The invasive plant species, some of them have been well recognized as weeds in agricultural production system, while weeds in natural ecosystem are recognized as environmental weed. Agricultural weeds problems have receive more attentions than environmental weed problems. Recently environmental weeds have received increasing attention in Indonesia, because their impact on native biodiversity, important alien invasive plants species have been documented, it is hoped that collections and dissemination of information on their spread alert people for their controls and managements.

Fifteen species of invasive alien species are discussed for their records and distribution in Indonesia.

## MATERIALS AND METHODS

The main sources for the selection of the species is the Compilation of alien plant species in Indonesia (Tjitrosoedirdjo & Tjitrosemito, 2005). All the alien plants species appear in the references were noted, the invasive and non invasive one were separated. Addition information was taken from the herbarium specimens and local floras. The decision as to which species to be discuss was based on the available important and species distribution and the species included here only a fraction of all invasive species in Indonesia. Only the species considered to as seriously problematic and regarded that deserve attention in terms of monitoring and control are included.

## RESULTS AND DISCUSSIONS

Important record base on the compilations by Tjitrosoedirdjo & Tjitrosemito, 2005, there are 12 species consider as important.

**1.** ***Acasia nilotica*** (L.) Willd. ex Del. (Mimosaceae)

Native Africa and continental Asia, in 1850 was introduced to Java, since long time out of cultivation and spread also outside of Java island (Backer & Bakhuizen van den Brink, 1965). Base on the collected specimens at the Herbarium Bogoriense (BO) it was found in Bogor and Jakarta in 1900, In East Java (Pasuruan) in 1931 and Timor island in 1985. It was also found at Wasur National Park, Papua (Mutazin, 2001).

Recently it is reported as important colonizer at Baluran National Park in East Java, the only savanna left in Java. *A. nilotica* was introduced in Baluran National Park in 1969 and planted as a fence to protect the teak forest nearby from fire in the savanna. In 1980 a little invasion of *A. nilotica* was noticed, but shortly after that a rapid expansion begun. In 1993 it was estimated to be 1200 ha and in 1996 the invaded areas was around 5 000 ha. The savanna providing the herbivore feeds especially for banteng (*Bos javanicus*, Bovidae) which is classified as vulnerable. The invasion of *A nilotica* reduces the herbage yield at the expend of reducing the population of banteng (Siregar & Tjitrosoedirdjo, 1999). *A. nilotica* is a serious environmental weeds.

**2.** ***Austroepatorium inulifolium*** (Kunth) R. M. King & H. Rob (Asteraceae)
Synonym: *Eupatorium inulifolium* Kunth

Native of Tropical America, it is come from the Bogor Botanical Garden, naturalized in West Java commonly found at the tea plantations, it is now commonly found in Cibodas, Mount Gede Pangranggo National Park and spread widely in West Java. In Bengkulu and West Sumatra it is found at the disturbed forest, plantations, perennial crops and road sides.

**3.** ***Chromolaena odorata*** (L.) R.M. King & H. Rob. (Asteraceae)

Native to Central and South America. It is an aggressive invader of pastures, plantation crops, and is a major environmental weed, suppressing and smothering underlying vegetation (Waterhouse, 2003).
In Indonesia it is found for the first time from Lubuk Pakam, North Sumatera in 1934. It spread very quickly, it is now spread all over the islands from Aceh to Papua. *C. odorata* invaded the Pananjung, Pangandaran Nature Reserve, Ujung Kulon Nature reserve in West Java, and grassland of East Nusa Tenggara and reducing the grazing area.

**4.** ***Clibadium surinamense*** L. (Asteraceae)

Native of Tropical America, it was naturalized since long time in Java and the first collection in Java was in 1888. It was reported for the first time in Sumatra in 1931, it is now commonly found in Sumatra and also recorded from Gimpu, Central Sulawesi. It can be found in abandoned agricultural fields, road sides, young secondary forest.

**5.** ***Eichhornia crassipes*** **(Mart.) Solms**

It was introduced to beautify the ponds of Bogor Botanical Garden in 1886, soon afterwards this plant had already spread all over the country (Tjitrosoedirdjo & Widjaja, 1991). In Sumatra: South Sumatra, Lampung, West Sumatra, Jambi and North Sumatra. Kalimantan: South Kalimantan. Nusa Tenggara: Bali, Lombok and Flores. Papua: Jayapura and Merauke. Almost all the open waters are invested by water hyacinth.

The most well known and striking example is the water hyacinth problems at Rawa Pening lake, Central Java where the problem up to now is still un-solve.

**6.** ***Eupatorium sordidum*** Less (Asteraceae)

Native of Mexico, it was introduced in West Java as ornamental plant. Running wild and become a problems at Gede Pangrango National Park in West Java at 1400-17000 m altitude at the forest trails, forest-borders and waste places.

**7.** ***Hydrilla verticillata*** **(L.f. Royle)**

It is recorded at the open water in three islands: Java, Sumatra and Sulawesi. Sumatra: Lampung, South Sumatra, West Sumatra and Jambi, Sulawesi: South Sulawesi. Kalimantan and no record yet from Papua.

**8.** ***Mikania micrantha*** Kunth (Asteraceae)
The genus *Mikania* has about 400 species mainly in the warmer parts in the New World. The only indigenous species in Asia is *M. cordata* while *M. micrantha* is the only New World species to have been introduced here. The latter species readily takes to disturbed areas and tends to be weedy (Parker, 1972). In 1949 it was imported from Paraguay and planted in the Bogor Botanical Garden, and in 1956 this species was introduced as non-legume ground cover in rubber plantations. Wirjahardja (1976) reported that by 1976 it occupied the greater part of rubber plantations and abundaned agricultural areas in West and East Java and South Sumatra.

*M. micrantha* is rapidly perennial vine, climbing to the canopy, dense infestation shade and inhibit the growth of native vegetation in forest gaps and margin, perennial crops. In Indonesia *M.*

*micrantha* displaced the native species *M. cordata* (Tjitrosoedirdjo, 2002). In Papua it is found in Merauke, Timika, Nabire and Sorong (Waterhouse, 2003).

**9. *Mimosa diplotrica*** C. Wright ex Sauvalle (Mimosaceae)
Synonym: *Mimosa invisa* Mart.
Native of Brazil, a scrambling shrub, in Java naturalized since long time ago and spread throughout Indonesia, found in sunny to lightly shaded sites; along drains and water-courses, in ravines, road-sides, pastures, cultivated grounds and moist waste places, mining reclamation area, sugar cane and coconut plantations from 0-2000 m alt (Tjitrosoedirdjo, 1992).

**10. *Mimosa pigra*** L. (Mimosaceae)

Native of tropical America, in Java it was already present in 1844, in Indonesia it is recorded in Java, Sumatra, Kalimantan and Papua. *Mimosa pigra* forms dense impenetrable thickets on moist localities, waste places, floodplains, along river banks, canal, waterholes and in reservoirs often forming a dense jungle and growth at 1-700 m altitude. It will also grow in drier habitats.

**11. *Passiflora edulis*** Sims (Passifloraceae)

Native of South America, it was naturalized in West and Central Java. In mount Gede Pangranggo in West Java, it was cultivated but running wild and become problems, climbing the canopy and suppresses the forest trees. Found at the hedges, brush-woods, open forest at 1-1400 m altitude

**12. *Penisetum polystachion*** (L.) Schult. (Poaceae)

Native of tropical Africa, a tuft grass it was first noticed in West Java in 1972 and Spread very quickly at the road sides, waste places, up land rice fields and plantations. It becomes dominant in cleared forests and spreads quickly after fires. Native plants are displaced and regeneration of trees and shrubs is prevented.

**13. *Piper aduncum*** L. (Piperaceae)

Native to Central and South America, a century ago introduced to Bogor Botanic Garden. The first herbarium specimen was found from Bogor in 1900 and spread out in Central Java, East Java, Sumatra, Kalimantan, Sulawesi, Maluku and Papua. *Piper aduncum* is a serious environmental weed, it is rapidly invades the disturbed sites and recently logged forest. At sites at Wanariset Semboja, East Kalimantan and Lore Lindu National Park, Central Sulawesi impenetrable dense infestations can be seen. It was also recorded at the low elevation in Jayapura, Nabire and Sorong in Papua (Waterhouse, 2003).

**14. *Salvinia molesta* D.S. Mitchell**

It is recorded at the open water in Java, Sumatra, Kalimantan, and Papua. It was not recorded yet from Sulawesi.

**15. Stachytarpeta jamaicensis** (L.) Vahl. (Verbenaceae)

Native to tropical America, commonly found in Java, known in Sulawesi and Timor, widely spread and cause many problems in Papua

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## Biology of a weedy rice Luolijing (*Oryza sativa*) in China

L. Yu[1], D.E. Johnson[2], Y. Zhou[1], and J. Zhang[1]
[1] State Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou,China, 10006 liuqyu53@yahoo.com.cn
[2] International Rice Research Institute, Los Baños, Laguna, Philippines
d.Johnson@cgiar.org

**Abstract:** Luolijing, a weedy rice (*Oryza sativa*) is distributed in the paddy area of Liaoning Province, and south-east region of Jilin Province, Northeast China. The weedy rice was found by a local farmer in 1990, but it wasn't reported on time, and it tends to be more serious year by year until recently. Samples of the weedy rice were collected from Donggang Town located at 39°4' north latitude, and 3.4 m above sea level, Dandong City, Liaoning province. The weedy rice density would be up to 100-110 plants $m^{-2}$, interfering with the growth of planted rice if no effective measure is adopted. Weedy rice is taller than normal cultivated rice. The morphological characteristics of seed are as follows: medium grain, drop easily, the hull is straw-colour or yellow alternate with black-gray; awns or awnless; the length of awn is 2 to 12 cm; the weight of 1000 seeds is 23.5g; the colour of seed capsule is nacarat. Its germination capacity is over 88% under the temperature of 13-38$^0$C. The weight of dry plant is reduced by 50-69% when submerged to 2.5-10 cm deep. Herbicides butachlor, oxadiazon (Ronstar), quinclorac, bentazone and pyrazosulfuron etc. were popularly used to control rice weeds. The herbicides provided about 50% control of the emergence weedy rice which stays in the soil less than 1 cm deep. However, it could not be controlled while the weedy rice seeds are more than 2 cm deep in the soil. Hand pulling is more effective than herbicide application for weedy rice control.

**Key words**: Biology, Luolijing (*Oryza sativa*), weedy rice

### INTRODUCTION

There were a few recordings of weedy rice in China. A 'Lu-Dao' was found earlier in 1950~1960's with the characteristic of weedy rice [4]. A *japonica* wild rice was determined as a cultivated rice turned into wilding by Ding Ying. He also indicated that O*ryza sativa* f. *spontanea* was a weedy rice which grew in Wu-Chuan, Guangdong Province [2]. Recently a weedy rice Luolijing was reported to seriously interfered with cultivated rice in Dandong, Liaoning Province (Chen et al. 2004, Yu et al. 2005).

Developing a research on weedy rice would formulate the correct strategy of weedy rice management, and accelerate the sustainable control of harmful living things. The objectives of this study were to determine the biological characteristics of the weedy rice, and provide scientific basis for the weedy rice control and utilization as resource.

### MATERIALS AND METHODS

The weedy rice Luolijing (*Oryza sativa* L.), and cultivars Chuenjiang 11 (*Oryza sativa* L. *japonica*) and Ganzaoxian (*Oryza sativa* L. *indica*) were used in these experiments. The rice seeds were observed in freezer (-20$^0$C) before seeding. The Loulijing seeds were collected from the paddy in Dandong, Liaoning Province. Other cultivated rice seeds were provided by the Bank of Rice Germplasm in the China National Rice Research Institute.

Germination characteristics of rice seeds

Ten rice seeds were placed into Petri dish (9 cm diameter) with 10 ml distilled water in four replications. It was cultured in aptitude climate chamber with 12 h light (7:00 to 9:00) and 12 h dark, light intensity of 700001x, temperature of 38$^0$C, 28$^0$C, 18$^0$C, 13$^0$C and 8$^0$C. Rice burgeon number were collected each day.

Characteristics of rice seeds on water absorption and cold tolerance

Ten rice seeds was placed into Petri dish (9 cm diameter) with 10 ml distilled water in four replications, and soaked for 0 h, 6 h, 12 h, 24 h and 48 h. The sample weights were quantified before and after soaking, and calculated the water contents. The rice seeds were treated at 20$^0$C below zero in a refrigerator for three days. The samples were taken out from the refrigerator and placed in room temperature for 2 h, then added with distilled water, 5 ml per Petri dish. The samples were moved in the aptitude climate chamber at 28$^0$C and cultured for four days. The germination number of rice seeds was investigated and the germination rate was calculated.

Control effects of weedy rice after adopting flooding

Pot experiments were conducted in the greenhouse at average temperature of max 37.8$^0$C and minimum 20.6$^0$C on sandy clay of pH 7.1 and 3.4% organic matter. Ten rice seeds after accelerating germination were planted in the soil 1cm deep in four replications. It was treated with the flooding of 0 cm, 2.5 cm, 5 cm, 7.5 cm and 10 cm at 1 day after rice broadcasting, replenished water each day, and maintained for six days. Then the normal management of 2-3 cm water depth was recovered, and observed it for 14 days. The data of rice seedling, plant height and biomass dry weight was collected.

## RESULTS AND DISCUSSION

Morphology of the weedy rice Luolijing

Samples of the weedy rice Luolijing were collected from Donggang Town located at 39°4' north latitude, and 3.4 m above sea level, Dandong City, Liaoning province, China. The weedy rice density was 100~110 plants m$^{-2}$, interfering with the cultivated rice if no effective measure was adopted. The weedy rice plant is taller than normal cultivated rice. The morphological characteristics of Luolijing seed are as follows: medium grain, easy to shatter after maturation, the hull is straw-colour or yellow alternate with black-gray, awns or awnless, the length of awn is 2 to 12 cm, the weight of 1000 seeds is 23.5 g, the seed capsule is nacarat.

Germination characteristics of rice seeds

The germination rate of Luolijing seeds was 95% after soaking at 28$^0$C and 38$^0$C for 3d, 88% given in the treatment of soaking at 18$^0$C for 7d, 95% for the treatment of soaking at 13$^0$C foe 14d. However, The Luolijing seeds didn't germinate after treatment at 8$^0$C for 28 d. The germination characteristic of Luolijing was similar comparing with cultivars japonica Chuenjiang 11 and indica Ganzaoxian. However, under the condition of 18$^0$C, the period of starting to germinate delayed 2 d than the two cultivars.

Characteristics of rice seeds on water absorption and cold tolerance

Water content of rice seeds, as an average value from three varieties of Luolijing, Chuenjiang 11 and Ganzaoxian, was raised significantly with prolonging the soaking period, and the germination rate of the rice seeds decreased significantly after treatment of low temperature of -20$^0$C for 3 h and growth at 28$^0$C for four days (Table 1).

Table 1. Relationship between cold-resistant and soaking period.

| Treatment | Water content of rice seeds (%) | Germination rate of rice seeds （%） |
|---|---|---|
| Soaking for 48 h, 3 rice varieties | 33.98 a | 0 d |
| Soaking for 24 h, 3 rice varieties | 28.11 b | 31.7 c |
| Soaking for 12 h, 3 rice varieties | 22.83 c | 46.7 c |
| Soaking for 6 h, 3 rice varieties | 19.54 d | 65.0 b |
| Soaking for 0 h, 3 rice varieties | 11.04 e | 91.7 a |

1 Values within each column followed by the same better are not significantly different at the 5% level by Tukey's (Tang and Feng 2002).

Among the three rice varieties, the average water content of the weedy rice Luolijing was lower than Chuenjiang 11 and Ganzaoxian after soaking for 0~48h, and higher germination rate of Luolijing seeds was given. It indicated that the husks of Luolijing caryopsis had stronger capacity obstructed from water than the two cultivars (Table 2).

Table 2. Characteristics of water content and cold-resistant from different rice varieties.

| Treatment | Water content of rice seeds (%) | Germination rate of rice seeds （%） |
|---|---|---|
| Luolijing, soaking for 0~48 h | 17.038 b | 63.3 a |
| Chuenjiang 11, soaking for 0~48 h | 20.533 a | 30.8 b |
| Ganzaoxian, soaking for 0~48 h | 20.180 a | 23.3 b |

1 Values within each column followed by the same better are not significantly different at the 5% level by Tukey's (Tang and Feng 2002).

Under water contents of 23.66~25.19%, the strength order of germination rate of rice seeds was as follow: Luolijing>> Chuenjiang 11>> Ganzaoxian. During the water content of 18.79~21.68%, the strength order of germination rate of rice seeds was as follow: Luolijing>> Chuenjiang 11 = Ganzaoxian. When the water contents were 9.99~12.63%, the germination rates of rice seeds didn't have significant difference among the three varieties (Table 3). Under the similar water contents, the germination rate of Luolijing seeds was significantly higher than Chuenjiang 11 and Ganzaoxian after treatment of low temperature at -20$^0$C for 3h and growth at 28$^0$C for four days. It indicated that the weedy rice Luolijing had stronger cold-tolerance function than the two cultivars.

Table 3. Characteristics of water absorbing and cold-tolerance of rice seeds.

| Treatment | Water content of rice seeds (%) | Germination rate of rice seeds （%） |
|---|---|---|
| Luolijing, soaking for 24 h | 23.66 cde | 87.5 a |
| Chuenjiang 11, soaking for 12 h | 24.51 cd | 45.0 c |
| Ganzaoxian, soaking for 12 h | 25.19 c | 0 d |
| Luolijing, soaking for 12 h | 18.79 fg | 95.0 a |
| Chuenjiang 11, soaking for 6 h | 21.68 def | 47.5 c |
| Ganzaoxian, soaking for 6 h | 20.77 ef | 50.0 bc |
| Luolijing, soaking for 0 h | 12.63 h | 100 a |
| Chuenjiang 11, soaking for 0 h | 10.49 h | 85.0 ab |
| Ganzaoxian, soaking for 0 h | 9.99 h | 90.0 a |

1 Values within each column followed by the same better are not significantly different at the 5% level by Tukey's (Tang and Feng 2002).

Control effects of weedy rice treated with flooding

Compared with the un-flooding chick, the flooding treatments of 2.5~10 cm for 6d reduced the seedling number and plant height of the weedy rice Luolijing significantly. However, it's not significant difference among the different depth of water. At 14d after re-irrigating with the water depth of 2~3 cm, the germination rate of rice seeds and its plant height were similar with the earlier flooding treatment. The treatment decreased the plant dry weight of Luolijing significantly and reached the weedy rice control of 50~69% (Table 4).

The seedling number and plant height of the Chuenjiang 11 flooded with 10 cm were similar as those in the un-flooding chick, showing that the cultivar seeds had more satisfactory characteristic of flooding-tolerance. However, Ganzaoxian, the cultivar indica rice, had poor function of flooding-tolerance, and the flooding treatment of 5cm killed its germinated seeds of 100% (Table 4). Apparently, the susceptibility of weedy rice Luolijing to the flooding was higher than Chunjiang 11, but lower than Ganzaoxian.

Although weedy rice had been reported in China (Vaughan et al. 2001), it didn't spread and caused the yield loss economically. However, the weedy rice recently infested Liaoning Province, and southeast region of Jilin Province, Northeast of China, and damaged the yield and quality of cultivated rice. It's difficult to determine where the weedy rice came from now. It's possible that it came from degraded cultivated rice after natural selection for long period (Chin 2001).. However, it is not possibly a result of crossing between cultivated rice and wild rice, because of wild rice can't live in the cold region, Northeast of China.

The seeds of weedy rice Luolijing had the characteristic of cold-tolerance, because its husks of caryopsis showed stronger function to obstruct water than the cultivated japonica rice Chunjiang 11 and indica rice Ganzaoxian, as well as its biological characteristics. However, whether the seedling plant of Luolijing had the function of cold-tolerance is desired to further research.

Table 4. Effects of water depth on rice seedling surviving and plant dry weight.

| Treatment (cm) | 6d after treatment | | 14d after irrigated with the depth of 2-3cm | | |
|---|---|---|---|---|---|
| | Seedling surviving (%) | Plant height (cm) | Seedling surviving (%) | Plant height (cm) | Dry weight of plants (g) |
| Luolijing, WD 0 | 100 a | 12.2 a | 100 a | 36.1 a | 0.54 a |
| Luolijing, WD 2.5 | 73 bc | 3.0 e | 80 ab | 26.8 ab | 0.27bcd |
| Luolijing, WD5 | 45 d | 3.0 e | 58 c | 24.7 b | 0.17 de |
| Luolijing, WD7.5 | 63 cd | 2.7 ef | 58 c | 25.6 ab | 0.17 de |
| Luolijing, WD 10 | 65 cd | 3.1 e | 60 c | 26.9 ab | 0.23 cd |
| Chunjiang 11, WD 0 | 100 a | 10.2 ab | 100 a | 26.4 ab | 0.54 a |
| Chunjiang 11, WD 2.5 | 93 ab | 7.7 cd | 95 ab | 23.6 b | 0.42 ab |
| Chunjiang 11, WD 5 | 98 ab | 8.7 bc | 93 ab | 22.8 b | 0.39 ab |
| Chunjiang 11, WD 7.5 | 98 ab | 6.3 cd | 93 ab | 18.7 bc | 0.29 bcd |
| Chunjiang 11, WD 10 | 100 a | 8.7 bc | 98 ab | 23.9 b | 0.37 bcd |
| Ganzaoxian, WD 0 | 88 abc | 5.8 d | 75 bc | 23.4 b | 0.29 bcd |
| Ganzaoxian, WD 2.5 | 15 e | 1.2 efg | 5 d | 9.8 cd | 0.02 ef |
| Ganzaoxian, WD 5 | 8 e | 1.2 efg | 0 d | 0 d | 0 f |
| Ganzaoxian, WD 7.5 | 0 e | 0 g | 0 d | 0 d | 0 f |
| Ganzaoxian, WD 10 | 3 e | 0.4 fg | 0 d | 0 d | 0 f |

1 Values within each column followed by the same better are not significantly different at the 5% level by Tukey's (Tang and Feng 2002). WD = water depth.

Flooding at early period of rice growth might be adopted to control the weedy rice in transplanted rice, which reduced the plant density and plant height of Luolijing significantly. The susceptibility of the weedy rice to flooding was higher than cultivated japonica rice Chunjiang 11. The cultivated japonica rice planted in the cold region of Northeast China had powerful flooding-tolerance. So that the weedy rice could be partly controlled and its seedling density significantly reduced, flooding should be done during the early period of rice growth.

Although the characteristics of biology and stress-resistance of the weedy rice Luolijing were investigated partly (Chen et al. 2004, Yu et al. 2005), the classification and distribution of the weedy rice, as well as its harm to the crop were not studied, measured and evaluated in China. The technologies of the weedy rice control are desired to research and extend which benefit farmers to reduce cost and increase income.

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**Study on the biology of field horsetail (*Equisetum arvense*)**

H. Zhang[1], X. Liu[1], X. Wu[1] , L. Tao[1], H. Ni[2]
[1]Institute for Control of Agrochemicals, Ministry of Agriculture, Beijing 100026, China
[2]College of Agriculture and Biotechnology, China Agricultural University
Beijing 100094, China.
hanwenni@cau.edu.cn

**Abstract:** Field horsetail (*Equisetum arvense*) is a perennial upland weed and very difficult to control. Experiments were conducted in the field and greenhouse in Heilongjiang, China, in 1999-2000 to study the biology of this weed. The results showed that this weed was an oxylophyte plant and favored to grow in neutral and slightly acid soils. Rhizome germination increased with increase in the number of nodes and length of internodes. Maximum growth rate was at 25°C. It could also regenerate by corm. The age of field horsetail could be determined by stem color and rhizome characteristics.

**Key words**: Age, biology, *Equisetum arvense,* field horsetail.

## INTRODUCTION

Field horsetail is a ferny perennial weed (Anderson 1996). It occurs principally in North America, Europe, Russia, England, and China (Bell 1988; Chen 1987; Tang 1988; Zhang 1998). This weed has brought much trouble to wheat, corn, soybean, and some horticultural crops (Anonymous 1988a, 1988b; Ramideide 1984; Daniel 1985).

Although the weed can reproduce via sexual mechanisms through germinated spores produced by male and female gametophytes, the rhizomes and corms are considered as the principal means of perennation, reproduction, and dissemination (Daniel 1985). It has an extensive rhizome system and produces corms as an important energy reservation for growth and survival. Willimas (1979) reported that 50% of the total rhizome weight of the plant was found in the top 25 cm of soil, 23% in the next 25 cm, and the rest in deeper soil, even in a depth of 100 cm or more (Daniel 1985; George 1986). Daniel (1985) researched the growth rates of summer stem, rhizome, and corm rate under greenhouse and field conditions and found that rhizome segments of 1 cm long with one-node easily regenerated from 15 cm deep.

There are many reports about control of field horsetail. A lot of herbicides, such as 2, 3, 6-TBA, dalapon, amitrole, MCPA, dicamba, picloram, dichlobenil, chlorthiamid, glyphosate, asulam, 2, 4-D, fosamine, linuron, simazine, paraquat, and atrazine, were applied to control this weed, but the most of them could not provide good control. Glyphosate, 2, 4-D, MCPA, amitrole, and dichlobenil could control the summer stems of this weed effectively, but could not control its rhizome (Nilsson 1991; Marshall 1980, 1987; Kuriyama 1992; Hallgren 1996; George 1986; Davison 1982). This weed could re-grew as well whether it was hoed once or 16 times during the growing season (Daniel 1985). The objective of this study was to study, through field survey and greenhouse experiments, the base biology that may be helpful for its effective control.

## MATERIALS AND METHODS

Effects of soil types and tillage on infestation of field horsetail

A number of fields with different soil types and tillage conditions in Baoquanling Farm, Heilongjiang province, China were selected to investigate effect of these factors on field horsetail growth during June 1999 - June 2000. Soil types and tillage conditions in the investigated fields see (Table 1 and 2). Four samples were taken from each field and the number of rhizomes counted in every site with 25 cm x 35 cm quadrat from surface to 15 cm depth. Density and height of spring stems were counted and measured when spores were mature.

Effects of node number and temperature on rhizome propagation

Rhizomes with different nodes (2, 4, 6) were selected and put into Petri dishes (12 m) with a piece of filter paper. Each petri dish was added distilled water of 30 ml (pH=7.04), sealed with paraffin and then put in growth chambers at different temperatures 7.2°C, 18°C, 25°C, and 32°C without light. Each treatment was replicated four times and arranged in a completely randomized design. For corms, only one temperature (25°C) was used due to limited materials. Shoot lengths and numbers were measured and counted every 2 days after incubation.

Determination of the age of perennial rhizomes

Rhizomes were dug out and their morphology was observed and the length of internodes measured. Then the age of rhizomes was determined based on stem color and rhizome characteristics.

## RESULTS AND DISCUSSION

Effects of soil type and tillage on the infestation of field horsetail

With increasing tillage intensity, the density of field horsetail rhizome became lower and lower. In continual 2-year tillage field, the density of field horsetail rhizome was 26 $m^{-2}$, and it was significantly less than others (Table 1). It means that subsoiling and deep plowing could effectively reduce the density of the weed and tillage is an effective control measure. Field horsetail favored bleached Baijiang soil where high density was observed and also could grow in sandy soils and black soils. However, there was no significant difference in number among three soil types (Table 2). When spring stem grew up to about 8.0 cm, it could disperse spores (Table 2). In order to prevent sexual reproduction, control measure should be taken in early stage.

Effects of node number and temperature on rhizome propagation

With the increase in the number of nodes and the length of internodes, the capacity of rhizome germination increased (Fig. 1). Field horsetail could germinate and grow from 7.2°C to 32°C, but maximum growth rate was at 25°C. Some rhizomes were two years old according to above determination. All of them could germinate at 25°C. Corms could germinate too at 25°C. The rhizomes with more nodes produced longer shoots because longer rhizomes reserve more energy and nutrients for their growth.

Table 1. Density of field horsetail's rhizomes (no. $m^{-2}$) in different tillage fields of bleached Baijiang soil in Baoquanling Farm (June 1999 and June 2000).

| Tillage system | Continual 2-year tillage | 1-year tillage | No tillage | Waste land |
|---|---|---|---|---|
| Density | $26^{c}$ | $174^{ab}$ | $209^{ab}$ | $223^{a}$ |

Note: Values within a row followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. Field horsetail growth in different types of soil in Baoquanling Farm (June 1999 and June 2000).

| Measure | Soil type | | | | | |
|---|---|---|---|---|---|---|
| | Bleached Baijiang | | Sandy | | Black | |
| Spores dispersed or not | No | Yes | No | Yes | No | Yes |
| Height (cm) | 3.471 | 7.789 | 5.082 | 8.396 | 3.154 | 8.600 |
| Density (no.$m^{-2}$) | $411^{ns}$ | | $434^{ns}$ | | $343^{ns}$ | |

Note: ns means no significant difference at the 5% level by Duncan's multiple range test

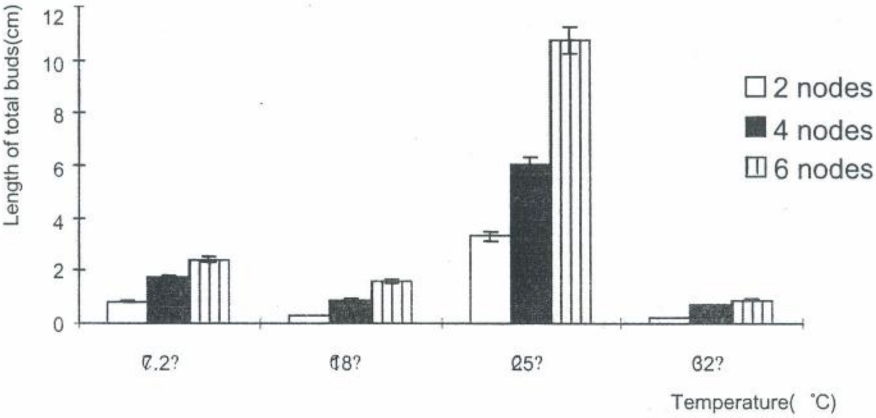


Figure 1. Effects of temperature and node number on the growth of field horsetail

Determination of the age of perennial rhizomes

The age of woody plants could be determined by their annual ring and the age of biennial weeds by their flowers, but it is difficult to determine the age of perennial weeds. In our field survey, we found that the age of field horsetail could be determined by stem color and rhizome characteristics. Younger rhizomes were green. With aging, the color became darker, from brown to black. For rhizome, top node rotted and dried out and new rhizome grew from the last second node in next year so that there was a dry and rotten node on the top of rhizomes segment grown in one year (Fig. 2). The age of the weed could be easily identified according to the dry and rotten nodes after dug out from soil.

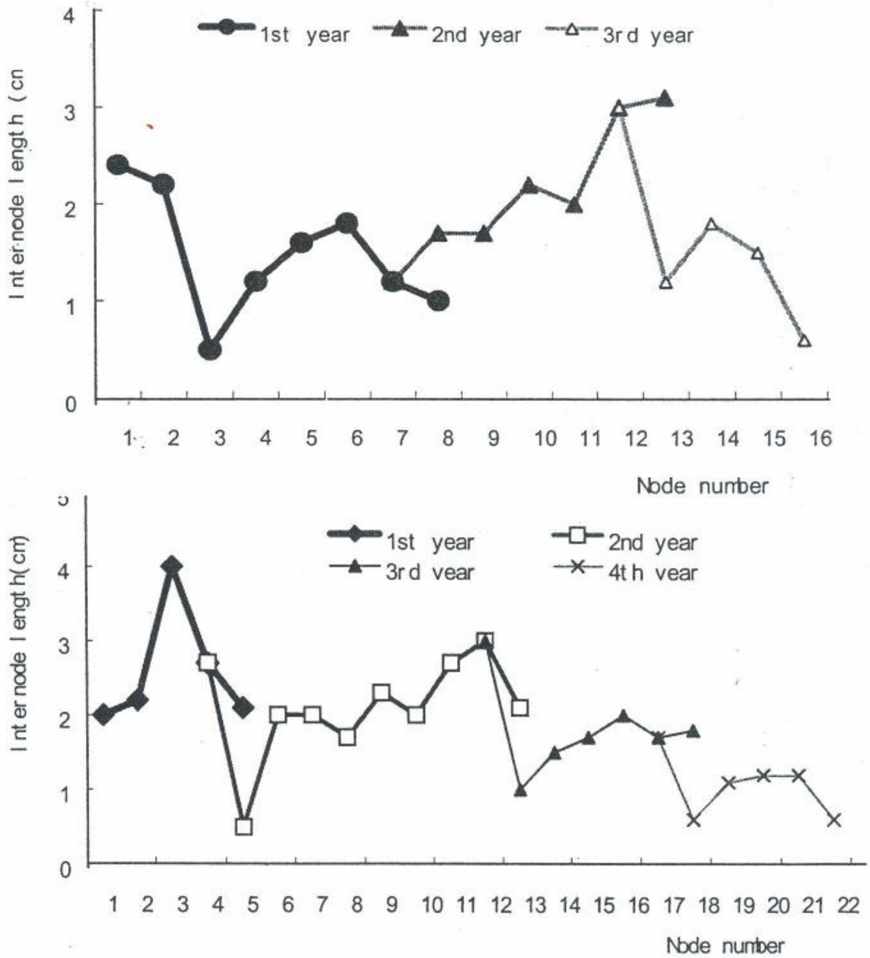


Figure 2. Growth characteristics of field horsetail rhizomes.

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# Monitoring forage production of Nilegrass using spectral remote sensing

C. M. Yang[1*], Y. J. Lee[1], K. Y. Hong[2], and F. H. Hsu[2]
[1] Agronomy Division, Taiwan Agricultural Research Institute, Wufeng, Taichung Hsien 41301, Taiwan ROC;
cmyang@wufeng.tari.gov.tw
[2] Forage Crops Division, Taiwan Livestock Research Institute, Hsinhua, Tainan Hsien 71246, Taiwan ROC

**Abstract:** Nilegrass (*Acroceras macrum* Stapf) is a $C_3$ perennial and has been commonly used as a forage grass in many areas including Taiwan. Field experiments were conducted at the Taiwan Livestock Research Institute Experimental Farm to measure seasonal changes in fresh weight of aboveground plant parts and ground-based remotely sensed reflectance spectra (350-2400 nm) of nilegrass vegetation during the growing seasons from June 2002 to October 2004. The data were used to establish spectral models for assessing and monitoring forage production along vegetation development. By linear correlation intensity analysis, results showed varied correlation coefficients between spectral reflectance and forage production in the measured spectral domain. Positive values of correlation coefficient were found from near-infrared (740-1300 nm) region to the front portion (1300-1400 nm) of short-wave infrared region (1300-1800 nm), while reflectance from the remaining wavebands showed negative correlation. The maximum absolute value of correlation coefficient was located at 439 nm, where reflectance-forage production relationship was better fitted to a quadratic function ($R^2$=0.503, P<0.001). Of the examined spectral indices, forage production most correlated with $R_{GREEN}/R_{NIR}$ ratio ($R^2$=0.654, P<0.001), where $R_{GREEN}$ is reflectance at green light (490-560 nm) maximum and $R_{NIR}$ is reflectance at near-infrared peak. Modeling of spectral characteristics and forage production was further improved by using a multiple linear regression (MLR) model. The best five-variable linear regression equation exhibited a greater sensitivity ($R^2$=0.726, P<0.001, Cp=6.000) to assess forage production. When validating the MLR model with other sets of data from different growing seasons, the model gave reasonable prediction values with the slope of 1.086 and root mean square error of 3.891 (N=21). Results suggest that forage production of nilegrass along phonological development may be assessed and monitored by models established from vegetation high-resolution reflectance data.

**Key words**: Correlation intensity analysis, forage production modeling, multiple linear regression analysis, nilegrass, spectral reflectance data.

## INTRODUCTION

A number of forage grasses have been selected and released from the Taiwan Livestock Research Institute to farmers to meet the local demand of forage for animal feeds recently (Buu et al. 1993; Shaug et al. 1999). Among them, nilegrass adapts to regional climate and is becoming a popular species with increasing cultivation acreage. In contrast to the other wide-cultivated $C_4$ type pangolagrass (*Digitaria decumbens*), nilegrass is a $C_3$ perennial and produces high forage yield with high forage quality throughout the year than its alternative. The newly released cultivar 'Taichi No.1' is also resistant to rust disease and grows well in winter if water is available to help solve forage shortage during the chilling seasons (Shaug et al. 1999, 2002).

Many reports have pointed out that forage quality may decrease after a certain growth stage during plant development. Lee et al. (1991) showed that nutrient digestibility and total digestible nutrient of pangolagrass decreased towards flowering. Buu et al. (1993) indicated that crude protein content of pangolagrass decreased while acid detergent fiber and crude fiber increased as growth stage advanced to maturity. Hsu *et al.* (2005) reported that crude protein content of both nilegrass and pangolagrass decreased yet neutral detergent fiber and acid detergent fiber increased with a delay of cutting. Since the timing of harvest determines forage quality, the criteria for harvest determination are important issues for growers in aid of growth management and yield production planning.

Canopy spectral data taken from remote sensing provide timely information on changes in plant biophysical parameters as indicators of crop growth and development. Such feature of remote sensing techniques provides an advantage over conventional field survey in monitoring and supervising crop growth status over a large area and under diversified environments (Bauer 1975). The extracted vital information can then be used for precision management of production processes. Especially, yield prediction in the early stages of growth provides an opportunity of an appropriate adjustment of farming operation in a more efficient and effective way. However, the spectral nature of crop vegetation should be defined and the relationships between spectral characteristics and biophysical parameters should be established before practical applications (Rouse et al. 1974; Wiegand et al. 1991).

The objectives were to take ground-based remotely sensed high-resolution reflectance spectra of nilegrass vegetation in conjunction with the aboveground plant parts measurements in order to select suitable spectral characteristics, and then used for establishing spectral models to assess forage production during the growing periods.

## MATERIALS AND METHODS

Field experiments were conducted in the experimental farm of Taiwan Livestock Research Institute (23°04'N, 120°26'N, elevation of 31 m) at Hsinhua headquarter from June 2002 to October 2004, with 8 growing seasons in total. Data acquired from Seasons 1 to 4, 5 and 7 were used for the establishment of models, while data from the other two seasons were used for validation of multiple linear regression (MLR) model. It was a 4-year-old nilegrass (*A. macrum* cv. Taishi No.1) pasture, and the soil was an acidic sandy loam with a pH of 4.8 and organic matter less than 0.8%. The experimental field was divided into 4 plots with 0.9 ha per plot. No pesticides were used during the experimental period and weed control was practiced by hand-weeding at 1-2 weeks after the previous cuttings.

On each day of spectral measurement, three1-$m^2$ areas per plot were harvested to obtain the fresh weight of aboveground plant parts (fresh-based forage production) and the plot average was used to couple with spectral data. Reflectance spectrum of nilegrass vegetation was taken by a portable spectroradiometer (model GER-2600, Geophysical & Environmental Research Corp., New York, USA) on near cloudless moments between 10:30 to 13:00 local standard time. There were 24 random spots of spectral measurements per plot on each sampling date and each measurement was set as an average of four full-range (350-2500 nm) spectral scans. The mean reflectance spectrum of a plot was calculated from those of 24 measurements and was used to correlate with the forage production of the plot. The spectroradiometer had a 10 degree field-of-view lens nadir-viewing over nilegrass vegetation, at a distance of 1.7 m, to acquire the reflected radiance. Reflectance spectrum was obtained by comparing radiance spectrum of the target vegetation with radiance spectrum of a standard reference panel, the so-called 'Spectralon' (Labsphere, Inc., North Sutton, NH, USA). Care was taken to prevent the influence of shadow and background.

Statistical analyses and graph plotting were performed by using Statistical Analysis System version 8.1 (SAS Institute 1998) and SigmaPlot 2001 (SPSS ASC BV, the Netherlands). Linear correlation intensity between spectral reflectance and forage production was analyzed and the narrow band of the maximum absolute value of correlation coefficient was identified. Among the spectral indices, the normalized difference vegetation index (NDVI) was calculated by the formula $(R_{NIR}-R_{RED})/(R_{NIR}+R_{RED})$, where $R_{NIR}$ is the reflectance at the near-infrared (740-1300 nm) peak and $R_{RED}$ is the reflectance at the red light (640-740 nm) minimum. The ratios of $R_{RED}/R_{NIR}$, $R_{GREEN}/R_{NIR}$, and $R_{RED}/R_{GREEN}$ were also computed, where $R_{GREEN}$ is the reflectance at the green light (490-560 nm) maximum. For MLR analysis, the Collinearity Diagnostics and the R-square Selection methods were used in combination to select suitable spectral characteristics (narrow bands), followed by the Step-wise Selection procedure. Changes of determination coefficient ($R^2$) with the number of characteristic wavebands used in MLR models were then examined to select the optimum waveband number and established an optimal model for forage production. The best five-variable MLR model was in the form of $Y_5=a+a_1R_{\lambda1}+a_2R_{\lambda2}+a_3R_{\lambda3}+a_4R_{\lambda4}+a_5R_{\lambda5}$, where dependent variable $Y_5$ is the forage production, and independent variables $R_{\lambda1}$, $R_{\lambda2}$, $R_{\lambda3}$, $R_{\lambda4}$ and $R_{\lambda5}$ are reflectance of the characteristic narrow bands λ1, λ2, λ3, λ4 and λ5 selected from the reflectance spectrum. The root mean square error (RMSE) was calculated as:

$$RMSE=\sqrt{\frac{1}{k}\sum_{i=1}^{k}(X_i-\hat{X}_i)^2}$$

and was used to compare the precision of estimation between the measured values ($X_i$) and the estimated values ($\hat{X}_i$).

