in rice-vegetable systems in the Philippines. Paper presented at the Symposium on IPM in Rice-Vegetable Systems.: Status, Constraints and Prospects. PCARRD, Los Banos, Laguna, Philippines. Oct. 2-4, 1996.

Baltazar, AM, EC Martin, MC Casimero, FV Bariaun, SR obien and SK De Datta. 1999. Agro-ecological approaches to managing weeds in rainfed rice-onion cropping systems. Paper presented during the 30th Pest Management Council of the Philippines Ann. ci Mtng. PhilRice, Maligaya, Munoz, Nua Ecija, Philippines. May 2-6, 1999.

Baltazar, AM, EC Martin, AM Mortimer, MC Casimero, SR Obien and SK De Datta. 2001. Reducing Cyperus rotundus tuber populations using stale-seedbed techniques in rice-onion cropping systems. In 18th Proc Asian Pacific Weed Sci Soc Conf. Pp. 215-223.

Brewster, SL. 1994. Onion and Other Vegetable Alliums. CAB International United Kingdom.

Casimero, MC, AM Baltazar, JS Manuel, SR Obien and SK De Datta. 2001. Population dynamics and growth of weeds in response to cultural and chemical control methods. In Proc. 18th Asian Pacific Weed Sci Soc Conf. Pp. 48-56.

Ooi, PAC. 2000. From passive observer to pest management expert: Science education and farmers. In Deepening the Basis of Rural Resource Management, Proceedings of a Workshop. Guijit, I., JA Berdegue and M. Loevinsohn (eds). Pp. 167-178.

Treatment of Chilean Needle-Grass (*Nassella neesiana*) in Victoria: A Benefit-Cost Analysis

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Abstract: The competitive ability and the efficient reproductive mechanisms of Chilean needle-grass (*Nassella neesiana*) have enabled it to dominate and cause damage in pastures in southeastern Australia including Victoria. However, the long-term economic impact of this grass to the Victorian community and the likely benefits and costs of alternative treatment strategies have not been estimated. A simple spreadsheet model that applies the discounting procedure of benefit-cost analysis (BCA) was used, given three likely rates of weed spread scenarios viz, 'medium' (best case), 'high' (base case) and 'very high' (worst case). Using data from a recent survey as inputs to the BCA model, the 815 ha of reported *N. neesiana* infestation was predicted to expand to around 1.5 million ha across Victoria in 30 years if control is uncoordinated. The BCA results show that the absence of government investment in the coordinated treatment of *N. neesiana*, under the worst-case scenario of weed spread rate, is likely to cost the Victorian community approximately $A464.0 million in 30 years. Government investment in *N. neesiana* control under the base-case 'high' rate of spread scenario was assessed to deliver to Victoria a net present value (NPV) of about $A82.0 million in terms of potential production loss saving and future control cost avoided. Under the 'medium' and 'very high' rates of spread scenarios, the same strategy was assessed to deliver an NPV of approximately $A44.0 million and $A230.0 million, respectively. The results are consistent in providing support for a coordinated treatment strategy involving both public and private land managers to significantly reduce the long-term economic impact of *N. neesiana* given the range of spread rate considered.

Key words: Australia, benefit-cost analysis, Chilean needle grass, spread rate, treatment strategies

INTRODUCTION

Chilean needle-grass (*Nassella neesiana*) was introduced to Australia from South America where it occurs in Argentina, Bolivia, Ecuador, Uruguay, Southern Brazil, and Chile (ARMCANZ, ANZECCFM 2000). *N. neesiana* is a tufted perennial grass that is capable of very rapid reproduction (McLaren *et al.* 1998).

Once well established in an area and allowed to seed, eradication rarely is an economic option (NSW Agriculture 2002). *N. neesiana* seeds are typically dispersed via vectors such as machinery, clothing, water or livestock (ARMCANZ, ANZECCFM 2000). *N. neesiana* produces two types of seeds. These are the normal seeds, borne on flower heads, and stem seeds, or cleistogenes, which are formed at the nodes and sheath base of the flowering stems (ARMCANZ, ANZECCFM 2000). The cleistogenes allow the plant to reproduce even if the normal panicle seeds have been destroyed (ARMCANZ, ANZECCFM 2000). The impacts and survival of *N. neesiana* are aided by its ability to develop a large, persistent seed bank, high seedling survival, tolerance to drought and heavy grazing, and effective dispersal (ARMCANZ, ANZECCFM 2000).

No herbicide is currently registered specifically for *N. neesiana* control in Australia. Rehabilitation of pastures through the use of competitive grasses and legumes and the addition of fertilizer are control options. *N. neesiana* has been nominated as a target species for biological control in Australia. McLaren *et al.* (2002) have found strong community support for biological control research to proceed.

Public and private landholders need to be aware of the potential economic impact that *N. neesiana* could cause if its control remains uncoordinated. A better understanding of the impact that this highly invasive grass species imposes on the Victorian community should lead public and private landholders to formulate and implement an economical and effective treatment strategy.

MATERIALS AND METHODS

It is reasonable to assume that the pace at which an invasive species expands is a crucial factor in influencing whether a particular treatment strategy is likely to be economical or not. However, due to the variability in the biology and environmental requirements of each species, obtaining a completely accurate prediction of the rate of expansion for all problem weeds is extremely difficult. Meanwhile, the implication of a variation in the weed's expansion rate on the potential impact of the weed and the economic outcome of a treatment strategy may be examined through scenario analysis.

Scenarios may be tested to provide some insights into two key weed management issues. First, which government-coordinated treatment strategy is likely to effectively reduce the potential economic impact of a particular weed? Effective reduction of impact would mean a strategy is able to realistically reduce overtime, the net rate of weed expansion to close to zero i.e. when the annual weed spread is negligible. Second, once an effective strategy is identified, would the benefits of the strategy to the broader community be enough compensation for the cost of implementing the strategy? Therefore, there is a need for a benefit-cost analysis from the community's point of view.

The long-term economic impact of *N. neesiana* to private and public landholders in Victoria in the absence of coordinated treatment and the likely benefits and costs of such a coordinated strategy are unknown. As an attempt to provide this information, benefit-cost analysis (BCA) that applies the discounting procedure is used, given three scenarios of rate of weed spread viz, 'medium' (best case), 'high' (base case), and 'very high' (worst case).

Current and potential weed distribution and rates of spread

Estimates of current distribution used in the study were based on the results of a recent survey by McLaren *et al.* (2002). To estimate the maximum potential spread of *N. neesiana* in each CMA region in Victoria, weed distribution data from Australia and overseas, and climate modeling overlayed on geographical information system (GIS) layers of susceptible land use, vegetation classes and soil properties were utilized. In particular, a climate-matching program that uses temperature and rainfall data from a set of geographical locations to construct a climate profile was used to indicate similar climatic regions in Victoria for the growth and survival of *N. neesiana*. This was then overlayed on land use types (public land and dryland pastures) and vegetation classes to determine where *N. neesiana* is highly likely to survive.

Using Victoria's pest plant prioritization process (Weiss and McLaren 2002), *N. neesiana* has been assessed to be a highly invasive species. The Victorian weed invasiveness model applied in this prioritization process can provide an estimate of time it would take for a weed species to reach its maximum distribution. Based on this model, it was predicted that *N. neesiana* would take around 75 years to completely cover its potential geographical range of distribution. For the 'medium' and 'very high' rates of spread, a 100 and 50 years were assumed to be reasonable, respectively.

Government investment, control techniques and their costs

The government investment in a coordinated weed treatment strategy was valued in terms of costs of administering the strategy and the on-ground costs of treatment on public land. Examples of administrative activities aimed at reducing the risk of 'human-induced' but presumed accidental weed spread may include community education, field inspection and extension. These activities may focus for instance, on reducing the likelihood of the weed spreading between properties and Catchment Management regions via its known spread vectors.

For this study, the base cost of weed control on dryland pastures is \$A228 ha^{-1} consisting of herbicide and its application i.e. through boom spraying (\$A182 ha^{-1}), extra fertilizer application (\$A20 ha^{-1}), and pasture re-sowing (\$A26 ha^{-1})(DNRE 1999). Herbicide application is the only weed control technique considered as likely to be applicable on the majority of public land, although strategic use of fire, slashing and rehabilitation with native species is being researched (e.g. Mason and Hocking 2002). The cost of herbicide application on public land i.e. through spot spraying (\$A228 ha^{-1}) was assumed to be around 25% higher than private land.

Treatment strategies and their benefits

The long-term goal of the Victorian government's weed treatment strategy may be stated as '*a significant reduction in the impact of the existing weed problem*'. In this study, two treatment strategies were considered achievable depending on the total size of the infestation in a CMA region. First, a 10-year total suppression strategy that is able to reduce the total infestation to 1% of the present size was assumed to be realistic only for infestation of no more than 10 ha in a CMA region e.g. Goulburn-Broken and Port Phillip (Table 1). Second, a 20-year 'containment' strategy that is able to reduce the infestation to 10% of its present size was considered feasible for up to 500 ha of infestation in a CMA region.

Each strategy's potential benefits that were quantified were the future costs of weed control on public land avoided, and the values of potential production loss saved and control costs avoided on private land. The present values of these benefits were derived by subtracting the discounted value of control costs and production losses associated with the 'with treatment' strategy from the present value of potential costs and losses associated with the 'do nothing' strategy (i.e. absence of government-coordinated strategy in a CMA region). For simplicity, other benefits and costs of treatment that are difficult to value in monetary terms e.g. biodiversity, spray drift, were not included.

A treatment strategy was considered to be economic if the net present value (NPV) of benefits over the life of the investment is greater than zero. For comparison, the benefit-cost ratios (BCRs) were also estimated. The BCR was computed by dividing the discounted dollar value of the potential stream of benefits by the discounted costs of the investment stream. The NPV was calculated using equation 1:

$$(1) \qquad \text{NPV} = \sum_{t=1}^{T} \frac{B_t - C_t}{(1+r)^t}$$

Where **Bt** is the benefit in year **t**, **Ct** is the investment cost in year **t**, **r** is the discount rate used to find the equivalent present value of sums receivable or payable in the future and **T** is the duration of the proposed weed treatment strategy. To remain consistent with benefit-cost analyses done in Victoria, the discount rate used was 4% and the evaluation period was 30 years.