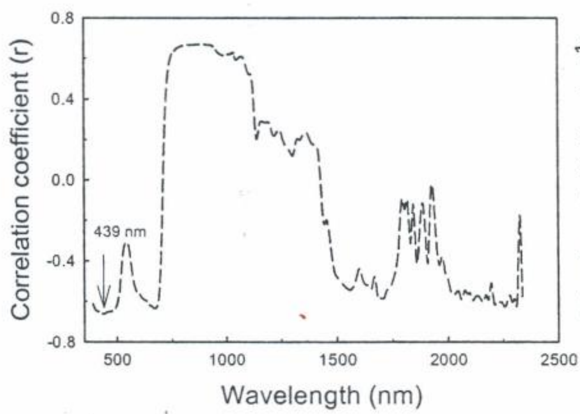


Fig. 1. Result of correlation intensity analysis between spectral reflectance in range of 350-2400 nm and forage production of nilegrass (*Acroceras macrum* Stapf).

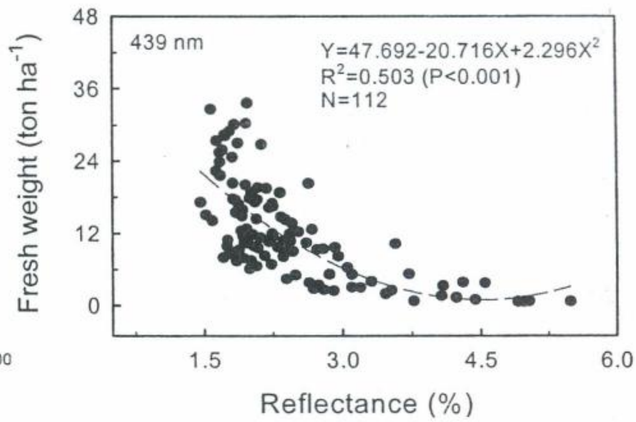


Fig. 2. The quadratic relationship between forage fresh weight and spectral reflectance at 439 nm of vegetation spectra of nilegrass (*Acroceras macrum* Stapf).

## RESULTS AND DISCUSSION

Results of linear correlation intensity analysis between spectral reflectance at 350-2400 nm range and forage fresh weight (fresh-based forage production) of nilegrass is plotted in Figure 1. There existed varied correlation coefficients along the measured spectral domain. A positive correlation coefficient was found in wavebands from the near-infrared (740-1300 nm) to the front portion (1300-1400 nm) of short-wave infrared region (1300-1800 nm), while reflectance of the remaining wavebands showed a negative value of correlation coefficient. The maximum absolute value of correlation coefficient located at 439 nm (r=-0.663**), where reflectance-forage production relationship was best fitted to a negative quadratic function ($R^2$=0.503, P<0.001) (Figure 2).

Of the examined four spectral indices, forage production most correlated with $R_{GREEN}/R_{NIR}$ ratio ($R^2$=0.654, P<0.001) (Fig. 3). It was a negative exponential function, the increase of forage production would accompany with the decrease of the $R_{GREEN}/R_{NIR}$ ratio. The commonly used spectral index NDVI had a positive exponential relationship ($R^2$=0.602, P<0.001) with forage production.

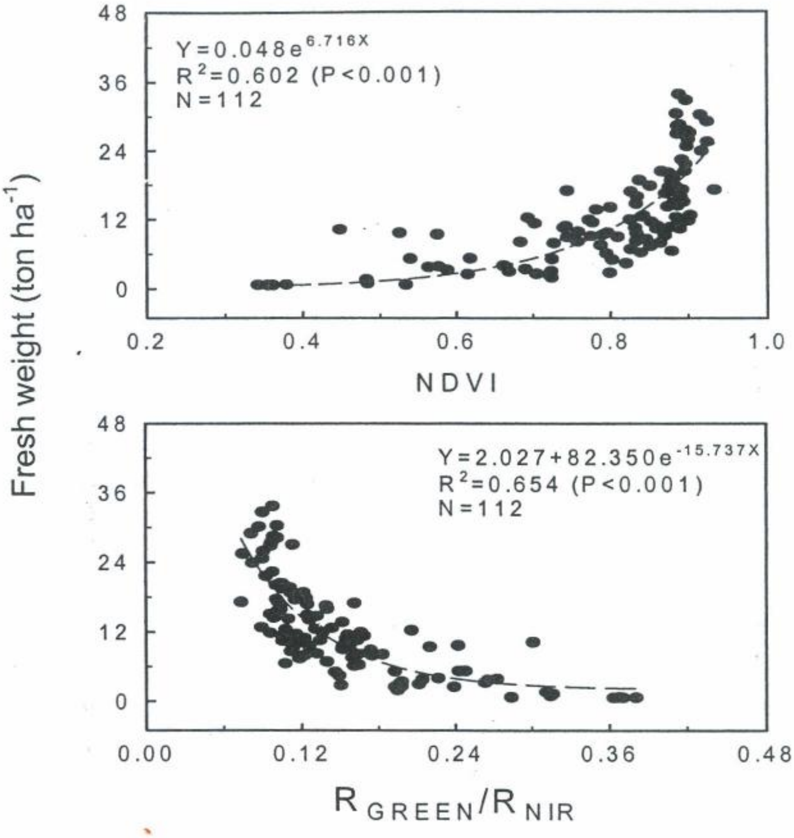


Fig. 3. The quadratic relationships between forage fresh weight and spectral indices (NDVI and $R_{GREEN}/R_{NIR}$ ratio) calculated from vegetation spectra of nilegrass (*A. macrum* Stapf).

Table 1. The best five-variable multiple linear regression equation for estimating forage fresh weight of *A.s macrum* Stapf from different narrow bands of vegetation spectra.

| Item | $Y=a+bR_{439}+cR_{891}+dR_{1709}+eR_{1909}+fR_{2255}$ | | | | | | $R^2$ | P | Cp |
|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | f | | | |
| Fresh weight | 18.175 | -2.499 | 0.618 | -2.232 | -0.219 | 2.542 | 0.726 | 0.0001 | 6.000 |
| Partial-$R^2$ | | 0.015 | 0.450 | 0.172 | 0.034 | 0.055 | | | |

$R_{439}$, $R_{439}$, $R_{891}$, $R_{1709}$, $R_{1909}$, and $R_{2255}$: the reflectance of at 439 nm, 891 nm, 1709 nm, 1909 nm, and 2255 nm, respectively.

Consistent with the reports by Yang et al. (2003) and Yang and Chen (2004), modeling of spectral characteristics to forage production was improved by incorporating multiple factors into the MLR model. The $R^2$ was promoted from 0.503 of the single spectral characteristics 439 nm to 0.602 of $R_{GREEN}/R_{NIR}$ ratio then to 0.726 (P<0.001, Cp=6.000) of the best five-variable linear regression model (Table 1). When validating the MLR model with data from two other growing seasons, the model gave reasonable prediction values with a slope of 1.086 and root mean square error of 3.891 (Fig. 4). Results suggest that forage production of nilegrass along phonological development may be assessed and monitored by models established from vegetation high-resolution reflectance data. More data containing the variability caused by cultivation management, soil background and climatic variation should be included to expand the applications.

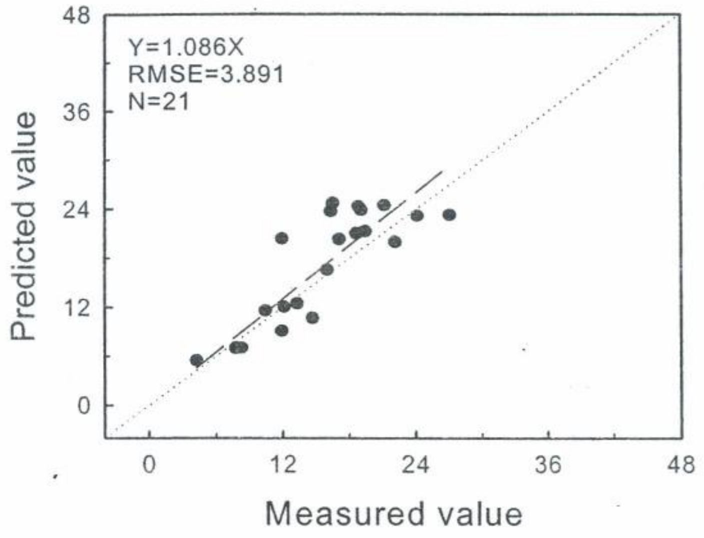


Fig. 4. Comparisons between the predicted values of forage fresh weight from the multiple linear regression model and the measured values of nilegrass (*A. macrum* Stapf).

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# Varietal Improvement

# Comparison four aromatic rice varieties with and without weed control conditions in pre-germinated seeded rice in Thailand

Pensee Nantasomsaran[1] Prasan Vongsaroj[2] Anuchat Kochasatit[3]
[1]Weed Science Group, Plant Protection Research and Development Office, Department of Agriculture, Bangkok, 10900, Thailand. Pen@doa.go.th
[2]Office of Experts, Department of Agriculture, Bangkok 10900, Thailand.
[3]Ubon Ratchathani Rice Research Center, Ubon Ratchathani, Thailand.

**Abstract:** Weeds and four aromatic rice competition were conducted in the field to determine plant traits that confer competitive ability against weeds in pre-germinated seeded rice. The experiments was laid out in 2x4 Factorial in RCB with 4 replications, first factors were weed control methods : 2,4-D/propanil at 2 Kg ai $ha^{-1}$ and untreated check plots, second factors comprised four rice aromatic varieties: Khao Dok Mali 105 (KDML 105), Hom Klongluangl (HKLG1), Hom Suphanburi (HSPR) and Patumthani 1 (PTT1). In untreated plots, HKLG1 gave the highest yield that was not significant with PTT1 and KDML105. HSPR and KDML 105 gave the lowest yield. At harvest, plant height of KDML105 was the highest than the others 3 varieties: HSPR, HKLG1 and PTT1 respectively. Therefore KDML105 and PTT1 had a good competitive ability with weeds in term of plant type and height. The second year trial gave the same results with the first year. Four cultivars gave a good response for weed control since grain yield of treated plots in each varieties showed higher yield than untreated plots.

**Key words:** Competition, aromatic rice, varieties, cultivar

## INTRODUCTION

Weeds are the one major constraint in rice production. Pre-germinated seeded rice (wet seeded rice) has a higher competition with weeds than transplanted rice. Weed and crop will be germinated at the same time in wet seeded rice.

There are many findings of International Rice Research Institute from 1977-1988, yield losses due to weeds in wet seeded rice was average 44 percent, transplanted rice 48 percent, dry seeded rice 74 percent and upland rice 48 percent (Ampong-Nyarko and De Datta, 1991). In Thailand, yield losses due to weed growth in different type of rice culture, wet seeded rice 20 percent, transplanted rice 16.5 percent, dry seeded rice 38.7 percent and upland rice 60.6 percent (Vongsaroj, 1997).

The availability of good water supply and short-duration modern rice and cost-efficient herbicides including high labor costs have motivated many farmers to shift from transplanting rice to broadcast seeding. Direct seeding has created new problems, such as major weed shifts and high density (De Datta and Nantasomsaran, 1991).

The differ of plant type causes the differ severe of weed and crop competition (Moody, 1991). The widespread replacement of traditional tall varieties with modern varieties may have increased weed problems. The traditional varieties have droopy leaves, the semi-dwarfs are shorter and have erect leaves. Therefore more light penetrates the crop canopy, and more weeds emerge and survive (De Datta, 1981).

In Thailand, the research works on weed and rice varieties competition have done for a long time. In 1976, Vongsaroj *et al* (1976) evaluated Thai traditional varieties; Leung Pratew 123, Khao Kaew, Puang Nak 16 with tall, droopy leaves, had lower weed density than modern varieties: $RD_1$, $RD_3$, $RD_7$ and $RD_9$. Nantasomsaran *et al* (1997) reported that KDML105 and $RD_6$ varieties with tall and high tillers had less weed weight than short IR72 variety. Naturally traditional varieties have more leaf area index, biomass and seed weight than modern varieties. Therefore weed and crop competition, the establishment of traditional varieties take more advantage than modern varieties. Weed and crop competition depend on many factors, however there are many variation of competition, depend on crop tolerance and weed suppression. The experiments should be done in many times and evaluated with many findings for the potential of those varieties (Caton *et al*, 2001).

The objective of this study is to find out the competitive ability of four rice cultivars under pre-germinated seeded rice.

## MATERIALS AND METHODS

The experiments was laid out in 2x4 Factorial in RCB with 4 replications at Chacheongsao Rice Research Section during July 2001 to January 2003 (wet season of 2 years), first factors comprise weed control methods: 2,4-D/propanil at 2 Kg ai ha$^{-1}$ and untreated check plots, second factors comprised four aroma rice varieties: Khao Dok Mali 105 (KDML105), Hom Klongluang1 (HKLG1), Hom Suphanburi (HSPR) and Patumthani1 (PTT1). Plot size was 5x6 m. Aromatic rice was sown in pre-germinated seeds. The treatment of 2,4-D/propanil was applied as the post emergence herbicide at 15 days after sowing (DAS).

Data of weed species and weed weights were recorded at 60 DAS by using the two quadrat of 50x50 cm sampling in each plot. Plant height was measured at 30, 60 DAS and at harvesting. Grain yields were recorded at 14 percent moisture content and yield component.

## RESULTS AND DISCUSSION

### First year experiment:

The dominant weed species in trial area were *Scirpus grossus* L.f. 30.29%, *Fimbristylis miliacea* (L.)Vahl 23.73%, *Sphenoclea zeylanica* Gaertn. 20.67%, *Cyperus difformis* L. 10.11% *Marsilea crenata* Presl 9.86%, *Echinochloa crus-galli* (L.) P.Beauv. 3.55%, *Ludwigia hyssooifolia* G.Don Exell 1.33%, *Nymphoides parvifolium* Ktze. 0.46%

In untreated plots, KDML 105 had the lowest weed number. Weed weight showed the same trend with weed density in untreated plots. The treated plot with 2,4-D/propanil HKLG1 had the lowest weed weight.

At harvest, plant height of KDML 105 was taller than three cultivars in two conditions of weed control and followed by HSPR, HKLG1 and PTT1 respectively. The advantage of tall plant is more competitive with weed than short stature plant

Patumthani1 had the highest panicle number in both untreated and treated plots whereas KDML 105 had the lowest panicle number

Grain yields were obtained from treated plots more than untreated plots

Hom Klongluang1 gave the higher yield than the others in untreated plots. Average of two weed control conditions, HKLG1 showed the highest yield and followed by PTT1, KDML105 and HSPR respectively.

### Second year experiment:

The experiment was conducted in the same area of the first year. The dominant weeds were sedge: *Fimbristylis miliacea* (L.) Vahl 90.82%, *Leptochloa chinensis* (L.) Nees 4.73%, *Scirpus grossus*

L.f. 2.07%, *Cyperus difformis* L. 1.31%, *Sphenoclea zeylanica* Gaertn.0.63%, *Echinochloa crus-galli* (L.) P.Beauv. 0.36% and *Aeschynomene aspera* L. 0.08%. Khao Dok Mali 105 had less weed number than three varieties (fig 1).

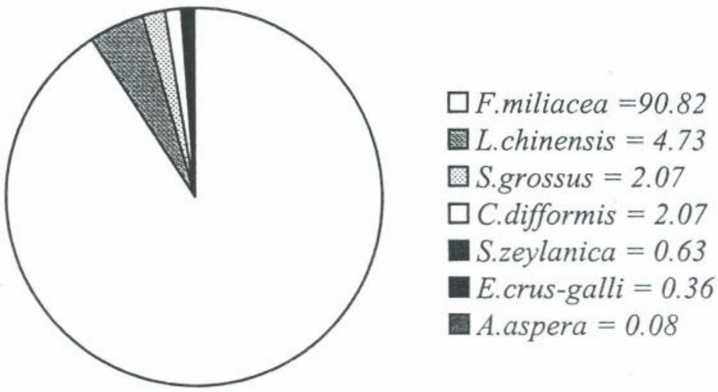


Fig 1. Weed species and density in untreated plots, 2002.

At 60 DAS, weed weight of treated plot was lower than untreated plots. In untreated plots, KDML 105 had the lowest weed population. In treated plots, HSPR had the lowest weed weight (fig 2). KDML105 was taller than three cultivars when average from two conditions of weed control methods (fig 3). Patumthani1 had the highest panicle number followed by HKLG1, KDML105 and HSPR respectively.

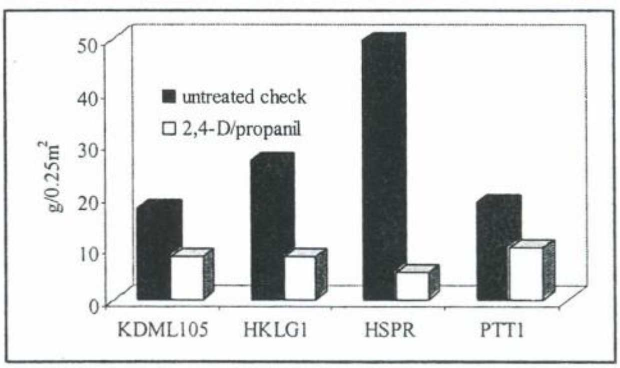


Fig 2 Weed weight at 60 DAS in ntreated and treated plots, 2002.

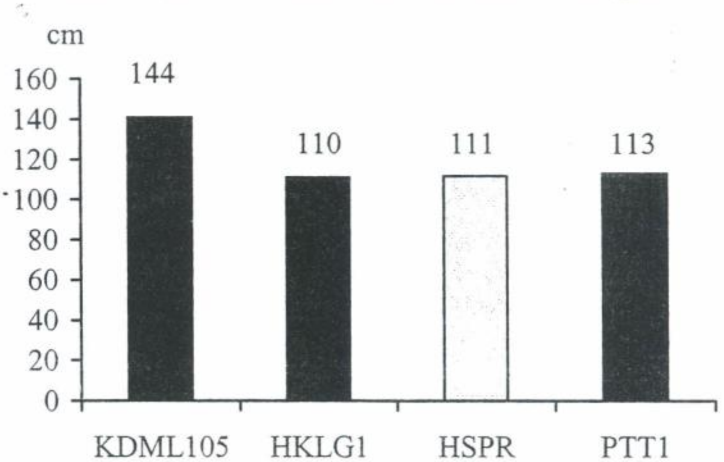


Fig 3. Plant height at harvest, 2002.

Grain yields of four cultivars gave the same trend with the first year. Hom Klongluang1 showed the highest yield and followed by PTT1, KDML105 and HSPR respectively (fig 4).

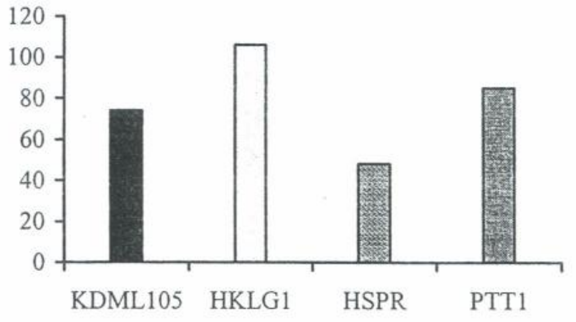


Fig 4. Grain yield, 2002.

## CONCLUSIONS

1. Hom Klongluang 1 gave the highest yield among four rice cultivars.
2. Patumthani 1 had a high tillering and panicle number.
3. Khao Dok Mali 105 had a tall, droopy leaves, and there were less weed population.
4. Hom Klongluang 1 and Khao Dok Mali 105 have a good trend to compete with weeds in pre-germinated seeded rice.


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# Allelopathy

# Effects of 6-methoxy-2-benzoxazolinone on induction of α-amylase in plant seeds

Hisashi Kato-Noguchi[1] and Francisco A. Macías[2]
[1]Department of Biochemistry and Food Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan
[2]Department of Organic Chemistry, Faculty of Science, University of Cádiz, Apdo 40, 11520 Puerto Real, Spain
hisashi@ag.kagawa-u.ac.jp

**Abstract:** 6-Methoxy-2-benzoxazolinone (MBOA) inhibited the germination of cress seeds at concentrations greater than 0.03 mM. Induction of α-amylase activity in the cress seeds was also inhibited by the MBOA at concentrations greater than 0.03 mM. Both inhibitions increased with increasing concentrations of MBOA, and the extent of the germination was positively correlated with the activity of α-amylase in their seeds. Rrespiration during germination accelerates to produce metabolic energy and biosynthetic precursors. Therefore, soluble sugars that can be readily used in respiration must be supplied constantly to maintain respiratory metabolism and germination. However, the amount of readily utilizable soluble sugars in plant seeds is usually very limited, with starch being the main reserve carbohydrate. α-Amylase was considered to play a major role in degradation of reserve carbohydrate to soluble sugars during germination. Thus, induction of α-amylase is essential to maintain active respiratory metabolism which allows germination of plant seeds. These results suggest that MBOA may inhibit the germination of cress seeds by inhibiting the induction of α-amylase activity. It may be one of the possible action mechanisms of MBOA on inhibition of the germination.

**Key words**: α-Amylase, 6-Methoxy-2-benzoxazolinone, allelopathy, germination, inhibitor, *Lepidium sativum*.

## INTRODUCTION

6-Methoxy-2-benzoxazolinone (MBOA) was found in several Graminaceous plant species such as wheat, corn and maize (Niemeyer 1988). MBOA and its precursor hydroxamic acids have been recognized as plant resistance agents because of their phytotoxic activity on insects (Dowd and Vega 1996; Bravo and Copaja 2002), and fungi and bacteria (Frey et al. 1997; Yue et al. 1998; Glenn et al. 2002).

MBOA and its related compounds have also attracted attention because of their involvement in allelopathic effects (Barnes and Putnam 1987; Inderjit and Duke 2003; Belz and Hurle 2004). MBOA was reported to inhibit the germination and growth of several plant species (Pérez 1990; Hayashi et al. 1994; Kato-Noguchi et al. 1998; Kato-Noguchi 2000). However, the physiological mechanism of MBOA on the inhibition is not fully understood.

α-Amylase is considered essential for seed germination because this enzyme triggers starch degradation in the endosperm of seeds and enables the seeds to germinate and grow (Perata et al. 1992; Perata et al. 1997; Vartapetian and Jackson 1997). It might be possible that MBOA inhibits the germination owing to the inhibition of α-amylase activity in the seeds. Thus, the effects of MBOA on germination and α-amylase activity in cress seeds were investigated.

## MATERIALS AND METHODS

### Plant material

Seeds of cress (*Lepidium sativum* L.) were sterilized in a 2 % (w/v) solution of sodium hypochlorite for 15 min and rinsed four times in sterile distilled water. All further manipulations were carried out under sterile conditions. MBOA was dissolved in a small volume of MeOH and added to two sheets of filter paper in a 9-cm Petri dish and dried. The filter paper in the Petri dish was moistened with 4 ml 0.05% (v/v) aqueous Tween 20. On the occasion, sucrose (1 mM) was added into the medium. Fifty seeds of cress were arranged on the filter paper in the Petri dish and germinated in the dark at 25 °C for 6 - 48 h. The lengths of emerged radicles of cress seeds were measured with a ruler. For determination of α-amylase activity, cress seeds were harvested, frozen immediately with liquid $N_2$ and freeze-dried.

### Extraction and assay of α-amylase

Freeze-dried cress seeds (10 seeds for one determination) were ground to a fine powder in a mortar using a pestle. Then the powder was homogenized with 1.5 ml of ice-cold solution of 100 mM HEPES-KOH (pH 7.5) containing 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 10 mM $NaHSO_3$ and 50 mM bovine serum albumin. The homogenate was centrifuged at 30,000 *g* for 30 min, and the supernatant was heated with 3 mM $CaCl_2$ at 75°C for 15 min to inactivate β-amylase and α-glucosidase (Sun and Henson 1991; Guglielminetti et al. 1995), and used for α-amylase assay.

α-Amylase was assayed by measuring the rate of generation of reducing sugars from soluble starch. Appropriate dilutions of the enzyme preparations were made, and 0.2 ml of the diluted preparations of the enzyme was added to 0.5 ml of 100 mM Na-acetate (pH 6.0) containing 10 mM $CaCl_2$. Retion was initiated with 0.5 ml 2.5 % (w/v) soluble starch. After incubation at 37 °C for 15 min, the reaction was terminated adding 0.5 ml of 40 mM dinitrosalicylic acid solution containing 400 mM NaOH and 1 M K-Na tartrate, and then placing immediately into a boiling $H_2O$ bath for 5 min. After dilution with distilled water, the $A_{530}$ of the reaction mixture was measured and reducing power evaluated using a standard curve obtained with glucose (Guglielminetti et al. 1995).

## RESULTS AND DISCUSSION

### Effects of MBOA on germination

The effects of exogenous applied MBOA on cress seed germination were determined. MBOA inhibited the germination and the germination was completely inhibited by 3 mM MBOA. When lengths of cress radicles were plotted against logarithm of MBOA concentrations, concentration-response curves were linear. At concentrations greater than 0.03 mM, MBOA inhibited the growth of cress radicles and the concentration required for 50 % inhibition on the radicle growth was 0.18 mM. These results suggest that MBOA inhibited the cress germination and the inhibition was increased with increasing MBOA concentrations. The growth inhibition by MBOA was recovered by addition of sucrose to the medium, which suggests that the inhibition may not cause by the toxicity of MBOA itself. The germination inhibition by MBOA was also found in several other plant species (Pérez 1990; Kato-Noguchi 2000), and uptake of MBOA by plant seeds was significantly faster than its precursor hydroxamic acid, 2,4-dihydroxy-7-methoxy- 1,4-benzoxazin-3-one (Pérez 1990).

When germinated cress seeds whose radicle emerged were transferred to the solution containing 3 mM MOBA from the plain solution (0 mM to 3 mM MBOA), the germination was inhibited, indicating that MBOA is able to inhibit the germination even after radicle emerged. On the other hand, when cress seeds were transferred to the solution containing 3 mM MOBA from the plain solution (3 mM to 0 mM MBOA), the germination of cress seeds initiated and their radicles began to grow. This result suggests that 3 mM MBOA did not killd the seeds, and also suggests that detoxification of MBOA absorbed by seeds may occur (Inderjit and Duke 2003; Glenn et al. 2003).

Effects of MBOA on α-amylase activity

MBOA inhibited the activity of α-amylase in cress seeds at concentrations greater than 0.03 mM. When α-amylase activities were plotted against logarithm of the concentrations, concentration-response curves were linear. The concentration required for 50 % inhibition on the activity was 0.16 mM as interpolated from the concentration-response curves, which value was almost the same as the concentration required 50 % inhibition on the radicle growth of the seeds. MBOA (0.001, 0.03, 0.1, 0.3, 1 or 3 mM) was added into the reaction mixture for α-amylase assay but the activity of α-amylase was not affected by presence of MBOA in the assay mixture, suggesting that MBOA dose not inhibit *in vitro* α-amylase activity. These results suggest that MBOA inhibited α-amylase induction at the protein level and the inhibition was increased with increasing MBOA concentrations. In addition, two concentration-response curves indicate that the length of cress radicles was positively correlated with the activity of α-amylase in their seeds.

Change in α-amylase activity in cress seeds after incubation was determined. The activity in control seeds (0 mM MBOA) was low at time 0 and increased as the process of germination occurred, where radicles of cress seeds emerged around 16 h after incubation. However, MBOA inhibited the increasing of α-amylase activity and the inhibition was greater with increasing MBOA concentrations. At 48 h, the activities in seeds treated with 0.1 and 0.3 mM MBOA, respectively, were 58 and 32 % of that in control seeds, and the activity in seeds treated with 3 mM MBOA remained almost unchanged.

Although the activity of α-amylase in control cress seeds (0 mM MBOA) was increased continually (0 to 0 mM MBOA), the increasing activity was reduced when cress seeds were transferred to the solution containing 3 mM MOBA from the plain solution (0 to 3 mM MBOA), suggesting that MBOA may well be able to inhibit increased activity in cress seeds after radicle emerged. On the contrary, the activity in cress seeds increased after transfer to the plain solution from the solution containing 3 mM MBOA (3 to 0 mM MBOA), where radicles of these seedlings resumed a growth rate similar to untreated seedlings. Thus, it seems that α-amylase activity in cress seeds correlates well with germination and growth.

Germination inhibition and α-amylase activity

Plant germination is a complex phenomenon, and many kinds of genes and enzymes are known to participate in this event (Beck and Ziegler 1989; Thomas 1993; Conley et al. 1999). During germination respiration accelerates to produce metabolic energy and biosynthetic precursors (Perata et al. 1997). Therefore, soluble sugars that can be readily used in respiration must be supplied constantly to maintain respiratory metabolism and germination. However, the amount of readily utilizable soluble sugars in plant seeds is usually very limited, with starch being the main reserve carbohydrate (Ricard et al. 1998; Saglio et al. 1999; Guglielminetti et al. 2000). α-Amylase was considered to play a major role in degradation of reserve carbohydrate to soluble sugars during germination (Perata et al. 1997; Vartapetian and Jackson 1997). Thus, induction of α-amylase is essential to maintain active respiratory metabolism which allows germination of plant seeds.

MBOA inhibited the germination of cress seeds and the induction of α-amylase in the cress seeds. Furthermore, α-amylase activity in the cress seeds reflected the extent of their germination. These results suggest that MBOA may inhibit the germination of cress seeds by inhibiting the induction of α-amylase. It may be one of the possible action mechanisms of MBOA on inhibition of the germination. The result that exogenously applied sucrose overcame the inhibiting effect of MBOA may support this hypothesis.

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# Momilactone B: a potent allelochemical in rice root exudates

Hisashi Kato-Noguchi and Takeshi Ino
Department of Biochemistry and Food Science, Faculty of Agriculture
Kagawa University, Miki, Kagawa 761-0795, Japan
hisashi@ag.kagawa-u.ac.jp

**Abstract:** A potent allelochemical causing an inhibitory effect on rice was recently isolated from rice root exudates, and the chemical structure of the inhibitor was determined by spectral data as momilactone B. Momilactone B was found to be released from rice roots into the neighboring environment, and its release level may be sufficient to cause growth inhibition of neighboring plants. The allelopathic potential of rice seedlings of seven cultivars was compared with the concentrations of momilactone B in the roots exudates of these rice seedlings. The allelopathic potential of each rice cultivar was closely correlated with the momilactone B concentration in the root exudates of the respective cultivar. Thus, the extent of the allelopathic potential of these rice varieties may be accounted for by the concentration of momilactone B in their roots exudates. These findings suggest that momilactone B may play an important role in rice allelopathy.

**Key words:** Allelopathy, donor-receiver bioassay, growth inhibitor, momilactone B, *Oryza sativa,* root exudates.

## INTRODUCTION

The negative impacts of commercial herbicide use on the environment make it desirable to diversify weed management options (Putnam 1988; Weston 1996; Narwal 1999). Many investigations have been attempted to exploit allelopathy of plants for weed control purposes in a variety of agricultural settings (Einhellig 1996; Seigler 1996; Duke et al. 2000), since allelopathy is regarded as the direct influence of an organic chemical released from one living plant on the growth and development of other plants (Rice 1984; Putnam and Tang 1986; Lovett 1991).

Rice (*Oryza sativa* L.) has been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management, such as breeding allelopathic rice strains. A large number of rice varieties were found to inhibit the growth of several plant species when these rice varieties were grown together with these plants under the field or/and laboratory conditions (Dilday et al. 1998; Kim et al. 1999; Olofsdotter et al. 1999; Azmi et al. 2000). These findings suggest that rice probably produces and releases allelochemical(s) into the environment. However, rice allelochemicals released from living rice plants have not yet been identified.

Aqueous extracts of rice plants inhibited the growth of several plant species (Tamak et al. 1994; Kawaguchi et al. 1997; Jung et al. 2004) 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; Chung et al. 2001; Rimando et al. 2001; Chung et al. 2002). It is not clear, however, whether these compounds are released from living rice plants into the neighboring environment.

In this paper, an allelochemical was isolated from rice root exudates and the chemical structure of the allelochemical was determined by spectral data. The release level of the allelochemical and allelopathic potential of seven rice cultivars were also determined to see whether their allelopathic potential is accounted for by the release level of the allelochemical.

## MATERIALS AND METHODS

Isolation and quantification of allelochemical

Seeds of rice (*Oryza sativa* cv. Koshihikari) were germinated and hydroponically grown for 14 days as described by Kato-Noguchi et al. (2002). Then, the culture solution was separated by several chromatographic fractionations keeping track of the biological activity and finally 2.1 mg of putative compound causing the inhibitory effect of the rice seedlings was isolated. The allelochemical in rice root exudates was quantified as procedure described by Kato-Noguchi et al. (2003a).

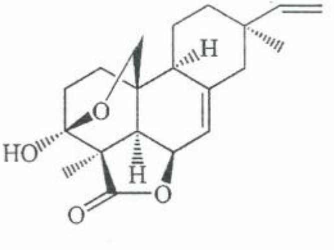
Donor-receiver bioassay

Seven cultivars of rice, cvs Hinohikari, Kamenoo, Koshihikari, Nipponbare, Norin 8, Sasanishiki, and Yukihikari were chosen for bioassay as donor plants. After surface sterilization, these seeds were grown at 25°C with a 12-h photoperiod in a growth chamber. After 4 days, uniform rice seedlings were transferred to 5.5-cm Petri dishes each containing a sheet of filter paper moistened with 3.5 ml of 1 mM MES buffer (pH 6.0) as described by Weidenhamer et al. (1987), and grown for further 3 days. Then, ten seeds of lettuce (*Lactuca sativa* L.) were arranged on the filter paper in the Petri dishes and allowed to germinate and grow with the rice seedlings under conditions as described above. After 3 days, the lengths of the hypocotyls and roots of the lettuce seedlings were measured. Control seedlings were incubated without rice seedlings.

## RESULTS AND DISCUSSION

Isolation and identification of rice allelochemical

About 5,000 rice seedlings, cv. Koshihikari, were hydroponically grown for 14 days in order to find out a potent allelochemical in rice root exudates. Keeping track of the biological activity, their culture solution was purified by several chromatographic fractionations and finally 2.1 mg of putative compound causing the inhibitory effect of the rice seedlings to be isolated (Kato-Noguchi et al. 2002; Kato-Noguchi and Ino 2003a). The chemical structure of the inhibitor was determined from its high-resolution MS, and $^{1}H$- and $^{13}C$-NMR spectral data as 3,20-epoxy-3α-hydroxy-9*β*-pimara-7,15-dien- 19,6*β*-olide (momilactone B; Fig. 1).




Momilactone B

Momilactone B was first isolated from rice husks together with momilactone A (Kato et al. 1973; Takahashi et al. 1976) and later found in rice leaves and straw (Cartwright et al. 1977; Kodama et al. 1988; Lee et al. 1999). The function of momilactone A as a phytoalexin has been extensively studied and several lines of evidence indicate that momilactone A has an important role in rice defense system against pathogens (Takahashi et al. 1999; Agrawal et al. 2002). Although the growth inhibitory activity of momilactone B was much greater than that of momilactone A (Takahashi et al. 1976; Kato et al. 1977), the function of momilactone B is obscure.

Allelopathic potentials of seven rice cultivars

Allelopathic potentials of seven rice cultivars were determined by "donner-receiver bioassay". In the bioassay, lettuce seeds were allowed to germinate and grow with 7-day-old rice seedlings for 3 days. The growth of hypocotyls and roots of the lettuce seedlings was inhibited by the presence of rice seedlings and the rice cultivars showed different range of the inhibitory activity. The length of lettuce hypocotyls relative to the length of control seedlings (grown without rice seedlings) was 75, 72, 63, 60, 51, 48 and 37 % for cvs Hinohikari, Nipponbare, Sasanishiki, Yukihikari, Norin 8, Kamenoo and Koshihikari, respectively, and the length of lettuce roots relative to the length of control seedlings was 65, 63, 61, 45, 36, 32 and 19 % for cvs Hinohikari, Nipponbare, Sasanishiki, Yukihikari, Norin 8, Kamenoo and Koshihikari, respectively. Thus, the inhibitory effect of cv. Koshihikari was the greatest, followed by that of cv. Kamenoo, Norin 8, Yukihikari, Sasanishiki, Nipponbare and Hinohikari.

The lettuce seedlings may grow with the rice seedlings without interspecies 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). In addition, during the bioassay any significant pH changes in the medium in all dishes did not found. Therefore, the inhibitory effect of rice seedlings against lettuce may not be due to the competitive interference and pH changes in the medium. These results suggest that all rice cultivars studied in this experiment may possess allelopathic potential and there are differences in allelopathic potential between these rice cultivars.

Release level of momilactone B from seven rice cultivars

Rice seedlings were hydroponically grown for 15 days and the concentrations of momilactone B in their culture solutions were determined. Momilactone B was found in all culture solutions, but the concentration in the solutions differed with rice cultivars. The highest concentration was in the solution of Koshihikari, followed by that of cv. Kamenoo, Norin 8, Yukihikari, Sasanishiki, Nipponbare and Hinohikari. It has been reported that momilactone B was produced in rice plants of cv. Koshihikari and released from the rice roots into the culture solution or neighboring environment (Kato-Noguchi et al. 2002; Kato-Noguchi and Ino 2003a; 2003b). Therefore, all rice cultivars studied may produce and release momilactone B into the culture solutions and the release level appears to depend on the cultivar.

Momilactone B inhibited the growth of roots and hypocotyls of cress and lettuce seedlings at concentrations greater than 3 μM, and the inhibition increased with increasing concentrations of momilactone B (Kato-Noguchi et al. 2002). Considering the inhibitory activity, the release level of momilactone B from rice seedlings of cv. Koshihikari into neighboring environment was sufficient to cause growth inhibition of neighboring plant species (Kato-Noguchi and Ino 2003a, 2003b).

Relationships between allelopathic activity and momolacton B

The relationships between the growth of lettuce hypocotyls and roots incubated with rice seedlings of seven cultivars and momilactone B concentrations in the culture solutions of corresponding rice cultivars are linear. Both regression coefficients (hypocotyls, - 0.9326; root, - 0.9166) are significant at 0.01 level, suggesting that momilactone B concentrations and allelopathic activities in their cultivars are closely correlated.

All rice cultivars studied in this experiment inhibited the growth of lettuce hypocotyls and roots when the lettuce was grown with the rice. Momilactone B was found in all culture solutions of their rice cultivars. The concentrations of momilactone B in the culture solutions reflected the extent of the growth inhibition of the lettuce seedlings by the rice seedlings. These results indicate that these rice cultivars possess various allelopathic activities and the extent of their allelopathic activity may be accounted for by their release level of momilactone B. Thus, momilactone B may play an important role in rice allelopathy or defense system against competition with neighboring plants for resources such as light, nutrients and water.

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# Allelochemicals from buckwheat and tatary buckwheat and practical weed control in the field

Yoshiharu Fujii, Anna Golisz, Akihiro Furubayashi, Zahida Iqbal and Habib Nasir
Chemical Ecology Unit, National Institute for Agro-Environmental Sciences,
3-1-3 Kan-non-dai, Tsukuba, Ibaraki 305-8604, Japan
yfujii@affrc.go.jp

**Abstract**: Bioactivity guided solvent extraction, chromatographic purification (silica gel, Sephadex gel and thin layer chromatography) and identification by Mass and NMR spectroscopy have yielded various allelochemicals from the aerial parts of common buckwheat (*Fagopyrum esculentum)* and tatary buckwheat *(Fagopyrum tataricum)*. In case of common buckwheat, gallic acid, rutin and fagomine were found important allelochemicals, and in case of tatary buckwheat, rutin was found to be the major allelochemical evaluated by total activity method. Field trials carried out at experimental fields showed strong weed inhibition by buckwheat. Plots where buckwheat was grown mixed with weeds, produced more than 75 % reduced weed biomass as compared to buckwheat free plots. Seeding of buckwheat in June increased the weed inhibitory effect than in July. These findings suggest that buckwheat can be used as cover crop as the allelochemicals released from the living or decaying plant material can reduce the growth of various co-occurring weeds effectively.

**Key words**: Allelopathy, catechol compounds, cover crop, fagomine, gallic acid, rutin.

## INTRODUCTION

Allelopathy is a phenomenon of chemical interaction and is probably of wide spread significance in the functioning of natural communities. In plant-plant interactions, allelopathy is generally used to denote the process by which plants release phytotoxic compounds (allelochemicals) to the environment, resulting in harmful or beneficial effects on neighboring plants. Utilization of the allelopathic potential of plants for weed control is given great emphasis, because it would reduce the risk of environmental toxicity. Crop species with allelopathic potential have been given greater attention during the last two decades. These include crops such as barley, oat, wheat, rye, canola, mustard species, sunflower, sweet clover, hairy vetch, creeping red fescue, tall fescue, perennial and rye grass [Fay and Duke (1977); Putnam and DeFrank (1979); Irons and Burnside (1982); Fujii (1999a, 1999b); Overland(1962); White et al. (1989)]. Cover crops are mostly used in no tillage production system because they provide surface residue which reduces soil erosion, conserves water retention and soil nutrients [Guenzi and Macalla (1962); Macalla and Norstadt (1974); Teasdale et al. (1991)]. Identification of cover crops that provide weed suppression may increase acceptance and utilization of these soil conserving tillage techniques [Leather (1982), Weston and Duke (2003)].

Buckwheat (*Fagopyrum esculentum* Moench) is a grain that is being eaten for hundreds of years in the Far East. China, Japan, Korea and other Asian countries have long enjoyed noodles made from buckwheat flour. In fact, Japan is the largest consumer of buckwheat and imports an annual of 100,000 metric tons of buckwheat. Buckwheat can also be used for a variety of baked products, including pancakes, breads, muffins, crackers, bagels, cookies, and tortillas among others. Buckwheat is an agronomic species of polygonaceae family that belongs to genus *Fagopyrum*. There are about 15 species in the genus *Fagopyrum*, which occur in temperate areas of Euro-Asia. Another member of this genus used as food is *Fagopyrum tataricum* Gaertner (tartary buckwheat). Weed suppression activity of buckwheat and tatary buckwheat were already known to farmers from the ancient times, but its allelopathic potential and allelochemicals were not fully studied. Recently, isolation of allelochemicals and evaluation of weed suppression in the field was done. This plant is known as "bitter buckwheat", and its allelopathic potential is also known Iqbal et al. (2002). Plants in this family produce a wide array of biologically active constituents. As fast-growing cover crop, buckwheat is most useful for weed suppression [Tsuzuki et al. (1987); Tsuzuki and Yamamoto (1987)]. Common buckwheat is known to Japanese farmers as cover crop since long for limiting soil erosion. There are some reports about its allelopathic activity in field and green house [Tominaga and Uezu (1995)]. Experiments conducted under field conditions have shown that buckwheat shades and smothers weeds, or competes them for soil moisture and nutrients. Both living buckwheat and buckwheat residue have an allelopathic effect on weed germination. Recently it was reported that aqueous extract of aerial part of buckwheat inhibited germination or seedling growth of lettuce and other weeds [Isojima et al. (2000)]. Buckwheat shoots produce and release a variety of bioactive chemicals, which are toxic to weeds [Iqbal et al. (2002, 2003)].

The present paper discusses the role of allelochemicals from the viewpoint of total activity and describes how these bioactive substances contribute towards the allelopathic activity of buckwheat and make it a successful candidate for allelopathic ground cover crop.

## MATERIALS AND METHODS

### Instruments

$^1H$ NMR spectra were recorded at 600 MHz, and $^{13}C$ NMR spectra at 150 MHz on JEOL JNM alpha-600 spectrometer in $CD_3OD$. Tetramethylsilane (TMS) was used as an internal reference. Silica gel $GF_{254}$, analytical chromatoplates, Sephadex-LH 20, and silica gel grade 60, 70-230 mesh for column chromatography were purchased from Wako, Japan and Merck, Germany.

### Isolation and identification of allelopathic substances

In this experiment, we focused on determination of the allelopathic potential of crude extract of buckwheat (*Fagopyrum esculentum)* and tatary buckwheat *(Fagopyrum tataricum)* shoot, along with all of its organic solvent fractions using seedling elongation test with pre germinated lettuce seeds. Freshly cut aerial parts of buckwheat were extracted with 80 % aqueous ethanol. The water suspension of the original aqueous ethanolic extract was subjected to a liquid–liquid partition to obtain hexane, ethyl acetate, and butanol sub-fractions. Various chromatographic purification techniques (silica gel, Sephadex gel, HPLC and thin layer chromatography) and advanced instrumentation (mass and NMR spectroscopy) were used to isolate, identify and quantify the allelochemicals from the aqueous ethanolic extract of aerial parts of common buckwheat following the same procedure as described earlier [Iqbal et al. (2002, 2003)]. Phytotoxins implicated as allelochemicals were identified from the most active fractions (ethyl acetate fraction) of tatary buckwheat. The allelopathic potential of all the isolated compounds was examined by testing their biological activities on lettuce and weeds.

## RESULTS AND DISCUSSION

### Allelopathic substances isolated from buckwheat *(Fagopyrum esculentum)*

Experiments conducted in field showed that the total number of weeds, which appeared in plots, were not different between buckwheat-weed plots and weed plots, however, the total weed dry wt. in buckwheat-weed plots was only 25% of that in the weed plots alone (Figure 1). The reduction rate of each weed was different among species suggesting differential sensitivity to buckwheat. The current experiments have shown that buckwheat is a successful competitor in the field against weeds and can effectively suppress weed growth. Although, buckwheat did not influence the number of the weeds that emerged, yet it reduced the weed biomass to considerable extent. Seeding of buckwheat in June increased the weed inhibitory effect than in July.

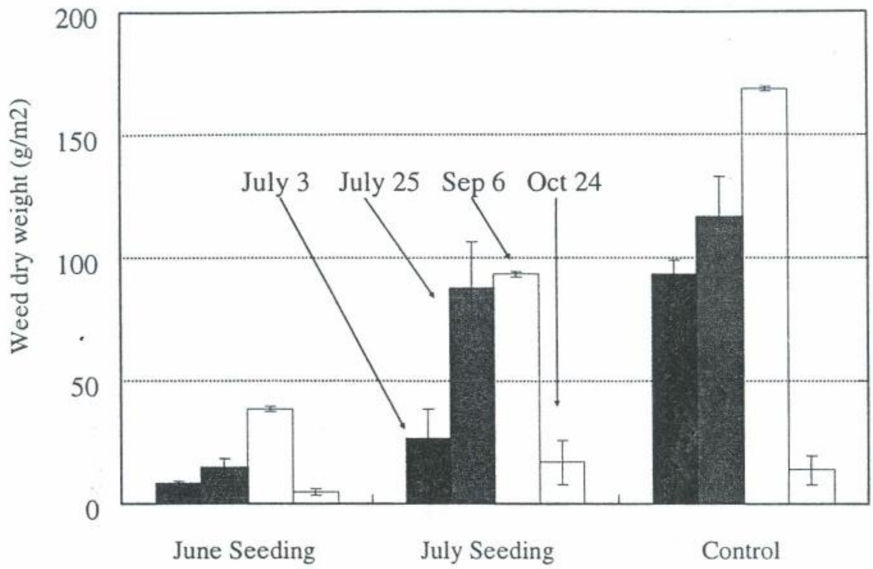
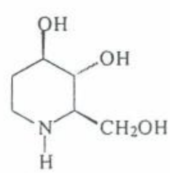
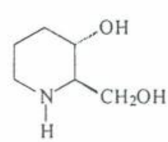



Figure 1. Effect of common buckwheat on the growth of weeds in the field.

To suppress weeds, the cover crop of buckwheat should be planted 30-50 days before crop planting. Two consecutive plantings of buckwheat will have a greater effect on weeds. One week after flowering, buckwheat can be mowed or cut and crops can directly be planted into the buckwheat mulch. These results confirm that buckwheat is a successful competitor against weeds and effectively suppress weed growth. It is not yet established whether the weed suppression is related to the production of allelopathic compounds released by buckwheat residues, or due to competition of resources e.g. reduction of light, moisture or nutrients at the soil surface.



**2-Hydroxymethyl-piperidin-3,4-diol (Fagomine)**



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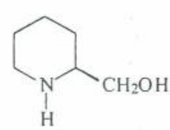
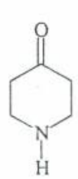
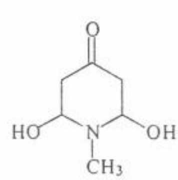


**2-Hydroxymethyl-piperidine**



**Piperidin-4-one**



**2,6-Dihydroxy-1-methyl-piperidin-4-one**

Figure 2. Piperidine type of allelochemicals isolated from buckwheat.

High plant growth inhibitory activity was observed by aqueous extract of aerial parts of buckwheat (*F. esculentum*). Bioactivity guided extraction, various chromatographic purification techniques (silica gel, sephadex gel, HPLC and thin layer chromatography) and advanced instrumentation (mass and NMR spectroscopy) have led to the isolation and identification of five phytotoxins belonging to piperidine type of alkaloids (Figure 2) and eight phenolics (Figure 3) as allelochemicals. Most of the phenolics contain catechol moiety in their structure. Catechol compounds have already been reported as allelopathic in the case of *Mucuna* and other important cover crops [Fujii (1999a, 1999b)]. Recently Callaway and Aschenhoug (2000) reported the importance of catechin in plant invasion. The catechol compounds isolated from buckwheat are also important and might have the same mode of action in the field as allelochemicals.

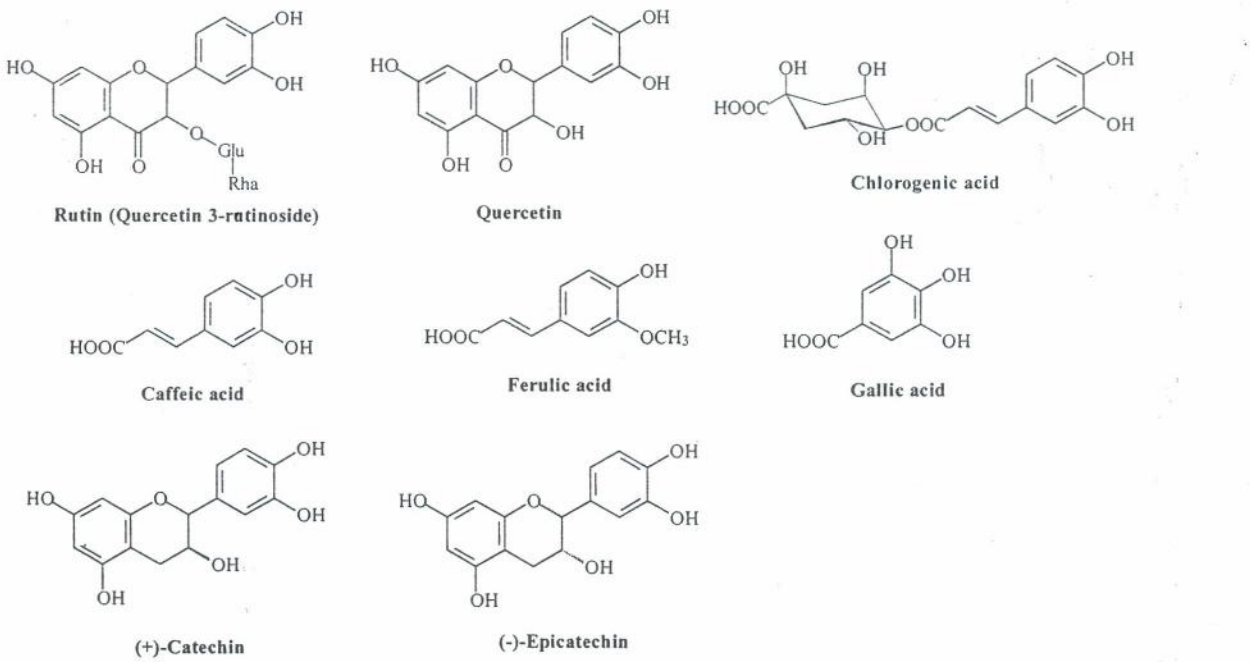



Figure 3. Structures of catechol moiety containing allelochemicals isolated from buckwheat.

The growth inhibitory activity of gallic acid on the roots of the test plant was as much as ten fold higher than on the hypocotyls indicating that root growth was initially more sensitive to the presence of this natural product. (+)-Catechin did not show strong inhibitory effects on the root and shoot growth of lettuce but was significantly inhibitory to various other weeds. The bioassays of other piperidine alkaloids revealed that the root and shoot growth was almost diminished at 500 ppm. It was observed that at a concentration of 10 ppm fagomine inhibited the elongation of roots of lettuce seedlings by 50%. Piperidin-4-one however was less potent. The concentrations of these compounds in aerial parts of buckwheat range from 0.01 %-0.02 % on fresh wt. basis. To consider the activity on the field, we introduce the concept of "Total Activity" [Hiradate (2004, 2005)], and calculate the total activity on field basis (Table 1). This is a simple idea of considering the ratio of concentration of each compound to specific activity of each compound. Since the concentration of gallic acid is much higher than other compounds, it was concluded that gallic acid plays the most important role in allelopathic activity of buckwheat. As for specificity, gallic acid inhibits the growth of broad leaf weeds, but not inhibitor for grass weeds. As the specific activity ($EC_{50}$) of rutin is smaller than that of gallic acid and fagomine, the concentration of rutin in buckwheat is much higher than other chemicals and about 0.2 % in fresh weight (2 % in dry weight), as compared with total activity, rutin is also important allelopathic factor next to gallic aicd.

Table 1: Total activity of buckwheat allelochemicals on the plant basis.

| | Content (C) (mg/kg-fw) | $EC_{50}$ (ppm) | Total Activity ($C/EC_{50}$) |
|---|---|---|---|
| Fagomine | 220 | 10 | 22 |
| 2-Hydorxymethylpiperidine-3-ol | 140 | 10 | 14 |
| Piperidine-4-one | 120 | 50 | 2 |
| Rutin | 2400 | 100 | 24 |
| Quercetin | 400 | 95 | 4 |
| Gallic acid | 400 | 5 | 80 |
| (+)-Catechin | 200 | 32 | 6 |

* EC50 means concentration of chemicals that inhibit 50 % growth.

**Allelopathic substances isolated from tatary buckwheat** *(Fagopyrum tataricum)*

Comparison of the inhibitory activity on lettuce root growth revealed that the major activity of the original extract of buckwheat tataricum was fractionated into the EtOAc fraction.
Purification of the ethyl acetate soluble fraction by repeated chromatographic procedures led to the isolation of allelochemicals, which were identified by NMR spectroscopic techniques as rutin, quercetin, and related catechol compounds (Figure 4). Piperidine alkaloids were also isolated and they are the derivatives of piperidin-2-carboxylic acid. The structures of these known compounds were confirmed by co-chromatography with authentic samples and comparison of their spectroscopic data with those reported in literature.

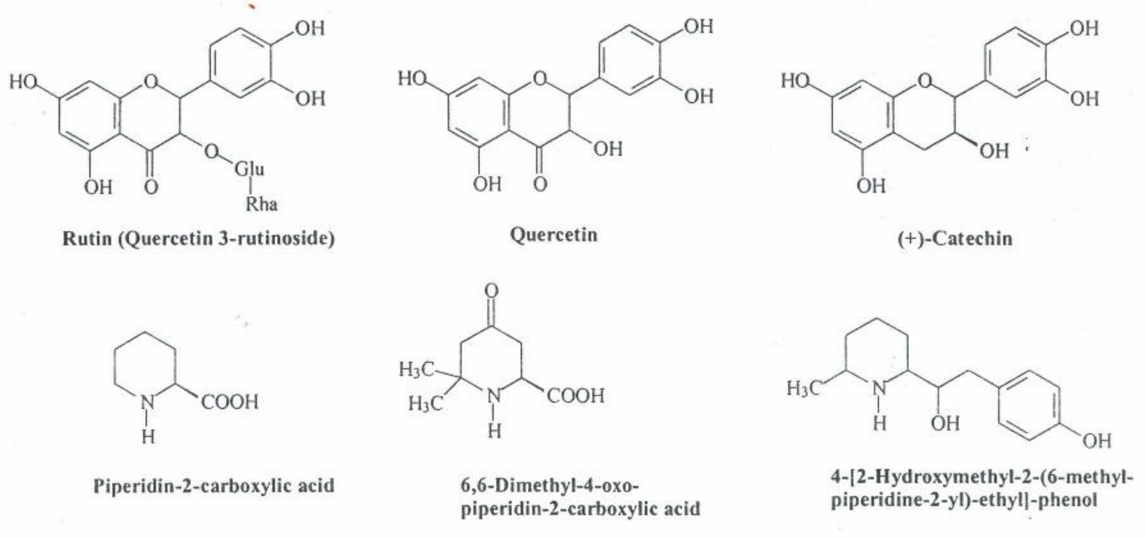



Figure 4. Structures of allelochemicals isolated from tatary buckwheat.

All the flavonoids reported here, especially rutin are known to have antibacterial, antiviral, antihypertensive and antioxidant activities. The plant growth inhibitory activity of all these compounds was tested on lettuce seedlings and all were found to be significantly inhibitory. $EC_{50}$ value of rutin was not as low as the other isolated compounds but it was found in much higher amount. This makes it evident that the purified compound of low specific inhibitory activity may play a greater role in the expressed inhibitory potential than those of high specific inhibitory activity by virtue of being present in larger quantity. In the present study Total Activity of all the inhibitory compounds was also calculated and among these, total activity of rutin was found to be the highest also in the case of tatary buckwheat. Among buckwheat species, *F. tataricum* is a species to note because it contains 50-130 times much higher amount of rutin.

These studies show that rutin is the potential allelochemical in tatary buckwheat. As regards the weed suppression activity in field it is not much different from common buckwheat (data not shown). This means that the concentration of gallic acid in buckwheat is high enough to suppress weeds in the field. Direct application of common buckwheat and tatary buckwheat can drastically reduce the biomass of weeds in the field (Figure 1). It is therefore suggested that in future buckwheat could be used as ground cover crop, this will allow long-term weed suppression in agro-economic system through the release of allelochemicals.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# Utilization of plant allelopathy for paddy weed control in Vietnam: An overview

T. D. Xuan*, S. Tawata, N. H. Hong, and T. D. Khanh
Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus,
1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan
xuan@vysa.jp

**Abstract:** Plant allelopathy plays an important role in agricultural production and is commonly exploited for biological control of pests and weeds. Allelopathy has been widely studied in Europe, the US, Australia, Japan, India, Taiwan, and Korea for decades. However, the term 'allelopathy' is only theoretically known in Vietnam, and research on the plant allelopathy has not much targeted since the recent ten years. With rich natural ecosystems, many plants may have strong allelopathic properties, which can be exploited in agricultural practice. The overuse of agrochemicals may cause problems for human health, environmental pollution, and unsafe agricultural products. A preliminary survey on searching plants with strong allelopathic potential on the ecosystems in Vietnam was conducted and their inhibition on emergence of noxious paddy weeds was examined before applying them to paddy fields. Nineteen species which controlled total paddy weeds biomass by 70% and increased rice yield by 20% at 1-2 tons $ha^{-1}$ were: *Ageratum conyzoides, Alocasia cucullata, Azadirachta indica, Bidens pilosa, Blechnum orientale, Eupatorium canabium, Euphobia hirta, Galactia pendula, Helianthus tuberosus, Leucaena glauca, Melia azedarach, Medicago sativa, Morus alba, Nerium oleander, Passiflora incarnate, Piper methysticum, Sophora japonica, Stylosanthes guianensis,* and *Tephrosia candela.* Their impacts on major paddy weeds were species dependent. These plants were useful source for paddy weed reduction and rice yield improvement. Numerous allelochemicals which involved in their strong activity were identified. The allelochemicals containing in these plants may be utilized for the development of biological herbicides.

**Key words**: Allelopathy, biological control, inhibition, paddy weeds, rice.

## INTRODUCTION

Rice is the staple crop in Vietnam and paddy field accounts for more than 6.7 million ha, or 82% of the arable land (IRRI 2003). Rice production in Vietnam has experienced dramatic changes in the past 30 years. Once a major rice-exporting country, Vietnam was a net importer of this staple for more than two decades. Vietnam is the world third largest rice exporting country (4 million t in 2004). With incentives to increase production and income with modern rice technology, Vietnamese farmers have gone from growing 15.9 million t in 1985 to 24.9 million t in 1995, and 34.5 million t in 2004 (Agroviet 2005). Larger agriculture production results in the greater use of agrochemicals such as synthetic fertilizers, herbicides, insecticides, and fungicides that cause environmental pollution, unsafe agricultural products and human health. Toxic agrochemicals should be minimized and replaced by the use of safer and bioactive chemicals isolated from plant with strong allelopathic potential.

The term 'allelopathy' is defined as a beneficial or detrimental effect from a donor plant to the recipient by the chemical pathway and the harmful impact of allelopathy may be used for biological weed and pest control (Rice 1984). Study on allelopathy was conducted sporadically in the early $20^{th}$ century. Research on allelopathic characteristic on major crops and plants including the determination of allelopathic potential, isolation and identification of allelochemicals as well as attempts to utilize their biological effectiveness in agricultural production, have been carried out widely in Europe, US, Australia, Japan, India, Taiwan, and Korea by the last decades of the $20^{th}$ century. The allelopathic characteristic and its mode in the plant and environment were further elucidated and various allelochemicals from common plants and crops were isolated and identified. However, in the plant ecosystem there may be many plants with strong allelopathic potential, which may be useful sources for biological control of weeds, pests, fungi and green manure that should be surveyed and evaluated.

The term 'allelopathy' was theoretically known by Vietnamese scientists. However, the research on this plant characteristic was not conducted, until the late 90s of the last century. Even though Vietnamese farmers have traditionally used several types of plant species such as *Melia azedarach, Ageratum conyzoides,* and *Eupatorium canabium* as green manures, clarification on applied doses, treated times, and their impacts on weeds and plant fungi were not carried out. Furthermore, some plant extracts were sprayed on rice and vegetables, which caused the reduction of pest and pathogen infestation (unpublished data). The chemicals from those plants responsible for their phytotoxic action were not described.

Vietnam has a rich plant ecosystem including both sub-tropical and tropical areas with abundant plant species which may have strong allelopathic potential. However the evaluation of their allelopathic activities has never been conducted. This note reviews our research in Vietnam from 1998-2004 on the search for plant species with strong allelopathic potential and their application for agriculture production as biological control of weed and green manures. Analysis of their chemical composition to isolate and to identify bioactive allelochemicals was also conducted.

## MATERIALS AND METHODS

### Screening for plants with strong allelopathic potential

In a preliminary survey, plants were selected from the plant ecosystems based on (a) their speed of invasiveness and have a great area in the plant ecosystems, (b) have lower natural weed density in their canopy and surroundings than the other plants in the ecosystem, and (c) are traditionally used as green manure, weed or pest management by local farmers, and medicinal purpose. Plant materials including their leaves, stems, and roots were collected and dried and their aqueous extracts were examined for the impacts on indicator plants. In this step, lettuce, radish, sesame, bean, and onion were commonly used as the indicator plants in bioassays.

The inhibitory levels on germination, hypocotyl and radicle elongation were noted and the magnitude of these inhibitory levels was determined as the inhibitory level of the plant. The plant species which exhibited strong allelopathic potential (greater than 20%) were selected and tested for their influence on emergence of major paddy weeds in laboratory and greenhouse trials.

### Effects on growth of paddy weeds

Noxious paddy weeds such as *Echinochloa crus-galli, Monochoria vaginalis, Rotala indica, Eleocharis acicularis, Scirpus juncoides, Doparium juncencum, Lindernia pyxidaria,* and *Cyperus difformis* were used. Seeds of these weed species were collected in previous years and dormancy breakage were done. Before the bioassay was conducted, the germination test of the weeds treated with distilled water was made and their germination was greater than 70%. Simultaneously, different applied doses and treated times, and frequency of treatment were carried out to determine which caused the maximum reduction on paddy weed biomass and showed the greatest promotion on rice growth and yield.

Field application

Based upon results from laboratory and greenhouse trials, plant species which were the most effective on weed reduction and rice yield promotion were selected and applied to paddy field. Commonly, the plant leaves were used because it can provide a great quantity for paddy field application. The plant leaves were chopped into 2 cm length and dried. Hand weeding and herbicide treatments were also prepared. Field experiments were conducted in a completely randomized design with at least three replicates and conducted in two cropping seasons in different paddy fields. Determination of weed biomass, weed type, and rice growth was conducted at 1 and 2 months after incorporation. Calculation on rice yield was also carried out.

Analysis of bioactive chemicals (allelochemicals)

Plant species which showed strong suppression on paddy weed growth and were effective to stimulate rice yield were selected in the search for bioactive chemicals (allelochemicals) which may be exploited for the development of biological herbicides. Their leaves, stems, and roots were used. Common methods for allelochemical extraction were applied, viz. 70% MeOH at $40^0$C and shaken for 1 day. Analytical instruments such as HPLC, TLC, GC-MS, LC-MS, IR, and NMR were used for identifying and isolating allelochemicals. Compounds which account for a large amount in the plant were purified and examined for their phytotoxic actions on weed emergence.

## RESULTS AND DISCUSSION

Screening for plants with strong allelopathic potential

During 1998-2004, some hundred plant species from the plant ecosystems in Vietnam were surveyed and collected for the screening. Their plant parts were separated and evaluated for the allelopathic potential. Their suppressive degrees were averaged and set as the inhibitory magnitude of the plant. Plants that exhibited an inhibitory magnitude greater than 20% were selected and examined for their impacts on noxious paddy weed growth. These plants are listed in Table 1, including some crops, legumes, and medicinal plants (total 25 plant species). Both rice (*Oryza sativa* L. Japonica cv. Hinohikari) hull and bran showed strong allelopathic potential. Alfalfa leaves, stem and pellets gave a great allelopathic potential (Table 1).

Effects on growth of paddy weeds

Plant materials of those 25 plant species were examined for their influence on weed and rice growth in laboratory and greenhouse. In laboratory trials, germination and growth of noxious paddy weeds against aqueous extracts of those plant species were conducted. Furthermore, paddy soil was taken to greenhouse. Plant materials were applied to the soil with different treated times, applied doses, and frequency of treatment. It was concluded that at 1-2 t $ha^{-1}$, applied plant species can significantly suppress major paddy weed emergences such as *E. crus-galli, R. indica, A. indica, E. acicularis, S. juncoides,* and *C. difformis,* and almost growth of the other species was completely inhibited.

The most effective treatment time that caused maximum weed reduction, was within 1 week after transplanting. Later incorporation of plant materials to soil provided less inhibition because at that period the weeds had already germinated and grown, and exhibited stronger resistance to the applied materials.

Table 1. Allelopathic potential of some crops and plants selected from the plant ecosystem (in decreasing order of activity).

| Plant species and common name | Inhibition (%) | Rank |
|---|---|---|
| *Helianthus tuberosus* (Jerusalem artichoke) | 100.0 | *** |
| *Medicago sativa* L. (Alfalfa) (cv. Rasen): leaves and stem | 100.0 | *** |
| *Medicago sativa* L. (Alfalfa) (cv. Yuba): leaves and stem | 100.0 | *** |
| *Nerium oleander* (Oleander) | 100.0 | *** |
| *Piper methysticum* L. (Kava): Root | 100.0 | *** |
| *Medicago sativa L.* (Alfalfa): pellets | 85.0 | *** |
| *Azadirachta indica A.* Juss (neem): leaves and bark | 81.3 | *** |
| *Leucaena glauca* Benth. (White lead-tree) | 80.7 | *** |
| *Sophora japonica* (Japanese pagoda tree) | 79.5 | *** |
| *Alocasia cucullata* (Chinese taro) | 67.0 | ** |
| *Ageratum conyzoides* L. (Billy goat weed) | 67.0 | ** |
| *Passiflora incarnate* (Passion flower) | 65.5 | ** |
| *Galactia pendula* Pers. (Galactia) | 65.4 | ** |
| *Eupatorium canabium* L. (Fragrant throughoutwort) | 64.9 | ** |
| *Oryza sativa* L. (rice) (cv. Koshihikari): bran | 60.7 | ** |
| *Melia azedarach* L. (Chinaberry) | 58.1 | ** |
| *Stylosanthes guianensis* (Stylo) | 54.6 | ** |
| *Blechnum orientale* L. (White fern) | 52.8 | ** |
| *Bidens pilosa* L. (Beggar tick) | 50.0 | * |
| *Fagopyrum esculentum* Moench (Buckwheat): pellets | 48.0 | * |
| *Euphorbia hirta* L. (Asthma weed) | 37.4 | * |
| *Alpinia speciosa* (Alpinia) | 34.6 | * |
| *Morus alba* L. (Mulberry) | 29.9 | * |
| *Tephrosia candida* Robx. (White tephrosia) | 25.7 | * |
| *Manihot esculenta* Crautz (Cassava) | 24.9 | * |
| *Desmodium rezoni* L. (Beggar weed) | 21.7 | * |
| *Cassia fistula* L. (Canafistula) | 16.8 | |
| *Oryza sativa* L. (rice) (cv. Koshihikari): hull | 15.9 | |

The inhibition is averaged from suppressive magnitude of different plant parts (leaves, stem, and root) on germination of the indicator plant. *, **, and *** indicate inhibition greater than 20, 50, and 80%, respectively. Numbers with (-) value indicate stimulation over control.

Field application

Results of the laboratory and greenhouse experiments indicated that 21 plant species showed strong reduction of noxious paddy weed and rice yield promotion. Their impacts on paddy weed biomass and rice yield are presented in Table 2. Table 2 shows that except for *Tephrosia candila* and rice bran which exhibited low weed suppression (1.9 and 25.1% of inhibition, respectively), other plant species gave strong weed reduction. Four treatments provided 50-65% weed control [rice bran, alfalfa (cv. Yuba) mixed with rice bran, and buckwheat pellets, the others exhibited very effective weed suppression by 70-90% (Table 2). Except for rice bran which slightly reduced rice yield (6.5%), other treatments promoted rice yield. *Galactia pendula* and *Melia azedarach* increased rice yield by lower than 10%. Furthermore, *Alocasia cucullata* and *Helianthus tuberosus* provided 17% rice yield promotion. Other plant species stimulated rice yield greater than 20% (Table 2). Herbicide had 11.6% weed reduction and handweeding had 25.6% . Total weed biomass reduction and nutrient composition in applied plant leaves may contribute to rice yield promotion

Analysis for allelochemicals

We found various phenolic acids and fatty acids from different plant parts from *A. conyzoides, A. indica, Fagopyrum* spp., *M. indica, M. sativa,* and *P. methysticum.* Two allelochemicals which showed strong herbicidal and fungicial activities and account for great amounts in the plant leaves are mimosine and 7,8-dihydro-5,6-dehydrokawain (DDK), which were found in Leucaena and *Alpinia speciosa*, respectively. These two compounds may be utilized as leading substances for bioactive herbicides and fungicides. Analysis for novel allelochemicals with strong herbicidal action is continuing.

Table 2. Effects of allelopathic plants on paddy weeds and rice in paddy fields as compared with the respective control.

| Plant speciesand common name | Weed reduction (%) | Increase in rice yield (%) |
|---|---|---|
| *Ageratum conyzoides* L. (billy goat weed) | 80.8 | 20.9 |
| *Alocasia cucullata* (Chinese taro) | 81.5 | 17.0 |
| *Azadirachta indica* A. Juss (neem) | 91.0* | - |
| *Bidens pilosa* L. (Beggar tick) | 81.8 | 23.3 |
| *Blechnum orientale* L. (White fern) | 74.7 | 23.3 |
| *Eupatorium canabium* L. (Fragrant throughoutwort) | 75.8 | 23.3 |
| *Euphobia hirta* L. (Asthma weed) | 87.9 | 23.3 |
| *Galactia pendula* Pers (Galactia) | 84.8 | 7.0 |
| *Fagopyrum esculentum* Moench (Buckwheat) | | |
| - pellets: | 51.7 | - |
| *Helianthus tuberosus* (Jerusalem artichoke) | 70.1 | 17.0 |
| *Leucaena glauca* L. (White lead-tree) | 85.9 | 23.3 |
| *Melia azedarach* L. (Chinaberry) | 86.9 | 4.7 |
| *Medicago sativa* L. (Alfalfa) | | |
| - Pellets | 70.0 | - |
| - cv. Rasen | 80.0 | 80.6 |
| - cv. Yuba | 65.0 | 29.0 |
| *Morus alba* L. (Mulberry) | 72.7 | 23.3 |
| *Nerium oleander* (Oleander) | 71.3 | 19.5 |
| *Oryza sativa* L. (Rice) | | |
| - Hull | 51.7 | 19.4 |
| - Bran | 25.1 | -6.5+ |
| - Hull+Rasen | 88.3 | 77.4 |
| - Bran+Yuba | 53.1 | 29.0 |
| *Passiflora incarnate* (Passion flower) | 74.5 | 21.5 |
| *Piper methysticum* L. (Kava) | 86.3* | - |
| *Sophora japonica* (Japanese pagoda tree) | 81.4 | 9.9 |
| *Stylosanthes guianensis* (Stylo) | 72.0 | 25.8 |
| *Tephrosia candila* L. (White tephrosia) | 1.9 | 23.3 |
| Herbicide (5 L $ha^{-1}$) | 77.8 | 11.6 |
| Hand weeding | 71.7 | 25.6 |

-: Calculation was not conducted. +: inhibited percentage compared with the control
Applied dose: 1-2 tons $ha^{-1}$ *: only greenhouse trial was conducted

Nineteen plant species which reduced paddy weed biomass and stimulated rice yield by more than 70 and 20%, respectively, were selected. These plants are important sources for biological weed control and they may contain numerous promising bioactive allelochemicals that may be useful for the development of novel biological herbicides. Beside the incorporation of plants with strong allelopathic potential to paddy field, the utilization of crop rotation with a rotational use of allelopathic crops may be very useful to reduce weed infestation and improve crop yield. In Northern Vietnam, between two annual rice cropping, various short-term crops such as maize, sweet potato, potato, beans, and vegetables are applied, which may cause very efficient impacts on weed reduction. In Southern Vietnam, three rice cropping can be conducted annually and a large area applies direct-seedling of rice that exacerbates weed problems because weeds and rice emerge at the same time, and causes a shift in weed flora towards predominantly grassy weeds. To resolve the increasing weed problems, herbicide use is increased that can result in hazardous effect to the environment, agricultural products and human health as well as concerns about the development of herbicide-resistant weeds. Therefore, a more appropriate cropping system (crop rotation, cover crop, mulch, and green manures) should be utilized to minimize the detrimental effects of agrochemicals.

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# Study on the allelopathy of Leuceana

S. Tawata[1], T. D. Xuan, and M. Fukuta
[1]Department of Bioscience and Biotechnology, Faculty of Agriculture
University of the Ryukyus, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan
b986097@agr.u-ryukyu.ac.jp

**Abstract:** Leucaena (*Leucaena leucocephala* de Wit) is a tropical legume that grows widely in subtropical and tropical zones. This plant contains high protein, and good annual yield, therefore it has a great potential as animal feeds. In Southeast Asia and Africa, Leucaena is grown for soil improvement and soil erosion prevention. Leucaena possesses mimosine [β-(N-(3-hydroxypyridone-4)-α-amino-propionic acid] that is responsible for the toxicity of Leucaena and preventing from the wide use of this plant. Mimosine helps Leucaena to defend itself from perpetrators, and expand its distribution in the ecosystem. The ingestion of Leucaena results in alopecia, growth retardation, cataract and infertility in animals. Here we review our research have conducted for last two decades on the reduction of mimosine from Leucaena, which can enhance usage of the legume as livestock feeds. Simultaneously, mimosine can be effectively exploited as a leading herbicidal compound. Various experiments carried out in our laboratory revealed that mimosine shows strong inhibition on numerous noxious weeds species and plant pathogens. Mimosine can be reduced by different ways: (1) hydrolosis by chemicals, (2) chelating by metals, (3) fermentation by microorganisms, (4) leaching by soaking water, and (5) exposure to heavy ion beams to produce new Leucaena varieties.

**Key words**: Leucaena, mimosine, DHP, degradation, toxicity

## INTRODUCTION

Leucaena is a legume tree with a potential use as protein in the subtropics and tropics. It has a wide environmental adaptability and a great use. It was estimated that some 2-5 million ha of Leucaena are planted world-wide. However, a large area of naturalized Leucaena in Southeast Asia and Pacific regions is difficult to verify (Brewbaker and Sorensson, 1990). In Japan, Leucaena appears only in the region of Okinawa. However it accounts for a large area in this Southern Islands of Japan.

Leucaena has been used as a high quality forage for ruminants. It supplies a balanced intake of protein, minerals and amino acids with a moderate tannin content which promotes by-pass protein value (Jones, 1979). However, the presence of a toxic amino acid, mimosine, prevents it wide use as animal feeds because animals get sick when ingested Leucaena. One of the degradation products of mimosine is 3-hydroxy-4-H-pyridone (HP), caused goiter, loss of hair and reduced productivity when fed to animals (Hegarty et al., 1979). It was found that mimosine binds with the ferrous ion to form a mimosine-ferrous complex that was not toxic to the growth of mice and rats, although the same amounts of free mimosine did inhibit their growth (Lin and Ling, 1961). Mimosine chelates iron with a binding constant of $10^{36}$, which may be important in its mechanism of action (Katoh et al., 1992). Furthermore, mimosine inhibits the DNA synthesis of many DNA viruses through the inactivation of ribonucleotide reductase, and this suppression was reversible by iron. The amendment of iron or copper has also been shown to reverse the inhibitory effect of mimosine on the DNA synthesis in Chinese hamster cells (Mosca et al., 1995).

We have conducted researches on Leuceana for more than 20 years with following purposes (i) to clarify the phytotoxic action, physiological and chemical modes, and regulation of mimosine in Leucaena and in the nature to utilize this compound for the development of bioactive fungicides and herbicides and other agronomic benefits, (ii) to reduce the mimosine content in Leucaena in order to promote its use for animal forage through the use of mimosine degradation enzyme, chemical action, and breeding pathway.

## PURIFICATION OF MIMOSINE FROM LEUCAENA

It was verified that young leaves possesses the greatest amount of mimosine. We developed a patented method to purify this compound on an industrial scale by the use of an ion-exchange resin method (Tawata, 1990). In which, Leucaena leaves were boiled and the solution was filtered. Ultra-filtration was conducted at 4 atm at 30°C, 700 rmp equipped with Filtron membrane. A column packed with acid form Amberlite IRA (technical grade) was employed and the resin was then washed with 2N $NH_4OH$ with pH adjusted to 4.5~5.0.

## MIMOSINE CONTENT FROM DIFFERENT PLANT PARTS OF LEUCAENA

Different plant parts (including bark, xylem, xylem of roots, mature leaves, immature leaves, mature stem, young stem, hairy roots, cortex of roots, mature seed pods, immature seed pods, immature seeds, mature seeds, flowers, flower buds) of Leucaena with four common varieties growing in the region of Okinawa were analyzed for mimosine content. It was concluded that younger plant parts of Leucaena produced a greater quantity of mimosine than the mature parts. The young leaves and seeds gave the greatest amount of mimosine.

## LEACHING OF MIMOSINE

Even though all plant parts of Leucaena possess mimosine but only young plant parts leached a great amount of mimosine by soaking water. The young leaves had the greatest quantity of mimosine through leaching.

## HERBICIDAL ACTIVITY OF MIMOSINE

Different dilutions of mimosine (1-1000 ppm) were prepared and tested for its impacts on various plants and weeds (viz. *Bidens pilosa, Brassica rapa, Mimosa pudica, Leucaena leucocephala, Lolium multiflorum, Phaseolus vulgaris*) to examine the herbicidal action of this compound. It showed that mimosine inhibited weed growth (*B. pilosa,* and *M. pudica*) at 25 ppm, suggesting that mimosine may be used as a herbicidal compound. However at 100 ppm, mimosine did not give any inhibition on Leucaena which implied that Leucaena has a greater resistance to the detrimental impacts of mimosine.

## MIMOSINE DETOXIFICATION

Combination between mimosine and $FeCl_3$ by different ratios were made and tested for their impacts on germination and growth of different indicator plants. It was observed that a mixture of mimosine and $FeCl_3$ at 6:4 ratio reduced the most toxicity of mimosine as compared with mimosine and $FeCl_3$ individually, and other treated ratios. This may be the most effective and economical method to lower both the toxicity of mimosine in Leucaena leaves giving a safer animal livestock and decrease the soil toxicity caused by mimosine in the area grown with Leucaena.