RESULTS AND DISCUSSION

Weed distribution

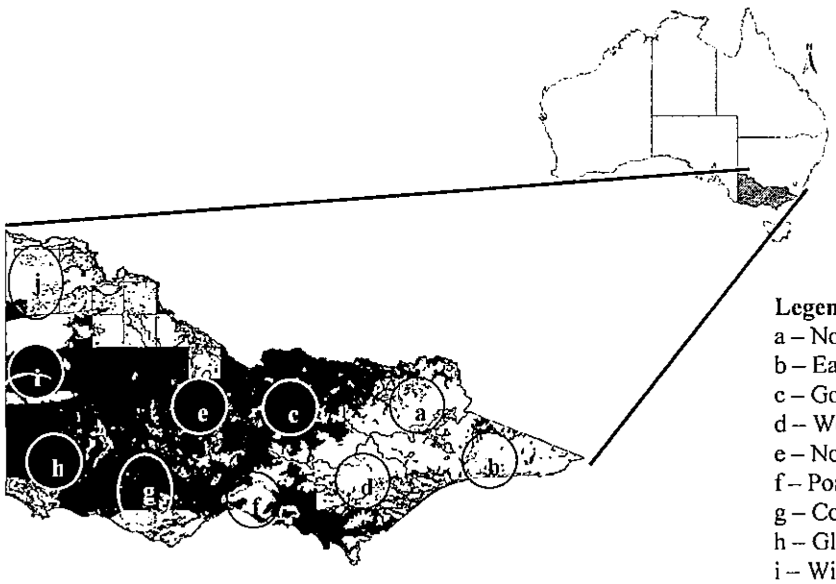
The un-shaded portion of the map of Victoria (Figure 1) indicates the areas that are unlikely to be susceptible to *N. neesiana* invasion. The dark shaded portions represent the areas where the weed is likely to establish. From Figure 1, the area estimates of the maximum potential distribution in each study region were then derived.

The recent survey of *N. neesiana* (McLaren *et al.* 2002) involved 450 land managers in six of the ten CMA regions in Victoria and found that 21%, 66% and 13% of respondents reported 'dense', 'scattered' and 'absent' infestation on their properties, respectively. The aggregate size of infestation reported by land managers was approximately 815 ha on an area of about 415 000 ha (Table 1).

Table 1. Current area infested, size of infestation by type, total size and maximum potential distribution of *N. neesiana* in six CMA regions in Victoria.

CMA Region	(ha) Current area infested	Size of infestation by type: Scattered	Heavy	Total size	Maximum Potential
1. North East	1,700	151	50	201	834 389
2. Glenelg-Hopkins	20	0	20	20	2 303 800
3. North Central	140,900	450	7	457	1 164 000
4. Corangamite	270,930	101	31	132	1 042 900
5. Goulburn-Broken	362	2	0	2	1 069 500
6. Port Phillip	435	1	0	1	468 000
TOTAL	**414 347**	**705**	**108**	**813**	**6 882 589**

Figure 1. Map of Victoria, Australia showing the CMA regions and areas assessed to be 'likely' (shaded) and 'unlikely' (un-shaded) to be susceptible to *N. neesiana* invasion.



Weed spread and economic impact without coordination, and the value of reduced impact with coordinated treatment

In the absence of government investment in a coordinated control program, *N. neesiana* was predicted to infest up to 1.5 million ha in Victoria (Table 2). The potential economic impact to public and private landholders was approximately $A93.0 million, $A169.0 million and $A464.0 million over 30 years for the 'medium', 'high' and 'very high' spread rate scenarios, respectively (Table 3). This estimate includes costs in CMA regions where no *N. neesiana* was reported to occur at the time of the survey but which included land which have been predicted to be highly likely susceptible to infestation.

Table 2. Predicted area infested (ha) by *N. neesiana* over 30 years in Victoria in the absence of coordination, by CMA region.

	Rates of Spread Scenarios		
	Medium High	**High**	**Very high**
1 North east	23,856	46,442	140,884
2 Glenelg-Hopkins	50,035	102,864	340,156
3 North Central	30,732	60,592	188,384
4 Corangamite	28,009	55,240	170,913
5 Goulburn Broken	28,376	56,188	174,545
6 Port Philip	15,412	29,301	85,083
7 Wimmera	19,644	37,955	113,208
8 Mallee	12,436	23,307	66,091
9 East Gippsland	4,931	8,688	22,240
10 West Gippsland	17,364	33,275	97,904
	230,795	**453,852**	**1,399,408**

Table 3. Discounted value of potential economic damage to the community associated with the 'no intervention' strategy for the management of *N. neesiana* in Victoria over 30 years, given three rates of weed spread scenarios.

CMA Region	Rates of Spread Scenarios: Medium	High	Very High
	Discounted Value of Economic Impact 'No Intervention' Strategy ($A million)		
1. North East	4.17	7.55	20.68
2. Glenelg-Hopkins	7.71	14.96	45.33
3. North Central	9.72	17.52	48.63
4. Corangamite	16.04	29.59	83.23
5. Wimmera*	4.92	8.99	24.61
6. East Gippsland*	4.24	7.09	16.67
7. West Gippsland*	13.30	24.10	65.07
8. Goulburn-Broken	10.90	20.41	58.14
9. Port Phillip	11.93	21.45	57.16
10. Mallee*	9.63	17.07	44.43
Total	**92.56**	**168.73**	**463.95**

The BCA results show that under the 'high' rate of spread (base-case) scenario, government investment in a coordinated treatment strategy, given the current infestation level of 815 ha, was assessed to deliver to Victoria a net present value (NPV) of about $A82.0 million over 30 years (Table 4). Under the 'medium' and 'very high' rates of spread scenarios, the same investment was assessed to generate an NPV of approximately $A44.0 million and $A230.0 million, respectively, in terms of production loss saved and control costs avoided.

Table 4. Present value of net benefits (NPVs) to the community and benefit-cost ratios (BCRs) associated with coordinated treatment strategy for *N. neesiana* in Victoria over 30 years, given three rates of weed spread scenarios.

CMA Region	Rates of Spread Scenarios: Medium	High	Very High	Medium	High	Very High
	NPV ($A million)			BCR		
1. North East	1.72	3.40	9.97	6.30	11.50	31.50
2. Glenelg-Hopkins	3.64	7.27	22.45	18.50	35.90	108.80
3. North Central	4.38	8.26	23.80	17.65	31.20	82.50
4. Corangamite	7.44	14.21	41.03	17.00	31.20	86.65
5. Wimmera*	2.37	4.40	12.21	26.55	48.45	132.65
6. East Gippsland*	1.94	3.36	8.14	11.45	19.10	44.90
7. West Gippsland*	6.50	11.90	32.39	44.75	81.10	219.00
8. Goulburn-Broken	5.30	10.05	28.92	36.70	68.65	195.55
9. Port Phillip	5.81	10.57	28.43	40.15	72.15	192.30
10. Mallee*	4.56	8.28	21.97	19.45	34.50	89.75
Total NPV	**43.66**	**81.70**	**229.31**	-	-	-

Note: *CMA regions where no land managers reported *N. neesiana* occurring at the time of the survey; the benefits and costs of treatment in these regions were also estimated assuming a one ha infestation. A 50% rate of re-infestation, 25% damage rate and 50% probability of successful achievement of the strategy's goal were assumed.

The total NPV being positive (Table 4) provides support for a statewide coordinated treatment strategy, involving both public and private land managers to significantly reduce the long-term economic impact from *N. neesiana*.

The BCA model applied in this study can be used to generate useful economic information given the existing data uncertainty and gaps in knowledge of invasiveness of weeds, particularly in relation to their maximum potential distribution range and rates of spread.

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LITERATURE CITED

ARMCANZ, ANZECCFM (2000). Weeds of National Significance Chilean Needle-Grass (*Nassella neesiana*) Strategic Plan. Agriculture & Resource Management Council of Australia & New Zealand, Australian & New Zealand Environment & Conservation Council and Forestry Ministers. National Weeds Strategy Executive Committee, Launceston.

DNRE (1999). Gross Margins (various regions). Farm Smart, Department of Natural Resources and Environment, East Melbourne.

Mason, B. and C. Hocking (2002). The potential for repair of exotic stipoid grass infested sites with kangaroo grass (*Themeda triandra* Forskk.) with special reference to remnant native grasslands. Plant Protection Quarterly, 17:98-101.

McLaren, D.A. *et al.* (1998). The distribution and impact of South/ North American stipoid grasses (Poaceae:Stipeae) in Australia. Plant Protection Quarterly, 13:62-70.

McLaren, D.A. *et al.* (2002). Distribution, economic impact and attitudes towards Chilean needle-grass in Australia. Proceedings of the 13th Australian Weeds Conference, 749-752.

NSW Agriculture (2002). Needle grass heads south. Media release New South Wales Agriculture, March 15, 2002.

Weiss, J. and D. McLaren (2002). Victoria's pest plant prioritisation process. Proceedings of the 13th Australian Weeds Conference, 509-512.

Social Impact Assessment of Weed Management Strategies in Rice-Onion Systems

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Abstract: The study assessed the possible social impacts of advocating weed management strategies for rice-onion systems, specifically, rice hull burning (RHB) and stale seedbed technique (SST) in Barangay Palestina, San Jose City, Nueva Ecija and Barangay Kaingin, Bongabon, Nueva Ecija, using Krawetz' social impact assessment (SIA) model. The study made use of comparative analysis between farmers in San Jose where RHB is one of the essential components in controlling weeds during the onion season and farmers in Bongabon where RHB is not practiced. The social variables assessed in this study included income, health and safety, household, social relations, community structure and processes, community resources, and support services. Results showed that RHB offers many advantages that include increased yield and reduced pesticide use. However, further studies should be conducted to verify its effect on health and the environment. Rice hull supply and road accessibility are factors that will influence its adoption. SST is socially acceptable and should be disseminated to rice-onion farmers and extension workers. Demonstration farms can be established in different barangays to further expedite the utilization or adaptation of the SST technology.