## SCREENING OF BACTERIAL DEGRADING MIMOSINE AND DHP

The impacts of mimosine and DHP on numerous bacteria was screened. Among 43 screened bacteria, growth of *Escherichia coli. Najjar, Corynebacterium pseudodiphterium, Aerobacter aerogenes, Bacillus natto Sawamura, Bacillus subtilis* var *Niger* were the least influenced by both mimosine and DHP at 1% concentration. The *Bacillus subtilis* var *Niger* showed the greatest reduction of mimosine content as compared with other treatments, which was confirmed by paper chromatography. It suggested that this bacterial has a potential to degrade mimosine.

## EXTRACTING MIMOSINE DEGRADING ENZYME

Our Laboratory has developed a method to extract mimosine degrading enzyme from young Leucaena leaves (total protein: 146.9 mg $ml^{-1}$, total activity: 7520 unit, specific activity: 51.4 u $mg^{-1}$, recovery: 1). The degrading potential of this enzyme on mimosine was examined. The biological characteristic of the enzyme was also studied. It was showed that this enzyme was very effective to degrade mimosine to DHP. Among 18 tested amino acids, glycine, leucine, isoleucine, valine, and asparagine had no impacts on the enzyme, whereas the other amino acids reduced its activity.

## MIMOSINE IN DIFFERENT VARIETIES OF LEUCAENA

The mimosine content from in Leucaena varieties in Okinawa was examined. It was found that the quantity of mimosine is varietal dependence. Varieties with low mimosine content are useful for forage, whereas the varieties with high mimosine quantity are beneficial to extract mimosine for multi-purpose uses.

## CONCLUSIONS

Leucaena is an important legume tree in Okinawa and many other subtropical and tropical regions. It can provide a greater amount of livestock feed when mimosine content in Leucaena is reduced. Mimosine is a potential compound which may be useful for the development of bioactive herbicides. Furthermore, the utilization of Leucaena leaves for soil improvement and weed control is very promising.

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# Herbicides

# The uptake and distribution of acid, ester and amine salt formulations of $^{14}$C-labelled 2,4-D in rubber vine (*Cryptostegia grandiflora*) with time

G.J. Harvey
Queensland Department of Natural Resources and Mines
Beenleigh, Queensland, Australia, 4207.

**Abstract:** Rubber vine (*Cryptostegia grandiflora*) was treated with $^{14}$C-2,4-D amine salt, emulsifiable acid and ester formulations in water as carrier using foliar application, and with the ester and emulsifiable acid in diesel distillate as carrier as basal bark treatments. Most of the herbicide was retained by the leaf or stem to which it was applied irrespective of formulation or carrier. Of the foliar treatments, uptake of the ester was greatest ($P<0.01$), while uptake of the amine salt and acid was approximately equal. Average total uptake of the ester was also statistically significantly greater ($P<0.01$) than of the acid following basal bark application. Translocation of the ester following both foliar and basal bark application, measured as the amount of $^{14}$C recovered from the roots and soil, was statistically significantly greater ($P<0.01$) than for the other treatments irrespective of application method. Surprisingly, and interestingly, the amount of $^{14}$C found in the roots and soil following treatment with ester and acid formulations did not differ significantly irrespective of the application method, although the concentration of $^{14}$C found in the roots was maintained longer following basal bark application of the ester. The implications of these findings are discussed.

**Keywords:** 2,4-D, carrier, *Cryptostegia,* formulation, rubber vine.

## INTRODUCTION

Rubber vine (*Cryptostegia grandiflora*), a major weed problem of the grazing industry and a major threat to native ecosystems in north Queensland, is effectively controlled by only a small number of herbicides, including 2,4-D [(2,4-dichlorophenoxy)acetic acid], picloram (4-amino-3,5,6-trichloropicolinic acid), metsulfuron [2-((((4-methoxy-6-methyl-1,3,5-trizin-2-yl)amino)carbonyl) sulfonyl)benzoic acid] and Krenite® (ammonium ethyl carbamoylphosphonate) (Harvey 1981a,b; 1987; McFadyen and Harvey 1990; Vitelli et al. 1994). Differences in uptake and translocation, together with differences in toxicity of the herbicides tested, are sufficient to account (Harvey 2005) for the differences in efficacy of the phenoxyacid and related herbicides in the field.

The efficacy of the above herbicides on rubber vine in the field is affected by their formulation as acid, ester or salt, and their placement as well as the carrier in which they are applied (Harvey 1981a,b; 1982). Although the efficacy of herbicide treatments generally is known to be affected by both formulation and carrier (e.g., Hull 1970; Burr and Warren 1971; Richardson 1977), very little information is available on the influence of these factors in the control of woody plants. All must be optimised for each situation (e.g., aerial vs ground application) for best results. Basal bark treatments are very effective against a wide range of woody species (King 1966; Bovey and Young 1980), and basal-bark treatment of rubber vine with 2,4-D esters in diesel distillate, or waste fuel, remains an effective control option for scattered and isolated infestations. However, there are very few quantitative studies on the effects of formulation on foliar absorption and translocation in woody plants, and quantitative assessment of the efficiency of basal-bark application is largely lacking. This study addresses the influence of aspects of formulation on absorption and translocation of 2,4-D in rubber vine.

## MATERIALS AND METHODS

Plant Material and Growth Conditions

Rubber vine seedlings were grown under natural light in a glasshouse at Sherwood, Brisbane, in individual nursery propagating tubes (ca. 8 cm high x 4 cm diameter) using a 2:1 mixture of sand and peat. Conditions in the glasshouse have been described earlier (Harvey 1990, 2005). All plants chosen for experimentation had a single, straight stem and were approximately 20 cm tall at 6 months of age and bore about 10 or 12 fully expanded leaves. Plants were removed from the glasshouse for acclimatisation at least 48 hours prior to treatment.

Purity and Activity of the 14C-2,4-D and Preparation of Formulations Containing Labelled Herbicide

Side-chain-labelled 2,4-D (2,4-D-2$^{14}$C, specific activity 1.04 GBq $mmol^{-1}$) was purchased from the Radiochemical Centre, Amersham (England). The triethylamine salt was prepared as described earlier (Harvey 2005). $^{14}$C-2,4-D acid was prepared by mixing 9.25 MBq of side-chain (2-$^{14}$C)-labelled 2,4-D with 5 mls of AF-201, a commercial formulation containing 300 g $L^{-1}$ emulsifiable 2,4-D acid. The $^{14}$C-2,4-D butoxyethyl ester was prepared (Harvey, 1989) using 9.25 MBq of side-chain (2-$^{14}$C)-labelled 2,4-D and 10 g pure 2,4-D. The ester, which was chromatographically pure by HPLC, was formulated as described earlier (Harvey 1990).

The identity and radiochemical purity of the acid, amine salt, and ester formulations were confirmed by TLC, visualized under UV light (254 nm) and exposed to x-ray film (Kodak RPS Royal Medical) for 24 hours as described earlier (Harvey 1990, 2005). All 2,4-D formulations gave single spots having identical mobility to authentic non-radioactive herbicides.

Table 1. Summary of details of herbicide concentration and carrier used in this experiment.

| Treatment Number[a] | Herbicide | Volume and concentration of herbicide plus carrier |
|---|---|---|
| 7 | 2,4-D butoxyethyl ester | 125 μL x 0.3%/water |
| 8 | 2,4-D acid | 125 μL x 0.3%/water |
| 9 | 2,4-D, TEA salt | 125 μL x 0.3%/water |
| 10 | 2,4-D butoxyethyl ester | 10 μL x 3%/diesel |
| 11 | 2,4-D acid | 10 μL x 3%/diesel |

[a] See Figure 1 for explanation of numbers

Application of the 14C-Herbicidal Solutions

125 μL x 0.3% solution of 2,4-D emulsifiable acid, ester, and the TEA salt formulations were applied to all leaves as before. 10 μL x 3% solutions of the 2,4-D acid and ester formulations in diesel distillate were applied to the lower 3 to 4 cm of the plant stems as basal bark treatments. Both plants were treated with the herbicide mixture, and plants were sampled at 3, 6 and 24 h, then at 2, 3 and 7 days.

Experimental design

The experimental design was a randomised complete block with 3 replications of each treatment at each time. Each plot consisted of two rubber vine seedlings in a single nursery propagating tube as described. Data were analysed by Analysis of Variance using SYSTAT® (Wilkinson 1986).

Counting Procedures

The procedure was as described earlier (Harvey 1990, 2005). Plants were harvested by cutting the stems at ground level. The leaves or treated stems, where appropriate, were then washed with distilled water/special methylated spirit [50:50 (v/v)], and the washings collected and pooled for later safe disposal. Soil was washed from the roots using the minimum amount of water needed to ensure fine rootlets were recovered (any fine rootlets recovered from the soil samples were added to the root fraction). The separate (treated) leaf, stem, root and soil samples or, in the case of the basal bark treatments, leaf plus stem and treated stem samples, were freeze-dried. Leaf, stem and root samples were weighed, then ground in a micro-hammer mill and subsamples carefully weighed into combustion thimbles (Packard Combusto-cones).

Dried soil samples were extracted for 24 to 48 h using commercial acetone, filtered, washed several more times with acetone, and the combined filtrate plus washings evaporated to near dryness over a warm hot-plate in a stream of dry air. The residue was taken up in ether and transferred to $^{14}C$-free combustible pads (Packard Combusto-pads). All samples were combusted in a Packard 306 sample oxidizer and counted in a Packard liquid scintillation spectrometer, corrected for background and quenching.

The herbicide treatment solutions were also evaporated to dryness on Combusto-pads, oxidised, and counted, except that the ester solutions were oxidised without drying (to avoid loss of ester through volatilisation).

Analysis of Results

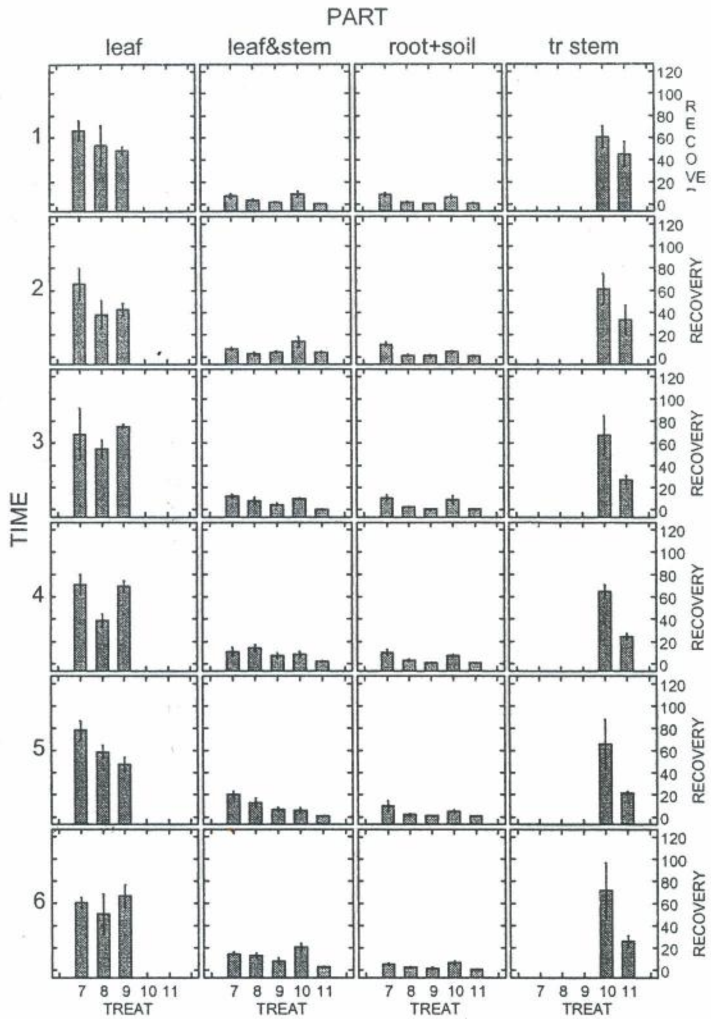
Corrected counts (disintegrations minute$^{-1}$; dpm) were entered into a computer program which calculated $^{14}C$-herbicide recoveries as μg herbicide and percent (%) recovery (as a percentage of herbicide applied). The data so generated were analysed by analysis of variance for main effects (treatment, time) using SYSTAT® (Wilkinson, 1986). Arcsine transformation of the recovered (%) herbicide data increased the precision of the analyses, and significance levels quoted refer to the transformed data, not the data shown. Soil and root data were analysed separately, but are presented graphically (Fig. 1) as combined data for purposes of simplicity and clarity.

## RESULTS AND DISCUSSION

Large differences in the proportions of 2,4-D in leaves and stems are to be expected following selective placement of the herbicide. Such large differences were found (Fig. 1) confirming the relative immobility of these herbicides, i.e. the greater proportion of the herbicide remains in that organ by which it is absorbed, except in the case of root absorption where some herbicides may be carried rapidly in the transpiration stream to the leaves, i.e. to a "transpiration sink" (Hay 1976; Shone and Wood 1974; Shone et al 1974; Briggs et al. 1982) or absorbed by cells along the LDT pathway (Loos 1975).

This experiment is, therefore, best regarded as a 3-part experiment where (a) differences in herbicide content between leaves and stems of leaf-treated plants, (b) differences between stems and leaves of stem-treated plants and (c) differences in herbicide content of roots and soil resulting both from formulation differences and from selective placement of the herbicide are examined.

Figure 1. Recovery of radioisotope following leaf or stem treatment of rubber vine with different formulations of $^{14}$C-labelled 2,4-D.




Leaf placement[a]

2,4-D as

7 2,4-D ester

8 2,4-D acid

9 2,4-D amine

Basal bark[a]

10 2,4-D ester

11 2,4-D acid

Time

1= 0.125 DAT[b]

2= 0.25 DAT

3= 1 DAT

4= 2 DAT

5= 3 DAT

6= 7 DAT

[b]DAT = Days after Treatment

[a]See Table 1 for details of treatments

(a) Leaf placement

All three formulations, ester, acid and amine salt of 2,4-D were absorbed rapidly. In absolute terms, absorption of the ester was faster and more complete (P<0.01) than uptake of the acid or amine formulations, average values over the period 1 to 7 days for the three formulations being 90, 65 and 72%, respectively. In relative terms, absorption of the acid was fastest, the absorption after 3 hours being 76, 80 and 63%, respectively, of the maximum attained for each formulation. The pattern of absorption was similar to that in earlier experiments on rubber vine and groundsel bush in that absorption of the acid and amine salt was apparently complete after 24 hours, but recovery of the ester was greatest after 3 days (Harvey 1990, 2005). Greater absorption of the oil-soluble ester than of the acid or amine conforms to the expected result ((Norris and Freed 1966a; Hull 1970; Que Hee and Sutherland 1973; Ashton and Crafts 1981), the ester being more effective in the field (Harvey 1987).

The amount of herbicide in the stems increased significantly with time for all formulations. The largest amount of radioisotope recovered in the stems was for the ester at 72 h, this value being statistically significantly higher (P<0.01) than for the other formulations or for the ester at other times. Otherwise,

the amount of radioisotope recovered from the stems was similar for the ester and acid, both being significantly greater than for the amine formulation.
The amount of herbicide found in the roots increased with time for all three formulations; only for the ester were these increases statistically significant. Conversely, the amount of radioactivity found in the soil decreased with time, possibly and at least partly because of herbicide metabolism and degradation by soil microbes. Soil microbes are, in general, more active than many plants in degrading 2,4-D and the other phenoxies both through ring cleavage and metabolism of the side chain (Loos 1975; Hatzios and Penner 1982).

None of the differences found in either roots or soil were statistically significant at any time for both the acid and amine formulations. By contrast, differences found for the ester tended to be significantly greater ($P<0.01$) for the ester at later times in the root and earlier times in the soil; where these differences were found they were also statistically significantly greater ($P<0.01$) than for the acid and amine at all times.

(b) Stem placement

Average total uptake for the ester (83%) was statistically significantly ($P<0.01$) greater than for the acid (32%) following basal bark application. This was due to greater stem uptake of the ester ($P<0.01$). The amount of radioisotope recovered from the roots was also significantly ($P<0.01$) greater for the ester than for the acid. Although the amount of radioisotope recovered from the soil was greater for the ester than the acid, and that recovered from the untreated leaf plus stem was much greater for the ester, the differences were not statistically significant.

As for the leaf treatments, the greater proportion (73 to 87% for the ester, 87 to 98% for the acid) of the herbicide remained in that part of the plant to which it was applied, in this case the stem.

(c) Root and soil differences

More radioisotope was recovered from the root after either leaf or stem application of ester than for any other treatment. The amounts recovered following leaf application reached maximum levels at 3 and 7 DAT; the amounts recovered following basal bark application were high and of the same order of magnitude after as little as 3 hours. These readings were statistically significantly ($P<0.01$) higher than for all other treatments at all times.

The amount of radioisotope recovered from soil was also greater for the ester than for the acid or amine formulations, though most of the differences were not significant. Surprisingly, the amount of radioisotope recovered from the soil was greatest for the leaf-applied ester. Recovery of $^{14}C$ from the roots after basal bark application of the ester was consistently high from 3 hours to 7 DAT, but recovery from roots in the foliar ester treatment rose substantially only between 2 and 3 DAT. Thus placement of the ester at the stem base increased the amount of translocated herbicide recovered from the roots at most times, but did not increase the amount of herbicide recovered from the soil.

General Discussion

Large differences in the proportions of 2,4-D in leaves and stems are to be expected following selective placement of the herbicide. Such large differences were found (Fig. 1) thus confirming earlier observations about the relative immobility of 2,4-D, i.e. the greater proportion of the herbicide remains in that organ by which it is absorbed (Hay 1976; Martin and Edgington 1981; Harvey 1990), except in the case of root absorption where some herbicides may be carried rapidly in the transpiration stream to the leaves (Hay 1976; Shone and Wood 1974; Shone et al. 1974; Briggs et al. 1982).

As noted earlier, foliar absorption of the ester was significantly greater ($P<0.01$) than for the acid and amine formulations, average values over the period 1 to 7 days for the three formulations being 90, 65 and 72%, respectively. These values may be compared with absorption values of 75-79% for various amine salts of 2,4-D in sunflower (*Helianthus annus*) (Que Hee and Sutherland 1973) and 1.7%, 2.9%, and 20.8% for 2,4-D amine, acid and ester formulations in the resistant bigleaf maple (*Acer macrophyllum*) (Norris and Freed 1966). Other values reported include 56 to 85% in pea (*Pisum sativum*) and 62 to 86% in tomato (*Lycopersicum esculentum*) (Fang 1958), 63% in ironweed (*Vernonia baldwinii*) (Linscott and McCarty 1962), 20 to 55% for sodium 2,4-D in wolftail (*Carex cherokeensis*) (Burns et al. 1969), 56 to 79% in honeyvine milkweed (Coble et al. 1970), and 64% for hemp dogbane (*Apocynum cannabinum*) and 84% for milkweed (*Asclepias syriaca*) (Wyrill and Burnside, 1976). However, direct comparisons should be treated with caution as in very few of the above reports were the herbicides formulated as they were in this experiment, as normal commercial concentrates, and most references are for the unformulated acid in acetone or alcohol as solvent, with or without added wetting agents.

Control of woody perennial plants is not successful if the crown and roots are not killed and the plant reshoots from the base. Basal bark application of phenoxy herbicides to woody perennial species is a particularly effective method of treatment (Coble et al. 1969; Upchurch et al. 1969), and basal bark application of 2,4-D ester or mixtures of 2,4-D plus 2,4,5-T or 2,4,5-T plus picloram esters gave 95-100% control of rubber vine in the field (Harvey unpublished). Pillmoor and Gaunt (1981) have suggested that the lethal action of 2,4-D may depend upon the length of time that the herbicide is maintained in tissue above a certain (critical) concentration. Such an explanation would be consistent with the results for the ester basal-bark treatments in this experiment, in which the amount of 14C in the roots following basal-bark treatment is, at all times, greater than the amount of 14C in the roots for all other treatments except for the ester leaf application at 3 and 7 DAT. However, the results obtained here do not seem consistent with this hypothesis for the basal-bark 2,4-D acid treatment, which is more effective than foliar application of 2,4-D ester (Harvey 1982), but for which the amount of 14C recovered from the roots is less than for the ester foliar treatment. We cannot, however, discard the hypothesis because we do not know the critical level of 2,4-D needed to cause death. Roots are very sensitive to auxin, including the synthetic auxins 2,4-D, 2,4,5-T, picloram etc. (Thimann 1969; Richardson and Amor 1975; Torrey 1976), and the amount of herbicide recovered from the roots (0.00005–0.00319% dry weight) is more than sufficient to produce a physiological response ($10^{-7}$-$10^{-11}$ M 2,4-D < 0.0002% dry weight).

The results do suggest that death of the crown and roots does not depend entirely on the amount of herbicide translocated to the roots, i.e. it would seem herbicide in the stem may kill the plant by girdling the stem and producing lethal physiological changes in the roots that are not dependent on the amount actually translocated to the roots. This surprisingly low translocation to the roots following basal bark application of phenoxy herbicides has previously been demonstrated by Hay (1956) and Sundaram (1965) in several species of tropical trees.

Esters of picloram and the phenoxyacids are generally more effective than the salts of these herbicides (Carpenter and Walker 1952; Krygier and Ruth 1961; Wiese and Rea 1962), a result confirmed earlier for rubber vine (Harvey 1981a, 1982, 1987b). We may conclude that (1) for any single herbicide, such as 2,4-D, the ester formulation is superior to acid or salt formulations for the control of rubber vine, and (2) this superior performance of the ester is due to greater absorption of the ester with resultant greater translocation of the herbicide or a toxic metabolite. These results provide an interesting contrast with those reported for absorption and translocation of the same formulations of the same herbicide in groundsel bush (*Baccharis halimifolia*), which is more susceptible to the amine salt, and in which the amine salt was more strongly absorbed and more mobile (Harvey 1990).

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# Protoporphyrinogen IX-oxidizing Activities involved in the Mode of Action of a New Compound N-[4-Chloro-2-fluoro-5-{3-(2-fluorophenyl)-5-methyl-4,5-dihydroisoxazol-5-ylmethoxy]-phenyl}]-3,4,5,6-tetrahydrophthalimide

Hwang In Taek*, Kyung Sik Hong, Jung Sup Choi, Hyoung Rae Kim,
Dong Ju Jeon, Kwang Yun Cho
Korea Research Institute of Chemical Technology, Jang-dong 100,
Yuseong-ku P.O. Box 107, Taejon, 305-606, Korea, ithwang@krict.re.kr

**Abstract**: N - [4 - Chloro- 2 -fluoro-5 - {3 - (2 - fluorophenyl) - 5 - methyl - 4,5 - dihydroisoxazol - 5 - y l-methoxy] - phenyl}]-3,4,5,6-tetrahydrophthalimide (EK-5385) is an experimental substituted bicyclic herbicide. Soil-applied EK-5385 showed good rice selectivity and potent herbicidal activity on barnyardgrass (*Echinochloa crus-galli* var. *oryzicola*) at rates of 3.9-250 g a.i. $ha^{-1}$. Barnyardgrass was exhibited normal growth under dark condition, however the growth of shoot and root was severely inhibited under light condition (14/10 hrs of light/ dark, 50 mol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation) when treated with EK-5385. $IC_{50}$ of EK-5385 to chlorophyll loss in cucumber cotyledons was approximate 0.7 and 0.3 M, respectively. $IC_{50}$ of EK-5385 to carotenoids loss in cucumber cotyledons was about 0.26 □M. $I_{50}$ concentration of EK-5385 on Protox activity was approximate 5.5 nM.

**Key words**: Carotenoids, chlorophyll, herbicidal activity, Protox, selectivity

## INTRODUCTION

Some of bicyclic herbicides cause rapid photobleaching and desiccation of green plant tissues. These herbicides exert their herbicidal effects by causing accumulation of a photosensitizing intermediate of porphyrin synthetic pathway, protoporphyrin IX (Proto IX). The Protox inhibitors have usually show foliar necrosis after 4-6 hours of sunlight following post-emergence applications. With pre-emergence applications, the tissues are damaged by contact with the herbicide as the plant emerges above the soil surface. As with post-emergence applications, the damage is tissue necrosis. Following absorption and movement to the site of action in chloroplast, light is an obligate requirement for herbicidal activity.

The purpose of this study was to elucidate the mode of action of novel compound EK-5385 (Fig. 1) on 1) herbicidal selectivity between transplanting rice and barnyardgrass, 2) EK-5385 required light for activity, 3) EK-5385, like oxadiazon and oxadiargyl, blocked porphyrin biosynthesis by inhibiting protoporphyrinogen oxidase.

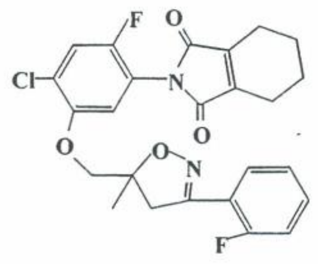


Fig. 1. Chemical structure of N-[4-chloro-2-fluoro-5-{3-(2-fluorophenyl)-5-methyl-4,5-dihydroisoxazol-5-ylmethoxy}-phenyl]-3,4,5,6-tetrahydrophthalimide (EK-5385).

## MATERIALS AND METHODS

Evaluation of herbicidal activity: Barnyardgrass seeds were sown 0.5-1.0 cm depth and 2.5-leaf stage of rice seedlings were transplanted 3 cm depth into plastic pot (surface area: $140cm^2$) filled with muddy loam soil. After seeding, pots were kept under 3 cm water depth in a glasshouse maintained at $28-33^0C$ (day) and $20-26^0C$ (night). Herbicide solutions were dropped into the water of submerged paddy condition at 2 days after transplanting, and application rates were different to the herbicides. These herbicide solutions were prepared by dissolving technical in acetone, and subsequently adding distilled water. The resulting solutions contained 500 ml $L^{-1}$ acetone and required amounts of technical. Herbicidal activity was evaluated visually at 2 weeks after treatment based on a scale of 0 to 100, where 0 indicated no visible effect and 100 indicated complete death of plants.

Effect of light on the herbicidal activity: Barnyardgrass seeds were seeded in test tube filled with 0.8% agar, and placed in growth chamber controlled with $30^oC$, 14/10 hrs (50□mol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation, PAR) and 0/24 hrs of light/dark condition. Plant height was evaluated at 2 days after setting. Herbicide application was conducted as dropping 1ml of 0.1, 0.3, 1, 3, and 10 □M herbicide solutions into test tube.

Photosynthetic pigments determination: Cucumber seeds were grown in vermiculite at $25^oC$ dark condition for 6 days. Etiolated cucumber cotyledon was floated on the Petri dish (4.5 cm) contained 7 mL of potassium phosphate buffer (pH 6.0) solution with or without herbicide. Petri dish was placed in growth chamber controlled with $25^oC$ dark conditions overnight and changed conditions to light with 50 □mol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR) for 14 hrs. Chlorophyll was extracted and assayed according to the procedure of Hiscox and Israelstam. The tissues from the dishes were soaked for 24 hrs in darkness in 10ml of dimethylsulfoxide at room temperature. Chlorophyll extraction was complete at this time. Total chlorophyll and carotinoid content of these samples were determined after extraction with Lichthenthler method.

Protophorpyrinogen IX determination: Protophoryrinogen IX (PPIX) was extracted from the above samples. One gram of etiolated cucumber cotyledon was grounded with 20 mL solutions of acetone and 0.1N $NH_4OH$ (9:1, v/v) and filtered with Mira cloth. The filtrates were centrifuged at $4^oC$, 20,000xg for 10 min. Supernatants were separated with n-hexane, and resolve HEAR layer in 3mL of n-hexane. PPIX contents in HEAR layer were determined with florescence spectrophotometer (Kontron Instruments SFM25, Ex 400 nm, Em 633nm, $25^oC$) compared with standard curve of PPIX sodium salt (Sigma). The photodiode array detector scanned from 300 to 700 nm to confirm the peak. Level of PPIX with triplicate was expressed on a molar basis per gram of fresh weight.

Etioplast isolation: All procedures were conducted under a dim, green light source. Etioplasts were isolated 7-day-old etiolated barley (*Hordeum vulgare* L. cv. Dongboree, 100 g fresh wt) leaves by differential centrifugation and Percoll-density centrifugation. The isolated etioplast fractions were highly enriched for etioplasts and had relatively little contaminated by other subcellular components. The isolated etioplast fractions were stored at $-80^oC$ until use.

Protox assay: Before assay, the extracts of etioplasts were thawed and sonicated twice for 5 s at $0^oC$ and were adjusted to 4 mg of protein $ml^{-1}$ in a suspension buffer consisting of 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, pH 7.8), 330 mM sucrose, 1 mM $MgCl_2$, 5 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT), and 0.1% (w/v) bovine serum albumin (BSA). Protein concentration was determined by the method of Bradford with BSA as a standard. When herbicide was utilized, it was added in a volume of 2 □L of acetone to 200 □L of the extract, and acetone was added to control treatments. The extracts were allowed to incubate on ice for 15 min with or without the herbicide, but the incubation time did not appear to be critical. The activity of Protox was determined by using a Kontron Instruments SFM25, temperature-controlled, recording luminescence spectrometer with excitation at 395 nm and emission monitored at 622 nm according to the method of Sherman et al. The assay mixture consisted of 100 mM HEPES (pH 7.5), 5 mM EDTA, 2 mM DTT, 1% Tween 20, and about 2 □M Protogen IX. Protogen IX utilized in the assay was prepared by the reduction of Proto IX with sodium amalgam as described by Jacobs and Jacobs. Autooxidation of Protogen IX to Proto IX in the presence of heat-inactivated extract was negligible.

## RESULTS

Herbicidal activity: Soil-applied EK-5385 showed good rice selectivity and potent herbicidal activity on barnyardgrass (*Echinochloa crus-galli* var. *oryzicola*) at rates of 3.9-250 g a.i. $ha^{-1}$. Soil-applied EK-5385 exerted 95% control efficacy to barnyardgrass at the rate of 3.9 g a.i. $ha^{-1}$, and the control efficacy was increased as increasing the rate of application. However, transplanting rice was safe to 250 g a.i. $ha^{-1}$ of soil-applied EK-5385 (Table 1).

Table. 1. Herbicidal Selectivity of EK-5385 and Oxadiazon between Transplanting Rice and Barnyardgrass

| Rate (g a.i./ha) | Rice Injury and Control Value (%) [a] | | | |
|---|---|---|---|---|
| | EK-5385 | | Oxadiazon | |
| | TR [b] | ECHOR | TR | ECHOR |
| 1 | 0 | 35 | 0 | 0 |
| 3.9 | 0 | 95 | 0 | 0 |
| 15.6 | 0 | 95 | 0 | 65 |
| 62.5 | 0 | 100 | 0 | 100 |
| 250 | 5 | 100 | 0 | 100 |

[a] a scale of 0 to 100, where 0 indicated no visible effect and 100 indicated complete death of plants

[b] TR; 2.5 leaf stage of transplanting rice, ECHOR; *Echinochloa crus-galli* var. *oryzicola*

Light requirement for EK-5385 phytotoxicity: Barnyardgrass was exhibited normal growth under dark condition (0/24 hrs of light/dark) of growth chamber, however, the growth was severely inhibited under light condition (14/10 hrs of light/dark, 50 mol $m^{-2} s^{-1}$ of photosynthetically active radiation, PAR) when treated with EK-5385. The growth inhibition was increased with increasing the rates of herbicide application. The inhibition was more sensitive in shoot growth than root growth to these herbicides. Application of 0.1 g a.i.$ha^{-1}$ EK-5385 inhibited by 50 and 30% of the normal shoot and root growth of barnyardgrass, respectively. Shoot growth was completely inhibited at 1 g a.i. $ha^{-1}$ of EK-5385 (Fig. 2).

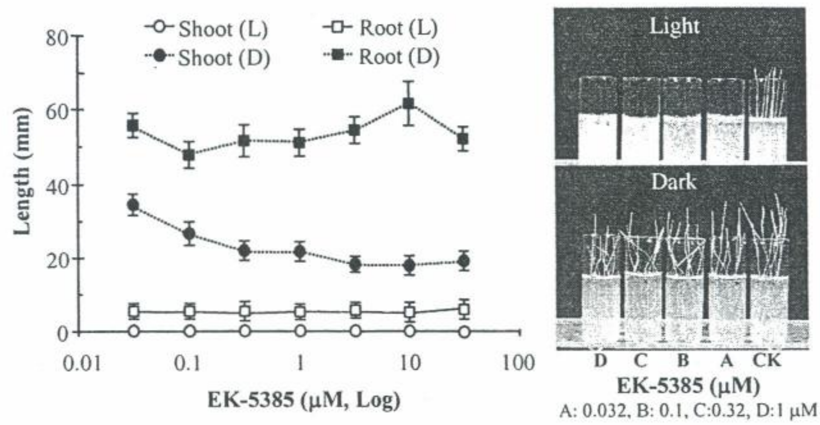


Fig. 2. Effect of light on the herbicidal activity of EK-5385. Error bars are standard error of the means. In some cases the error bar is obscured by the symbol.

Loss of chlorophyll and carotinoids: EK-5385 gradually reduced chlorophyll contents in cucumber cotyledons with increasing the application rate. $IC_{50}$ of EK-5385 to chlorophyll loss in cucumber cotyledons was approximate 0.7 □M. Carotenoids contents in etiolated cucumber cotyledons treated with EK-5385 was decreased with increasing rates EK-5385 application. $IC_{50}$ of EK-5385 to carotenoids loss in cucumber cotyledons was approximately 0.26 □M (Fig. 3).

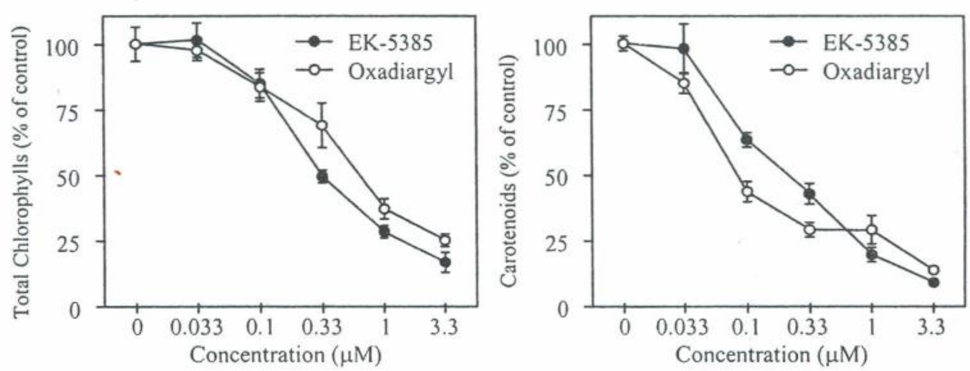


Fig. 3. Total Chlorophylls and Carotenoids in Etiolated Cucumber Cotyledons treated with EK-5385 and Oxadiargyl. Error bars are standard error of the means. In some cases the error bar is obscured by the symbol.

Effect on Protox IX accumulation and Protox activity: Protox IX accumulated in the cucumber cotyledons treated with EK-5385 (Fig. 4). EK-5385 effectively inhibited Protox activities in etioplast from barley leaves (Fig 5). $I_{50}$ concentrations of EK-5385 on Protox activity were approximately 5.5 nM. The Protox inhibition in barley etioplasts increased with increasing concentration of EK-5385, but no further inhibition of Protox in barley ethioplasts occurred beyond 0.01 □M EK-5385 concentration.

Thus, the fact that EK-5385 has shown the same pattern with the references oxadiazon and oxadiargyl in these conditions suggested these compounds have the same mode of action of Protox inhibition in chlorophyll biosynthesis. In additions to this result, the potential for foliar treatment, cellular leakage, and lipid peroxidation remains to be evaluated.

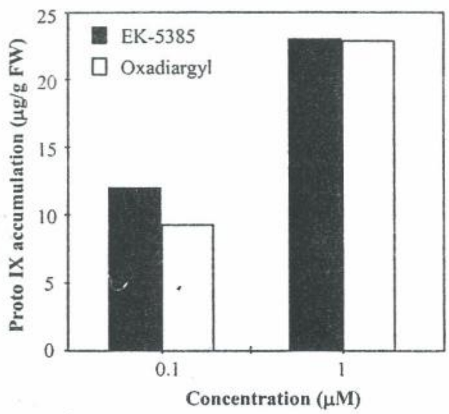


Fig. 4. Protophorphyrin IX accumulation in cucumber cotyledon treated with EK-5385 and Oxadiargyl.

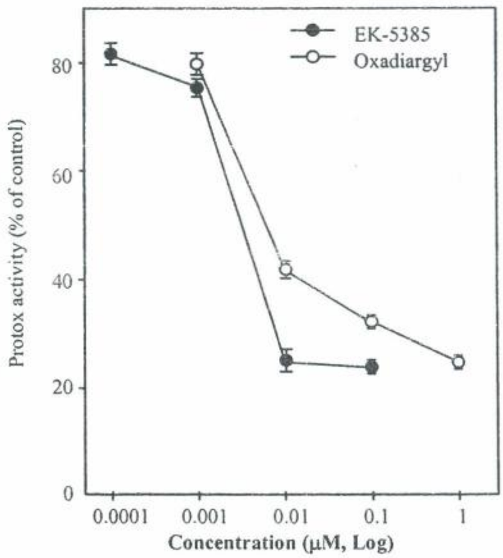


Fig. 5. Protophorphyrinogen Oxidase Activity of Extract from Barley treated with EK-5385 and Oxadiargyl. Error bars are standard error of the means.

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## Influence of eco-conditions on the selectivity and efficacy of major paddy rice herbicides used in Vietnam

N. V. Tuat , N. H. Son*
National Institute of Plant Protection, Hanoi, Vietnam
tuat@hn.vnn.vn; hanhson@hn.vnn.vn

**Abstract:** Herbicides have become more and more important in agriculture production in Vietnam since 1995. With the advantages of low cost and labor saving, the quantity of herbicides used has sharply increased from several thousand tons annually before 1990 to more than 10 thousand tons in 2003 to put herbicide in equal proportion with insecticides and fungicides. Up to now, herbicides are mainly used in paddy rice since it takes the largest area among agricultural crops. However, technical miss-use of herbicide due to inadequate knowledge on the complex interaction between crops, herbicides and environment conditions, is constraining the progress of herbicide application. There have been many cases of herbicide damage, e.g., 2,4-D, butachlor or oxadiazon, due to the farmer's misunderstanding of application scope in some specific eco-conditions. Information on these is still inadequate. This paper is concerned on the research findings on herbicide impact of some abiotic factors, such as temperature, humidity, water stand and irrigation timing, soil structure, and the selectivity and efficacy of major herbicide groups mainly used in paddy rice. The study was conducted by the researchers of the National Institute of Plant Protection, at the Red River Delta of Vietnam.

**Key words**: Abiotic factors, efficacy, herbicides, selectivity.

### INTRODUCTION

As a tropical country, Vietnam has a favorable condition for the diversification and development of both agriculture crops and weeds. There have been more than 400 weed species recorded to be dominant in agri-eco systems throughout the country and they have become a real constraint in intensive farming. Thus, weed management is necessary in order to maintain and increase crop yield. Presently, weed control is mainly dependent on chemical herbicides that have been introduced into Vietnam since the 60s. A dramatic change in farmers' attitude towards the use of herbicides in recent years due to changing agricultural labor structure and rural urbanization, increasing need for agricultural labor, has fostered the use of herbicides for weed control.

A good herbicide requires high selectivity that is highly dependent on the complex interaction between crop, herbicide, and environmental conditions (Ashton and Harvey 1971). According to this review, the seven main abiotic factors involved affecting the response of plants to herbicides are plant growth stage, growth rate, plant morphology, plant physiology, chemi-biology, biochemistry and genetic. Molecule structure and concentration of herbicide, active ingredient, formulation, and application mode of herbicides will determine their selectivity to plants. In addition, abiotic factors such as soil structure, precipitation and/or water level and ambient temperature also play an important role in selectivity of herbicides. Since herbicides used in Vietnam are mainly imported, there had been very few studies on herbicide selectivity and its application options appropriate for the eco-condition in Vietnam. These may result in some recent poisoning cases in 2000 and 2002 attributed to butachlor activity due to low temperature and rainfall during the application.

This study aimed mainly to critically evaluate the effect of abiotic factors on the efficacy and safety of some major herbicide activities used in paddy rice, to select herbicides with the most wide application range, and to optimize their usage in certain ecological places in Vietnam. The research was concerened with some of major abiotic factors, i.e., temperature, water regime and soil type.

## MATERIALS AND METHODS

Trials were carried out using Khangdan, a Chinese hybrid rice variety, and eight most common herbicides, i.e., pretilachlor; butachlor; oxadiazon; metsulfuron methyl; bensulfuron methyl; Eehoxysulfuron; quinclorac and fenoxaprop– ethyl. Seeds of four most common weeds such as *Echinochloa crus-galli; Leptochloa chinensis; Fimbristylis miliaceae and Cyperus difformis*, were also broadcast in the experimental plots to enrich their populations.