Key words: rice hull burning, rice-onion systems, social impact assessment, stale seedbed technique, weed management

INTRODUCTION

Cultural practices, beliefs, traditions, and other social factors determine if a technology being introduced is suited to the community. It is easier to transfer technologies once they are perceived to be socially acceptable. In this study, the possible social impacts of rice hull burning (RHB) and stale seedbed technique (SST) were assessed. They were weed management strategies developed by the Integrated Pest Management Collaborative Research Support Program (IPM-CRSP) at the Philippine Rice Research Institute. The impact of RHB and SST on factors like income, health and safety, household, social relations, community structure and processes, community resources, and support services will be considered before these weed control strategies are advocated on a wider scale.

Objectives of the Study

1) To determine the communities' perception on the use/adoption of rice hull burning and stale seedbed technique;
2) To identify and assess the possible social effects and impacts of rice hull burning and stale seedbed technique on the community;

3) To identify and suggest possible mitigation and enhancement measures for the negative impacts of rice hull burning and stale seedbed technique to properly suit the needs of the community.

MATERIALS AND METHODS

A combination of research techniques such as focus group discussion (FGD), documentary analysis, and key informant interviews were used in doing the social impact assessment of RHB and SST. Krawetz' model (1991), which involves projecting, assessing and evaluating, mitigating, enhancing, and stating residual impacts of an introduced technology was employed. In projecting, the researcher describes the anticipated social effects in the environment of the proposed technology in the future. Effects that are significant are called impacts. The researchers then mitigate impacts by suggesting means of reducing or ridding the technology of its negative impacts and at the same time enhancing its positive impacts. Please refer to Figure 1 below.

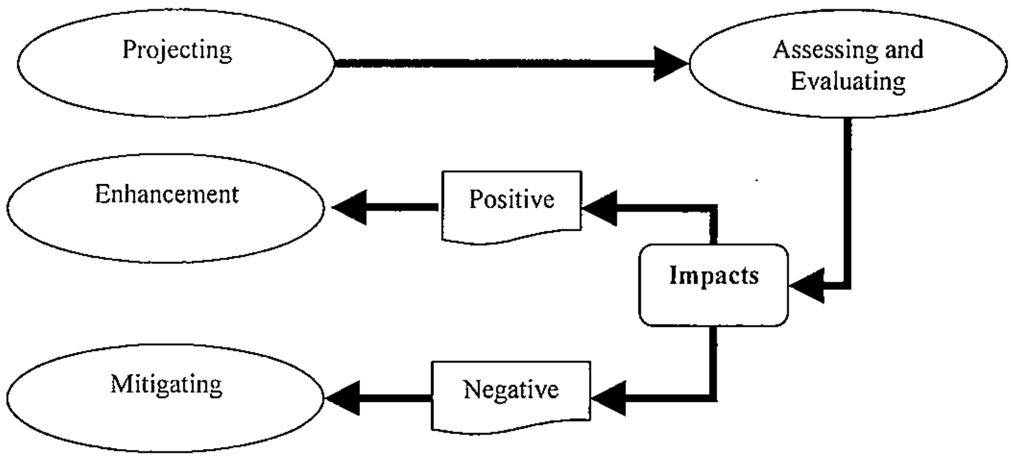

Figure 1. Krawetz' SIA Model

Barangay Kaingin, Bongabon, Nueva Ecija is one of the experimental sites of IPM-CRSP where farmers do not practice RHB. Their onion production practices were compared with the farmers in Barangay Palestina, San Jose City, Nueva Ecija who practice RHB. A comparative analysis of the two barangays was made to approximate the possible impact when a community adopts RHB. Comparative analysis was not employed on the SST because it was assumed to be a new technology and both communities do not practice it yet. The researchers, however, compared and contrasted the weed control methods that farmers employ relative to SST so that they can approximate the changes that would take place within a given community *if* and *when* it adopts the technology.

A focus group discussion (FGD) was used to obtain the current practices of farmers on onion production, particularly on weed management. The assistance of the agricultural technicians assigned in the barangays was sought in identifying the respondents. Three FGDs were conducted; one in Barangay Kaingin, Bongabon, where distinction was made between farmers who plant both Red Creole or Red Pinoy (red) and Yellow

Granex (yellow) onion cultivars and those who plant only yellow onions. Ten (10) respondents were involved in the FGD where nine (9) of them were males and one (1) female.

Two FGDs were conducted in Barangay Palestina with two kinds of farmers: those utilizing RHB (6 participants; all males) and those who do not utilize it (10 participants; all males). The groups were also divided into two: those who plant both red and yellow onions and those who solely plant yellow onion. Farmers planting the same varieties were seated together during the FGD. After the farmers presented their practices, the IPM-CRSP technologies were then introduced and the benefits that the farmers could possibly get from these technologies were discussed. The farmers were also asked why a given technology may or may not suit their farming practices.

The researchers also gathered secondary data to substantiate and validate the claims given by the respondents during the FGD and to properly assess the acceptability and suitability of the technologies being introduced in both barangays. The SIA was conducted in August, 2001.

RESULTS AND DISCUSSION

Rice Hull Burning in San Jose, Nueva Ecija

The economic analysis done by Francisco and Norton(1999) in Barangay Palestina on farmers practicing RHB revealed that the economic benefit that can be derived from RHB ranges from P77, 867 ($1,557.34) up to P116,797 ($2335.94). Profit largely depends on thickness of the rice hull (15 cm – 30 cm), where the thicker the rice hull burned, the higher the income. The farmers interviewed used 15 cm rice hull in their field. During the FGD, the farmers mentioned that with the Yellow Granex variety, they need around P40,000 or $800 for capital. With this, they profit approximately P72,500 or $1450, which is not very far from the results of the study of Francisco. In the case of red onion, they need a capital of not less than P30,000 or $600 and have an approximate net incremental benefit of about P50,000 or $1000.

The researchers observed that most of the farmers were knowledgeable as to why they practice RHB. The primary reason cited was that it is a valuable method for controlling weeds. With RHB, they have also noted that the onion bulbs that they harvested were much bigger; they attributed this to the soil being loose. The bulbs produced command a better price in the market because they can qualify for export.

Gergon and Miller (2000) obtained similar results in their study. RHB significantly reduced the population of *Meloidogyne graminicola* and the bulbs produced were bigger and heavier. The ash of the burned rice hull served as fertilizer and increased the P and K content of the soil.

Farmers in Palestina who do not practice RHB prefer to plant bulblets since they are more competitive against weeds, compared to varieties grown through seeds. With yellow cultivars, the bulbs would be relatively smaller yet heavier than the ones planted with RHB. Farmers attributed this to the fact that with RHB, onion bulbs absorb more water, which is then mostly stored in the bulb, and make them bigger. The onions bulbs produced without RHB are more compact but not as big. They also mentioned that the keeping quality of onions in RHB is not as good as onions produced without RHB.

Inaccessibility of roads to transport rice hull is the major constraint for not practicing RHB. Another factor is the distance, which results in higher transportation cost. Also, they do not have enough capital to purchase rice hull. Farmers mentioned some negative effects brought about by RHB activity to their community. According to them, during the peak of onion season, when the supply of rice hull is low and the demand is high, stiff competition may lead to conflicts among farmers.

Another problem is the smoke coming from the burning rice hull, which is also a source of hostility around the neighborhood. According to them, the smoke causes cough, cold, and allergies of children. The farmers reported that a new city ordinance, which prohibits farmers to burn rice hull in their fields has been passed in San Jose City. However, such ordinance is not yet implemented. The RHB practice is widespread, particularly in areas away from houses. The farmers are aware of its benefits so the practice continues despite complaints from their neighbors.

Rice hull disposal has always been a big problem among millers. With RHB, they get additional income. Millers are able to sell it for P70 - P100 per truckload depending on the demand. If the rice hull is not utilized in the field, millers just dump and burn it on the roadside. In this case, it will still cause a problem to the environment. Others claim that it would be better if the rice hull were burned in the farmers' fields where it will be beneficial.

Rice Hull Burning in Bongabon, Nueva Ecija

Most farmers in Bongabon plant Red Creole and Yellow Granex cultivars. They are aware of the economic and agricultural benefits that can be derived from RHB. However, farmers do not practice it because they lack supply of rice hull; there are few rice mills in their area. The other reason is road inaccessibility. Big trucks hauling rice hull cannot go to the farmers' field. People who have access to rice hull are those living along highways and provincial roads where transportation is accessible, thus RHB may further widen the gap of social stratification.

As mentioned previously, the other negative impacts of RHB is on health and the environment. Children within the locality might get sick due to continuous smoke inhalation. Burning rice hull may also contribute to the greenhouse gases (GHG) being released to the atmosphere, specifically carbon dioxide (CO_2). Up to what extent, it is still subject to verification. The summary of the impacts of RHB and their mitigations/recommendations are shown in Table 1.

Table1. Impacts, constraints and mitigations for RHB.

Positive Impact	Negative Impact/Constraint	Mitigation/Recommendations
• Farmers find it useful for controlling weeds	• Smoke from the rice hull being burned contribute to health problems: coughing and colds (children are the ones mostly affected); it is possible that the smoke could also lead to other respiratory problems • Conflicts arise among neighboring farmers due to smoke	• Verify if synchronous burning is possible to avoid conflicts brought about by the smoke • Obtain records from municipal health officer on the incidence of respiratory problems during RHB to quantify possible negative impact on health • Hold information campaigns on the advantages and disadvantages of rice hull burning to aid farmers in decision- making (It is possible that they can offer more insights on mitigating the negative effects of the RHB).
• Lessen farmers' exposure to herbicides		
• Cost of onion production is reduced since number of hand weeding, herbicides and fertilizer application is reduced	• Possible displacement of labor for hand weeding	
• Soil fertility is enhanced • Soil borne pathogens are controlled	• Biodiversity is affected • Release GHG to the atmosphere which contribute to global warming	• Quantify effect on biodiversity to verify impact of RHB • Conduct soil analysis to check for presence of soil borne pathogens
• Higher profit because Yellow Granex onion qualify the export grade	• Length of storage for Yellow Granex is reduced	
• Extra income for millers • Millers find it a beneficial way of disposing rice hulls rather than just burning it along the roadsides	• Road damages due to the size of trucks, specifically in Bongabon, where roads are not in good condition as well as some barangay roads in San Jose City • Conflict/competition among neighbors arise if supply of rice hull is limited • Greater social stratification due to road inaccessibility and unequal distribution of rice hull	• CRSP should establish link with LGUs and inform policy makers of the advantages of RHB and the importance of road accessibility for its utilization

Stale Seedbed Technique

SST is a weed management strategy that has been found to be effective in controlling weeds, specifically purple nutsedge (*Cyperus rotundus* L.). The technique is done either through plowing and repeated harrowing during fallow period or by a single harrowing followed by herbicide application with 2-3 weeks interval between each operation. As reported by Baltazar et al. (2000), this method can reduce the number of hand weeding operations and herbicide applications in one cropping season. From the farmers' description of their practices, however, it was learned that SST is basically the method that they employ when planting bulb onions. One farmer in San Jose pointed out that this was actually their practice before but they abandoned it when they learned about the benefits of RHB.

During the FGD, the researchers noted that the IPM-CRSP SST and that of the farmers' practice differ. Farmers prefer to employ two to three herbicide applications and two to three hand weeding in one cropping season. The researchers have also noticed that farmers tend to combine the different herbicides in order to save labor, i.e., fluazifop-p-butyl and oxyflourfen. The researchers do not advocate this practice since tank mixing of herbicides might produce chemical reactions, which could injure the crop.

Since the technology is almost similar to the farmers' practice (especially those planting bulb onions), the technology should be very easy for farmers to adopt. The only problem would be its proper implementation because farmers are spending too much for hand weeding (especially the latter part of the season) for fear that the weeds may still affect their yield. IPM-CRSP can sponsor a seminar on how farmers can manage their weeds efficiently. The possible negative impact of SST is reduction of labor. Several men, women, and children might receive lesser income since the number of hand weeding will be reduced.

In the case of Bongabon farmers who plant red onions and also plant rice, they cannot properly implement the two harrowing at two-week interval between each operation because of the short fallow periods between cropping seasons. Farmers need to prepare their land for less than a month to plant in time for the onion-cropping season. Except for this constraint, there should be no major difficulty in adopting this technology. The impacts of SST and their mitigations are shown in Table 2.

CONCLUSIONS AND RECOMMENDATIONS

Based on economic analysis and mitigations to lessen the negative impacts on several social factors, the following conclusions and recommendations are presented:

1. RHB and SST are effective weed management strategies, however, they both have positive and negative impacts.
2. RHB offers many advantages including increased yield and reduced pesticide use, thus contributing to higher income of farmers. Moreover, it enhances soil fertility and kills soil-borne pathogens. While it lessens farmers' exposure to herbicide, its effect on health, particularly of children needs to be verified. It

also brings negative effects on social relationship in the community and the environment, though. Nevertheless, positive impact of RHB seemingly outweighs its negative effects.

3. Rice hull availability and road accessibility play a crucial role in the adoption of RHB in onion-producing areas. If these two factors were present, farmers would tend to adopt and practice RHB. The importance of good road infrastructures for agricultural development cannot be overemphasized.
4. Information campaigns must be conducted on the advantages and disadvantages of RHB to aid farmers in decision-making. It is possible that they can offer more insights to mitigate the negative effects of RHB.
5. The advantages of SST far outweigh its negative effects, so the researchers find it a socially acceptable technology. SST should be disseminated to rice-onion farmers and extension workers to expedite its utilization. Demonstration farms should be established in different barangays in cooperation with local government units or with farmers' organization, for possible adaptation of the technology, particularly in areas where there is intensive cropping system.

Table 2. Impacts, constraints, and mitigations for SST.

Positive impact	**Negative Impact/Constraints**	**Mitigation**
• Effective way of controlling weeds	• Possible reduction of labor; especially among women and children engaged in hand weeding • Timing for land preparation is reduced for farmers planting red onion varieties	
• Women can have more time to attend to their children and other household chores • Use of child labor for hand weeding will be reduced • Children will have more time to study		
• Reduced herbicide and hand weeding (cost-effective) • Farmers' exposure to chemicals is reduced		• CRSP should establish linkage with LGUs in conducting information campaign • Demonstration plots may be established in the area

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LITERATURE CITED

Baltazar, A.M.,et al. 2000. Reducing herbicide use with agronomic practices in onions (Allium cepa) grown after rice (Oryza sativa). The Philippine Agricultural Scientist. 83 (1): 34-44.

Francisco, S.R. and G.W. Norton. 1999. Economic impacts of IPM practices in rice-vegetable systems. Sixth IPM CRSP Annual Report, Virginia Tech, VA.

Gergon, E.B. and S. A. Miller. 2000. Effect of rice hull burning and deep plowing on the rice root knot nematode in rice-onion cropping system with supplemental nematode control using soil amendments. Seventh Annual Report IPM CRSP, Virginia Tech., Blacksburg, VA.

Krawetz, Natalia M. 1991. Social impact assessment: an introductory handbook. Environmental Management in Indonesia Project, Jakarta

Mechanism and Management of Herbicide Resistance in Weeds

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Abstract: Herbicide resistance has evolved to many important groups of herbicides, encompassing almost all of the major herbicide mode-of-action groups for the past three decades. Resistance can occur as a result of heritable changes to biochemical processes that enable plants to survive when treated with herbicide. These changes can occur at a target site such that binding of the herbicide is reduced, or a reduction in the amount of herbicide that reaches the target enzyme through detoxification or sequestration of herbicides into the vacuole and reduced absorption of herbicide. Factors that can impact weed resistance include residual soil activity and mode of action of the herbicides. Herbicide resistance can be exploited for the development of detection tools for resistant plants and herbicide resistant crops (HRCs). Multiple strategies should be adopted to reduce the spread of herbicide resistant (HR) weeds and selection pressure from herbicides. However, these strategies must be functionally integrated so that they complement each other and increase the negative impact on the HR weed populations.

Key words: Herbicide resistant weeds, herbicide resistance mechanism, herbicide resistant crops, management of herbicide resistance

INTRODUCTION

Soon after herbicides became part of the environment to which weeds were exposed, they began to demonstrate their ability to adapt to various kinds of herbicides. Herbicide resistance refers to survival and reproduction of some biotypes; within a population following after herbicidal treatment due to their inherited special characteristics (Gressel 2002). Resistant weeds have been reported for three decades since Ryan who first reported *Senecio vulgaris* resistance to simazine in 1968 (Ryan 1970). To date, herbicide resistance has evolved to many important groups of herbicides, encompassing almost all of the major herbicide mode-of-action groups and about 272 resistant biotypes from 162 species have been reported worldwide (Heap 2003).

RESISTANCE MECHANISM

Resistance occurs as a result of heritable changes to biochemical processes that enable plants to survive when treated with herbicide. These changes can be occurring at a target site by modification of the herbicide-binding site that prevents effective herbicide binding. This has been documented for herbicides that target most major sites of action, including those inhibit photosynthetic electron transfer at photosystem II (PS II), acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS), tubulin polymerization and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Besides, resistance can also be due to increased rate of herbicide detoxification, sequestration of herbicides into the vacuole and reduced absorption of herbicide.

Altered target-site-based resistance

The enzyme EPSPS in the biosynthetic pathway for the production of aromatic amino acids is an enzyme inhibited by glyphosate (Haslam 1993). Resistance in the *Eleusine indica* population appears to be due to a Pro_{106} to Ser mutation in EPSPS that results in approximately 2- to 4-fold increased resistance to glyphosate (Baerson et al. 2002). Besides, our studies detected another Pro_{106} to Thr mutation in another *E. indica* population. Two mutations have been detected in the R biotype of *E. indica* indicates that resistance to glyphosate can be conferred by a number of different point mutations. Different point mutations have also been identified in *Kochia scoparia* which is resistant to the ALS-inhibiting herbicide (Guttieri et al. 1995). ALS is the first common enzyme in the biosynthetic pathway for the production of the branched-chain amino acids (Singh & Shaner 1995). Resistance to the ALS-inhibiting herbicides is due to a less sensitive ALS.

History shows resistance to ALS-inhibitors developed in forty weed species within a few years of use. Conversely, after three decades of use, resistance to glyphosate has developed in only four weed species (Heap, 2003). Resistance to glyphosate is rare due to its unique mode of action and low residual activity.

Resistance to PS II in triazine is due to a single amino acid substitution of Ser_{264} to Gly in the D1 protein encoded by the chloroplastic *psb*A gene (Trebst 1996). This amino acid substitution removes a hydrogen bond that is important for binding herbicides. ACCase is the target site of the aryloxyphenoxy-propanoate (APP) and cyclohexanedione (CHD) herbicides which inhibit lipid synthesis (Incledon & Hall 1997). Resistance to the ACCase-inhibiting herbicides has been attributed to an insensitive ACCase. Resistance to microtubule assembly-inhibiting herbicides by dinitroaniline family has been caused by single amino acid substitutions in α-tubulin (designated as TUA1) (Yamamoto et al. 1998), causing altered hydrophobicity, which may alter the binding of herbicides to the protein.

Non target-site-based resistance

Plants contain certain mechanisms to detoxify the herbicides or by decreased translocation rate of herbicide or sequestration of the herbicide away from the target site. Our studies on paraquat-resistant *Crassocephalum crepidioides* showed 10% of the absorbed ^{14}C-paraquat into the susceptible (S) biotype was translocated basipetally, but not in the R biotype (Ismail et al. 2001a) and suggest that differential translocation may contribute to the mechanism of resistance at ten-leaf stage. Besides, at ten-leaf stage, paraquat treatment did not change superoxide dismutase (SOD) in the S biotype but the activity increased 3-fold in the treated R biotype. These observations suggest that SOD may play a part in the mechanism of paraquat resistance (Ismail et al. 2001b). Besides, herbicide metabolism such as cytochrome P450 monooxygenases (P450s), glutathione transferases (GSTs) and aryl acylamidases are also available in plants.

Exploitation from herbicide resistance

Herbicide resistance can be exploited for the development of detection tools for resistant plants and herbicide resistance crops (HRCs). In the understanding of Pro_{106} to Ser or The mutation in glyphosate-resistant *E. indica*, we have successfully developed a polymerase chain reaction (PCR) amplification of specific alleles (PASA) method for screening R biotype of *E. indica* by showing a single DNA fragment for S biotype and 2 fragments for R biotype in agarose gel electrophoresis. The PASA procedure to distinguish S and R biotypes described in the study is rapid without involving greenhouse trials and offers the advantages of a faster and easy separation of resistant plants at an early stage in plant development.