Laboratory tests were used for evaluating the influence of temparature. Rice and weed seeds were sown on three major typical soil types (heavy clay, slightly and sandy soil) contained in trays (10 x 20 cm). The study had five replicates. The trays were kept in growing chambers controlled at seven different temperatures from 10 to 40°C with interval of 5°C. Herbicides were sprayed at recommended time and concentration .

Green house tests were conducted for evaluating the influence of water stand. Rice and weeds seeds were sown in cement pools (2.5 x 3.0 m), with three replicates, in the greenhouse. Three water levels were imposed (1). no water stand; (2). water level maintained at one third of rice plant height; and (3) water level maintained at two third of plant height within 15-20 days after spraying. Herbicides were sprayed at recommended time and concentration.

Field tests were conducted for evaluating the influence of soil and irrigation regimes on selectivity of herbicides to direct seeding rice and their efficacy.

*Soil test.* Three major typical soil types consisted of (1) heavy clay soil; (2) slightly clay soil; and (3) sandy soil were selected for evaluation. Seeds of rice and weeds were randomly broadcast and herbicides were applied, at recommended time and concentration, in a large trial plot (300 $m^{-2}$) for each typical soil type without replications.

*Irrigation regimes*. The test was designed as randomized complete block (RCB) in three replicates with four irrigation treatments: (1). flooding before applying herbicides; (2), water drained at application and reflooded after 1 day, (3) water drained at application and reflooded after 2 days and (4) water drained at application and reflooded after 3 days. Plot size was 15 $m^{-2}$ (3 m x 5 m). Water level remained at one third of plant height at appropriate growing stage of rice when herbicides applied.

*Soil types tests*. Done with three above major soil structures in large scale trials without replicates (plot size is 300 $m^{-2}$).

*Observation.* Phytotoxity of rice was recorded at 1, 3, 5, and 7 days (for Lab. tests) and 3, 5, 7, and 14 days (for green house and field tests) after treatment, evaluated by EWRS (European Weed research Societys) score: (1) no symptom; (2). Very mild symptoms, slightly stunting; (3). Mild but clearly recogniable symptoms; (4). More severe symptoms (e.g.chlorosis); (5). Thinning out, heavy chlorosis or stunting, reduction in the yield to be expected; (6); (7); (8); (9) heavy damage to total kill.

Herbicide bio-efficacy was evaluated at 20 days (for Lab. tests) and 30 days (for green house and field tests) after treament by % bio – mass dicreased.

## RESULTS AND DISCUSSIONS

Effects of temperature on the selectivitiy and efficacy of tested herbicides applied in different soil types

Ambient temperature proved to have significant effect on the selectivity of eight tested herbicides towards rice. In general, all the tested herbicides, except oxadiazon applied at 5 days after sowing, showed relative safety to rice, with the damage levels ranging from 2 to 3 under 20 to 30°C. The higher and or lower the temperatures out of that range resulted in more severe damage to rice. The most severe rice damages were recorded in oxadiazon, then in butachlor and bensulfuron methyl caused damages at levels 6, 7 and 8 at 10°C; and levels 5 to 6 at 40°C, respectively (Figure 1). Major phytotoxity symptoms were yellowish leaves, if severe leaves were burned, plants stunted or even died.

However this effect also depended on the soil structure (Figures 1, 2 and 3) with more profound damage in heavy clay soil than in slightly clay and sandy soil. The damages caused by post-emergence herbicides like quinclorac and fenoxapro, were less varied in interaction with temperature than pre-emergence herbicides, pretilachlor and butachlor (Figure 1).

**Figure 1: Effect of temparature on herbicides phytotoxity (Lab. test with slightly soil – data recorded at 7 days after treatment)**

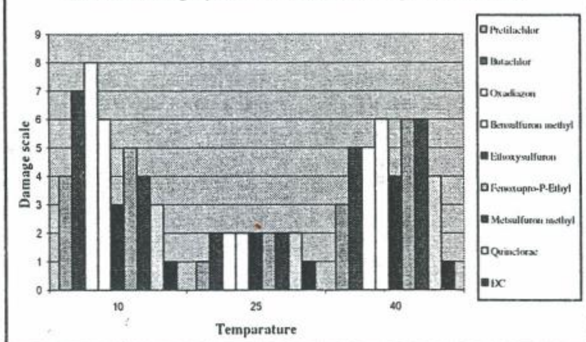
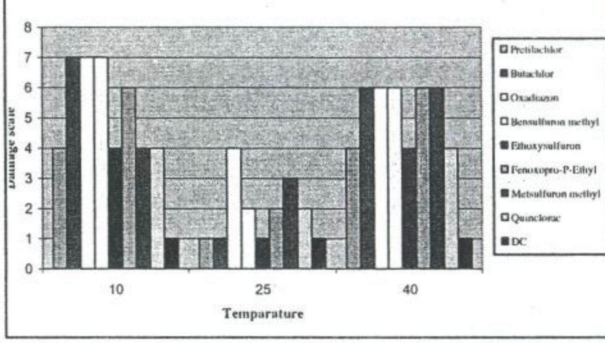
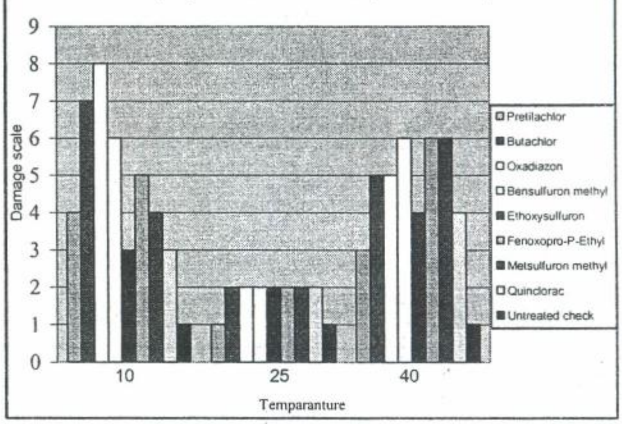



**Figure 2: Effect of temparature on herbicides phytotoxity (Lab. test with sandy soil – data recorded at 7 days after treatment)**




Additionally, ambient temperature also influenced the efficacy of herbicides against grass (Table 1) and sedge weeds (Table 2). To both groups, the highest efficacy of all eight tested herbicides was recorded when applied at temperature greater than 20°C. It showed slightly varying efficacy at 20 to 40°C. On the contrary, a dramatic decrease in efficacy (about 10-15%) of all tested herbicides was evident at temperatures lower than 20°C.

**Figure 3: Effect of temparature on herbicides phytotoxity (lab. test with heavy clay soil - data recorded at 7 days after treatment)**




However, these reductions in herbicide efficacy also depended on appropriate herbicide groups (Tables 1 and 2). More significant reductions were recorded with pre and/or early-post-emergence herbicides like pretilachlor, butachlor and oxadiazon compared to post-emergence ones.

Pretilachlor and quinclorac were most effective against grass weeds, controlling about 90% of the weeds at optimum application temperature greater than 20°C, followed by butachlor, oxadiazon and fenoxaprop-ethyl. Fair to high efficacy (54.7-96.0%) were also found on sedges exposed to all tested herbicides except metsulfuron methyl. This herbicide and ethoxysulfuron also caused very low efficacy (15.3-37.3%) against grasses across tested application temperatures.

Table 1. Effect of temparature on bio-efficacy of herbicides to control grass weeds (Lab. test with slight clay soil – data recorded at 20 days after treatment).

| | Bio-efficacy to control grass weeds (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Activity | $10^0C$ | $15^0C$ | $20^0C$ | $25^0C$ | $30^0C$ | $35^0C$ | $40^0C$ |
| Pretilachlor – Applied at 3DAS | 72.8 | 77.3 | 88.8 | 92.2 | 91.7 | 90.2 | 83.0 |
| Butachlor – Applied at 3DAS | 68.3 | 70.0 | 81.6 | 78.9 | 76.8 | 82.5 | 77.5 |
| Oxadiazon – Applied at 5DAS | 65.9 | 68.4 | 79.0 | 81.4 | 82.3 | 83.9 | 76.1 |
| Bensulfuron methyl – Applied at 5DAS | 53.1 | 60.8 | 72.1 | 75.6 | 77.2 | 68.2 | 63.0 |
| Ethoxysulfuron – Applied at 7DAS | 20.3 | 21.0 | 32.1 | 35.8 | 40.7 | 45.3 | 28.3 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 64.7 | 70.1 | 81.9 | 84.7 | 86.0 | 88.7 | 75.2 |
| Metsulfuron methyl – 20DAS | 15.3 | 22.9 | 30.2 | 26.3 | 28.3 | 32.1 | 28.3 |
| Quinclorac – 5DAS | 69.5 | 77.9 | 85.9 | 83.1 | 87.0 | 89.6 | 80.5 |
| Untreated check | - | - | - | - | - | - | - |

The decrease in herbicide efficacy at lower temperature can be attributed to some reasons. On the one hand, low temperature inhibits penetration and degradation of herbicides, thereby delaying their effectiveness. While slowly moving to the targeted active sites in plant, part of the herbicides may be lost due to degradation into soil and plant. On the other hand, low temperature may postpone the germination of weeds, preventing the contact of new susceptible seedlings to herbicides at their most effective stage of high concentration after application.

Table 2. Effect of temparature on bio-efficacy of herbicides to control sedges Lab. test with slight soil – data recorded at 20 days after treatment).

| | Bio-efficacy to control grass weeds (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Activity | $10^0C$ | $15^0C$ | $20^0C$ | $25^0C$ | $30^0C$ | $35^0C$ | $40^0C$ |
| Pretilachlor – Applied at 3DAS | 72.4 | 80.1 | 92.1 | 93.3 | 95.7 | 94.0 | 86.5 |
| Butachlor – Applied at 3DAS | 65.1 | 71.3 | 85.6 | 87.9 | 83.2 | 89.7 | 79.8 |
| Oxadiazon – Applied at 5DAS | 68.7 | 72.2 | 83.7 | 86.9 | 85.5 | 87.1 | 80.0 |
| Bensulfuron methyl – Applied at 5DAS | 70.0 | 75.3 | 82.5 | 86.0 | 84.9 | 89.1 | 81.0 |
| Ethoxysulfuron – Applied at 7DAS | 64.3 | 70.1 | 77.5 | 75.8 | 82.5 | 80.1 | 78.6 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 54.7 | 60.8 | 70.5 | 73.2 | 76.4 | 79.2 | 75.7 |
| Metsulfuron methyl – 20DAS | 24.9 | 30.2 | 42.1 | 46.5 | 50.4 | 53.5 | 44.8 |
| Quinclorac – 5DAS | 72.5 | 80.3 | 94.6 | 92.0 | 96.3 | 97.8 | 90.6 |
| Untreated check | - | - | - | - | - | - | - |

Effects of irrigation timings to selectivity and efficacy of tested herbicides

When exposed to herbicides, rice as well as weeds were damaged at different levels, which depended on not only the herbicides but also on the timing of water supply at application in both spring season (Table 4). All herbicides except ethoxysulfuron caused damage to rice to some extent in all four tested water timings. In general, the earlier the water flooding after herbicide application, the more damage was found in rice by pre-emergence herbicides (pretilachlor and butachlor) and early-post-emergence herbicide (oxadiazon). The most severe damage levels were recorded in the treatments with pretilachlor (level 9) and butachlor (level 8) in early flooding condition in spring season (Table 3). In contrast, the

Table 3. Effect of irrigation timing on selectivity of herbicides (Field test data collected at 15 days after spraying in spring season).

| Activity | Level of crop damage (scale) at various temperatures | | | |
|---|---|---|---|---|
| | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
| Pretilachlor – Applied at 3 DAS | 9 | 7 | 3 | 1 |
| Butachlor – Applied at 3DAS | 8 | 5 | 4 | 2 |
| Oxadiazon – Applied at 5DAS | 5 | 3 | 3 | 2 |
| Bensulfuron methyl – Applied at 5DAS | 2 | 3 | 2 | 2 |
| Ethoxysulfuron – Applied at 7DAS | 1 | 1 | 1 | 1 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 3 | 3 | 3 | 5 |
| Metsulfuron methyl – Applied at 20DAS | 3 | 3 | 2 | 2 |
| Quinclorac – applied at 5DAS | 3 | 3 | 2 | 4 |
| Untreated check | 1 | 1 | 1 | 1 |

DAS - days after sowing; Treatment 1 - flooding before applying herbicides; Treatment 2, 3 and 4 - Drain water at application and reflood at 1, 2 and 3 days after, respectively.

post-emergence herbicides (quinclorac and fenoxaprop) caused more severe damage to rice when the field was irrigated late, 3 days after spraying. More severe damages were also recorded in spring than in summer.

The efficacy of herbicides against weeds also varied under different water timing regimes (Figures 4, 5, and 6). Early and late flooding at 3 days after herbicide application inhibited their effectiveness. In almost all cases, flooding at 1 and 2 days after herbicide application produced highest control of all grass, sedge and broad leaf weed groups. Among 8 tested herbicides, quinclorac and fenoxapro – p – ethyl were more slightly varied in efficacy than others.

Figure 4: Effect of irrigation timing on bio-efficacy of herbicides to control grass weeds (Date collected at 30 days after spraying of the field test)

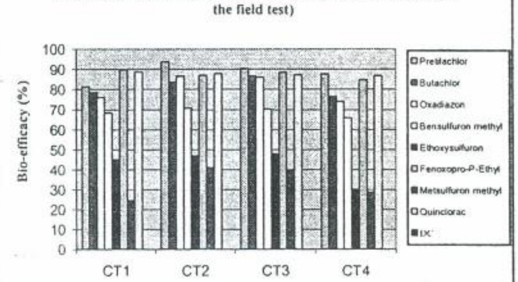



Figure 5: Effect of irrigation timing on bio-efficacy of herbicide to control sedges (Data collected at 30 days after treatment from the field test)

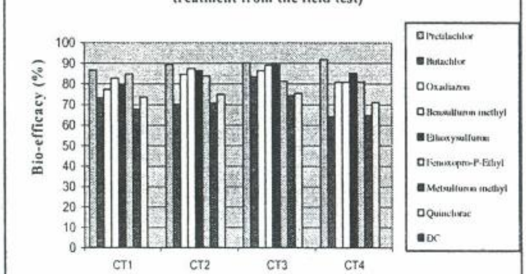
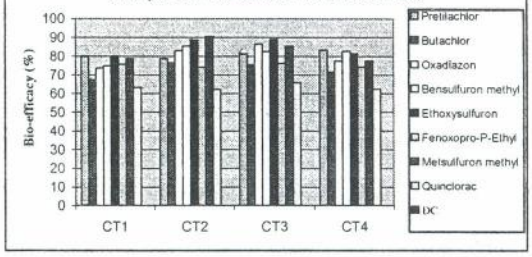



Figure 6: Effect of irrigation timing on bio-efficacy of herbicide to control broad leaf weeds (Data collected at 30 days after treatment from the field test)




Effect of water stands on selectivity and efficacy of post - emergence herbicides

All five tested post-emergence herbicides proved to be safe to rice (Table 5) when water level in the field was one-third of the height of the rice plants during application. In contrast, severe damages were found in non-free water or higher water stands except treatments with ethoxysulfuron, which seemed to be safe regardless of the field water level. Highest damage level of 6 and 5 were found in rice treated with metsulfuron methyl and fenoxoprop-ethyl, respectively, when water was up to two third of rice height at application.

However, herbicides demonstrated most effectiveness against grass weeds in no water stand experimental treatments, followed by flooding at one third of plant height (Table 6). The same trend was also found for the efficacy of herbicides on sedge weed control except bensulfuron methyl (Table 7). Ethoxysulfuron showed the highest level of efficacy decrease (from 38.1 to 23.3%), next to bensulfuron methyl (from 74.8 to 64.2%); fenoxaprop-ethyl (from 91.2 to 83.2%) and quinclorac (from 91.7 to 82.6%).

Table 5. Effect of water stand on the selectivity of post-emergency herbicides (Data recorded at 15 days after application from green house tests)

| Activity | Level of crop damage (scale) at different water levels | | |
|---|---|---|---|
| | Treatment 1 | Treatment 2 | Treatment 3 |
| Bensulfuron methyl – Applied at 5DAS | 3 | 1 | 4 |
| Ethoxysulfuron – Applied at 7DAS | 1 | 1 | 2 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 5 | 1 | 5 |
| Metsulfuron methyl – Applied at 20DAS | 4 | 1 | 6 |
| Quinclorac – Applied at 5DAS | 4 | 1 | 4 |
| Untreated check | 1 | 1 | 1 |

Treatment 1 - no water stand; Treatment 2 - water level maintained at one third rice plant height; and Treatment 3 - water level maintained at two third plant height within 15-20 days after spraying.

Table 6. Effect of water stand on the bio-efficacy of post-emergency herbicides to control grass weeds (Data recorded at 30 days after application from green house tests).

| Activity | Bio-efficacy to control grasses (%) | | |
|---|---|---|---|
| | Treatment 1 | Treatment 2 | Treatment 3 |
| Bensulfuron methyl – Applied at 5DAS | 74.8 | 69.7 | 64.2 |
| Ethoxysulfuron – Applied at 7DAS | 38.1 | 31.1 | 23.3 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 91.2 | 83.1 | 83.2 |
| Metsulfuron methyl – Applied at 20DAS | 41.2 | 36.6 | 28.4 |
| Quinclorac – Applied at 5DAS | 91.7 | 88.3 | 82.6 |
| Untreated check | - | - | - |

Table 7. Effect of water stand on the bio-efficacy of post-emergency herbicides to control sedges (Data recorded at 30 days after application from green house tests).

| Activity | Bio-efficacy to control sedges (%) | | |
|---|---|---|---|
| | Treatment 1 | Treatment 2 | Treatment 3 |
| Bensulfuron methyl – Applied at 5DAS | 86.1 | 938 | 97.0 |
| Ethoxysulfuron – Applied at 7DAS | 90.1 | 80.8 | 78.2 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 81.1 | 76.4 | 72.1 |
| Metsulfuron methyl – Applied at 20DAS | 64.1 | 56.3 | 50.1 |
| Quinclorac – Applied at 5DAS | 80.9 | 80.9 | 74.9 |
| Untreated check | - | - | - |

Effect of soil structures on selectivity and efficacy of herbicides

As in temperature, the selectivity and bio- efficacy of herbicides also changed in accordance with soil structures. This effect is more clearly recognized with pre-emergence herbicides such as butachlor or early post-emergence herbicides like oxadiazon. On the other hand, phytotoxicity of herbicides tended to be more severe in sandy soil while the difference was not clear in slightly clay and heavy clay soil (Figure 7). In contrast, bio-efficacy on grasses, sedges, and broadleaf weeds considerably increased in slightly and heavy clay soil compared with that in sandy soil (Table 8).

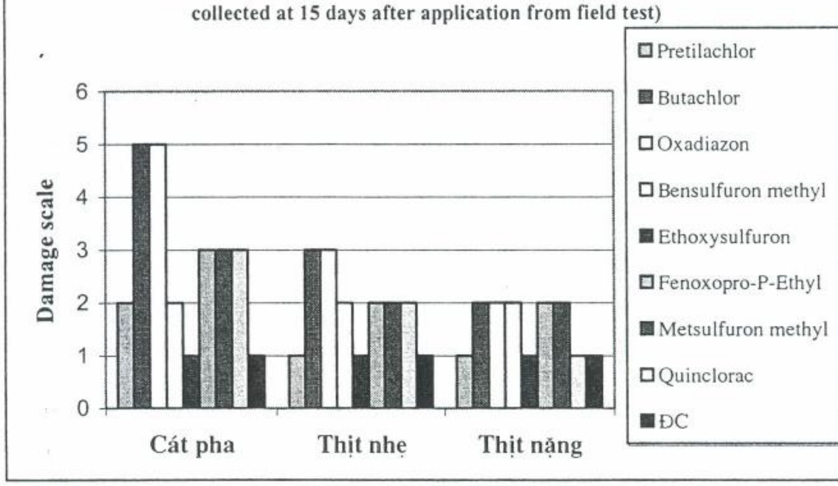



**Figure 7: Effect of soil structure on selectivity of herbicides (data collected at 15 days after application from field test)**

Table 8. Effect of soil structure on the bio-efficacy of herbicides (Data recorded at 30 days after application from field test).

| Activity | Bio-efficacy of herbicides in various soil structures | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Sandy soil | | | Slightly soil | | | Heavy clay soil | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Pretilachlor – Applied at 3DAS | 82.3 | 875 | 801 | 92.7 | 94.5 | 880 | 91.3 | 96.8 | 87.6 |
| Butachlor – Applied at 3DAS | 70.5 | 72.1 | 786 | 76.4 | 70.6 | 75.4 | 77.1 | 68.9 | 67.8 |
| Oxadiazon – Applied at 5DAS | 80.9 | 80.4 | 84.2 | 86.3 | 79.0 | 81.2 | 88.9 | 75.5 | 80.1 |
| Bensulfuron methyl – Applied at 5DAS | 70.3 | 81.3 | 83.2 | 76.7 | 78.6 | 81.5 | 75.3 | 75.0 | 78.6 |
| Ethoxysulfuron – Applied at 7DAS | 42.9 | 83.5 | 86.5 | 44.3 | 78.2 | 83.2 | 46.2 | 75.2 | 79.3 |
| Fenoxopro-P-Ethyl – Applied at 15DAS | 78.4 | 642 | 79.3 | 80.1 | 61.8 | 73.2 | 84.4 | 59.2 | 71.0 |
| Metsulfuron methyl – Applied at 20DAS | 29.3 | 80.2 | 92.4 | 397 | 76.8 | 91.0 | 35.9 | 71.0 | 87.6 |
| Quinclorac – Applied at 5DAS | 77.7 | 76.5 | 81.7 | 80.4 | 73.1 | 80.6 | 80.1 | 67.5 | 78.5 |
| Untreated check | - | - | - | - | - | - | - | - | - |

1 - grasses; 2 - sedges; 3 - broad leaves

Temperature caused significant effect on efficacy and selectivity of herbicides applied in direct seeding rice. Temperature ranging from 20 to 35°C proved to be optimum for herbicide application in terms of weed control and safety. Considerable damages of rice and dramatic reduction of herbicides efficacy were recorded if herbicides were applied at 15°C downward with the pre-emergence herbicides showing more profound effect than post-emergent ones.

The earlier the field was flooded, the higher the efficacy of pre- and/or early-post-emergence herbicides against weeds and the more severe the damage on rice. A reverse effect was recorded for post-emergence herbicides, quinclorac and fenoxaprop-ethyl.

Though the highest effectiveness of tested herbicides against both grass and sedge weeds were recorded when treated in no water stand experimental plots, the most severe effects on rice were also found here plus in flooded treatments of two third rice height. A considerably high control of weeds in flooded treatments of one-third rice height was recorded with complete safety to rice.

In sandy soil, all the herbicides got lower selectivity and bio-efficacy than in slightly clay and heavy clay soil. However this effect also depended on the nature of herbicides with more profound damage found in pre-germination herbicides.

Although pre-germination herbicides showed a wider spectrum of weed control, they are more likely varied in their effect with changes in abiotic factors. Thus, the risk caused by undesired conditions, such as raining or change of temperature, during or just after application is unavoidable.

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# Factors affecting the herbicidal performance of flucetosulfuron

J. N. Lee[1], D. S. Kim[1], K. H. Hwang[1], S. J. Koo[1], Y. W. Kwon[2]
[1]R&D Park, LG Life Sciences, Ltd, Yuseong-gu, Daejeon 305-380, Korea
[2]Division of Plant Science, Seoul National University, Seoul, Korea
dosoonkim@lgls.co.kr

**Abstract**: Experiments were conducted in a glasshouse to investigate the effects of temperature, light, water depth, rainfall, run-off, N-fertilizer and weed growth stage on the activity of flucetosulfuron (LGC-42153). The herbicidal activity of flucetosulfuron against *Echinochloa* spp. was significantly reduced when plants were subjected to low temperature or shaded conditions. The activity was also reduced at deep water after soil application, while it was not significantly affected by water depth at the time of foliar application. Water run-off significantly decreased the activity, with $LT_{90}$ values of 3.4 and 6.7 days for 50% and 100% run-off, respectively, indicating that at least 4-7 days of run-off free periods are required to get more than 90% weed control depending on the amount of run-off. Rainfall after foliar application also affected the activity but not so great as expected. Soil fertility also affected the activity, which increased with increasing the level of basal N-fertilizer. Although the activity of flucetosulfuron depended on the growth stage of weeds, flucetosulfuron at 25 g a.i.$ha^{-1}$ controlled *Echinochloa crus-galli* and *Aeschynomene indica* up to 5 and 6 leaf stage, respectively.

**Key words**: *Aeschynomene, Echinochloa*, flucetosulfuron, growth stage, light, N-fertilizer, rainfall, rice, run-off, temperature, water depth.

## INTRODUCTION

Flucetosulfuron (LGC-42153) is a sulfonylurea herbicide developed by LG Life Sciences, Ltd (Koo et al. 2003). When applied to soil or foliage, flucetosulfuron provides broad-spectrum weed control including annual broad leaf weeds, sedges, some grasses such as *Echinochloa* spp.. and perennial weeds in paddy rice field (Kim et al. 2003). Due to its broad-spectrum weed control including *Echinochloa* spp., flucetosulfuron was registered as a solo one-shot herbicide (Fluxo®) in Korea. Like other sulfonylurea herbicides, flucetosulfuron inhibited acetolactate synthase (ALS) (Hwang et al. 2003), the first committed enzyme for the biosynthesis of the branched-chain amino acids. This compound was absorbed via roots, stem and leaf, and its translocation via leaf was faster than glyphosate and pyribenzoxim (Lee et al. 2003).

Herbicide performance is usually affected by various environmental factors. This report summarizes the results of a series of experiments to investigate factors related to the herbicidal performance of flucetosulfuron, including temperature, light intensity, water depth, rainfall, run-off, N-fertilizer, and weed growth stage.

## MATERIALS AND METHODS

Pot experiments were conducted in a glasshouse at LG Life Sciences R&D Park, Daejeon, Korea. Rice was grown in 10 cm x 10 cm plastic pots containing paddy soil (clay loam soil), while *Echinochloa crus-galli*, *Aeschynomene indica* and *Brassica napus* were grown in 10 cm in diameter or 10 cm x 10 cm plastic pots. Twelve days-old rice seedlings were transplanted, while weeds were sown. The pots were maintained at appropriate temperature and light conditions. Foliar application was made using $CO_2$-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8002E flat fan nozzle adjusted to deliver 1000 L $ha^{-1}$, while soil application was made by direct application of the herbicide formulation to flooded soil in the pots. Formulations of flucetosulfuron were 50%

WG and 0.07% GR for foliar and soil application, respectively. Fresh weight was measured at 20 or 30 days after application. All experiments consisted of three or four replicates of a completely randomized design.

**Climatic factor**: To evaluate the effects of temperature and light, plants were maintained at different temperature (15~25 and 25~35°C) and light (0, 50 and 75% shading) conditions, immediately after herbicide application was made. For the temperature test, soil application was used, while for the shading test, foliar application was used.

**Water factor**: To evaluate factors related to water, different water depths and levels of run-off were imposed after application. For water depth study, rice and *E. crus-galli* were grown at about 1 cm water depth initially, and 1 day before application, the pots were maintained at different water depth regimes: 0 or 6 cm for soil application and 0 or 5 cm for foliar application. For run-off study, 50% and 100% of total flooded water was drained at 1, 2, 4, 8 days after soil application. Rainfastness study was also conducted, and for this study, artificial rainfall at 5 mm $hour^{-1}$ was given at 1, 2, 4, 8 hours after foliar application.

**N-fertilizer**: To evaluate the effect of N-fertilizer, *E. crus-galli* and rice were grown at various N-fertilizer levels (0, 27.5, 55.0, 110 N kg $ha^{-1}$ as a basal N-fertilizer), and then flucetosulfuron (0.07% GR) was applied directly to flooded soil at 12 days after transplanting (rice) or sowing (*E. crus-galli*).

**Growth stage**: To evaluate the effect of plant growth stage, flucetosulfuron was applied to foliages of *E crus-galli* and *A. indica* at three different growth stages (3, 5 and 7 leaf stages for *E. crus-galli* and 2, 3, 6 leaf stages of *A. indica*)

**Statistical analysis**: Fresh weight was converted to percentages of control for each test, and these percent values were used for statistical analysis. To quantify the herbicide dose-response, nonlinear regression analysis was conducted to fit the standard dose-response curve (Streibig 1981). All statistical analyses were conducted using Genstat 5 (1993).

## RESULTS AND DISCUSSION

### Effects of temperature and light

Flucetosulfuron was more active against *E. crus-galli* at 25~35°C than 15~25°C (Fig 1A.). Similarly flucetosulfuron caused greater damage on rice at 25~35°C than 15~25°C (Fig 1B.). The $LD_{50}$ values for *E. crus-galli* and rice were 3.58 and 292.38 g a.i.$ha^{-1}$, respectively, at 15~25°C, while those subjected at 25~35°C, had 2.33 and 70.06 g a.i.$ha^{-1}$ respectively. Therefore, it can be concluded that flucetosulfuron is more active at higher temperature.

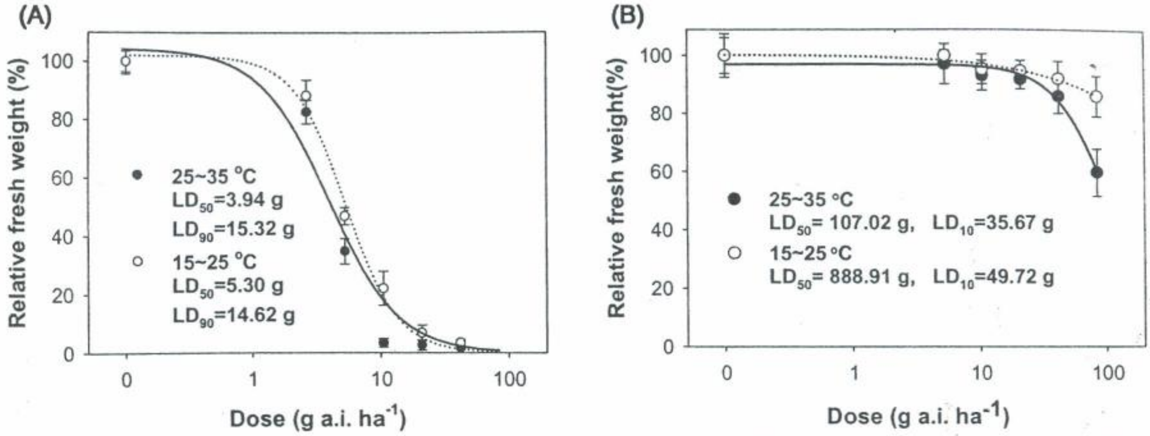


Figure 1. Dose-responses for the relative fresh weight of *E. crus-galli* (A) and rice (B) to flucetosulfuron applied directly to soil and maintained at different temperatures, 15~25°C(○) and 25~35°C (●).

Light intensity also affected the herbicidal activity of flucetosulfuron. Figure 2 shows that the herbicidal activity of flucetosulfuron on both *E. crus-galli* and *B. napus* decreases with light shading. The $LD_{50}$ value for *E. crus-galli* was 0.015 g a.i.ha$^{-1}$ at 0% shading condition, while it was 0.048 and 0.169 g a.i.ha$^{-1}$ at 50 and 75% shading condition, respectively. The $LD_{50}$ value for *B. napus* was 0.054 g a.i.ha$^{-1}$ at 0% shading condition, while it was 0.165 and 0.577 g a.i.ha$^{-1}$ at 50 and 75% shading condition, respectively. This result indicates that shading decreases activity of flucetosulfuron significantly.

**Effects of water**

Water depth after herbicide application affected the herbicidal activity of flucetosulfuron.. The activity of flucetosulfuron was greater at 1 cm than 6 cm to both rice and *E. crus-galli*. The $LD_{50}$ values for *E. crus-galli* and rice were 9.59 and 187.19 g a.i.ha$^{-1}$ at 6 cm, while those at 1 cm were 5.10 and 104.20 g a.i.ha$^{-1}$, respectively. Shallow water depth increases the herbicide concentration in water, and this is likely to increase absorption into plants and thereby increase herbicidal activity of flucetosulfuron. However, when *E. crus-galli* was grown at 6 cm water depth, the plant was more susceptible to flucetosulfuron than the plant grown at 1 cm water depth (data not shown).

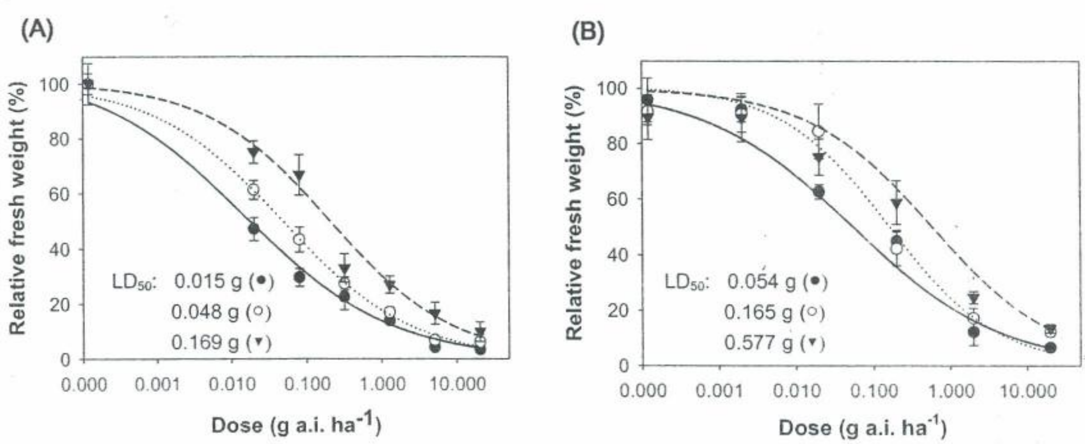


Figure 2. Dose-responses for the relative fresh weight of *E. crus-galli* (A) and *B. napus* (B) treated with flucetosulfuron at 0% (●), 50% (○) and 75% (▾) shading.

Deep water prior to herbicide application affects the growth of *E. crus-galli* morphologically and physiologically, rendering the plant more susceptible; however, the deep water after herbicide application is likely to reduce herbicide absorption due to reduced herbicide concentration. Therefore, it is important to maintain flooding water at a proper depth before or after application of flucetosulfuron.

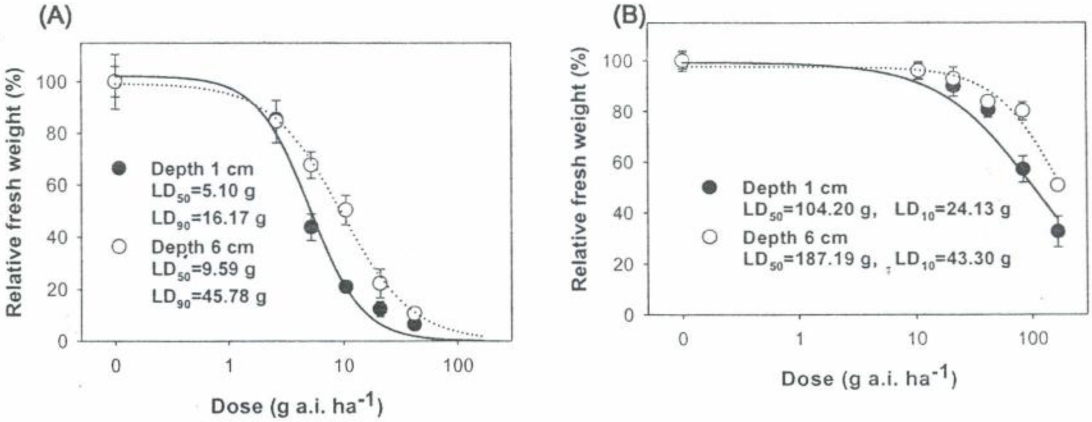


Figure 3. Dose-responses for the relative fresh weight of *E. crus-galli* (A) and rice (B) treated with flucetosulfuron by soil application and maintained at 1 cm and 6 cm water depths.

In the case of foliar application, the activity of flucetosulfuron, against *A. indica,* was not significantly affected by water depth (Figure 4); however the activity of bentazon decreased at flooded (5 cm) condition. This result indicates that for foliar application, flucetosulfuron does not require complete drainage but bentazon requires complete drainage to achieve consistent weed control.

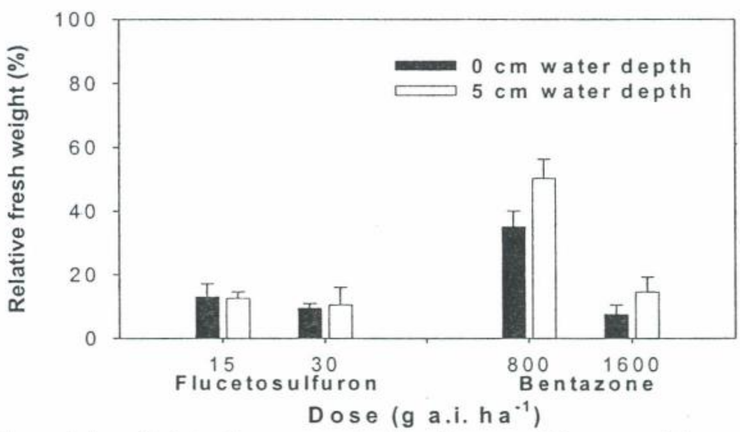


Figure 4. Fresh weight of *A. indica* treated with flucetosulfuron and bentazone at 0 and 5 cm water depths, given immediately prior to herbicide application and maintained thereafter.

Artificial water run-off given after herbicide soil application caused significant loss of herbicidal activity against *E. crus-galli* (Figure 5). The loss was affected by the amount of run-off and time when run-off was made. Sensitivity to run-off appeared to be similar between flucetosulfuron and pyrazosulfuron-ethyl + molinate mixture, and both herbicides seemed to require at least 4 days of run-off free period to achieve sufficient weed control.

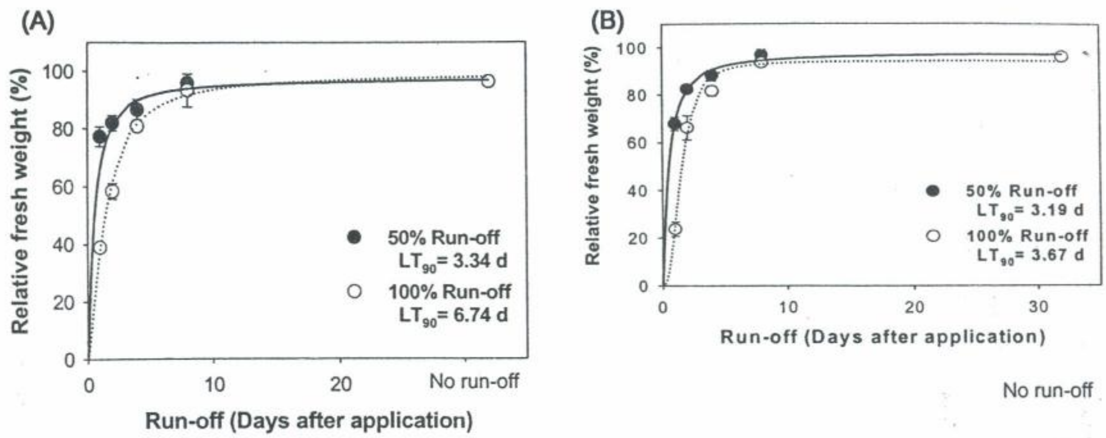


Figure 5. Herbicidal efficacy of flucetosulfuron (A) and pyrazosulfuron+molinate mixture (B) on *E.crus-galli* under different run-off conditions. The $LT_{90}$ indicates days required to achieve 90% weed control

Rainfall after foliar application affects herbicidal performance (Coupland et al. 1987). The length of time required between herbicide application and the onset of rain for effective weed control is important for post-emergence herbicides. In our study, rainfall (5 mm hour$^{-1}$) tended to reduce the efficacy of flucetosufluron, but no significant reduction was observed even in the case of rain onset after 1 h (Figure 6), indicating that flucetosulfuron is not susceptible to rainfall. Our previous work showed that flucetosulfuron uptake into and translocation in plants were very rapid (Lee et al. 2003). The result of the present rainfastness study confirms and is consistent with the previous findings.