Herbicide resistance can also be exploited through the development of HRCs. Over the past few years, several HRCs including both transgenic and non-transgenic have been commercialized in the market. Most of the ALS-resistant crops are derived from selection of natural mutations. For example, imidazolinone-resistant corn can be developed by *in vitro* selection in tissue culture to identify resistant cell lines and plant regeneration (Newhouse et al. 1991). All resistant selections have HR forms of ALS, the known site of action of imidazolinone herbicides.

Two approaches were taken to introduce glyphosate resistance into plants either by overproduction of EPSPS or by use of a mutated target site. Shah et al. (1986) reported the construction of a chimeric EPSPS gene to attain high level expression of EPSPS and introduced into petunia cells. Transformed petunia cells as well as regenerated transgenic plants were resistance to glyphosate. A second approach to obtain resistant plants was to use a gene for a modified target enzyme (Padgette et al. 1995). *Agrobacterium* strain CP4 has an EPSPS that has almost the same magnitude of resistance to glyphosate as the *E. coli aroA* mutation. Plants such as soybeans, cotton and oilseed rape with a hybrid gene construct containing the CP4 EPSPS, are totally resistant to all agriculturally used rates of glyphosate.

Glyphosate is biodegraded in the soil and utilized by various organisms as a sole phosphorus source, mainly by C-P lyases that degrade glyphosate to sarcosine and pyrophosphate or by glyphosate oxidoreductase (GOX) to glyoxylate and aminomethylphosphonate (Hallas et al. 1988). Both the carbon skeleton and the phosphorous are eventually recycled by the microorganisms into their own system. Genes for such degradation might provide the next series of glyphosate-resistant engineered plants.

One of the first applications of genetic engineering in rice has been the development of tolerance to glufosinate by incorporating the bialaphos resistance (BAR) gene (Christou et al. 1991). The BAR gene encodes for the enzyme phosphinothricin acetyl transferase (PAT), which is used as an assayable marker gene (D'Halluin et al. 1992). Besides, detoxification of glufosinate by acetylation of the amino group, PAT disrupts glufosinate's inhibitory activity of glutamine synthetase making the plant resistant to glufosinate.

MANAGEMENT OF HERBICIDE-RESISTANT WEED

Although there have been many cases of herbicide resistance throughout the world, control of the R biotypes usually can be obtained by alternative herbicide with different mechanism of action without involving other management strategies. However, this has led to multiple herbicide resistance cases such as *K. scoparia* (Foes et al. 1999). Hence, multiple strategies should be adopted to reduce the spread of herbicide resistant (HR) weeds and selection pressure from herbicides. These include innovative herbicide use, cultural, mechanical practices and adoption of HR crop cultivars. Besides, these strategies must be functionally integrated so that each strategy complements other strategies, thus increasing the negative impact on the HR weed populations (Jordon 1996).

Innovative herbicide use

Herbicide application timing and rate Herbicide application timing can be adjusted to improve the management of HR weed population. An effective strategy would be to target herbicide application during the most sensitive stage of HR weed development. Reduced rate of effective herbicides along with other management strategies to manage HR weed populations is a viable management option. This may provide economic control of target weed population, but consistency of activity is somewhat variable. Thus, when reduced rates of herbicides are used, other strategies such as cultivation and rotary hoeing should be included (Buhler et al. 1995).

Herbicide tank mixtures and rotation The basic for using herbicide tank mixtures and rotations to manage HR weed populations focuses on the need to include herbicides with different mode of action that similarly control the target weed and have similar persistence in the agroecosystem (Wrubel and Gressel 1994).

Herbicide application method One of the ways to reduce the selection pressure on weed populations is to minimize the treated area of the field. Banding herbicides reduces the amount of active ingredient per hectare without reducing the effective herbicide application rate (Eadie et al. 1992). However, banding must be combined with mechanical cultivation for broad-spectrum weed management. The application of herbicides as a seed dressing also serves as weed management utility. While only a small region surrounding the germinating crop seed is weed free, interference by weed is sufficiently delayed to allow the application of other strategies such as cultivation or postemergence herbicide application. Site-specific application of herbicides may be used as an option for management of HR weed population. As weed occurs in aggregated spatial patterns, treating only those weed patches will significantly reduce the amount of herbicide applied to field (Wallinga et al. 1998).

Tillage and mechanical management

Mechanical cultivation can lessen herbicide selection pressure on the crop field. However, the combination of herbicides and mechanical strategies provide more effective and consistent weed management than either strategy alone (Mohler et al. 1997).

Cultural management

Crop rotations The concept of using crop rotations to manage HR weed population is to diversify the crop sequence and spread the risk of economic loss in any one crop (Matthews 1994). Diverse crop rotations create an unfavorable environment for specific weed and thus delay or deter the adaptation of that population to agroecosystem (Ikerd 1996).

Crop seeding timing and rate, crop planting configuration, crop cultivars Managing HR weed by adjusting crop seeding timing and rate (Powles 1997) as well as planting configuration (Boerboom 1999) can increase the competitive ability of the crop at the expense of the weed population. Increasing crop competitive ability may reduce the selection pressure imposed by the herbicide on the agroecosystem. Using highly competitive crop cultivars whilst integrated with other cultural strategies could reduce dependence on herbicides for weed management further aiding in HR weed management (Linquist et al. 1998).

Cover crops, mulch and intercrop systems The inclusion of cover crop, mulches, living mulches, smother crop and intercropping system other than the crop represent a potentially important strategy for management of HR weed population (Liebman & Dyck 1993). The goal of this strategy is to replace an unmanageable weed population with a manageable plant population (Teasdate 1996). Furthermore, the inherent weed suppression that results from this strategy allows the reduction of herbicide use.

Weed seedbank management Knowledge of weed seedbank dynamics can be used to predict weed infestations better and to evaluate effectiveness of integrated weed management which involves multiple strategies such as mechanical, cultural practices and herbicide usage.

Fertilization strategies Understanding basic mechanism and timing of nutrient uptake by weeds and crop can lead to fertilization strategies that will enhance the competitive ability of crops while reducing interference from weed. Such strategies can include deep band application of fertilizers to crop row, as opposed to broadcast applications (Di Tomaso 1995).

Biological control HR weed population could be key targets for development of biological weed management strategy. Weed seed predators and pathogens are promising strategies to reduce weed seed population in the soil (Povey 1993). Plant pathogens, which act as mycoherbicides can be combined with herbicide to control weed population (Quimby and Boyett 1986).

HRC cultivars

The adoption of HRC cultivars improves weed management and provided flexibility to crop production system (Wilcut et al. 1996). However, continuous use of HR crop alone may lead to the selection pressure of HR weed population. Therefore, adoption of HR crop cultivars in weed management should be combined with other strategies such as cultural and mechanical practices as well as herbicide usage.

CONCLUSION

Herbicide resistance has evolved to many important groups of herbicides, encompassing almost all of the major herbicide mode-of-action groups for the past three decades. Understanding of resistance mechanism and management of herbicide resistance in all agricultural situations is imperative. Herbicide resistance can be exploited for the development of detection tools for resistant plants and HRCs. The adoption of HRCs offer the farmer a powerful new tool that, if used wisely, can be incorporated into an integrated weed management. All of the available management tools, including an integrated management approach should be used to manage weed to stop rapid worldwide increase in evolution of herbicide resistance.

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LITERATURE CITED

Baerson, S.R. et al. 2002. Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiology, 129: 1265-1275.

Bhowmik, P.C. 1997. Weed biology: importance to weed management. Weed Science, 45:349-356.

Boerboom, C.M. 1999. Nonchemical options for delaying weed resistance to herbicides in Midwest cropping systems. Weed Technology, 13:636-642.

Buhler, D.D, et al. 1995. Integrating mechanical weeding with reduced herbicide use in conservation tillage corn production systems. Agronomy Journal, 87:507-512.

Christou, P. et al. 1991. Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important *indica* varieties via electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. Biotechnology, 9: 957-962.

D'Halluin, K. et al. 1992. The BAR gene as a selectable marker in plant engineering. Methods in Enzymology, 216: 415-441.

Di Tomaso, J.M. 1995. Approaches for improving crop competitiveness through the manipulation of fertilization strategies. Weed Science, 43:491-497.

Eadie, A.G. et al. 1992. Banded herbicide applications and cultivation in a modified no-till corn (*Zea mays*) system. Weed Technology, 6:535-542.

Foes, M.J. et al.. 1999. A kohcia (*Kochia scoparia*) biotypes resistant to triazine and ALS-inhibiting herbicides. Weed Science, 42:20-27.

Gressel, J. 2002. Molecular biology of weed control. Taylor & Francis, New York

Guttieri, M.J. et al. 1995. Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in kochia (*Kochia scoparia*) biotypes. Weed Science, 43: 175-181

Hallas, L. E. et al. 1988. Characterization of microbial traits associated with glyphosate biodegradation in industrial activated sludge. Journal of Industrial Microbiology, 3: 377-385.

Heap, I. 2003. International survey of herbicide-resistant weeds. Annual Report Internet, http://www.weedscience.com.html February 2003.

Incledon, B.J. and Hall, J.C. 1997. Acetyl-coenzyme A carboxylase: quanternary structure and inhibition by graminicidal herbicides. Pesticide Biochemistry Physiology, 57: 255-262.

Ismail, B.S. et al. 2001a. Metabolism, uptake and translocation of 14-paraquat in resistant and susceptible biotypes of *Crassocephalum crepidioides* (n.) S. Moore. Weed Biology and Management, 1:176-181.

Ismail, B.S. et al. 2001b. Role of superoxide dismutase and peroxidase activities in paraquat resistant regflower ragleaf (*Crassocephalum crepidioides* (Benth.) S. Moore). Australian Journal of Agriculture Research, 52: 583-586.

Ikerd, J.E. 1996. Government policy options: implications for weed management. Journal of Productivity of Agriculture, 9(4):491-495.

Jordon, N. 1996. Weed prevention: priority research for alternative weed management, Journal of Productivity of Agriculture, 9:485-490.

Liebman, M. and Dyck, E. 1993. Crop rotation and intercropping strategies for weed management. Ecological Application, 3(1): 92-97.

Lindquist, J.L. et al. 1998. Mechanism for crop tolerance and velvetleaf suppressive ability. Agronomy Journal, 90:787-791.

Matthews, J.M. 1994. Management of herbicide resistant weed populations. Pages 315-323 in S.B. Powles and J.A.M. Holtum, eds. Herbicide Resistance in Plants: Biology and Biochemistry. CRC Press, Boca Raton.

Mohler, C.L. et al. 1997. Evaluation of mechanical weed management programme for corn (*Zea mays*). Weed Technology, 11:123-131.

Newhouse, K. et al. 1991. Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides. Theoretical Applied Genetics, 83: 65-70.

Padgette, S.R. et al. 1995. Development and characterization of a glyphosate-tolerant soybean line. Crop Science, 35: 1451-1461.

Povey, R.D. et al. 1993. Predation of annual grass weed seeds in arable field margin. Annals Applied Biology, 122(2): 324-330.

Powels, S.B. 1997. Success from adversity: herbicide resistance can drive changes to sustainable weed management system, Brit. Crop Protection Conference, 3:1119-1124.

Quimby, P.C. and Boyette, C.D. 1986. *Alternaria cassiae* can be integrated with selected herbicides Proceeding of Weed Science Society America, 26. Weed Science Society of America, Champaign, II, 52.

Shah, D.M. et al. (1986) Engineering herbicide tolerance in transgenic plants. Science, 233: 478-481.

Singh, B.K. and Shaner, D.L. 1995. Biosynthesis of branched chain amino acids: from test tube to field. Plant Cell, 7: 935-941.

Ryan, G.F. 1970. Resistance of common groundsel to simazine and atrazine. Weed Science, 18: 614-617.

Teasdale, J.R. 1995. Influence of narrow row/high population corn (*Zea mays*) on weed control and light transmittance. Weed Technology, 9:113-118.

Trebst, A. 1996. The molecular basis of plant resistance to photosystem II herbicides. Pages 5-14 in T.M. Brown, ed. Molecular genetics and evolution of pesticide resistance. American Chemical Society, Washington.

Wallinga, J. et al. 1998. Measures that describe weed spatial patterns at different levels of resolution and their application for patch spraying of weed. Weed Research, 38:351-355.

Wilcut, J.W. et al. 1996. The niche for herbicide-resistant crops in U. S. agriculture. In. Herbicide-resistant crops-agricultural, environmental, economic, Regulatory, and technical aspects. Duk, S.O. Eds., Lewis Publishers, Boca Raton Fl. 213.

Wrubel, R.P. and Gressel, J. 1994. Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technology, 8:635-648.

Yamamoto, E. et al. 1998. α–Tubulin missense mutations correlate with antimicrotubule drug resistance in *Eleusine indica.* Plant Cell, 10: 297-305.

Changes In Problem Weeds with Growth Stages of Rice (*Oryza sativa*) in Rainfed Paddy Fields of Northeast Thailand

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Abstract: The problem weeds were surveyed in 1999 to 2001 to establish an effective weed management system for direct sown rice, Khao Dawk Mali-105 an aromatic cultivar, under rainfed condition in the northeast Thailand,. Ten to twenty species emerged, with dry matter weights ranging from 140 to 328 g m^{-2}, emerged before plowing at the beginning of the rainy season. These weeds can be controlled by plowing and puddling in transplanted rice and by herbicides in non-tilled direct sown rice. At one month after transplanting, *Cyperus iria*, *C. compactus*, *Fimbristylis miliacea*, *F. dichotoma*, *Fuirena ciliaris* and *Ludwigia hyssopifolia* were the dominant species which grew higher than the young rice plants. *Monochoria vaginalis* and *Paspalum scrobiculatum* were also considered as noxious. *Echinochloa crus-galli*, *E. colonum* and *Sphenoclea zeylanica*, major weeds in the Central Plain were considered to be negligible. At ripening, 91% of *Gramineae*, 10% of *Cyperaceae* and 24% of broadleaf weed species observed were considered as competitors for solar radiation because they had plant height more than 80% of the culm length of rice plants. Further investigations are necessary on the changes in weed damage at different growth stages of photo-sensitive rice cultivars, such as Khao Dawk Mali-105, under rainfed conditions.

Key words: direct sowing rice cultivation, Northeast Thailand, problem weed, rainfed paddy field

INTRODUCTION

An aromatic, photo-sensitive and late maturing rice cultivar, Khao Dawk Mali-105, is cultivated widely in Northeast Thailand as lowland rice under rainfed condition. In the recent years, direct dry sowing rice cultivation has been introduced to this area in order to resolve problems on labor shortage for transplanting. Unstable precipitation and difficulty in water management in this area make weed problem serious not only for the direct dry sown rice but also for the transplanted rice. Though the strategy of weed management has been developed for rice production (Vongsaroj 1996), information on weeds in rainfed paddy fields is inadequate in this country (Morita & Kabaki 2000). We investigated the problem weeds associated with the various stages of cultivation of Khao Dawk Mali-105 to identify the target weeds to be controlled.

MATERIALS AND METHODS

In order to determine problem weed species in rainfed paddy fields, the following observations were carried out at the different stages of rice cultivation during 1999 to 2001 in Khon Kaen and Roiet Provinces, Thailand where an aromatic rice cultivar, Khao Dawk Mali-105, was cropped widely.

Before plowing

The number of individual plant per weed species, culm or inflorescence with maximum plant height and dry matter weight of aerial part were measured from a 50 x 50 cm quadrat in two farmers' paddy fields near Khao Suan Kwang Demonstration Farm of ITCAD in Khon Kaen Province and at three experimental paddy fields of the Land Development Station, Tungkuralonghai, Suwanaphum, Roiet Province, on 19 and 21 of June, 1999 respectively.

After puddling

Weed seedlings that emerged in a paddy field puddled on 25th July 1999, at the Land Development Station, Tungkuralonghai,, were identified at one and two weeks after puddling. The paddy field was puddled and kept flooded with 3 cm water for two weeks.

Tillering stage of rice plant

The number of individuals per species and coverage of weed species were measured in four dry direct sown fields at 42 days after sowing and in one transplanted field at 14 days after transplanting, at the Land Development Station, Tungkuralonghai. Hand weeding was practiced in direct sown fields at 28 days after sowing. Measurement was conducted using a 60 x 60 cm quadrat.

Ripening stage of rice plant

Culm length of rice plant and plant height of weeds were measured in farmers' direct sown fields in Phaykkhphumphisai and Tungkularonghai and in the direct sown field at the Land Development Station, and in a transplanted field of the Khao Suan Kwang Demonstration Farm at the ripening stage of rice during 12th to 20th October, 2001. Ten culms of rice plants, with high and low plant height were measured in the field .

In addition, a weed survey was conducted in farmers' fields in the southern part of Roiet Province on 22nd July to 10th August, 1999. The survey was done when rice plants reached around 50 cm height. Weed species exceeding the height of rice and those that occurred abundantly, though shorter than rice, were recorded as noxious weeds which could compete with rice plant.

RESULTS AND DISCUSSIONS

Before plowing

Ten species of weeds from the fields of Thungkularonghai and 20 species from Khao Suan Kwang were identified. Dry matter weight ranged from 140 to 328g m^{-2}, while the number of plant m^{-2} ranged from 200 to 240 in Tungkuiaronghai and from 360 to 800 in Khao Suan Kwang, respectively. Cyperaceae species such as *Fimbristylis miliacea* were dominant in both locations as shown in Table 1. The paddy fields of Tungkularonghai were flooded with a water depth of around 20 cm, while those in Khao Suan Kwang were not flooded at the time of measurement.

Weeds that grew at the beginning of the rainy season to plowing or sowing time could be controlled by plowing and puddling in transplanted paddy fields and by herbicide application before or after plowing in direct dry sown fields respectively. However, these weed species could re-grow when the efficacy of weeding was inadequate.

After puddling

Ten species of weed seedlings were identified according to their morphological characteristics at one week after puddling as shown in Fig.1. Among them, *Fimbristylis miliacea* and *Monochoria vaginalis* were considered as dominant species. *M. vaginalis* and *Cyperus* spp. emerged earlier than the others such as *F. miliacea*, *Fuirena ciliaris* and *Rotala indica*. The maximum leaf stage at two weeks after puddling was 4.1 in *M. vaginalis* and 5.5 in *F. miliacea* as given in Table 2.

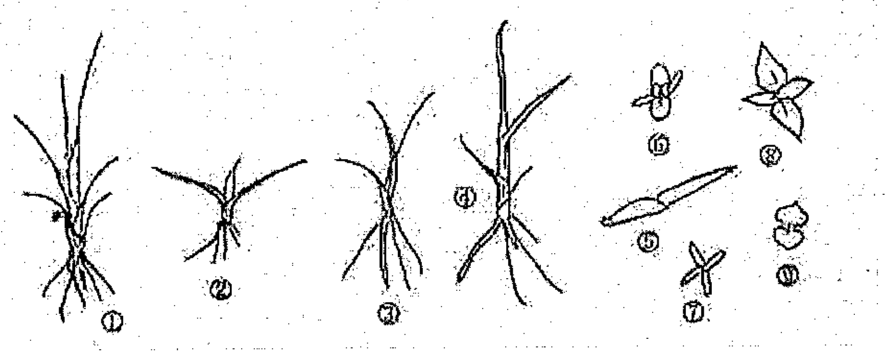

Figure 1. Seedling of weed species emerged after puddling in rainfed paddy field in Tungkuralonghai, North-east Thailand. 1: *Finbrystilis miliacea*, 2:*Scirpus* sp., 3:*Cyperus iria*, 4:*Fuirena cilialis*, 5:*Monochoria vaginalis*, 6:*Rotala indica*, 7:*Limnophilla laotica*, 8:*L. aromatica*, 9:*Ludwigia hyssopifolia*.

Tillering stage of rice plant

As shown in Table 3, the coverage of rice plant was greater in direct sown fields than in transplanted field, while there was no difference in the plant height of rice between both cultivation methods. However, weed infestation was more conspicuous in direct sown fields than in transplanted field, even though hand weeding was practiced in direct sown fields. *Finbristylis miliacea* was the dominant species in direct sown field in terms of number of plants m^{-2}, coverage and height of plant. *Limnophila laotica* and *Dopatrium* sp. ranked next to *F. miliacea* in terms of number of plant m^{-2}.

Table 1. Species and amounts of weeds m^{-2} just before plowing in rainfed paddy fields in North-east Thailand.