**Effects of N-Fertilizer**

Fertilizer also affects herbicidal activity. Kim (1999) reported that weeds grown at high levels of N-fertilizer were more susceptible to herbicide. The activity of flucetosulfuron was also clearly influenced by N-fertilizer (Figure 7). The $LD_{50}$ values for *E. crus-galli* and rice were 21.22 and 1019.43 g a.i. ha$^{-1}$ at 0 kg ha$^{-1}$ of basal N-fertilizer while those were 11.49 and 222.07 g a.i. ha$^{-1}$ at 110 kg a.i.ha$^{-1}$ of basal N-fertilizer, respectively (Table 1). As the basal N-fertilizer increases, the herbicidal efficacy and phytotoxicity increased. Therefore, the use of N-fertilizer at optimum level is also important to achieve good weed control without crop phytotoxicity.

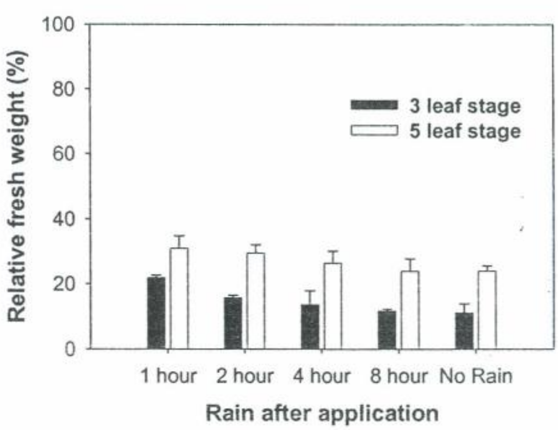


Figure 6. Relative fresh weight of *E. crus-galli* applied with flucetosulfuron at 3 and 5 leaf stages. Rain treatment (5 mm hour$^{-1}$) was made at 1, 2, 4, 8 hours after herbicide application.

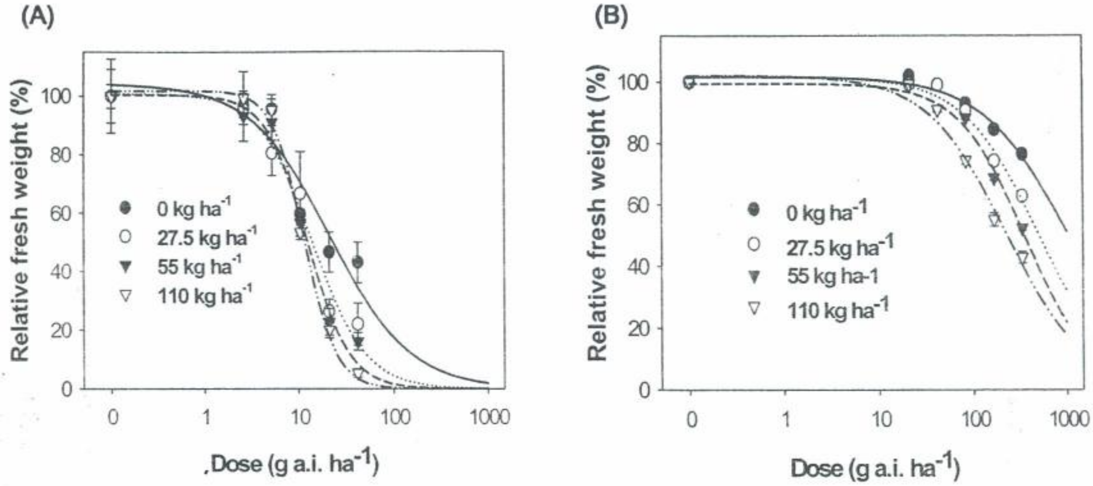


Figure 7. Dose-response for the relative fresh of *E. crus-galli* (A) and rice (B) applied directly with flucetosulfuron applied directly to flooded soil. The plants were grown at different levels of basal N-fertilizer.

Table 1. $LD_{50}$ and $LD_{90}$ values (g a.i. $ha^{-1}$) of flucetosulfuron for *E. crus-galli* and rice at different levels of basal N-fertilizer.

| N-fertilizer ($kg\ ha^{-1}$) | *Echinochloa crus-galli* | | Rice | |
|---|---|---|---|---|
| | $LD_{50}$ | $LD_{90}$ | $LD_{50}$ | $LD_{10}$ |
| 0 | 21.22 | 177.31 | 1019.43 | 100.90 |
| 27.5 | 13.85 | 56.94 | 493.24 | 65.46 |
| 55.0 | 12.43 | 37.74 | 356.74 | 59.34 |
| 110 | 11.49 | 26.76 | 222.07 | 26.74 |

**Effects of weed growth stage**

In general, bigger and older plants require a higher dose of herbicide to achieve the same efficacy than that for smaller and younger plants (Chandrasena et al. 1986). In the case of *E. crus-galli*, $LD_{90}$ values of flucetosulfuron were 15.73 and 19.40 g a.i.$ha^{-1}$ at 3- and 5-leaf stage, respectively, but the value increased rapidly to 60.36 g a.i.$ha^{-1}$ at 7 leaf stage (Figure 8A and Table 2). The increase of $LD_{90}$ with growth stage was also seen in *A. indica* with its $LD_{90}$ values being 5.82, 7.95 and 25.50 g a.i.$ha^{-1}$ at 2, 4 and 6 leaf stage, respectively (Figure 8B and Table 2). This result indicates that flucetosulfuron provides similar control up to about 5-leaf stage for *E. crus-gaill* and 4-leaf stage for *A. indica*, but advanced stages above those will require significantly higher doses.

Table 2. $LD_{50}$ and $LD_{90}$ values of flucetosulfuron for *E. crus-galli* and *A. indica* at different growth stages.

| *Echinochloa crus-galli* (g a.i. $ha^{-1}$) | | | *Aeschynomene indica* (g a.i. $ha^{-1}$) | | |
|---|---|---|---|---|---|
| Stage | $LD_{50}$ | $LD_{90}$ | Stage | $LD_{50}$ | $LD_{90}$ |
| 3 leaf | 3.73 | 15.73 | 2 leaf | 1.78 | 5.82 |
| 5 leaf | 4.54 | 19.40 | 4 leaf | 2.39 | 7.95 |
| 7 leaf | 5.85 | 60.36 | 6 leaf | 5.34 | 25.50 |

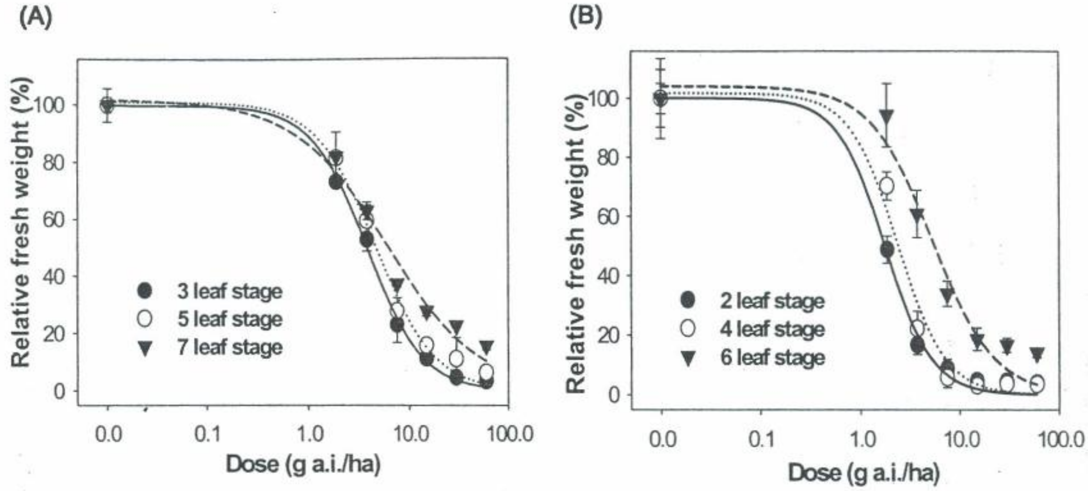


Figure 8. Dose-response for Relative fresh weight of *E. crus-galli* (A) and *A. indica* (B) to flucetosulfuron applied to foliar at different growth stages.

Collectively, these results show that herbicidal activity of flucetosulfuron was influenced by temperature, water depth, run-off, level of N-fertilizer, and growth stage of weeds. Among these factors, impact of water depth and run-off in soil application seem to be most significant. Although the level of N-fertilizer also affects herbicidal efficacy and phytotoxicity, practical impact is likely to be minimal since fertilization usually follows a certain determined program. As for foliar application, influences of various factors were very little indicating flucetosulfuron will provide consistent performance across widely different conditions.

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# Reducing herbicide rate by exploiting water regimes

J. D. Janiya*, and D. E. Johnson
Crop, Soil and Water Sciences Division, International Rice Research Institute (IRRI)
DAPO Box 7777, Metro Manila, Philippines
J.Janiya@cgiar.org

**Abstract**: The studies were conducted at the lowland field site and screen-house of the International Rice Research Institute, Philippines, to determine if flood management could allow application rates of herbicide Sofit (pretilachlor + fenclorim as safener) to be reduced while maintaining satisfactory weed control. Three water regimes (saturated conditions, alternate flooding and draining, and continuous flooding) were combined with two intervals of water introduction (early-3 days after seeding [DAS] and late-7 DAS) and four herbicide rates (no herbicide, full rate, 75% of recommended rate [RR], and 50% of RR). In field and screen house experiments, rice yield was similar among three water regimes when full rate of herbicide was applied. Reduced application of pretilachlor at 50% of recommended rate reduced weed growth regardless of water regime. Weed density and biomass were greatest where the soil was maintained saturated rather than flooded. The different water regimes favored growth of certain weed species. *Cyperus difformis* L., *Ischaemum rugosum* Salisb. and *Echinochloa* spp. established well in the saturated soil while *Sphenoclea zeylanica* Gaertn. established well in both saturated and continuously flooded soils.

**Key words**: Pretilachlor, reduced herbicide rate, water regimes

## INTRODUCTION

Water management is a strong determinant of the weed flora in irrigated direct seeded rice. The timing, depth, and duration of flooding may determine the successful establishment of weeds (Mortimer et al. 1997) and may switch the weed flora from exclusively obligate aquatic to entirely terrestrial species or a mixture of both (Hill et al. 2001). Timing of flooding in direct seeded rice is critical in the establishment of rice seedling and the control of weeds through flooding (Pablico et al. 1996; Tuong et al. 2000). Seeding of rice under water or early flooding however, while preventing germination of many weed species can reduce the rice stand due to poor anchorage and mortality. Water applied during the early stage of weed growth prevents many weed seeds from germinating and suppresses weed establishment, and the longer the flooding is delayed after weed emergence the deeper the water required to achieve control (Moody 1993). Tuong et al. (2000) found that flooding the field 3 days after seeding (DAS) reduced weed growth by 73-88% compared with the farmer's practice of flooding 10 DAS.

Flooding and soil moisture level after the introduction of water may play an important role on weed seedling survival and growth or mortality. Moon et al. (1999) found that different weed species respond differently to water regimes. Emergence of *Echinochloa crus-galli* (L.) P. Beauv. and *Ludwigia octovalvis* (Jacq.) Raven was greater in saturated soil conditions while *Sphenoclea zeylanica* (Gaertn.) showed greater emergence under flooded conditions. Tuong et al. (2000) reported that *Echinochloa glabrescens* Munro ex Hook. f. dominated the field when flooding of direct seeded rice was delayed until 7 DAS whereas *Monochoria vaginalis* (Burm. f.) Presl were dominant either when fields were flooded at 3 DAS or when rice was sown in standing water. Williams et al. (1990) compared different water depth on its effect on weed establishment and growth. Without herbicides, 20 cm water depth provided good control of *Echinochloa* spp and *Cyperus difformis* L. compared to poor control at 5-cm-deep flooding. Weed control was improved by herbicide treatment but weeds were not controlled with shallow flooding even when this was combined with herbicide. Tuong et al. (2000) reported that flooding direct seeded rice at 3 DAS sustained yield and water productivity without having to use herbicide or with only half of the recommended rate (0.15 kg a.i. $ha^{-1}$). Williams et al. (1990) found that weed control was improved at all water depth (2-8 inches) by herbicide treatments, but weeds were not controlled in shallow water (2 inches), even with herbicides. Water management can therefore play a critical role in weed management in direct seeded and will have an influential role on appropriate herbicide usage and both water depth and herbicides were necessary for consistent weed control. Thus this study was conducted to determine if the rate of herbicide could be reduced when combined with judicious water management.

## MATERIALS AND METHODS

One field experiment (1) was conducted from March to June 2004 at the International Rice Research Institute, Philippines. The field was plowed and harrowed once, and then plots (3 m x 12 m) were established. The soil within the plots was leveled using a wooden plank pulled by a hand-tractor. The experiment was laid out in a split-split-plot design with four replications. Herbicide rate: no herbicide, recommended rate [RR, 0.3 kg a.i. $ha^{-1}$], 75% RR, and 50% RR was assigned to the main plot. Time of flooding (early-3days after seeding (DAS) and late-7 DAS) was assigned to the subplot and water regimes [continuous saturation (SAT), while alternate flooding and draining (AWD) and flooded conditions (FLD)] were assigned to the sub-subplots. Pre-germinated rice seeds (IR72) were broadcast at 100 kg $ha^{-1}$. The herbicide (pretilachlor + fenclorim) was applied at 2 DAS. Forty kilograms per ha each of N, $P_2O_5$ and $K_2O$ were applied at 10 DAS and additional 30 kg $ha^{-1}$ N was applied at 30 and at 52 DAS. Water was applied at a prescribed time to a depth of 5 cm. Weeds were counted as broadleaf, grass, sedge at 10 and 18 DAS, and counted and weighed by species at 31, 80 and 117 DAS using two 50 cm x 50 cm quadrats per plot. Weed samples were dried at 80°C for 48 h then weighed. Grain yields at harvest were taken from a 5 $m^2$ sampling area and these adjusted to 14% moisture.

Two screen house experiments (2 & 3) were conducted in August to September 2004 and February to March 2005. Plastic trays measuring 32 cm x 24 cm x 10 cm were filled with soil taken from the unweeded plots of the field experiment above. Soil in the trays were soaked in water and puddle manually and leveled. Holes were bored at each side of the trays 1 cm above the base of the tray to allow entrance of water and another bored at each side of the tray 2 cm above the soil level to maintain 2 cm water depth. Metal basins with four division measuring 120 cm x 60 cm x 40 cm were filled with water to a desired depth to meet the 2 cm flooding depth. The treatments were arranged in a split-split-plot design with four replications. Pre-germinated rice seeds (IR72) were sown in rows (3 rows $tray^{-1}$) at 11 seeds $row^{-1}$. Pretilachlor + fenclorim was applied using a precision spray chamber using the same rates as in the field experiment. Water was introduced at a prescribed time. Trays for the continuously flooded treatment were positioned such that the depth of water was maintained at 2 cm. For the alternate flooding and draining the trays were submerged to 2 cm water for 3.5 days then raised to drain the water for 3.5 days. For saturated treatment the water level was kept at about 1cm below the soil surface. Weed density count was done at 15 and 30 DAS. The experiment was terminated after 30 days.

## RESULTS AND DISCUSSION

Field experiment

Total weed density from 10 to 80 DAS and weed biomass from 31-117 DAS were reduced by all herbicide rates compared to the control (0) (Table 1). Fifty percent RR effectively controlled weeds and resulted in similar yield to RR. Similar results with the use of pretilachlor in wet-seeded rice were reported by Pablico and Moody (1993). Early flooding increased the density of sedges ($P<0.05$) at 10 DAS but not at the later stages of crop growth (Table 2). Density of grasses and sedges at 18DAS, and grass density, dry weight of grasses and sedges at 31 DAS were greater (P<0.01) when the soil was kept saturated compared to AWD or FLD.

Table 1. Effect of herbicide rate on weed density (number $m^{-2}$), dry weight (g $m^{-2}$) and rice yield (kg $ha^{-1}$). Expt.1.

| Herbicide rate (kg a.i. $ha^{-1}$) | Weed density (no $m^{-2}$) (days after seeding) | | | | | Weed biomass (g $m^{-2}$) (days after seeding) | | | Grain yield |
|---|---|---|---|---|---|---|---|---|---|
| | 10 | 18 | 31 | 80 | 117 | 31 | 80 | 117 | (kg $ha^{-1}$) |
| 0 | 989 | 1097 | 776 | 238 | 117 | 55 | 371 | 260 | 617 |
| 0.15 | 52 | 202 | 63 | 39 | 139 | 13 | 96 | 139 | 2017 |
| 0.225 | 20 | 117 | 25 | 32 | 124 | 8 | 107 | 108 | 1939 |
| 0.3 | 22 | 119 | 19 | 20 | 105 | 8 | 48 | 66 | 2581 |
| SE ± | 70 | 78 | 74 | 13 | 19 | 5 | 53 | 36 | 210 |

Table 2. Density and dry weight of grasses and sedges at 10, 18 and 31 DAS as affected by time of flooding and water regimes. Expt. 1.

| Flooding time | Weed density (no $m^{-2}$) 10 DAS | Water regime | Weed density (no $m^{-2}$) 18 DAS | | Weed biomass (g $m^{-2}$) 31 DAS | |
|---|---|---|---|---|---|---|
| | Sedges | | Grasses | Sedges | Grasses | Sedges |
| Early | 95.9 | SAT | 40.9 | 134.4 | 6.3 | 10.8 |
| Late | 50.7 | AWD | 35 | 100.7 | 2.6 | 5.5 |
| | | FLD | 35.8 | 88.2 | 2.6 | 5.3 |
| SE ± | 13.8 | SE ± | 1.1 | 11 | 0.7 | 1.4 |

The total weed biomass at 31 DAS showed interaction among herbicide rate time of flooding and water regime (Table 3). Herbicide application at 50% RR combined with early flooding reduced weed growth by more than 75% compared to the control. Without herbicide applied, early introduction of water (3 DAS) and maintaining the field either SAT or AWD resulted in greater weed growth compared to FLD. Weed biomass at a given herbicide application rate did not differ among water regimes when water was introduced at 7DAS except at the 50% RR in with saturated soil conditions and where weed growth was at a similar level to the no herbicide treatment. Among the 24 weed species collected four major species *C. difformis*, *S. zeylanica*, *Ammania baccifera* L. and *Ischaemum rugosum* Salisb were the dominant species in the experiment. There were significant effects of herbicide application (P<0.01) on the growth of *S. zeylanica, C. difformis* and *A. baccifera* but no significant interaction effects between water management and herbicide rate (Table 4). With these species, application of 50% RR reduced growth by about 90% and there was no apparent advantage of applying a higher rate. *I. rugosum* had significantly more biomass where the plots had been maintained saturated (SAT). Grain yield was greatest where the herbicide was applied at the recommended rate and least where no herbicide was applied (Table 5). There were significant interactions between water management and herbicide rate with the benefit of the higher rates being most apparent in the saturated treatment. At the 50% RR alternate and continuous flooding showed a yield advantage compared to where the soil was only saturated.

Table 3. Weed biomass at 31 days after seeding as affected by herbicide rate, time of flooding and water regime. Expt. 1.

| Time of flooding and water regime | Herbicide rate | | | | |
|---|---|---|---|---|---|
| | NH | RR | 75% RR | 50% RR | SE ± |
| Early flooding | | | | | |
| SAT | 77.7 | 21.7 | 18.9 | 9.4 | |
| AWD | 61.7 | 1.8 | 10.8 | 11.7 | |
| FLD | 49.8 | 11.8 | 5.1 | 21.4 | |
| Late flooding | | | | | 6.52 |
| SAT | 43.9 | 4.7 | 8.5 | 33.4 | |
| AWD | 45.6 | 3.4 | 0.7 | 1.7 | |
| FLD | 51 | 5.1 | 4.5 | 5.8 | |

Table 4. Weed biomass (g $m^{-2}$) of major weed species at 31 days after seeding as affected by herbicide rate, time of flooding and water regime. Expt. 1.

| | Herbicide rate | | | | |
|---|---|---|---|---|---|
| Weed species | NH | RR | 75% RR | 50% RR | SE ± |
| *Sphenoclea zeylanica* | 18.3 | 3.3 | 1.3 | 1.9 | 1.8 |
| *Cyperus difformis* | 16.9 | 1 | 0.5 | 1.9 | 3.3 |
| *Ammania baccifera* | 1.6 | 0.1 | 0 | 0.1 | 0.5 |
| Water regime | SAT | AWD | FLD | | |
| *Ischaemum rugosum* | 5.1 | 1.5 | 1.9 | | 0.65 |

Table 5. Grain yield (kg $ha^{-1}$) as affected by herbicide rate and water regime. Expt.1.

| | Herbicide rate | | | | |
|---|---|---|---|---|---|
| Water regime | NH | RR | 75% RR | 50% RR | SE ± |
| SAT | 0.48 | 2.36 | 1.56 | 1.36 | |
| AFD | 0.36 | 2.6 | 1.76 | 2.53 | 0.2 |
| FLD | 1.01 | 2.78 | 2.51 | 2.16 | |

Screenhouse experiments

Water control in the field had been problematic due to leaks and movement through cracks in the bunds. Thus screenhouse experiments were conducted to assess the treatments with better water control.

As observed in experiment 1, herbicide application generally had a greatest effect on weeds than water management in experiments 2 & 3. There were interactions between herbicide rate and water regimes in both experiments 2 & 3 (Table 6). With no herbicide applied, keeping the field at saturation (SAT) led to greatest weed biomass. There was 49 and 47% less weed biomass in AWD and FLD plots compared to SAT in experiment 2 and 47 and 67% less than SAT in experiment 3. In plots treated with herbicide weed biomass was reduced regardless of water regime. In experiment 2, the greatest weed biomass occurred where 50% RR was followed by soil saturation and least when plots were flooded. These data generally support the results of the field experiment. Dominant weed species in experiment 2 were *C. difformis*, *S. zeylanica*, *M. vaginalis* and *Echinochloa* spp. while in experiment 3 they were *Cyperus iria* L., *I. rugosum*, *S. zeylanica* and *A. baccifera*. In experiment 2, there was a clear advantage of applying herbicide for control of *S. zeylanica* and *M. vaginalis* but the advantage of applying RR rather than 50% RR was unclear (Table 7). In experiments 2 & 3, biomass of *C. difformis* was greatest where no herbicide was applied and the soil maintained saturated. Where 50% RR was applied, *C. difformis* had similar levels of growth whether flooding was continuous or not. In experiment 3, *I. rugosum* was reduced by herbicide application regardless of rate while there was no significant effect on *A. baccifera* or *S. zeylanica*. Herbicide rate and time of flooding did not affect rice biomass; however, higher rice biomass was obtained from the AWD treatment in Expt 2 and FLD in Expt 3 (Table 8)

Table 6. Effect of herbicide and water regime on total weed biomass (mg tray$^{-1}$) at 30 days after seeding. Expt. 2 and 3.

| Water regime | Herbicide rate | | | | |
|---|---|---|---|---|---|
| | NH | RR | 75% RR | 50% RR | SE ± |
| Expt 2 | | | | | |
| SAT | 906.9 | 62.3 | 51.4 | 146.3 | |
| AFD | 461.5 | 43.3 | 17.4 | 72.5 | 33.6 |
| FLD | 473.5 | 2.3 | 5.5 | 48.8 | |
| Expt 3 | | | | | |
| SAT | 1841.7 | 20.9 | 21.1 | 89.7 | |
| AFD | 973.1 | 11.9 | 20.1 | 34.3 | 131.3 |
| FLD | 608.2 | 28.6 | 6.3 | 33.4 | |

Table 7. Effect of herbicide rate and water regime on dry weight (mg tray$^{-1}$) of major weed species 30 days after seeding. Expt. 2 and 3.

| Weed species | Herbicide rate | | | | |
|---|---|---|---|---|---|
| | NH | RR | 75% RR | 50% RR | SE ± |
| Expt 2 | | | | | |
| *Sphenoclea zeylanica* | 228 | 6.6 | 10 | 36.4 | 39.7 |
| *Monochoria vaginalis* | 62.6 | 4.4 | 5 | 29.2 | 13.3 |
| *Echinohloa* spp. | 29.4 | 6.5 | 3.6 | 6 | 8.6 |
| *Cyperus difformis* | | | | | |
| SAT | 306.7 | 0 | 0.5 | 1.3 | |
| AFD | 97.4 | 0.6 | 0.4 | 1 | 22.3 |
| FLD | 47.6 | 0 | 0 | 0 | |
| Expt 3 | | | | | |
| *Ischaemum rugosum* | 62.3 | 0.5 | 1.3 | 5 | 4.7 |
| *Ammania baccifera* | 50.2 | 1.7 | 2.2 | 22.8 | ns |
| *Sphenoclea zeylanica* | 81.3 | 29.7 | 27.2 | 45.5 | ns |
| *Cyperus difformis* | | | | | |
| SAT | 257.1 | 0.6 | 0.5 | 0.6 | |
| AFD | 128.6 | 0 | 0 | 0.4 | 22.9 |
| FLD | 94.5 | 0 | 0 | 0 | |

Table 8. Effect of water regimes on rice biomass (g tray$^{-1}$) 30DAS.

| Water Regimes | Expt 2 | Expt 3 |
|---|---|---|
| SAT | 5.4 | 7.6 |
| AFD | 5.9 | 7.6 |
| FLD | 5.6 | 8.2 |
| SE ± | 0.1 | 0.2 |

The results obtained from these experiment suggest that herbicide rates can be reduced from the recommended rate and still provide adequate control of weeds. Moody (1995) stated that farmers in most countries in south and Southeast Asia apply herbicides less than the recommended rate. Experiment 1 suggests, however, that yields could only be maintained where the field can be subsequently flooded. The suppressive effect of water on weeds is dependent on depth and combination of water depth and herbicide would further improve weed control (Williams et al. 1990; Hill et al. 2001). The results of our studies suggest that there were differences in the levels of weed control depending on whether the fields had been flooded at 3 or 7 DAS with better control with earlier flooding after 50% RR application under saturated condition. Selection of weeds species commences very early in the life of the crop and the selection is caused by imposed water regime (Hill et al. 2001). Weed seeds may germinate before flooding however the survival of these species will be determined by their ability to withstand flooding or the size and age of the seedlings during flooding. In these experiments, *C. difformis* was an example of a species that was able to establish particularly well in saturated soil. The use of water management in conjunction with reduced herbicide application rates may provoke a shift to aquatic species but this may be averted by the rotation of management regimes.

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# Evaluation of imidazolinone herbicides for weedy rice (*Oryza sativa*) control in Clearfield™ paddy

T. C. Thien[1]*, D. V. Chin[2], T. V. Hien[2], N. T. Nhiem[2]
[1]BASF Rep. Office in HCM city, Vietnam
[2]Cuu Long Delta Rice Research Institute, Omon, Cantho, Vietnam
thientc@vietnam.basf.com.sg

**Abstract:** Weedy rice (*Oryza sativa*) infestation recently has become more serious in the Mekong delta rice areas. This study was conducted to determine whether the imidazolinone herbicides can be a good alternative measure for general weed control (including weedy rice) in paddy field of herbicide tolerant rice variety. The bio-efficacy, crop selectivity, and carry-over risk of selective imidazolinone herbicides were investigated at paddy field trials of Cuu Long Delta Rice Research Institute in the 2003 wet and 2004 dry season. The rice seeds of CL 161 at rate 100 kg $ha^{-1}$, japonica type containing imidazolinone tolerance trait PWC-16 supplied by the Lousiana State University Agricultural Center were mixed with weedy rice seeds (50 kg $ha^{-1}$) and grass seeds (3.5 kg $ha^{-1}$), then sown on puddled soil. Four compounds of Imazapic, Imazapyr, Imazapic + Imazapyr 1:3, Imazethapyr + Imazapyr 3:1 at rate of 80 to 160 g a.i $ha^{-1}$ were applied at 7 or 12 days after sowing (DAS) on drained field. The Imazapic solo and ready mixture with Imazapyr used at the rate of 120 – 160 g a.i $ha^{-1}$, applied at 7-12 DAS, appeared to be promising products due to their excellent control of weedy rice as well as other general weeds, good selectivity on CL161 rice (slightly yellowing of leaf were observed at 3-4 days after applied, but rice plants totally recovered after one week), and no carry over risk on following rice crop sown with non-tolerance variety observed.

**Key words:** Carry-over risk, imidazolinone, herbicide tolerant variety, selectivity, weed control efficacy.

## INTRODUCTION

Weedy rice, commonly considered to be ecotypes of *Oryza sativa*, is one of most harmful weeds in lowland fields, particularly in direct-seeded cultivation. Weedy rice infestation recently is one of the biggest threats to rice growers in many countries such as Malaysia (Azmi et al. 1998), Central Americas, and Mekong Delta of Vietnam (Chin et al. 2000). Their characteristics of easy shattering, long viable of seed in soil, and taller than cultivated rice make these unwanted plants difficult to manage. Being the same genus and species as rice grown for consumption makes weedy rice nearly impossible to control by conventional means. So far, selective herbicide for weedy rice control is still non-existent. Also no effective and economical method is reported, especially in Mekong Delta where rice is cultivated all year round. This study was conducted to determine whether the integration of imidazolinone herbicides and tolerant trait containing variety (which is called CLEARFIELD™ rice) can be a good alternative measure for general weed control (including weedy rice) in paddy rice field.

Imidazolinone herbicides control weeds by inhibiting the plant specific enzyme acetohydroxyacid synthase (AHAS), which is involved in the biosynthesis pathway of the branched-chain amino acids - valine, leucine and isoleucine. This inhibition causes a disruption of protein synthesis which, in turn, interferes with DNA synthesis and cell growth. AHAS, and therefore the biosynthesis of these three amino acids, does not occur in animals, which partially explains the generally low toxicity to mammals and other non-target animal species.

CLEARFIELD™ rice has been developed by Lousiana State University Agricultural Center breeders through a combination of mutagenesis and conventional plant breeding, which is tolerant to imidazolinone herbicides. Technology does not involve introduction of genetic material from other sources and, thus, is characterized as a non-GMO (genetically modified organism) process, and grain harvested from field applied with this technique are approved for food and feed use in global market with no restriction.

## MATERIALS AND METHODS

The study was conducted in clay soil lowland fields with good water supply in the 2003 wet and 2003-2004 dry seasons. The rice variety CL 161, a japonica type with IMI tolerance trait PWC, supplied by LSU-AC through BASF Corporation, was sown at 100 kg $ha^{-1}$. Seeds of different ecotype weedy rice (*Oryza sativa*), *Echinoloa crus-galli* and *Leptochloa chinensis*, were mixed with the germinated CL 161 rice seed and broadcasted randomly in the experimental field at the rate of 50 kg $ha^{-1}$, 2.5 kg $ha^{-1}$, and 1 kg $ha^{-1}$, respectively.

Field trials with plot size of 25 $m^2$ were established using randomized complete block design with four replications and 15 treatments. Three major active ingredients of Imazapic, Imazapyr, and Imazethapyr were used as solo and ready-mix formulations at various dose rate from 80 to 160 g a.i $ha^{-1}$ applied at 7 DAS or 12 DAS on drain off water field. Crop oil is added at 0.5% water volume as non-ionic surfactant.

To identify the carry-over risk of herbicides on the next crop, non-tolerance rice varieties, OM 2517 at seed rate of 200 kg $ha^{-1}$ and OM 1490 at 150 kg $ha^{-1}$, were used in year of 2003 and 2004 respectively, on no-plough soil right after harvesting the first rice crop. For weed control, pre-emergence treatment of pretilachlor at at 360 g a.i $ha^{-1}$ was applied followed by Bispyribac-sodium at 25 g a.i $ha^{-1}$ at 7 DAS.

## RESULTS AND DISCUSSION

### Crop selectivity

Imidazolinone products at recommended rates plus an additive (crop oil concentrate) exhibits good crop safety in both application timing of 7 and 12 DAS. Some temporary symptoms (stunting, yellowing of leaves, and slow tillering) of rice plant occurred at 3 days after treatment, but the plants totally recovered after two weeks without causing any negative effect to plant growth and grain yield (Table 1).

Besides, the IMI products gave excellent control of weedy rice. In case of untreated or standard herbicides, the weedy rice surpassed CL 161 rice plants in height and caused significant reduction of grain yield (Table 2).

### Herbicidal activity

Imidazolinone herbicides used solo or combined at rate of 120 – 160 g a.i. $ha^{-1}$ provided effective control of the most important weeds in rice field, including grass weeds (*Echinochloa crus-galli*, *Lepchtochoa chinensis*), important sedges (*Cyperus difformis, Fimbristylis miliacea*), and broadleaf weed (*Ludwigia octovalvis*), superior or equal to other reference products (Tables 3 and 4). In addition, they showed special activity on weedy rice bio-types that are insufficiently controlled by reference active ingredients such as pendimethalin + cyclosulfamuron, bispyribac sodium + fenoxaprop-P-ethyl, or pretilachlor (in residue trial – Table 6). Meanwhile, pot experiments showed that in both application timing of 7 DAS and 12 DAS, IMI herbicides at 80 g a.i $ha^{-1}$ and at lower rates were not enough to guarantee the herbicidal efficacy (Table 5).

Among the IMI products, the Imazapic and combination of Imazapic and Imazapyr at ratio of 1:3 showed better weed control herbicidal efficacy than the others. Besides, application timing of 12 DAS gave more consistent herbicidal efficacy. It is possible that the post-emergence applied IMI herbicides are primarily absorbed through leaves and, hence, more leaf surface area was exposed to herbicide droplet at later application.

Table 1. Crop selectivity of imidazolinone herbicides on rice variety of CL161 (O Mon, Can Tho, combined of 4 trials in 2 seasons 2003-2004)

| Treatment | Application rate a.i. $ha^{-1}$ (g) | % Rice injury | | No. of tiller | Plant height (cm) |
|---|---|---|---|---|---|
| | | 3 DAA | 15 DAA | 30 DAS | 60 DAS |
| Untreated | - | 0 | 0 | 3.9 | 112[2] |
| Imazapic | 80 | 10 | 0 | 3.1 | 69 |
| Imazapic | 100 | 10 | 0 | 3.3 | 65 |
| Imazapic | 120 | 20 | 0 | 3.2 | 69 |
| Imazapic | 160 | 30 | 0 | 3.1 | 74 |
| Imazapyr | 80 | 10 | 0 | 3.2 | 72 |
| Imazapyr | 100 | 10 | 0 | 3.9 | 67 |
| Imazapyr | 120 | 20 | 0 | 3.6 | 73 |
| Imazapyr | 160 | 20 | 0 | 4.2 | 76 |
| Imazapic + Imazapyr[1] | 20 + 60 | 10 | 0 | 3.7 | 72 |
| Imazapic + Imazapyr[1] | 25 + 75 | 10 | 0 | 3.1 | 66 |
| Imazapic + Imazapyr[1] | 30 + 90 | 10 | 0 | 3.3 | 69 |
| Imazapic + Imazapyr[1] | 40 +120 | 20 | 0 | 3.5 | 73 |
| Imazethapyr + Imazapyr[1] | 60 + 20 | 10 | 0 | 3.3 | 70 |
| Imazethapyr + Imazapyr[1] | 75 + 25 | 10 | 0 | 3.1 | 67 |
| Imazethapyr + Imazapyr[1] | 90 + 30 | 10 | 0 | 3.3 | 72 |
| Imazethapyr + Imazapyr[1] | 120 + 40 | 20 | 0 | 3.5 | 74 |
| Pendimethalin + Cyclosulfamuron | 495 + 15 | 10 | 0 | 3.8 | 114[2] |
| Bispyribac sodium + Fenoxaprop-P-ethyl | 25 + 30 | 30 | 0 | 3.7 | 102[2] |
| LSD (0.05) | | | | ns | 9.4 |

DAA = days after appl.; DAS = days after sowing; [1]ready-mix formulation; [2]height of weedy rice.

Table 2. Grain yield of CL161 treated with various imidazolinone herbicides (O Mon, Can Tho, 2003-2004); DAA = days after appl. ; DAS = days after sowing; [1]ready-mix formulation.

| Treatment | a.i. ha$^{-1}$ (g) | Grain Yield (kg ha$^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Wet season 2003 | | Dry season 2003-2004 | |
| | Application timing | 7 DAS | 12 DAS | 7 DAS | 12 DAS |
| Untreated | - | 19 | 133 | 1425 | 1327 |
| Imazapic | 80 | 218 | 920 | 3355 | 3200 |
| Imazapic | 100 | - | - | 3130 | 3050 |
| Imazapic | 120 | 424 | 900 | 3100 | 3070 |
| Imazapic | 160 | 740 | 860 | - | - |
| Imazapyr | 80 | 144 | 850 | 3710 | 3490 |
| Imazapyr | 100 | - | - | 3425 | 3440 |
| Imazapyr | 120 | 279 | 835 | 3465 | 3320 |
| Imazapyr | 160 | 671 | 830 | - | - |
| Imazapic + Imazapyr[1] | 20 + 60 | 181 | 954 | 3410 | 3320 |
| Imazapic + Imazapyr[1] | 25 + 75 | - | - | 3320 | 2910 |
| Imazapic + Imazapyr[1] | 30 + 90 | 351 | 970 | 3510 | 3520 |
| Imazapic + Imazapyr[1] | 40 +120 | 544 | 847 | - | - |
| Imazethapyr + Imazapyr[1] | 60 + 20 | 125 | 1000 | 3600 | 3200 |
| Imazethapyr + Imazapyr[1] | 75 + 25 | - | - | 3370 | 3270 |
| Imazethapyr + Imazapyr[1] | 90 + 30 | 291 | 973 | 3520 | 3110 |
| Imazethapyr + Imazapyr[1] | 120 + 40 | 647 | 887 | - | - |
| Pendimethalin + Cyclosulfamuron | 495 + 15 | 27 | 169 | 1540 | 1370 |
| Bispyribac sodium + Fenoxaprop-P-ethyl | 25 + 30 | 27 | 135 | 1585 | 780 |
| LSD (0.05) | | 130 | 115 | 658 | 527 |

Table 3. Post-emergence weed control with imidazolinone herbicides on CL161 rice (O Mon, Cantho, Wet season 2003).

| Treatment | g a.i. ha$^{-1}$ | % weed control at 60 DAS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 7 DAS treatment | | | | 12 DAS treatment | | | | |
| | | WR | ECH | LEP | SED | WR | ECH | LEP | SED | LUD |
| Untreated | - | (113) | (201) | (182) | (53) | (159) | (128) | (208) | (50) | (19) |
| Imazapic | 80 | 75 | 100 | 79 | 95 | 99 | 95 | 90 | 80 | 72 |
| Imazapic | 120 | 96 | 95 | 63 | 52 | 100 | 97 | 96 | 81 | 88 |
| Imazapic | 160 | 100 | 100 | 100 | 99 | 100 | 96 | 97 | 95 | 100 |
| Imazapyr | 80 | 38 | 62 | 16 | 0 | 100 | 93 | 95 | 78 | 99 |
| Imazapyr | 120 | 45 | 71 | 99 | 0 | 100 | 97 | 95 | 86 | 97 |
| Imazapyr | 160 | 77 | 82 | 70 | 5 | 100 | 99 | 97 | 86 | 94 |
| Imazapic+Imazapyr[1] | 20 + 60 | 57 | 80 | 77 | 0 | 100 | 96 | 96 | 94 | 93 |
| Imazapic+Imazapyr[1] | 30 + 90 | 91 | 84 | 75 | 15 | 100 | 95 | 100 | 95 | 91 |
| Imazapic + Imazapyr[1] | 40+120 | 93 | 91 | 92 | 41 | 100 | 100 | 100 | 100 | 100 |
| Imazethapyr+Imazapyr[1] | 60 + 20 | 73 | 32 | 44 | 0 | 100 | 97 | 98 | 88 | 96 |
| Imazethapyr+Imazapyr[1] | 90 + 30 | 79 | 89 | 71 | 65 | 100 | 99 | 98 | 97 | 97 |
| Imazethapyr+Imazapyr[1] | 120+40 | 91 | 82 | 65 | 86 | 100 | 99 | 99 | 98 | 94 |
| Pendimethalin + Cyclosulfamuron | 495+15 | 0 | 44 | 95 | 63 | 0 | 25 | 50 | 95 | 100 |
| Bispyribac sodium + Fenoxaprop-P-ethyl | 25 + 30 | 0 | 99 | 99 | 15 | 0 | 96 | 100 | 98 | 100 |

Table 4. Post emergence weed control with imidazolinone herbicides on CL161 rice (O Mon, Cantho, Dry season 2004).

| Treatment | g a.i. ha $^{-1}$ | % weed control at 60 DAS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 7 DAS treatment | | | | 12 DAS treatment | | | |
| | | WR | ECH | LEP | SED | WR | ECH | LEP | SED |
| Untreated | - | (176) | (15) | (10) | (56) | (145) | (26) | (10) | (48) |
| Imazapic | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazapic | 100 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazapic | 120 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazapyr | 80 | 93 | 100 | 100 | 100 | 98 | 100 | 100 | 100 |
| Imazapyr | 100 | 96 | 100 | 100 | 100 | 98 | 100 | 100 | 100 |
| Imazapyr | 120 | 94 | 79 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazapic+Imazapyr[1] | 20 + 60 | 96 | 93 | 97 | 100 | 97 | 100 | 100 | 100 |
| Imazapic+Imazapyr[1] | 25 + 75 | 98 | 100 | 100 | 100 | 98 | 100 | 100 | 100 |
| Imazapic + Imazapyr[1] | 30 + 90 | 96 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazethapyr+Imazapyr[1] | 60 + 20 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazethapyr+Imazapyr[1] | 75 + 25 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Imazethapyr+Imazapyr[1] | 90 + 30 | 100 | 100 | 100 | 100 | 99 | 100 | 100 | 100 |
| Pendimethalin + Cyclosulfamuron | 495 + 15 | 15 | 48 | 61 | 100 | 0 | 5 | 71 | 68 |
| Bispyribac sodium + Fenoxaprop-P-ethyl | 25 + 30 | 0 | 41 | 100 | 100 | 2 | 100 | 100 | 100 |

* WR: *Oryza sativa* (weedy rice) ; ECH: *Echinochloa crus-galli*; LEP: *Leptochloa chinensis*; SED: combined of *Cyperus difformis* and *Fimbristylis miliacea*.
DAA = days after appl. ; DAS = days after sowing; [1] ready-mix formulation; (n) No of weed $m^{-2}$; avr. of 4 replications

Table 5. Post emergence weed control with imidazolinone herbicides at low rate (Pot trial - CLRRI, Can Tho, 2004)

| Treatment | g a.i. ha$^{-1}$ | % weed control at 30 DAA | | | | | |
|---|---|---|---|---|---|---|---|
| | | 7 DAS treatment | | | 12 DAS treatment | | |
| | | WR | ECH | LEP | WR | ECH | LEP |
| Untreated | - | (18) | (54) | (44) | (21) | (54) | (25) |
| Imazapic | 60 | 100 | 32 | 74 | 70 | 43 | 0 |
| Imazapic | 80 | 100 | 78 | 66 | 85 | 59 | 0 |
| Imazapic + Imazapyr [1] | 15 + 45 | 100 | 71 | 61 | 89 | 36 | 69 |
| Imazapic + Imazapyr [1] | 20 + 60 | 100 | 33 | 83 | 82 | 49 | 74 |
| Imazethapyr + Imazapyr [1] | 45 + 15 | 100 | 73 | 60 | 90 | 0 | 0 |
| Imazethapyr + Imazapyr [1] | 60 + 20 | 100 | 52 | 42 | 100 | 44 | 43 |

DAA = days after appl.; DAS = days after sowing; [1] ready formulation; (n) No of weed pot$^{-1}$; avr. of 3 replications.