Weed species	Khao Suan Kwang (2 rep.)			Roiet (3 rep.)		
	No.plants or culms*	Max.height cm**	Dry matter g*	No.plants or culms*	Max.height cm**	Dry matter g*
Echibochloa colonum	2.0	40	16.6			
Cyperus iria	80.0	55	72.8	24.0	54	15.1
Cyperus compactus	34.0	60	46.2			
Cyperus compressus				1.3	30	0.8
Cyperus sp. 3				108.0	40	26.8
Furiena ciliaris				20.0	42	5.1
Fimbristylis miliacea	90.0	30	9.6	54.7	47	38.1
Fimbristylis sp. 2				9.3	32	2.7
Fimbristylis sp. 3				6.7	32	3.3
Fimbristylis sp. 4				1.3	22	0.8
Panicum repens				162.7	70	93.5
Paspalum scrobiculatum				2.7	37	1.2
Brachiaria decumbens				1.3	26	0.5
Eragrostis sp. 1				45.3	50	22.0
Eragrostis sp. 2				1.3	95	5.3
Perennial grass	4.0	35	15.2			
Digitaria sp.				1.3	30	1.7
Cyanotis axillaris				33.3	45	12.0
Alternanthera sessilis	4.0	7	0.2			
Ludwigia hyssopifolia				4.0	15	0.9
Dopatorium sp.?				1.3	10	0.0
Limnophilla sp.				9.3	10	0.1
Vandellia anagallis						
Melochia corchorifolia	2.0	8	0.2	16.0	30	8.8
Hedyotis tenelliflora?				20.0	33	5.2
Sphaeranthus africanus	2.0	4	0.0			
Epaltes divaricata	2.0	8	0.0			
Marsilea crenata	-	20	15.4			
Oryza sativa				5.3	34	1.1
Total	220.0		176.2	529.3		245.0

*:Average and **:Maximum value of replications.

Table2. Weed species emerged after puddling on 24th July, 1999 under flooded condition in rainfed paddy field in Tungkuralonghai.

Weed species	Obsevation after puddling			
	7 days		14 days	
	No.	Stage	No.	Stage
Fimbristylis miliacea	4	2	70	4
Fuirena ciliaris			12	2.2
Cyperus iria	42	1	76	3.5
Monochoria vaginalis	56	1	104	3.2
Rotala indica			12	1.5
Ludwigia hyssopifolia	10	1	46	2
Limnophila laotica	18	1	34	2.5
Cynodon dactylon	28	7	14	5

Ripening stage of rice plant

The culm length of Khao Dawk Mali 105 fluctuated within the range of approximately 30 cm in the direct sown field. Culm length was longer than 92cm in all direct sown fields examined. On the supposition that weed species which had plant height higher than 80% of culm length of lower rice plants in a field were competitive to rice for solar radiation, the problem weeds at ripening stage were determined. Weeds capable of competing with rice for solar radiation include 10 among the 11 species examined in Graminae, one among the 10 species in Cyperaceae and five among the 21 species in the broadleaf weeds, respectively (Table 4). This suggests that Gramineous species are more noxious at the ripening stage of rice than the other weed groups.

Through the weed survey in farmers' paddy fields at the vegetative stage of rice, *Cyperus iria*, *C. compactus*, *Fimbristylis miliacea*, *F. dichotoma*, *Fuirena cilaris* and *Ludwigia hyssopifolia* exceeded the height of rice plants. *Monochoria vaginalis* and *Paspalum scrobiculatum* were observed frequently though their plant height was lower than that of rice. Infestation of *Echinochloa crus-galli*, *E. colonum* and *Sphenoclea zeylanica* which are popular in irrigated paddy fields especially in the Central Thailand was considered to be not so serious in these provinces.

Table 3. Weed species at tillering stage of rice in rainfed paddy field of Tungkuralonghai.

Species	Direct dry sown field			Transplanted field		
	No. plant (m^{-2})	Coverage (%)	Plant height (cm)	No. plant (m^{-2})	Coverage (%)	Plant height (cm)
Panicum repens	0	0	0	2.8	1	24
Fimbristylis miliacea	131.9	10.8	31.0			
Fuirena ciliaris	17.5	1.5	29.5			
Cyperus difformis	0.8	0.3	7.0			
Cyperus haspan	1.4	0.3	4.3			
Cyperus sp.	3.6	0.3	7.5	2.8	1	9
Monochoria vaginalis	20.3	1.3	11.8			
Rotala indica	60.6	1.3	15.5			
Ludwigia hyssopifolia	40.3	1.8	10.0			
Vandellia anagalis	52.8	1.5	20.5			
Limnophila laotica	111.1	3.8	12.3			
Dopatrium sp.	106.9	2.5	11.8			
Rice plant		40.0	41.0		15.0	43.0

Measured on 3rd Aug., 1999, 42 days after sowing for DS fields (hand-weeded at 28 DAS) and 14 days after transplanting for TP field.

Table 4. Culm length of rice plant, Khao Dawk Mali-105 (cm, S.D.) of hiher and lower plants at four locations, and weed species exceeding 80% of culm lengh of lower rice plants (above) at ripening stage in North-east Thailand at October 2001.

Phayakkaphumphisai		Thungkuralonghai		Swanaphum		Khao Suan Kwang	
Higher	Lower	Higher	Lower	Higher	Lower	Higher	Lower
144.5(9.8)	110.5(6.5)	110.7(5.2)	92.6(7.2)	129.2(6.2)	92.4(4.5)	135.4(6.1)	124.7(5.9)

	Weed species exceeding 80% of culm length of lower rice plants
Gramineae	*Echinochloa crus-galli, Eragrostis* sp., *Hymenachne actigluma, Leersia hexandra, Leptochloa chinensis, Oryza rufipogon, O. sativa, Panicum repens, Rottboellia exeltata, Sacciolepis indica*
Cyperaceae	*Cyperus compactus*
Broadleaf	*Aeschynomene indica, Sesbania bispinosa, Melochia corchorifolia, Ludwigia hyssopifolia, Cyanotis axilaris*
	Other weed species growing in paddy fields at ripening stage of rice
Gramineae	*Echinochloa colonum*
Cyperaceae	*Cyperus difformis, C. haspan, C.iria, Crperus* sp., *Eleocharis dulcis, Fimbristylis miliacea, F. dichotoma, Fuirena cilialis, Scirpus juncoides*
Broadleaf	*Rotala indica, Ludwigia adescendens, L. octovalvis, Hydrolea zeylanica, Limnophila aromatica, Lindernia* sp., *Ipomoea aquatica, Hedyotis diffusa, H. tenelliflora, Monochoria vaginalis, Aneilema nudiflorum, Limnocharis flava, Ottelia alismoides, Ceratopteris thalictoriodes, Marsilea crenata*

In conclusion, the problem weeds were Cyperaceae and broadleaf species during vegetative growth stages and Graminae species during ripening stage. Soil moisture condition affects weed emergence from rainfed paddy soils of Northeast Thailand (Morita & Kabaki 2002). Factors affecting the changes in problem weeds, the evaluation of damages by weeds and the effective control methods at the various growth stages of rice plant are necessary to stabilize production of Khao Dawk Mali-105 in Northeast Thailand.

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LITERATURE CITED

Morita, H. and N. Kabaki. 2000. Observation of weed flora in arable lands and some topics to control *Richardia scabra* in North-eastern Thailand. J. of Weed Sci. and Tech.,45(Suppl.):171-172 (in Japanese).

Morita, H. and N. Kabaki. 2002. Effects of soil moisture conditions on the emergence of weeds and rice plants from rainfed paddy soils in north-east Thailand. Weed Biology and Management,2:209-212.

Vongsaroj, P. 1996. Implementation of integrated weed management for sustainable rice production. Proc. Innovative Weed Management Strategy for Sustainable Agriculture, JIRCAS:38-47.

APPENDIX I. LIST OF PAST AND PRESENT OFFICERS AT THE ASIAN-PACIFIC WEED SCIENCE SOCIETY, SINCE ITS INCEPTION IN 1967

Term of Office	President	Vice-President	Secretary
1967 The 1st APWSS	The conference organizers were Dr. Donald L. Pluckenett, Dr. Roman R. Romanowski Jr. "APWSS" was proposed to be established in the Philippines after this conference	(No Post)	(No Post)
1967-1969 The 2nd APWSS	Marcos R. Vega University of the Philippines at Los Baños, College, Laguna 4031 Philippines	(No Post)	Roman R. Romanowski Jr. University of Hawaii Honolulu, Hawaii 96822 USA
1969-1971 The 3rd APWSS	Cornelius Van der Schans ELI LILLY	Les J. Matthews Ministry of Agriculture and Fisheries New Zealand	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1971-1973 The 4th APWSS	Les J. Matthews Ministry of Agriculture and Fisheries New Zealand	Kenji Noda Weed Science Society of Japan	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1973-1975 The 5th APWSS	Kenji Noda Weed Science Society of Japan	Mohammad Soerjani Weed Science Society of Indonesia Biotrop, Bogor, Indonesia	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1975-1977 The 6th APWSS	Mohammad Soerjani Weed Science Society of Indonesia Biotrop, Bogor, Indonesia	Peter W. Michael University of Sydney Sydney, Australia	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1977-1979 The 7th APWSS	Peter W. Michael University of Sydney Sydney, Australia	H.R. Arakeki ASRB, Nirmal Towers New Delhi, India	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1979-1981 The 8th APWSS	H.R. Arakeki ASRB, Nirmal Towers New Delhi, India	Beatriz L. Mercado College of Agriculture University of the Philippines at Los Baños, College, Laguna 4031 Philippines	Donald L. Plucknett University of Hawaii Honolulu, Hawaii 96822 USA
1981-1983 The 9th APWSS	Beatriz L. Mercado College of Agriculture University of the Philippines at Los Baños, College, Laguna 4031 Philippines	Tanongchit Wongsiri Dept. of Agriculture Bangkhen, Bangkok Thailand	Roy K. Nishimoto University of Hawaii Honolulu, Hawaii 96822 USA
1983-1985 The 10th APWSS	Tanongchit Wongsiri Dept. of Agriculture Bangkhen, Bangkok Thailand	Yuh-Lin Chen Dept. of Agricultural Chemistry National Taiwan University Taipei, Taiwan, China	Beatriz L. Mercado College of Agriculture University of the Philippines at Los Baños, College, Laguna 4031 Philippines
1985-1987 The 11th APWSS	Yuh-Lin Chen Dept. of Agricultural Chemistry National Taiwan University Taipei, Taiwan, China	Doong Soo Kim Rural Development Administration 250 Seodun-Dong Suwon 441-707 Korea	Beatriz L. Mercado College of Agriculture University of the Philippines at Los Baños, College, Laguna 4031 Philippines Acting Secretary-Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4301 Philippines