## Carry-over risk on following rice crop

Under good water supply condition, imidazolinone herbicides applied at rates from 80 - 160 g a.i ha$^{-1}$ showed no negative carry-over effect on the next non-tolerance rice crop. Meanwhile, good control of weedy rice in the previous season also reduced weedy rice infestation in the following season by preventing seed accumulation in the soil. The build up of weedy rice seed bank in plots where unfavorable control was obtained also led to suppressed height and lodging of weedy rice on the cultivated rice plants, resulting in drastic grain yield loss even in the following season (Table 6).

Table 6. Carry-over risk identification of imidazolinone herbicides on following rice crop with non-tolerance varieties (O Mon, Can Tho, 2003-2004).

| Treatment | g a.i. $ha^{-1}$ | 7 DAS Phyto. (%) | 30 DAS No. of tiller* | 30 DAS Plt. Height* (cm) | 60 DAS Plt. Height* (cm) | Grain Yield* (tons $ha^{-1}$) |
|---|---|---|---|---|---|---|
| Untreated | - | 0 | 3.2 | 45.6 | 112.8** | 1.3 |
| Imazapic | 80 | 0 | 3.7 | 39.5 | 70.2 | 2.9 |
| Imazapic | 120 | 0 | 3.9 | 40.4 | 73.5 | 3.0 |
| Imazapyr | 80 | 0 | 3.6 | 39.0 | 70.1 | 3.1 |
| Imazapyr | 120 | 0 | 4.0 | 40.4 | 73.2 | 3.0 |
| Imazapic+Imazapyr[1] | 20 + 60 | 0 | 4.4 | 41.3 | 72.9 | 3.0 |
| Imazapic+Imazapyr[1] | 30 + 90 | 0 | 4.3 | 41.0 | 74.2 | 3.1 |
| Imazethapyr+Imazapyr[1] | 60 + 20 | 0 | 4.5 | 40.5 | 73.6 | 3.0 |
| Imazethapyr+Imazapyr[1] | 90 + 30 | 0 | 4.1 | 40.9 | 74.2 | 3.1 |
| Pendimethalin+ Cyclosulfamuron | 495 + 15 | 0 | 3.2 | 45.9 | 110.9** | 1.3 |
| Bispyribac sodium+ Fenoxaprop-P-ethyl | 25 + 30 | 0 | 3.1 | 45.4 | 113.4** | 1.4 |
| LSD (0.05) | | | ns | ns | 12.3 | 0.6 |

DAS = days after sowing; [1]ready formulation; *avr.of 2 trials, 4 reps. per trial; ** height of weedy rice.

Formulation available & Registration status

CLEARFIELD™ Production System for Rice, which combines IMI herbicides and herbicide-tolerant varieties, has been recently introduced in US, Brazil, Colombia, and Central America (BASF internal sources 2004). Not considered as a GMO variety, the CLEARFIELD™ rice product offers growers access to all local and international markets with no restrictions. Many formulations are registered and commercialized suitable for local market demand (Table 7). Registration in Vietnam is expected soon for treatment on the local rice varieties that contain herbicide tolerant trait through intensive breeding work.

Table 7. Some products used in CLEARFIELD™ Production System for Rice.

| Active Ingredient | Product name | Country |
|---|---|---|
| Imazapic | Masterkey 70 DG | Colombia, US, Brazil, Central America |
| Imazethapyr | Newpath 24 SL | |
| Imazapic + Imazapyr 1:3 | Kifix 70 DG | |

The introduction of IMI-tolerance varieties offers rice growers a good opportunity to manage weedy rice and other common weeds. However, more studies in Mekong Delta area are required to evaluate the carry-over risk on light texture soil and/or on rotation field crops (e.g., watermelon, corn, pulse, …).

Besides, its success much depends on how well the cultivation and management strategies can reduce the potential for weed resistance, maximizing the agronomic and yield potential of seed, and ensure sustainability of the system through managing the use of the trait (s).

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# Penoxsulam: a new global rice herbicide

R. Mann*[1], L. Nguyen[2], V. Patil[3], D. Larelle[4], C. Mavrotas[5],
H. Nonino[6], M. Morell [7], Y.K. Min[8], I. Shiraishi[9]
[1]Dow AgroSciences LLC, Indianapolis, IN, USA, 46131, [2]Dow AgroSciences BV, Ho Chi Minh City, Vietnam, [3]Dow AgroSciences Asia, Kuala Lumpur, Malaysia, [4]Dow AgroSciences, Le Chesnay Cedex, France, [5]Dow AgroSciences Export SAS, Athens, Greece, [6]Dow AgroSciences, Mogi Mirim, Brazil, [7]Dow AgroSciences, Bogota, Colombia, [8]Dow AgroSciences, Seoul, Korea, [9]Dow AgroSciences, Tokyo, Japan
rkmann@dow.com

**Abstract:** Penoxsulam (code name DE-638) is a new triazolopyrimidine sulfonamide herbicide currently being developed by Dow AgroSciences for use in rice around the world. Penoxsulam provides post-emergence cross-spectrum control of *Echinochloa* grasses and many broadleaf and sedge weeds in rice with 2 to 4 weeks residual weed control in most areas. Penoxsulam is a systemic, phloem and xylem mobile herbicide that is absorbed via leaves, shoots and roots. Penoxsulam has been field tested in over 20 countries in over 1,900 field trials from 1998 to 2004. Penoxsulam can be applied to dry-seeded, water-seeded and transplanted rice, and provides excellent post-emergence weed control activity of all *Echinochloa* spp (ECHSS), the major grass weed in rice around the world. Penoxsulam use rates range from 10-15 g $ha^{-1}$ in ASEAN countries, 30-40 g $ha^{-1}$ in European/Eufrasian countries, 26-50 g $ha^{-1}$ in Southern/Central American countries and 35-50 g $ha^{-1}$ in North America, Japan and Korea. Rice tolerance to penoxsulam has been excellent in indica and japonica rice varieties in all countries when applied at the correct timing of application. Penoxsulam is typically applied from BBCH12 to BBCH22 stage of ECHSS weeds. The low use rates reduce potential for water quality issues and there are no rotation crop issues. Due to its favorable acute, chronic and ecotox profile as compared to other commercially available rice herbicides, penoxsulam was registered under U.S. EPA's Reduced Risk Pesticide Program. Penoxsulam has excellent formulation flexibility; formulated as a low concentrate OD with adjuvant built in, a high concentrate SC and as a clay or cellulose based granule for use in transplanted and water-seeded rice depending on customers needs. The liquid formulations have a 1 hour rainfastness requirement and can be tankmixed with other herbicides and insecticides.

**Key words:** DE-638, *Echinochloa crus-galli*, penoxsulam, rice, weed control.

## INTRODUCTION

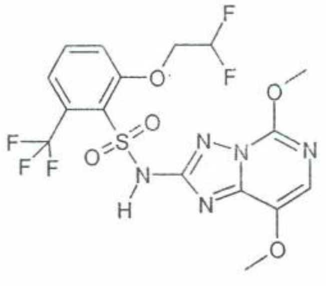
Penoxsulam is being developed to control economically important weeds in dry-seeded, water-seeded and transplanted rice crops around the world. Depending on local application methods, penoxsulam is a versatile herbicide with formulation flexibility that can be applied with a backpack sprayer, mist-blower, tractor sprayer and aerial application (airplane and helicopter). Penoxsulam granules can be applied by hand, blower, or by aerial application. Penoxsulam has been tested under the following code names: DE-638, XDE-638, XR-638, DASH-001 and DASH-1100. Penoxsulam was designated a Reduced Risk Pesticide by US EPA in January, 2003, since it met the 4 criteria for Reduced Risk: 1) reduces risk to human health, 2) reduces risk to non-target organisms, 3) reduces the potential for contamination of surface and ground water due to very low use rates, and 4) broadens the adoption of integrated pest management practices while reducing environmental loading.

This paper presents information on the active ingredient: physico-chemical properties, toxicological and environmental profiles, as well as biological performance summary of results for major rice growing countries around the world.

## CHEMICAL AND PHYSICAL PROPERTIES

Common name (ISO)........... Penoxsulam

Structure:



Chemical name (CAS) ......... 2-(2,2-difluoroethoxy)-*N*-(5,8- dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide

Chemical family ................... Triazolopyrimidine sulfonamide

Code names tested ................ DE-638, XDE-638, XR-638, DASH-001, DASH-1100

Empirical formula ................ $C_{16} H_{14} F_5 N_5 O_5 S$

Molecular weight ................. 483.373

Vapor pressure ..................... $7.16 \times 10^{-16}$ mm Hg or $9.55 \times 10^{-14}$ Pa at 25 °C
$1.87 \times 10^{-16}$ mm Hg or $2.49 \times 10^{-14}$ Pa at 20 °C

Dissociation Constant (*p*Ka) 5.1

Water solubility at 20 °C ..... 5.7 mg $L^{-1}$ @ *p*H 5
408 mg $L^{-1}$ @ *p*H 7
1460 mg $L^{-1}$ @ *p*H 9

Octanol/water partition coefficient at 19 °C: log $K_{OW}$ = -0.354 (unbuffered water)

## FORMULATIONS

Penoxsulam is formulated to meet the needs of growers and their cultural practices. For use in ASEAN countries, penoxsulam products will be formulated as an Oil Dispersion (OD) product containing 25 g ai $L^{-1}$ penoxsulam or as a combination product containing 10 + 50 g ai $L^{-1}$ of penoxsulam + cyhalofop-butyl, respectively. The OD formulation uses a vegetable oil carrier which optimizes the biological performance without the need for additional tankmix adjuvants. The 25OD formulation is also used in parts of Latin America, Eufrasia, and Greater China rice growing countries, whereas a 20 g ai $L^{-1}$ formulation is use in the European Union. A 240 g ai $L^{-1}$ SC (Suspension Concentrate) formulation is used in North America and many countries in Latin America, as well as India and Pakistan, whereas a diluted 37.5 g ai $L^{-1}$ SC formulation is used in Japan and Korea. Several clay-based granules have been developed for use in Japan and Korea, whereas cellulose-based granule has been developed for use in water-seeded rice in California, USA.

## TOXICOLOGY

Acute Oral $LD_{50}$ (rat) .................................. > 5000 mg $kg^{-1}$ b.w.

Acute Dermal $LD_{50}$ (rabbit) ......................... > 5000 mg $kg^{-1}$ b.w.

Eye Irritation (rabbit) .................................. Mild transient ocular irritation

Skin Irritation (rabbit) ................................. Very slight and transient skin irritation

Skin Sensitization (guinea pig) .................... Non-sensitizer

Teratogenicty (Ames test, CHO-HGPRT, micronucleus assay, mouse lymphoma assay)
................................................................... Negative

Mutagenicity..........................................Negative

Chronic 2 yr (rat, mouse, dog)..................Not carcinogenic

## ECOTOXICOLOGY

| | | |
|---|---|---|
| Aquatic Plants $EC_{50}$ | Green algae 96 hr | 0.086 mg $L^{-1}$ |
| | Blue-green algae 120 hr | 0.49 mg $L^{-1}$ |
| | Duckweed 14 day | 0.003 mg $L^{-1}$ |
| | Freshwater diatom 120 hr | >49 mg $L^{-1}$ |
| Avian Oral $LD_{50}$ | Bobwhite Quail | > 2025 mg $kg^{-1}$ b.w. |
| | Mallard Duck | > 2000 mg $kg^{-1}$ b.w. |
| Avian Dietary $LC_{50}$ (8 day) | Bobwhite Quail | > 4411 ppm |
| | Mallard Duck | > 4310 ppm |
| Fish* 96 hr $LC_{50}$ | Rainbow trout | > 102 mg $L^{-1}$ |
| | Bluegill Sunfish | > 103 mg $L^{-1}$ |
| | Common Carp | > 101 mg $L^{-1}$ |
| | Silverside | > 129 mg $L^{-1}$ |
| Aquatic invertebrates $LC_{50}$(96 hr) | Ramshorn snail | >126 mg $L^{-1}$ |
| | Mysid shrimp | >114 mg $L^{-1}$ |
| | *G. pseudolimnaeus* | > 126 mg $L^{-1}$ |
| $EC_{50}$ | *Daphnia magna* | 48 hr > 98 mg $L^{-1}$ |
| | Eastern oyster | 96 hr > 127 mg $L^{-1}$ |
| Earthworm 14 day $LC_{50}$ | | > 1000 mg $kg^{-1}$ |
| Bees | 48-h oral $LD_{50}$ > 110 $\mu$g $bee^{-1}$; 48-h contact $LC_{50}$ > 100 $\mu$g $bee^{-1}$ | |

*No acute toxicity was observed in any fish species at any concentration. Penoxsulam has an extremely low bioaccumulation factor (BCF) of 0.02 ml $g^{-1}$ and will not bioaccumulate.

## ENVIRONMENTAL FATE

In water, the major route of degradation is through a combination of photolysis and biological degradation. Laboratory studies demonstrate photolysis half-life in water of two days under conditions simulating summer sunlight at 40°N latitude. Half life of the herbicide under laboratory aerobic and anaerobic aquatic conditions averaged 25 and 7 days, respectively. Field studies in flooded water-seeded rice paddies using typical agronomic practices provided half-life in water of approximately 6.5 days, whereas penoxsulam half-life was approximately 14.6 days in dry-seeded rice cultural practices where penoxsulam is applied prior to permanent water flood.

In soil, penoxsulam degradation is mainly a microbiological process, influenced by temperature. Soil half-lives under laboratory aerobic conditions averaged 42 days at 20°C and 7.3 days under anaerobic conditions (20°C).

The rapid degradation of penoxsulam minimizes the risk of damage for cultivated crops following rice harvest. Many field carry-over studies have been completed under normal rice cultural practices around the world without any carry-over or negative effect seen to many crops, including but not limited to cereals, corn, sorghum, soybean, sunflower, and cotton.

## RESIDUES IN RICE AND GRAIN QUALITY

Penoxsulam residues in the harvest straw were approximately 1-2 ppb, whereas penoxsulam residues in the rice grain were 1 ppb or less. Four studies were conducted in Italy, France and Greece to evaluate grain quality at harvest following an application of penoxsulam at 40 gr ai $ha^{-1}$. It was shown that penoxsulam did not induce any negative effect on the following attributes: 1000 rice caryopsis weight, milling yield, immature and damaged kernels, protein and amylose content and gelation time.

## TRANSLOCATION, MODE OF ACTION AND RESISTANCE MANAGEMENT

Penoxsulam is a systemic, phloem and xylem mobile herbicide that is absorbed primarily via leaves and shoots and secondarily via roots. The compound is translocated in plants to meristematic tissues and induces plant chlorosis, necrosis and ultimately control of susceptible plant species. Complete desiccation of susceptible plants may occur in 7-10 days under ideal growing conditions, but may take longer under less ideal conditions.

Penoxsulam is a triazolopyrimidine sulfonamide whose mode of action is the inhibition of the Acetolactate Synthase (ALS) enzyme in plants. Several other ALS mode of action products are already used for weed control in rice. To minimize the risk of development of ALS-resistant weed biotypes to penoxsulam in rice, it is recommended to apply penoxsulam only once per season under a rigorous resistance management program that utilizes the following procedures: use of appropriate cultural practices that minimize weed seed generation and carryover in the seed bank, alternate the use of herbicide products with different modes of action for weed control, use tankmixes and combination products with multiple and different modes of action, and use mechanical means to control escaped weeds.

## INTENDED USES GLOBALLY

Penoxsulam is a post-emergence herbicide to be used in dry and water-seeded rice as well as transplanted rice. Minimum and maximum effective single use application rates vary from as little as 10 g ai $ha^{-1}$ to a maximum of 50 g ai $ha^{-1}$, depending on the country. There is no need to add additional adjuvant to any of the OD formulations, as the formulation performance is optimized, saving additional costs and labor to the applicator. Typical applications of the liquid penoxsulam formulations would be an application to non-flooded dry-seeded rice or to a drained rice paddy to exposed weeds, and in some conditions application of penoxsulam to a shallow flooded paddy (3 to 10 cm, depending on the country practices) with a diluted liquid formulation or a granule formulation will provide commercial control of important weeds.

The following three tables provide a summary of the results of testing penoxsulam as a foliar application in rice from 1998 to 2004. These tables are not an endorsement of weed control and should only be used as a guide. Different rates of penoxsulam may be required to provide effective control of the same weed species in different countries, and there are different biotype responses to penoxsulam globally. Different cultural practices and difference in environmental conditions will also impact effective foliar use rates and residual weed control activity.

Table 1. Plant species that have been found to be susceptible to foliar applications of penoxsulam.

| | | | |
|---|---|---|---|
| *Acalypha australis* | *Cardamine parviflora* | *Kyllinga squamulate* | *Portulaca oleracea* |
| *Aeschynomene spp* | *Chenopodium spp* | *Lindernia spp* | *Rotala indica* |
| *Alisma plantago-aquatica* | *Cleome viscosa* | *Lythrum junceum* | *Sagittaria spp* |
| *Alternanthera philoxeroides* | *Commelina communis* | *Malachra alceifolia* | *Scirpus juncoides* |
| *Amaranthus spp* | *Cyperus spp (annual)* | *Melochia spp* | *Scirpus mucronatus* |
| *Ammannia spp* | *Echinochloa spp (annual)* | *Monochoria spp* | *Scleria pterota* |
| *Bacopa spp* | *Echinochloa polystachya* | *Nasturtium officinale* | *Sesbania exaltata* |
| *Baltimora recta* | *Eclipta alba* | *Nymphaea spp* | *Sphenoclea zeylanica* |
| *Bergia capensis* | *Elatine trianda* | *Oenanthe javanica* | *Spilanthes acmella* |
| *Bidens tripartita* | *Eleocharis spp* | *Phyllanthus niruri* | *Tridax procumbens* |
| *Boerhaavia diffusa* | *Fimbristylis spp* | *Physalis angulata* | *Xanthium strumarium* |
| *Caperonia spp* | *Heteranthera limosa* | *Polygonum spp* | |

Table 2. Plant species that have been found to be moderately susceptible to foliar applications of penoxsulam.

| | | | |
|---|---|---|---|
| *Butomus umbellatus* | *Cyperus serotinus* | *Hyssopus spp* | *Marsilea spp* |
| *Commelina benghalensis* | *Eriochloa spp* | *Ipomoea spp* | *Potamogeton distinctum* |
| *Corchorus spp* | *Erucaria spp* | *Ischaemum rugosum* | *Scirpus maritimus* |
| *Cyperus esculentus* | *Euphorbia nutans* | *Jussiaea abyssinica* | *Sida spp* |
| *Cyperus rotundus* | *Glyceria declinata* | *Ludwigia spp* | *Spigelia anthelmia* |

Table 3. Plant species that have been found to be tolerant to foliar applications of penoxsulam.

| | | | |
|---|---|---|---|
| *Aneilema keisak* | *Digitaria spp* | *Leptochloa spp* | *Paspalum spp* |
| *Brachiaria spp* | *Eleusine indica* | *Murdannia nudiflora* | *Rottboellia spp* |
| *Cenchrus echinatus* | *Eriochloa punctata* | *Oryza sativa* | *Scirpus planiculmis* |
| *Dactyloctenium spp* | *Euphorbia heterophylla* | *Panicum spp* | *Setaria spp* |

## CROP SELECTIVITY

Tolerance of rice to penoxsulam is due to differential metabolism compared to susceptible weeds. The speed of penoxsulam metabolism to inactive molecules contributes to the differential selectivity between tolerant and susceptible plant species. Under greenhouse conditions, penoxsulam's half-life in japonica rice was 1.6 day, in indica rice 0.6 day, whereas in *Echinochloa crus-galli* the penoxsulam half-life was 4.4 days.

## REGISTRATION STATUS

As of early 2005, penoxsulam has been registered in over 15 countries including Argentina, China, Colombia, Indonesia, Italy, Korea, Malaysia, Morocco, Philippines, Turkey, United States, and Vietnam. Registration activity is ongoing in additional rice growing countries.

## CONCLUSION

Penoxsulam is a new post-emergence herbicide for broad-spectrum weed control in dry-seeded, water-seeded, and transplanted rice. It fits the cultural practices of many rice growing countries very well, showing a low use rate product with very favorable toxicological and environmental profile, no grain residues, no anticipated crop rotation restriction, water management flexibility, excellent selectivity and very good control of many of the most important weeds (*Echinochloa* spp., sedges, and broadleaf weeds) in rice. Penoxsulam is a flexible product that can be formulated to meet local cultural practice needs, and applied alone or in combination with other herbicide products to meet grower needs.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# Penoxsulam, a new rice herbicide for use in direct seeded and transplanted rice in Asean countries

N. Lap[1], S. Lerwit[2], A. Criador IV [3], S. Djoko [4], Vasant L.Patil [5], and R.K. Mann [6]
[1]DowAgroSciences BV, Ho Chi Minh City, Vietnam; [2]DowAgroSciences Thailand Ltd, Bangkok , Thailand; [3]DowAgroSciences Makati, Philippines; [4] DowAgroSciences Indonesia PT, Jakarta, Indonesia; [5]DowAgroSciences Asia, Kuala Lumpur, Malaysia; [6]DowAgroSciences LLC USA

**Abstract:** Penoxsulam, code name DE-638, is a new triazolopyrimidine sulfonamide rice herbicide that is being developed by Dow AgroSciences for the control of rice weeds in rice growing countries around the world. It is a broad-spectrum herbicide with post-emergence and residual activity that controls many important grasses, broadleaf and sedge weeds with excellent rice tolerance. This paper discusses the value of penoxsulam, a new unique herbicide that can be used to increase rice productivity under different rice cultures in direct-seeded as well as transplanted rice in five ASEAN countries, viz., Malaysia, Philippines, Indonesia, Thailand and Vietnam. Small plot field research trials and on-farm demonstration trials were completed from 1998 to 2004. Multi-location and multi-season field trials across ASEAN countries over a 7 year period and large plot size on-farm demonstrations on grower fields in 2003-2005 demonstrated that when penoxsulam was applied as a delayed pre-emergence to foliar applied post-emergence at 0 to 18 days after sowing in direct wet-seeded rice, 10 to 15 g a.i. ha$^{-1}$ of penoxsulam provided >90% control of ECHCG, ECHCO, FIMMI, CYPIR, CYPDI, MOOVA and SPDZE, resulting in up to 200% yield increase compared with non-herbicide application. When applied as post-emergence at 7 to 18 days after transplanting in transplanted rice, 12.5 to 15 g a.i. ha$^{-1}$ of penoxsulam provided >90% control of ECHCG, FIMMI, CYPIR, CYPDI, MOOVA and SPDZE, resulting in up to 200% yield increase.

Penoxsulam is not effective for control of LEFCH, an important grass weed in some areas causing reduction in rice yield. Penoxsulam + cyhalofop-butyl tank mix or as a premix oil dispersion (OD) formulation at 10+50 to 12.5 + 62.5 g a.i. ha$^{-1}$, respectively, when applied at 4-18 DAS can be used to obtain >90% control of LEFCH without reducing control of other weeds. Penoxsulam has excellent crop safety, with rates up to 5X rate, i.e., 60 g a.i. ha$^{-1}$, when applied at 0-18 days after sowing or transplanting it did not cause crop injury to rice or reduce yields.

**Key words**: Common weeds in rice, direct seeded rice, penoxsulam, performance transplanted rice.

## INTRODUCTION

Penoxsulam (DE-638) is a new Dow AgroSciences rice herbicide that has had been widely researched and field tested since 1998 throughout rice growing countries around the world before introduction to the market. Extensive and intensive testing under varied rice growing conditions of temperate and tropical rice areas around the globe has resulted in generating very robust data based recommendation. Trial recommendations have been subjected further to rigorous reconfirmation under "on farm demonstrations" essentially carried out by growers themselves (typically under local agronomic conditions that are widely adopted by farmers in respective area) since 2003. This approach of taking science-based solution from lab/ field trials directly to rice growers certainly reaffirms the robustness of recommendations with clearly defined biological performance deliverables. Based on this approach, within Southeast Asia area, during the pre-development and development periods of 1999-2004, many studies of 216 small- scale field trials and 252 large-scale demonstrations of penoxsulam on rice were completed in 5 countries in the area. They included field studies in normal farming practices and variable field conditions such as studies under different soil moisture, different watering regimes to field after application of rice herbicidal treatment, different water spray volumes, different soil types and different application methods, and demonstrations to reconfirm result in scientific trials.

This paper focuses on the performance of penoxsulam alone and its pre-mix product penoxsulam + cyhalofop-butyl in 166 bioefficacy and rice crop tolerance trials under normal rice farming conditions conducted from 1998-2004 and in 246 large-scale demonstrations (on-farm demonstrations) conducted from 2003-2005 in the five ASEAN countries.

## MATERIALS AND METHODS

Field trials were conducted on field research stations of Rice Research Institutes/Centers in Malaysia, Philippines, Indonesia and Vietnam and on farmer fields in Thailand and Philippines. Trials were randomized complete block design (RCBD) with 3 or 4 replications with plot 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 made to exposed weeds, and the paddy re-flooded within 24 - 48 h after application. Other agronomic practices followed were as per local farming conditions in respective country.

Penoxsulam formulations tested in these trials were GF-237 and GF-1075 containing 25 g a.i. $L^{-1}$ penoxsulam OD formulation; and GF-981 OD, containing10 g a.i. + 50 g a.i. $L^{-1}$ penoxsulam and cyhalofop-butyl, respectively. The rates tested of penoxsulam ranged from 5 to 60 g a.i. $ha^{-1}$. Each tested rate was diluted in a spray volume of 150- 400 L $ha^{-1}$ water and applied by knapsack sprayer with fan nozzle. No additional adjuvant tank mix was required for use with GF-237, GF-1075 and GF-981 due to the OD formulation having adjuvant already built in.

Large scale demonstrations were conducted on farmer's fields. The average sizes were 1,000 $m^2$ (ranging 500 $m^2$-5,000 $m^2$). DowAgroSciences delivered samples to farmers and they applied penoxsulam (GF-1075) or mixture of penoxsulam + cyhalofop butyl (GF-1075 + Clincher* 10EC or GF-981) themselves following their own normal farming practices. The rates of penoxsulam were 10-12.5 g a.i. $ha^{-1}$ and there was only one application of tested chemical made per demonstration.

Individual weed control evaluation 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 and confirmed by each specie weed count at 42 DAA. Phytotoxicity was recorded at 1, 3, 7, 14 and 28 DAA by visual assessment of percent injury.

Collected data were statistical analyzed by PRM5 and Minitab software. * Trademark of Dow AgroSciences LLC.

## RESULTS AND DISCUSSIONS

Rice Crop Phytotoxicity: The tested rates of 5-60 g a.i. $ha^{-1}$ penoxsulam in 216 small-scale field trials and 252 large-scale demonstrations carried out in five ASEAN countries under normal or abnormal conditions did not cause any injury symptom to indica rice when applied at 0-24 DAS or 7-18 DAT.

Efficacy of penoxsulam on *Echinocloa sp.* (ECHSS) in small-scale field trials

Table 1 demonstrates that all tested rates of 7.5-15 g a.i. $ha^{-1}$ penoxsulam provided herbicidal activity on ECHSS. The average of 93 trial results on ECHCG showed that penoxsulam at 10-12.5 g a.i. $ha^{-1}$ provided >90% control of this species when applied at 0-18 DAS. When applied at 10-18 DAS, >90% control of ECHCO was achieved with 15 g a.i. $ha^{-1}$ penoxsulam across 14 trials on this weed species.

Table 1. Efficacy of penoxsulam on ECHSS at 42 DAA (% Biomass Reduction) *Echinocloa crus - galli* (ECHCG).

| Days After Sowing | Country | No. of replications | Penoxsulam (g a.i. $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | 7.5 | 10 | 12.5 | 15 |
| 0-3 DAS | Thailand | n = 12 | 87.8 | 93.6 | 95.5 | - |
| *(16 trials)* | Vietnam | n = 48 | 94.7 | 94.2 | 94.6 | 95.4 |
| 4 -9 DAS | Malaysia | n = 8 | 71.0 | 90 | 89.1 | 90.5 |
| *(25 trials)* | Philippines | n = 36 | 87.8 | 94.9 | 97.2 | 98.1 |
| | Thailand | n = 26 | 79.7 | 91.8 | 94 | 97.3 |
| | Vietnam | n = 24 | 87.6 | 93.3 | 95.6 | 97.9 |
| 10 –14 DAS | Malaysia | n = 20 | - | 87 | 93.1 | 92.3 |
| *(40 trials)* | Philippines | n = 24 | 82.5 | 84.6 | 91.4 | 93.8 |
| | Thailand | n = 38 | 71.3 | 90.2 | 93.5 | 95.8 |
| | Vietnam | n = 50 | - | 96.2 | 94.9 | 94.5 |
| | Indonesia * | n = 15 | - | 97.8 | - | 99.7 |
| 15-18 DAS | Philippines | n = 8 | 55.0 | 68.8 | 93.3 | 92.1 |
| *(12 trials)* | Thailand | n = 9 | - | 78.9 | - | 91.7 |
| | Vietnam | n = 11 | - | 86.4 | 85.6 | 93.6 |
| | Indonesia * | n = 12 | - | 97.2 | 100 | 100 |
| *Echinocloa colona* (ECHCO) | | | | | | |
| 10 –14 DAS | Philippines | n = 12 | 86 | 91.9 | 92.5 | 94 |
| *(9 trials)* | Thailand | n = 3 | - | 86 | - | 95 |
| | Vietnam | n = 18 | - | 89.7 | - | 94.5 |
| 15-18 DAS *(5 trials)* | Philippines | n = 4 | - | 81.5 | 84.3 | 85.8 |
| | Thailand | n = 3 | - | 86 | - | 95 |
| | Vietnam | n = 12 | - | 92.1 | - | 94.4 |

* Transplanted rice

Efficacy of penoxsulam on *Fimbristylis miliacea* (FIMMI) in small-scale field trials

Table 2 demonstrates that across 80 trials, 10-12.5 g a.i. $ha^{-1}$ penoxsulam provided >90 % control of FIMMI when applied at 0-18 DAS across ASEAN countries.

Table 2. Efficacy of Penoxsulam on FIMMI at 42 DAA (% Biomass Reduction).

| Days after Sowing | Country | No. of replications | Penoxsulam (g a.i. $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | 7.5 | 10 | 12.5 | 15 |
| 0-3 DAS | Philippines | n = 24 | 93.4 | 96.1 | 95.3 | 90.5 |
| *(18 trials)* | Vietnam | n = 32 | 99 | 98.3 | 97.6 | 97.9 |
| | Thailand | n = 16 | 86.8 | 94.9 | 96.1 | - |
| 4 -9 DAS | Malaysia | n = 8 | 90.8 | 89 | 90.7 | 93.4 |
| *(22 trials)* | Philippines | n = 44 | 92.7 | 96.1 | 97 | 97.1 |
| | Thailand | n = 6 | - | 100 | 99.6 | - |
| | Vietnam | n = 24 | 91.3 | 94.8 | 95.8 | 98.8 |
| 10 –14 DAS | Malaysia | n = 16 | - | 91.9 | 94.2 | 92.5 |
| *(35 trials)* | Philippines | n = 32 | 90.8 | 92.5 | 92.2 | 96.6 |
| | Thailand | n = 16 | - | 99.4 | 99.4 | 100 |
| | Vietnam | n = 70 | 98.8 | 93.6 | 95.5 | 96.3 |
| 15-18 DAS | Philippines | n = 8 | - | 92.8 | 93.5 | 94.8 |
| *(5 trials)* | Vietnam | n = 12 | - | 89.3 | 85 | 90.9 |
| | Indonesia * | n = 2 | - | 100 | 100 | - |

*Transplanted rice

Efficacy of penoxsulam on *Cyperus sp.* (CYPSS) in small-scale field trials

Table 3 demonstrates that across 68 trials treated at 0-18 DAS, 10 g a.i. $ha^{-1}$penoxsulam provided >90% control of CYPDI, and in 22 trials treated at 4-18 DAS, 12.5 g ai $ha^{-1}$ provided >90% control of CYPIR.

Table 3. Efficacy of penoxsulam on CYPDI &CYPIR at 42 DAA (% Biomass Reduction) *Cyperus difformis* (CYPDI).

| Days After Sowing | Country | No. of replications | Penoxsulam (g a.i. $ha^{-1}$) 7.5 | 10 | 12.5 | 15 |
|---|---|---|---|---|---|---|
| 0-3 DAS (7 trials) | | | | | | |
| | Vietnam | n = 28 | 99.6 | 98.2 | 96.8 | 97.9 |
| 4-9 DAS | Philippines | n = 60 | 96.1 | 95.9 | 100 | 99.2 |
| *(22 trials)* | Thailand | n = 19 | 90.9 | 96 | 96.6 | 96 |
| | Vietnam | n = 4 | - | 98.8 | 100 | 100 |
| 10 –14 DAS | Malaysia | n = 4 | - | 86.3 | - | 86.3 |
| *(34 trials)* | Philippines | n = 60 | 95.4 | 96.2 | 97.6 | 98.8 |
| | Thailand | n = 39 | 95.8 | 93.3 | 97 | 95.4 |
| | Vietnam | n = 28 | 100 | 97 | 96.9 | 98 |
| 15-18 DAS | Philippines | n = 8 | 100 | 100 | 100 | 100 |
| | Thailand | n = 3 | - | - | 100 | 100 |
| *(5 trials)* | Vietnam * | n = 4 | 99.9 | 99.9 | 100 | 100 |
| | Indonesia * | n = 4 | - | 98.1 | 100 | 100 |
| *Cyperus iria (*CYPIR) | | | | | | |
| 4 -9 DAS | Malaysia | n = 7 | 85 | 87.9 | 88.9 | 88.3 |
| (9 trials) | Philippines | n = 16 | 86.3 | 85.9 | - | - |
| | Vietnam | n = 12 | 87.5 | 91.7 | 91.3 | 97.5 |
| 10 –14 DAS | Malaysia | n = 12 | - | 90.4 | 90.6 | 92.1 |
| (11 trials) | Philippines | n = 4 | 85 | 86.3 | 88.3 | 87.3 |
| | Vietnam | n = 28 | - | 89 | 91.7 | 92.5 |
| 15-18 DAS | Vietnam | n = 8 | - | 83.3 | 87.5 | 90.6 |
| (2 trials) | | | | | | |

* Transplanted rice

Efficacy of penoxsulam on *Sphenoclea zeylanica* (SPDZE) in small-scale field trials

Table 4 demonstrates that in 81 trials across ASEAN countries, 10 g a.i. $ha^{-1}$ penoxsulam provided >90% control of SPDZE at the application window of 0-18 DAS.

Table 4. Efficacy of penoxsulam on SPDZE at 42 DAA (% Biomass Reduction).

| Days after Sowing | Country | No. of replications | Penoxulam (g a.i. ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | 7.5 | 10 | 12.5 | 15 |
| 0-3 DAS | Philippines | n = 8 | 94.8 | 96.5 | - | - |
| *(10 trials)* | Vietnam | n = 24 | 96.2 | 97.8 | 97.2 | 98 |
| | Thailand | n = 8 | 91.2 | 96 | 100 | - |
| 4 -9 DAS | Philippines | n = 60 | 94.9 | 93.3 | 99.6 | 97.9 |
| *(26 trials)* | Thailand | n = 26 | 85.9 | 96 | 95.7 | 99 |
| | Vietnam | n = 16 | 83.8 | 92.5 | 95.9 | 97.5 |
| 10 –14 DAS | Malaysia | n = 12 | - | 94.3 | 99.8 | 95.7 |
| *(38 trials)* | Philippines | n = 56 | 93.8 | 97.4 | 95.8 | 100 |
| | Thailand | n = 37 | 93.8 | 98.1 | 98.8 | 98.2 |
| | Vietnam | n = 42 | - | 94.8 | 96.4 | 96.8 |
| 15-18 DAS | Philippines | n = 8 | 100 | 100 | 100 | 100 |
| *(7 trials)* | Thailand | n = 3 | - | 100 | 100 | 100 |
| | Vietnam | n = 11 | - | 89.3 | 92.5 | 93.6 |

Efficacy of penoxsulam on *Monochoria vaginalis* (MOOVA) in small-scale field trials

Table 5 demonstrates that in 34 trials, 10-12.5 g a.i. ha$^{-1}$ penoxsulam provided >90% control of MOOVA at application window of 0-18 DAS across ASEAN countries.

Table 5. Efficacy of penoxsulam on MOOVA at 42 DAA (% Biomass Reduction).

| Days After Sowing | Country | No. of replications | Penoxsulam (g a.i. ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | 7.5 | 10 | 12.5 | 15 |
| 0-3 DAS (2 trials) | Vietnam | n = 8 | - | 93.5 | 89 | 90.3 |
| 4 -9 DAS | Malaysia | n = 4 | 82.5 | 86.5 | 96.8 | 93.5 |
| *(13 trials)* | Philippines | n = 48 | 97.4 | 98.1 | 99.4 | 97.1 |
| 10 –14 DAS | Malaysia | n = 7 | - | 96.3 | 95.7 | 97.5 |
| *(22 trials)* | Philippines | n = 40 | 98.3 | 98.1 | 99.4 | 98 |
| | Vietnam | n = 38 | - | 95.1 | 95.4 | 95.6 |
| 15-18 DAS | Philippines | n = 12 | 100 | 100 | 100 | 100 |
| *(7 trials)* | Vietnam | n = 3 | - | 96.7 | - | 93 |
| | Indonesia * | n = 10 | - | 82 | 100 | 98 |

* Transplanted rice

Efficacy of penoxsulam on common weeds in rice in large-scale field demonstrations

Weeds that were evaluated in demonstration fields were *Echinocloa crus-galli*, *Echinocloa colona*, *Fimbristylis miliacea*, *Cyperus difformis*, *Cyperus iria*, *Cyperus rotundus*, *Sphenoclea zeylanica*, *Ludwidia octovalvis* and *Monochoria vaginalis*. Table 6 provides a summary of results across 252 demonstration trials where penoxsulam was applied at 0-20 DAS and demonstrates that 10-12.5 g a.i. $ha^{-1}$ provided 90-99 % control of *Echinocloa* sp. and 85-99% control of sedges and broadleaf weeds. Results also show that across 6 demonstration trials treated at 14 & 21 DAT in transplanted rice that penoxsulam at 12.5-15 g a.i. $ha^{-1}$ provided >90% control of *Echinocloa crus-galli* and *Monochoria vaginalis*.