Term of Office	President	Vice-President	Secretary
1987-1989 The 12th APWSS	Doong Soo Kim Rural Development Administration 250 Seodun-Dong Suwon 441-707 Korea	Achmad Soedarsan Bogor Research Institute for Estate Crops Jalan Taman Kencana No. 1 P.O. Box 81, Bogor, Indonesia	Acting Secretary-Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4301 Philippines Secretaries of the Conference; Organizing Committee, Yong Woong Kwon and Kyung H. Lee Department of Agronomy Kyungpook National University Taegu 702-701 Korea.
1989-1991 The 13th APWSS	Achmad Soedarsan Bogor Research Institute for Estate Crops Jalan Taman Kencana No. 1 P.O. Box 81, Bogor, Indonesia	John Theodore. Swabrick Department of Plant Industries Queensland Agricultural College Lawes, Queensland 4345 Australia	Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4301 Philippines
1991-1993 The 14th APWSS	John Theodore. swabrick Department of Plant Industries Queensland Agricultural College Lawes, Queensland 4345 Australia	Kozo Ishizuka Institute of Applied Biochemistry University of Tsukuba Tsukuba, Ibaraki 305 Japan	Jenny A. Bibo Water Resources Commission G.P.O. Box:2454 Brisbane Queensland 4001, Australia
1993-1995 The 15th APWSS	Kozo Ishizuka Institute of Applied Biochemistry University of Tsukuba Tsukuba, Ibaraki 305 Japan	Ahmed Anwar Ismail Malaysian Plant Protection Society MARDI, Box 12301 Kuala Lumpur, Malaysia	Yuji Yamsue Faculty of Agriculture Kyoto University, Kyoto 606 Japan
1995-1997 The 16th APWSS	Ahmed Anwar Ismail Malaysian Plant Protection Society MARDI, Box 12301 Kuala Lumpur, Malaysia	Rungsit Suwanketnikom Dept. of Agronomy Kasetsart University Chatuchak, Bangkok 10903 Thailand	Baki bin Bakar Institute of Biological Science University of Malaya Kuala Lumpur, Malaysia
1997-1999 The 17th APWSS	Rungsit Suwanketnikom Dept. of Agronomy Kasetsart University Chatuchak, Bangkok 10903 Thailand	Ze Pu Zhang Analytical and Testing Center Chinese Academy of Agricultural Sciences 12 Zhongguancun Nandajie Hai Dian, Beijing 100081 China	Sombat Chinawong Dept. of Agronomy Kasetsart University Chatuchak, Bangkok 10903 Thailand
1999-2001 The 18th APWSS	Ze Pu Zhang Analytical and Testing Center Chinese Academy of Agricultural Sciences 12 Zhongguancun Nandajie Hai Dian, Beijing 100081 China	Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4301 Philippines	Chao Xian Zhang Institute of Plant Protection Chinese Academy of Agricultural Sciences 2 West Yuan Ming Yuan Road Beijing 100094 China
2001-2003 The 19th APWSS	Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4301 Philippines	Duong Van Chin Dept of Weed Science & Farming System at Cuulong Delta Rice Research Institute Omon, Cantho, Vietnam	Analisa Henedina M. Ramirez Dept. of Agronomy University of the Philippines at Los Baños, College, Laguna 4301 Philippines

Term of Office	Treasurer	Newsletter Editor	Date and Place APWSS Conference Held
1967 The 1st APWSS	(No Post)	(No Post)	12-22 June 1967, Meeting for "The First Asian-Pacific Weed Control Interchange" held at East-West Center, University of Hawaii in Honolulu, Hawaii, USA 87 participants from 22 countries
1967-1969 The 2nd APWSS	(No Post)	(No Post)	16-20 June 1969, The 2nd APWSS Conference held at Los Baños College, Laguna, Philippines 146 participants from 20 countries
1969-1971 The 3rd APWSS	Roger C. Billman MONSANTO Far East Ltd.	(No Post)	7-12 June 1971, The 3rd APWSS Conference held at Kuala Lumpur Malaysia 200 participants from 20 countries
1971-1973 The 4th APWSS	R.H. Ferguson DOW Chemical	(No Post)	**12-16 March 1973, The 4th APWSS Conference held in Roturua New Zealand 300 participants from 18 countries.**
1973-1975 The 5th APWSS	Roy K. Nishimoto University of Hawaii Honolulu Hawaii 96822 USA	(No Post)	5-11 October 1975, The 5th APWSS Conference held in Tokyo, Japan 303 participants from 21 countries
1975-1977 The 6th APWSS	Roy K. Nishimoto University of Hawaii Honolulu Hawaii 96822 USA	(No Post) Philip S. Motooka prepared everything for Newsletter. Oregon State University, Corvallis, Oregon 97331 USA	11-17 July 1977, The 6th APWSS Conference held in Jakarta, Indonesia 314 participants from 22 countries
1977-1979 The 7th APWSS	Roy K. Nishimoto University of Hawaii Honolulu Hawaii 96822 USA	(No Post) Philip S. Motooka prepared everything for Newsletter. Oregon State University, Corvallis, Oregon 97331 USA	26-30 November 1979, The 7th APWSS Conference held in Sydney, Australia 250 participants from 18 countries
1979-1981 The 8th APWSS	Roy K. Nishimoto University of Hawaii Honolulu Hawaii 96822 USA	(No Post) Philip S. Motooka prepared everything for Newsletter. Oregon State University, Corvallis, Oregon 97331 USA	22-29 November 1981, The 8th APWSS Conference held in Bangalore, India, 298 participants from 20 countries
1981-1983 The 9th APWSS	Philip S. Motooka University of Hawaii Honolulu, Hawaii 96822 USA	(No Post) Treasurer Philip S. Motooka prepared everything for Newsletter. Oregon State University, Corvallis, Oregon 97331 USA	28 Nov.-2 Dec. 1983, The 9th APWSS Conference held in Manila, Philippines 250 participants from 20 countries
1983-1985 The 10th APWSS	Keith Moody International Rice Research Institute PO Box 933, Manila, Philippines	(No Post) Secretary Beatriz L. Mercado prepared everything for Newsletter. College of Agriculture, University of the Philippines at Los Baños, College, Laguna 4031 Philippines	24-30 November 1985, The 10th APWSS Conference held in Chiangmai, Thailand 530 participants from 22 countries

Term of Office	Treasurer	Newsletter Editor	Date and Place APWSS Conference Held
1985-1987 The 11th APWSS	Keith Moody International Rice Research Institute PO Box 933, Manila, Philippines	(No Post) Secretary Aurora M. Baltazar prepared everything for the Newsletter. National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4031 Philippines	29 Nov.-5 dec. 1987, The 11th APWSS Conference held in Taipei, Taiwan,China: 462 participants from 22 countries
1987-1989 The 12th APWSS	Keith Moody International Rice Research Institute PO Box 933, Manila, Philippines	(No Post) Secretary Aurora M. Baltazar prepared everything for the Newsletter. National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4031 Philippines	21-26 August 1989, The 12th APWSS Conference held in Seoul, Korea, 585 participants from 21 countries
1989-1991 The 13th APWSS	Keith Moody International Rice Research Institute PO Box 933, Manila, Philippines	(No Post) Secretary Aurora M. Baltazar prepared everything for the Newsletter. National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4031 Philippines	15-18 October 1991 The 13th APWSS Conference held in Jakarta, Indonesia 199 participants from 16 countries
1991-1993 The 14th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	(No Post)	6-10 September 1997, The 14th APWSS Conference held in Brisbane, Australia 453 participants from 18 countries
1993-1995 The 15th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	(No Post)	24-28 July 1995, The 15th APWSS Conference held in Tsukuba, Japan 450 participants from 20 countries
1995-1997 The 16th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	John Theodore. Swarbick Dept. of Plant Industries Queensland Agricultural College Lawes, Queensland 4345 Australia	8-12 September 1997 The 16th APWSS Conference held in Kuala Lumpur, Malaysia 375 participants from 22 countries
1997-1999 The 17th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4031 Philippines	22-27 November 1999 The 17th APWSS Conference held in Bangkok, Thailand 392 participants from 22 countries
1999-2001 The 18th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	Aurora M. Baltazar National Crop Protection Center University of the Philippines at Los Baños, College, Laguna 4031 Philippines	28 May-2 June 2001 The 18th APWSS Conference held in Beijing, China
2001-2003 The 19th APWSS	Anis Rahman AgResearch, Ruakura Agricultural Research Center Private Bag.3123, Hamilton	Yasuhiro Yogo National Agricultural Research Center Tsukuba, Ibaraki 305-8666 Japan	The 19th APWSS Conference held in Manila, Philippines

APPENDIX II. LIST OF OFFICERS OF THE WEED SCIENCE SOCIETY OF THE PHILIPPINES.

Post	Name of Officers	Mailing Address
President	Mr. Jose J. Cruz	Agchem Manufacturing Corporation Liberty Building Arnaiz Avenue Makati, Metro Manila
Vice-President	Dr. Madonna C. Casimero	Philippine Rice Research Institute Maligaya, Science City of Muñoz Nueva Ecija
Secretary	Ms. Analisa Henedina M. Ramirez	University of the Philippines at Los Baños, College, Laguna
Treasurer	Mr. Joel D. Janiya	International Rice Research Institute Los Baños, Laguna
Auditor	Dr. Gil L. Magsino	National Crop Protection Center University of the Philippines at Los Baños, College, Laguna
Business Manager	Mr. Napoleon T. Saavedra	Syngenta Philippines Incorporated Legaspi Village Makati, Metro Manila
Board Members	Dr. Aurora M. Baltazar	Philippine Rice Research Institute Maligaya, Science City of Muñoz Nueva Ecija
	Dr. Enrique C. Paller	Dept. of Agronomy University of the Philippines at Los Baños , College, Laguna

AUTHOR INDEX