Results of penoxsulam large field demonstration trials confirmed the results generated in small scale replicated field trials that 10-12.5 g ai $ha^{-1}$ penoxsulam in both conditions provided 85 to 99% control of the common weeds in ASEAN rice.

Table 6. Efficacy of penoxsulam on common weeds at 42 DAA (% Biomass Reduction).

| Country | Application Window | Penoxsulam rate (g a.i. $ha^{-1}$) | No. of demonstrations | % control | | |
|---|---|---|---|---|---|---|
| | | | | ECHSS | Sedges | Broad leaves |
| Philippines | 2-5 DAS | 7.5 | 47 | 90 | 91 | 87 |
| | 6-10 DAS | 10 | 52 | 91 | 92 | 86 |
| | 15-20 DAS | 12.5 | 60 | 92 | 89 | 80 |
| Vietnam | 0-3 DAS | 10-12.5 | 15 | 95-99 | 95-99 | 85-90 |
| | 4-9 DAS | 10-12.5 | 22 | 95-99 | 95-99 | 95-99 |
| | 10-14 DAS | 10-12.5 | 30 | 95-99 | 95-99 | 95-99 |
| | 15-18 DAS | 10-12.5 | 20 | 90-95 | 85-90 | 90-95 |
| Indonesia* | 14 DATr | 12.5-15.0 | 4 | 90-95 | - | 90-95 |
| | 21 DATr | 15 | 2 | 90-95 | - | 90-95 |
| | | *Total* | *252 demos* | *90-99* | *85-99* | *85-99* |

* Transplanted rice

Summary on efficacy of penoxsulam on common weeds listed

Overall, the small and large scale field trial results shown in tables 1-6 demonstrate that penoxsulam was very efficacious on common weeds in rice. Farmers can apply a very low use rate of 10-12.5 g a.i. $ha^{-1}$ penoxsulam to attain 85-99% control of key important weeds except LEFCH. The penoxsulam OD product will provide farmers a very flexible timing of application product compared to other current products that provides one of three choices, either pre-emergence at 0-3DAS, early post application at 4-9 DAS or middle to late post application at 10-18 DAS.

Efficacy of mixture of penoxsulam + cyhalofop on *Leptochloa chinenses* (LEFCH)

Penoxsulam at 10-30 g a.i. $ha^{-1}$ does not provide commercial control of *Leptochloa chinenses*. However, cyhalofop-butyl makes an excellent tank mix or premix partner for controlling *L. chinenses* without antagonizing the control of *E. crus-galli* and other weeds listed. The results of these mixtures shown in Table 7 demonstrate that tank mixing or premixing at 10+50 g a.i. $ha^{-1}$ penoxsulam + cyhalofop-butyl, respectively, provided 85-100% control of this weed without antagonizing *E. crus-galli* control.

Table 7. Efficacy of penoxsulam+cyhalofop on LEFCH at 42 DAA (% Biomass Reduction).

| Days After Sowing | Country | No. of replications | Penoxsulam+ Cyhalofop (g a.i. $ha^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 10 + 50 | | 12.5 + 62.5 | | 15 + 75 | |
| | | | *Tank mix* | *Pre mix* | *Tank mix* | *Pre mix* | *Tank mix* | *Pre mix* |
| 9-Apr | Thailand | n = 15 &26 | 91.3 | 92.8 | 99.1 | 94.8 | 96.5 | 95.6 |
| *(9 &10 trials)* | Vietnam | n = 16 &12 | 91.7 | 91.8 | 94.4 | 98.8 | 97.8 | 95.8 |
| 10 –14 | Philippines | n = 44 &20 | 98.2 | 94.3 | 98.2 | 95.5 | 99.9 | 97.4 |
| *(19 &22 trials)* | Thailand | n = 24 &30 | 84 | 95.6 | 82.4 | 94.7 | 88 | 97.1 |
| | Vietnam | n = 8 &32 | 91.3 | 94.7 | 93.8 | 98.8 | 100 | 98.3 |
| 15-18 | Philippines | n = 12 &8 | 98 | 95.3 | 100 | 94.5 | 99 | 97.5 |
| *(5 & 3 trials)* | Vietnam | n = 8 &4 | 93,8 | - | 83.8 | 87.5 | 93.8 | 100 |

n = tankmix and prepack, respectively.

Effect of penoxsulam and penoxsulam + cyhalofop on rice grain production

Table 8 demonstrates that in 64 trials across ASEAN countries, the application of penoxsulam at 10-15 g a.i. $ha^{-1}$ or penoxsulam + cyhalofop at 10+50 g a.i. $ha^{-1}$, increased rice grain yield up to 200% and 300 %, respectively, when compared with untreated treatments.

Table 8. Yield comparison among penoxsulam and untreated treatments.

| No. of replications | Treatments | Rate (g a.i. $ha^{-1}$) | Average Yield (kg a.i. $ha^{-1}$) | % against Untreated |
|---|---|---|---|---|
| 239 | Penoxsulam | 10 | 3,223 | *190* |
| (64 trials ) | (g a.i. $ha^{-1}$) | 12.5 | 3,523 | *207* |
| | | 15 | 3,445 | *203* |
| | Untreated | | 1,699 | *100* |
| 91 | Penoxsulam + Cyhalofop-Butyl | 10+50 | 3,603 | *316* |
| (24 trials) | Untreated | | 1,139 | *100* |

In summary, penoxsulam at 10-15 g a.i. $ha^{-1}$ provided 85 to 99% control of above common weeds in direct-seeded and transplanted rice in tropical rice culture. Penoxsulam + cyhalofop-butyl is an efficacious mixture for truly broad-spectrum grass, broadleaf and sedge control in ASEAN rice. The application of penoxsulam or penoxsulam + cyhalofop-butyl mixtures are very safe to rice and they protect rice from yield loss caused by common weeds in the crop.

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# New application technique of pretilachlor/fenclorim for weedy rice control in direct seeded wet sown rice in South East Asia

Jean-Louis Allard[1], Wongyala Pradith[2], and Ruediger Kotzian[3]
[1]Syngenta Asia Pacific Pt.e Ltd. 250 Northbridge Road #39-00 Raffles City Tower Singapore 179101
[2]Syngenta Thailand; [3]Syngenta Crop Protection AG, Schwarzwaldallee 215, CH 4002 Basel

**Abstract**: Direct seeded wet-sown rice is widely cultivated in South East Asia, the Southern provinces of China, and is increasing in South Korea. The technique has been known since the 1980s and after a rapid initial development, further expansion has slowed down because of several constraints of this cropping system. In addition to high infestations of grasses such as *Echinochloa crus-galli* (L.) Beauv and *Leptochloa chinensis* (L.), weedy rice (*Oryza sativa L.* fp. *spontanea*) infestations are increasing in areas where wet-sown rice is grown continuously, such as Vietnam, Malaysia, Sri Lanka, Thailand and Korea. The rapid increase of weedy rice infestations causes high yield losses and reduces farmers' income. Farmers currently manage weedy rice infestations by using the stale-seedbed technique and hand weeding. These techniques delay rice planting, increase water use and require high labour cost. The application of pretilachlor/fenclorim (SOFIT EC 300) by dripping the concentrate or slightly diluted product directly into water during the last levelling, offers a unique way to achieve reliable broad-spectrum weed control including *Leptochloa* sp. and weedy rice, thereby maximizing overall yield potential.

**Key words**: Direct seeded rice; South East Asia; weed control; pretilachlor; fenclorim; weedy rice

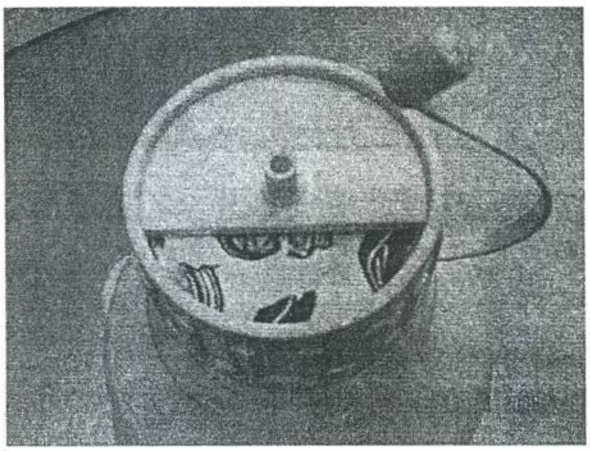
## INTRODUCTION

Pretilachlor/fenclorim was launched in the mid-80s in South East Asia as a pre-emergence herbicide in direct seeded wet-sown rice. The recommended application timing is 0-4 days after sowing. Spraying of pre-emergence herbicides requires applicators to walk through a saturated field which is labour intensive. Further the increasing infestations of Golden Apple Snail has forced farmers to maintain dry conditions as long as possible after rice seeding thereby reducing the performance of pre-emergence herbicides. These conditions considerably limited the use of pretilachlor/fenclorim. The presence of weedy rice had already been reported in 1983 (H. Hyakutake and S. Zungsontiporn, 1985). Since 2002 weedy rice infestations have become a serious problem in the direct seeded rice areas. The authors reported that infestations of "weedy types" reduce yield of cultivated rice and the presence of red grains lower the quality of rice. The seeds of weedy rice shatter readily at maturity, thus building up a seed bank. Current recommended herbicides do not control weedy rice. Syngenta now offers a solution to control weedy rice through a novel technique for the application of pretilachlor/fenclorim. A herbicide application by dripping the product into water during levelling of the paddy field 2-3 days before sowing was evaluated during several seasons in the Chao Praya region of Thailand.

Picture 1: Early emergence of weedy rice panicles

**Dripping application**

Farmers usually perform the last puddling or land levelling by using a levelling board attached behind the tractor. During this working process pretilachlor/fenclorim was dripped without additional equipment directly into paddy water. A bottle was attached with a hanging device upside down in front of the tractor or in front of the levelling board. A hole was pricked into the seal of the bottle by using a special device (picture 2). The size of the hole was designed to provide dripping rates between 600-750 g ai $ha^{-1}$ of product at normal tractor speed of 1 meter /sec. Tractor motion and the levelling board distributed the product evenly over the whole paddy field. A minimum water level of 5-10 cm was required to ensure that all areas of the field were well covered with water. The paddy water remained in the field for 2-4 days before it was drained and pre-germinated rice was broadcasted.



Picture 2: Dripping lid

Picture 3: Dripping of pretilachlor/fenclorim during last levelling

**Trial design and evaluations**

Two large-scale (2-3 ha) demonstrations were conducted in 2004 in Suphanburi and Nonthaburi provinces, central plain, Thailand. SOFIT EC 300 (Pretilachlor/ fenclorim) was applied at 2 l $ha^{-1}$ (600 gai $ha^{-1}$). Crop tolerance, control of weedy rice and other weed species were assessed visually using a 100 % scale. (0% = no control, no crop damage, 100 % = full control, complete crop destruction). Rice yield obtained after treatment was compared with the yield of the previous season. Further small plot trials were conducted with 4x6 $m^2$ plots and 4 replicates. A micropipette and a hand levelling board were used to simulate drip application. Together with manual land levelling the product was dripped into 5-10 cm standing water. The water was maintained for 2-4 days, and then the incubated Suphanburi 1 rice variety was seeded into the paddy water. The water was drained immediately after rice sowing. Water was re-introduced 7-10 days after sowing.

## RESULTS AND DISCUSSION

The pretilachlor/fenclorim treatment increased rice yield by one-third in the Nonthaburi location and almost doubled rice yield in Suphanburi site (table 1). Despite the heavy weedy rice infestation at both locations the treatment achieved a 70-80 % reduction of the weedy rice population. Good crop tolerance with minor transient phytotoxicity to rice was reported after the drip and spray application (table 2). The higher rate of 750 g ai $ha^{-1}$ was better than the 600gai $ha^{-1}$ in the overall weed control including *Echinochloa crus-galli* (L.) Beauv (Table 3). The results show the reduction of both number of weeds and amount of dry weight of weeds after dripping and spray applications. Dripping pretilachlor/fenclorim at rates of 600-750gai $ha^{-1}$ provided broad-spectrum weed control, including *Echinochloa crus-galli* (L.) Beauv., *Leptochloa chinensis* (L.), *Sphenoclea zeylanica* Gaertn. and *Cyperus difformis* Linn and gave comparable or better results than the spray application. Best weed control was obtained with pretilaclor/fenclorim at 750gai $ha^{-1}$ dripped 2-4 days before sowing the rice (table 3). This treatment also provided the highest rice yield (table 4).

Table 1. Effect of herbicide on weedy rice control and rice yield before and after dripping under heavy weedy rice infestation in demonstration trials (2 locations), July 2004-March 2005

| Treatment | Application method And timing | Locations | | | |
|---|---|---|---|---|---|
| | | Suphanburi province | | Nonthaburi province | |
| | | Weedy rice control (%) | Rice yield (Kg $ha^{-1}$) | Weedy rice control (%) | Rice yield (Kg $ha^{-1}$) |
| 1. Early-post practice under heavy weedy rice infestation | Conventional spray in previous season | 0.0 | 3,645 | 0.0 | 3,333 |
| 2. pretilachlor 30% EC 600 gai $ha^{-1}$ | Dripping at 2 DBS[1/] in the following season | 80.0 | 6,770 | 70.0 | 4,166 |

[1/] Days before Sowing

Table 2. Effect of herbicide application timing and method on wet sown rice in small plot trials (average of 2 locations), May-December 2004

| Treatment | Application method and timing | Phytotoxicity (%)[1/] | | No. of rice tiller $m^{-2}$ | | Rice height (cm) | | |
|---|---|---|---|---|---|---|---|---|
| | | 15 DAA[2/] | 30 DAA | 20 DAS[3/] | 105 DAS | 30 DAS | 60 DAS | 111 DAS |
| 1. pretilachlor 30% EC 600 gai $ha^{-1}$ | Dripping at 4 DBS[4/] | 6 | 0.0 | 294a[5/] | 336 a | 44.7 c | 78.6 d | 112.9 cd |
| 2. pretilachlor 30% EC 750 gai $ha^{-1}$ | Dripping at 4 DBS | 10 | 0.0 | 308a | 315 a | 47.4 ab | 85.0 ab | 120.0 a |
| 3. pretilachlor 30% EC 600 gai $ha^{-1}$ | Dripping at 2 DBS | 9.5 | 0.0 | 305a | 322 a | 45.7 bc | 82.5 abc | 116.6 abc |
| 4. pretilachlor 30% EC 750 gai $ha^{-1}$ | Dripping at 2 DBS | 11 | 0.0 | 291a | 321 a | 46.2 abc | 85.4 a | 119.2 ab |
| 5. pretilachlor 30% EC 412 gai $ha^{-1}$ | Spray at 2 DAS | 8 | 0.0 | 312a | 318 a | 46.1 abc | 80.8 cd | 116.2 bc |
| 6. Hand weeding (at 30 DAS) | | 0.0 | 0.0 | 285a | 312 a | 47.7 a | 83.4 abc | 116.6 abc |
| 7. Weedy check | | 0.0 | 0.0 | 242a | 255 b | 46.7 ab | 81.2 bcd | 110.9 d |
| C.V. (%) | | - | - | 10.71 | 8.67 | 2.66 | 3.15 | 2.16 |
| F-test | | - | - | Ns | * | * | * | ** |

[1/]Visual assessment; [2/] Days after Application; [3/] Days after Sowing; [4/] Days before Sowing;
[5/] In each column, means followed by the same letter are not significantly different at 5 % level by DMRT

Table 3. Effect of herbicide application timing and method on number of weeds at 40 days after sowing (small plot trials, average of 2 locations), May-December 2004

| Treatment | Application method and timing | Number of weed $m^{-2}$ and dry weed weight in gm $m^{-2}$ at 40 DAA[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ECHCG | | LEPCH | | SPHZE | | CYPDI | |
| | | Number | Dry weight | Number | Dry weight | Number | Dry weight | Number | Dry weight |
| 1. pretilachlor 600 gai $ha^{-1}$ | Dripping at 4 DBS[2] | 10.0 b[3] | 12.7 b | 0.0 a | 0.0 a | 43.3 b | 2.9 a | 7.0 a | 0.5 a |
| 2. pretilachlor 750 gai $ha^{-1}$ | Dripping at 4 DBS | 1.8 a | 1.5 a | 0.0 a | 0.0 a | 12.3 a | 1.0 a | 2.0 a | 0.1 a |
| 3. pretilachlor 600 gai $ha^{-1}$ | Dripping at 2 DBS | 5.0 ab | 7.1 ab | 0.5 a | 0.0 a | 28.5 ab | 3.3 a | 1.0 a | 0.0 a |
| 4. pretilachlor 750 gai $ha^{-1}$ | Dripping at 2 DBS | 4.3 ab | 6.0 ab | 1.0 a | 0.1 a | 20.0 a | 2.5 a | 0.0 a | 0.0 a |
| 5. pretilachlor 412 gai $ha^{-1}$ | Spray at 2 DAS | 4.3 ab | 3.1 a | 0.0 a | 0.0 a | 28.3 ab | 2.5 a | 1.5 a | 0.2 a |
| 6. Hand weeding (at 30 DAS) | | 1.8 a | 0.0 a | 0.5 a | 0.1 a | 7.8 a | 0.4 a | 16.0 a | 1.3 a |
| 7. Weedy check | | 27.8 c | 44.0 c | 36.0 a | 3.6 b | 141.0 c | 23.2 b | 396.0 b | 53.7 b |
| C.V. (%) | | 50.52 | 50.16 | 318.21 | 267.65 | 35.26 | 52.82 | 58.91 | 62.20 |
| F-test | | ** | ** | Ns | * | ** | ** | ** | ** |

[1] Days after Application; [2] Days before Sowing , [3] In each column, means followed by the same letter are not significantly different at 5 % level by DMRT

Table 4. Effect of herbicide on rice yield (small plot trials, average of 2 locations), May-December 2004

| Treatment | Application method and timing | Rice yield (kg $ha^{-1}$)[1] |
|---|---|---|
| 1. pretilachlor 600 gai $ha^{-1}$ | Dripping at 4 DBS[2] | 5555 c[3] |
| 2. pretilachlor 750 gai $ha^{-1}$ | Dripping at 4 DBS | 6326 a |
| 3. pretilachlor 600 gai $ha^{-1}$ | Dripping at 2 DBS | 5907 bc |
| 4. pretilachlor 750 gai $ha^{-1}$ | Dripping at 2 DBS | 6262 ab |
| 5. pretilachlor 412 gai $ha^{-1}$ | Spray at 2 DAS | 6078 ab |
| 6. Hand weeding (at 30 DAS) | | 6261 ab |
| 7. Weedy check | | 4291 d |
| C.V. (%) | | 4.59 |
| F-test | | ** |

[1] At 14% moisture content at 105 days after sowing; [2] Days before Sowing , [3] In each column, means followed by the same letter are not significantly different at 5 % level by DMRT

## CONCLUSION

SOFIT EC 300 (pretilachlor/fenclorim) dripping during the last field leveling is an effective solution for the control of weedy rice infestations in tropical direct seeded wet-sown rice. The specially designed dripping device facilitates the combination of herbicide application and land leveling into one operation. This innovative approach delivers a new application method, broad-spectrum weed control and a unique solution to manage the increasing infestations of weedy rice in direct seeded wet-sown rice in South East Asia.

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# Impact of different application methods of pretilachlor (Sofit®) on weed control in wet-seeded rice

A.S.K Abeysekera[1], R.M. Bandara[2], B.Marambe[2]
[1] Rice Research and Development Institute, Batalagoda, Sri Lanka
[2] Faculty of Agriculture, University of Peradeniya, Sri Lanka
anuru@sltnet.lk, bmarambe@pdn.ac.lk

**Abstract:** Weeds are major biotic stresses resulting in 20% to 40% yield losses in wet-seeded rice. Use of herbicides is the commonly used weed control method of rice farmers in Sri Lanka. The present study was conducted at Rice Research and Development Institute in 2004 during the minor cultivating season (April-August) using randomized complete block design with 4 replicates, to investigate the weed control efficacy of pretilachlor as a pre- emergence herbicide under different application methods, and its phytotoxicity on rice. Recommended rate of pretilachlor (1.6 L $ha^{-1}$) was applied at just before sowing with a knapsack sprayer, as a sand mix and with a splash bottle. Hand weeding and no weeding treatments were used as controls. The results revealed that different application methods effectively controlled major grasses, broadleaves and sedges in the respective treatments except *Aeschynomene indica* and *Cyperus difformis*. Different application methods of pretilachlor did not show any negative impact on germination, plant height, leaf area, biomass production, yield, and yield components of rice. Results indicated that the three application methods were effective in controlling weeds associated with wet-seeded rice.

**Key words**: Application method, pretilachlor, weeds, wet-seeded rice.

## INTRODUCTION

Weeds are major biotic stress in rice cultivation in Sri Lanka. Yield losses caused by weeds in rice (*Oryza sativa* L.) under Sri Lankan conditions have been estimated by various researchers to be 20% (Velmurugu 1980), 20-30% (Darmarathna and Jayawardena 1989; Gunasena 1992), and 20-40% (Herath Banda et al. 1998, Amarasinghe and Marambe 1998). At present, grassy weeds have become the main biotic problem in rice cultivation in the dry and intermediate zones whereas broadleaves and sedges are dominant in wet zones of Sri Lanka. Although integrated weed management methods promoted among farmers include cultural as well as chemical methods, use of chemicals has become the predominantly adopted cost-effective option among 90% of farmers in the dry and intermediate zones and 50 to70% farmers in the wet zone.

Over the past four to five decades, more than 20 herbicides have been recommended for rice cultivation in Sri Lanka. However, propanil has been most widely used as early-post emergence grass herbicide in rice due to its effectiveness on local weed composition and agro-climatic conditions. In the late 1990's bispyribac sodium was recommended and has gained island-wide acceptance among rice farmers within a short period, resulting in effective weed control under both research and farmer managed conditions (Abeysekera 1999).

In the 1980's, the first group of pre-emergence herbicides was introduced in Sri Lanka comprising of butachlor, pretilachlor and molinate. However, these herbicides were not popular among farmers due to rapid spread of broadcast crop establishment technique and poor crop management practices including land preparation, leveling and water management. Phytotoxicity problems were observed in rice exposed to pre-emergence herbicide (Abeysekera 1999). The farmers also perceived that crop damage could be caused to rice seedlings by walking in the field when applying herbicides during the first week of crop establishment. However, with the advancement in farming education programs and farmer's own experience, pretilachlor being a pre-emergence herbicide is becoming popular among rice farmers in Sri Lanka.

Use of knapsack sprayers with 8 to16 L capacity is the most widely used herbicide application technique in Sri Lanka. A more selective, effective, easy, and economical application technique, with an accurate dosage of herbicide is important in achieving higher weed control efficacy. Therefore, the objectives of this study were to compare the weed control efficacy among three different application methods of pretilachlor in wet seeded rice and their phytotoxicity on rice.

## MATERIALS AND METHODS

The experiment was carried out at the Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka, located in the low country intermediate zone ($IL_1$) during the minor cultivating season, April-August 2004. The site receives an annual rainfall ranging from 1500 to 2285 mm, and the daily mean temperature varies from 23°C to 28°C. The experimental field was a low land containing low humic gley (LHG) soil. A three-month old rice variety, Bg 300, was used for the experiment. Pretilachlor (Sofit®) was used as a pre-emergence herbicide at 1.6 L $ha^{-1}$. The application methods used are T1: Sand mixture – 1.6 L $ha^{-1}$ herbicide diluted in 2.5 to 3 L of water, mixed with 60 to 70 kg of coarse river sand and applied just before sowing, T2: Splash application technique – 1.6 L $ha^{-1}$ herbicide diluted in 10 l of water and splashed evenly on the paddy field with 3-5 cm standing water by using a Splash Pack® 2 days before sowing (the herbicide was retained for 2 days in the field and water was drained just before sowing), T3: Spraying using a knapsack sprayer – 1.6 L $ha^{-1}$ herbicide diluted in 350 L of water and applied using 16 L capacity knapsack sprayer, T4: Hand Weeding, and T5: No Weeding.

The experiment was laid out in a randomized complete block design with 4 replicates. Cultural practices, fertilizer and pest management were done according to the recommendations of the Department of Agriculture. Weed counts and dry weights of individual species were taken at 6 weeks after sowing (WAS). Growth and development of rice were recorded at 4, 6, and 8 WAS, together with the yield components and final yield. Visual phytotoxic symptoms on rice plants were obtained at 2, 4 and 6 weeks after application of herbicide. The data were analyzed using the SAS computer software package and mean separation was done by Duncan multiple range test (DMRT).

## RESULTS AND DISCUSSION

The major weed flora found in the experimental area is shown in Table 1. The dominant weed group was grasses, followed by broadleaves and sedges. Among grasses *Leptochloa chinensis, Echinochloa crus-galli* and *Isachne globosa* were the dominant species.

Table 1. The major weed flora found in experimental area.

| Grasses | | Sedges | | Broadleaves | |
|---|---|---|---|---|---|
| Species | (%) | Species | (%) | Species | (%) |
| *L. chinensis* | 14.5 | *C difformis* | 6.0 | *M. vaginalis* | 9.2 |
| *E. crus-galli* | 7.2 | *C. iria* | 5.8 | *C. diffusa* | 7.9 |
| *I. globosa* | 6.8 | *F. miliacea* | 5.4 | *E. alba* | 5.9 |
| *P. distichum* | 4.3 | *F. dichotoma* | 2.4 | *M. nudiflora* | 5.4 |
| *E. colona* | 0.9 | *C. rotundus* | 1.2 | *M. quadrifolia* | 3.9 |
| | | | | *L. octovalvis* | 2.2 |
| | | | | *A. indica* | 2.2 |

Weed control efficacy

Significantly higher number of grass, broadleaves and sedges were observed in the no- weeding treatment when compared to the rest. Pretilachlor effectively controlled grasses, broadleaves and sedge weeds, but the results were not significantly different from the hand weeded treatments (Table 2). The three application techniques did not show any significant difference in weed control efficacy of the herbicide. However, the results revealed that pretilachlor has relatively poor control over *Aeschynomene indica, Cyperus difformis* and *Isachne globosa*.

Table 2. Effect of different method of application of Pretilachlor on weed count and dry weight at 6WAS.

| Treatment | Weed count (no $m^{-2}$) | | | Weed dry weight (g $m^{-2}$) | | |
|---|---|---|---|---|---|---|
| | Grasses | Broad leaves | Sedges | Grasses | Broad leaves | Sedges |
| Sand Mixture | 17.6 b | 3.2 c | 8.7 b | 6.3 b | 1.1 b | 1.0 b |
| Splash | 24.5 b | 6.7 bc | 5.4 b | 6.7 b | 1.1 b | 0.85 b |
| Spray | 18.4 b | 11.3 b | 8.4 b | 6.4 b | 1.2 b | 0.83 b |
| Hand Weeding | 4.5 c | 3.4 c | 2.2 c | 0.5 c | 0.0 b | 0.32 b |
| No Weeding | 59.9 a | 24.6 a | 28.3 a | 18.6 a | 17.0 a | 11.77 a |

Within a column, means followed by same letters are not significantly different by the DMRT at p=0.05.

In general, the weed control efficacy of pretilachor was lower on broadleaf weeds than on grasses and sedges (Table 2). The results also revealed that different spray volumes adopted in three different herbicide application techniques did not affect the weed control efficacy of pretilachlor. However, increase in spray volume of propanil has been reported to increase the weed control efficacy (Nakornsri 1996; Anonymous 1985). This contradiction in results may be due to the different modes of action and time of application of the herbicide.

Phytotoxicity on rice plant

(a) Germination and plant count of rice

At 2 WAS rice seedling count did not show any significant difference among the treatments (Table 3), suggesting that different application methods of pretilachlor did not affect the germination of rice seeds in wet-seeded conditions. Similar results were observed at 4 and 6 WAS. In the no-weeding treatment, a significantly lower plant count was observed at 6 WAS, which could be the result of severe competition exerted on rice plants by weeds.

Table 3. Effect of different application methods of pretilachlor on rice plant count at 2, 4, and 6 weeks after sowing (WAS).

| | Rice plant count (No $m^{-2}$) | | |
|---|---|---|---|
| Treatments | 2WAS | 4WAS | 6WAS |
| Sand Mixture | 265.9 a | 319.4 a | 564.6 a |
| Splash | 298.3 a | 313.2 a | 451.4 a |
| Spray | 302.4 a | 306.2 a | 454.3 a. |
| Hand Weeding | 286.3 a | 327.6 a | 512.5 a |
| No Weeding | 301.5 a | 289.0 a | 315.7 b |

Within a column, means followed by same letters are not significantly different by the DMRT at p=0.05.

(b) Growth and development of rice

The herbicide treatments did not have any detrimental impact on the growth and development of rice plants. Plant height, leaf area index (LAI) and dry matter production obtained at 6 WAS showed no significant difference among the herbicide-treated plots and hand weeded treatments (Table 4). However the lowest dry matter weight and LAI were observed in the no-weeding treatment, probably due to the heavy weed infestation.

Table 4. Effect of different application methods of pretilachlor on plant height, dry matter production and LAI of rice at 6 WAS.

| Treatments | Growth parameters | | |
|---|---|---|---|
| | Plant height (cm) | LAI | Dry matter ($g\ m^{-2}$) |
| Sand Mixture | 55.5 a | 4.24 a | 517.4 a |
| Splash | 51.0 a | 4.57 a | 546.8 a |
| Spray | 51.3 a | 4.59 a | 505.4 a |
| Hand Weeding | 52.9 a | 4.64 a | 496.3 a |
| No Weeding | 50.5 a | 2.23 b | 217.4 b |

Within a column, means followed by same letters are not significantly different by the DMRT at p=0.05.

(c) Yield and yield components of rice

No significant differences in yield and yield components of rice were observed between pretilachlor-treated plots and hand-weeded plots. However, yield and yield components were significantly lower in the no-weeding treatment, which could be due to the higher density of weeds (Table 5). The percentage grain filling and 1000-grain weight were not significantly different. However, the number of grains per panicle and panicles per square meter were significantly low in no-weeding plots than the other treatments (Table 5). These results clearly showed that pretilachlor, applied using the three techniques, had no detrimental effect on early growth and yield of rice. No visual phytotoxicity symptoms of rice plants were observed with pretilachlor in rice fields.

Table 5. Effect of different methods of application of pretilachlor on yield and yield components of rice.

| Treatment | Yield and yield components | | | | |
|---|---|---|---|---|---|
| | Number of Panicles $m^{-2}$ | Number of Grains $panicle^{-1}$ | Grain filling (%) | 1000-grain weight (g) | Yield ($t\ ha^{-1}$) |
| Sand Mixture | 592.5 a | 88.3 a | 86.8 a | 26.65 a | 3.90 a |
| Splash | 547.5 a | 91.2 a | 90.7 a | 26.25 a | 4.15 a |
| Spray | 588.6 a | 85.4 a | 89.1 a | 26.15 a | 4.18 a |
| Hand Weeding | 608.3 a | 94.5 a | 93.3 a | 26.41 a | 4.40 a |
| No Weeding | 302.4 b | 51.3 b | 89.7 a | 25.74 a | 2.20 b |

Within a column, means followed by same letters are not significantly different by the DMRT at p=0.05.

Pretilachlor is an effective herbicide for controlling annual grasses, broadleaves and sedges in wet-seeded rice fields. The efficacy of control of broadleaves by the herbicide is relatively lower when compared to that of grasses and sedges. However, this was not reflected in total weed biomass and grain yield of rice. Weed control efficacy of pretilachlor was not significantly different when the chemical was applied using a knapsack sprayer, splash pack or as a sand mixture. The three different methods of pretilachlor application did not cause any visual phytotoxic symptoms and also showed no significant effect on early growth, yield and yield components of rice. The splash pack and sand mixture techniques are easy, and cost effective for the farmer. However, further studies are needed to verify the effectiveness and farmer acceptance of these methods under different management practices.

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# Herbicidal performance of flucetosulfuron in controlling *Echinochloa* species in rice cultivation

Do-Soon Kim[1], K. H. Hwang[1], J. N. Lee[1], J. S. Lim[1], S. J. Koo[1], D. V. Chin[2]
[1]R&D Park, LG Life Sciences, Ltd, Yuseong-gu, Daejeon 305-380, Korea
[2]Department of Weed Science and Farming System, Cuulong Delta Rice Research Institute, Omon, Cantho, Vietnam
Correspondence to dosoonkim@lgls.co.kr

**Abstract**: Field trials were conducted to test efficacy of flucetosulfuron on *Echinochloa crus-galli* in many countries including Korea, Japan, China and Vietnam from YR1 to YR2. Flucetosulfuron provided excellent control of *Echinochloa crus-galli* in various rice cultivation systems by soil and foliar application. By soil application, flucetosulfuron controlled *E. crus-galli* up to 5 leaf stage and its appropriate dose ranged from 10 to 30 g a.i. $ha^{-1}$. By foliar application, flucetosulfuron controlled *E. crus-galli* up to 6 leaf stage and its appropriate dose ranged from 20 to 50 g a.i. $ha^{-1}$ Collectively, flucetosulfuron demonstrated stable performance across widely different field conditions at very low application rates (10 to 50 g ai. $ha^{-1}$), and showed flexibility in terms that both soil and foliar application were possible in a very wide application window.

**Key words**: Flucetosulfuron, *Echinochloa crus-galli*, barnyardgrass, rice

## INTRODUCTION

Flucetosulfuron (LGC-42153) is a new sulfonylurea herbicide developed for rice (Koo et al., 2003). Like other sulfonylurea herbicides, flucetosulfuron inhibited acetolactate synthase (ALS) (Hwang et al., 2003) and was absorbed via roots, stem and leaf, and its translocation via leaf was faster than glyphosate and pyribenzoxim (Lee et al., 2003). When applied to soil or foliage, flucetosulfuron provides broad-spectrum weed control including annual broad leaf weeds, sedges, some grasses such as *Echinochloa* spp. and perennial weeds in paddy rice field (Kim et al., 2003). In particular, strong activity of flucetosulfuron against *Echinochloa* spp. may render flucetosulfuron different from other sulfonylurea herbicides. Due to its broad-spectrum weed control including *Echinochloa* spp., flucetosulfuron was registered as a solo one-shot herbicide (Fluxo®) in Korea.

From the early stage of flucetosulfuron development, we conducted various field studies to evaluate field performance of this herbicide not only in Korea but also in rice-cultivating countries. Therefore, in this report, we summarize results of field trials in rice-cultivating countries including Korea, Japan, China, Vietnam, Thailand and so on.

## MATERIALS AND METHODS

To evaluate the herbicidal performance of flucetosulfuron in controlling barnyardgrass (*Echinochlo crus-galli*), pot experiments were conducted in a glasshouse at LG Life Sciences R&D Park, Daejeon, Korea. *Echinochloa crus-galli* was grown in 10 cm x 10 cm plastic pots. Foliar application was made at 3, 5 and 7 leaf stages using $CO_2$-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8002E flat fan nozzle adjusted to deliver 1000 L $ha^{-1}$, while soil application was made at 1~6 leaf stages by direct application of the herbicide formulation to flooded soil in the pots. Formulations of flucetosulfuron were 50% WG and 0.07% GR for foliar and soil application, respectively. Fresh weight was measured at 30 days after application. All experiments consisted of three replicates of a completely randomized design.

To evaluate the field performance of flucetosulfuron, field experiments were conducted in rice fields in Korea, Japan, China, Vietnam, Thailand, Italy, Spain and Brazil. Foliar application was made using a $CO_2$-pressurized knapsack sprayer, while soil application was made at 1~6 leaf stages by direct application of the herbicide formulation to flooded soil. Formulations of flucetosulfuron were 10% or 50% WG for foliar application and 0.07% GR for soil application. Visual assessment was made at 30~50 days after application.

## RESULTS AND DISSCUSSION

### Performance in the glasshouse

Flucetosulfuron controlled *E. crus-galli* very effectively by soil or foliar application. Flucetosulfuron applied to flooded soil controlled effectively *E. crus-galli* up to 6 leaf stage at 20 g a.i.ha$^{-1}$, while molinate, a well-known grass killer in transplanted rice cultivation, controlled only up to 2 leaf stage (Figure 1A). Foliar applied flucetosulfuron also performed well, providing more than 90% control of *E. crus-galli* up to 5 leaf stage with less than 20 g a.i. ha$^{-1}$ of flucetosulfuron (Figure 1B). This result thus indicates that flucetosulfuron at 20 g a.i.ha$^{-1}$ provides similar control up to about 5-leaf stage for *E. crus-gaill* by soil or foliar application.

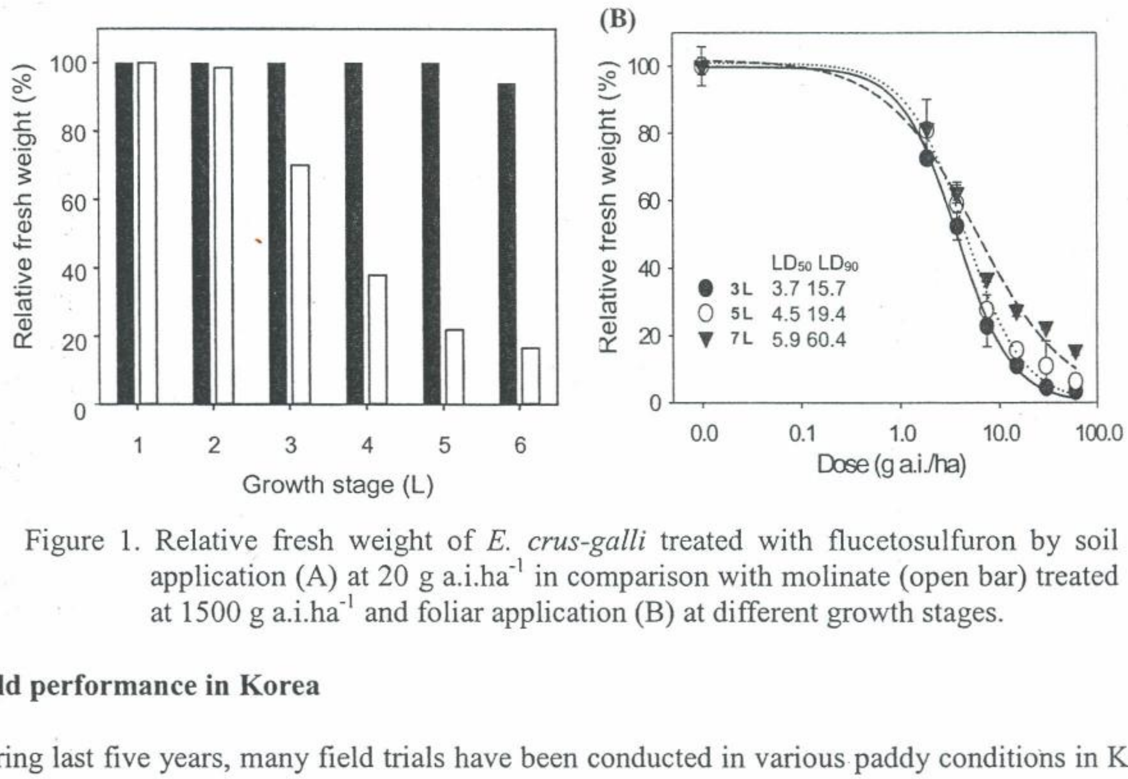


Figure 1. Relative fresh weight of *E. crus-galli* treated with flucetosulfuron by soil application (A) at 20 g a.i.ha$^{-1}$ in comparison with molinate (open bar) treated at 1500 g a.i.ha$^{-1}$ and foliar application (B) at different growth stages.

### Field performance in Korea

During last five years, many field trials have been conducted in various paddy conditions in Korea. In the case of soil application, flucetosulfuron showed very good efficacy to *E. crus-galli* up to 4 leaf stage in paddy fields (Figure 2A). Flucetosulfuron of 10 g a.i.ha$^{-1}$ controlled more than 95% of *E. crus-galli* at 1~2 leaf stage, showing the similar efficacy to commercial standards including pretilachlor mixtures. At 20 g a.i ha$^{-1}$, flucetosulfuron controlled effectively *E. crus-galli* up to 4 leaf stage with superiority to commercial standards including molinate + pyrazosulfuron-ethyl mixture. In the case of foliar application, flucetosulfuron showed good efficacy to *E. crus-galli* in paddy fields (Figure 2B). Flucetosulfuron at 15 g a.i. ha$^{-1}$ achieved about 90% control of *E. crus-galli* up to 4 leaf stage. When increased the dose to 30 g, the herbicide controlled *E. crus-galli* up to 5 leaf stage with superiority to commercial standard, bentazone + cyhalofop-ethyl.

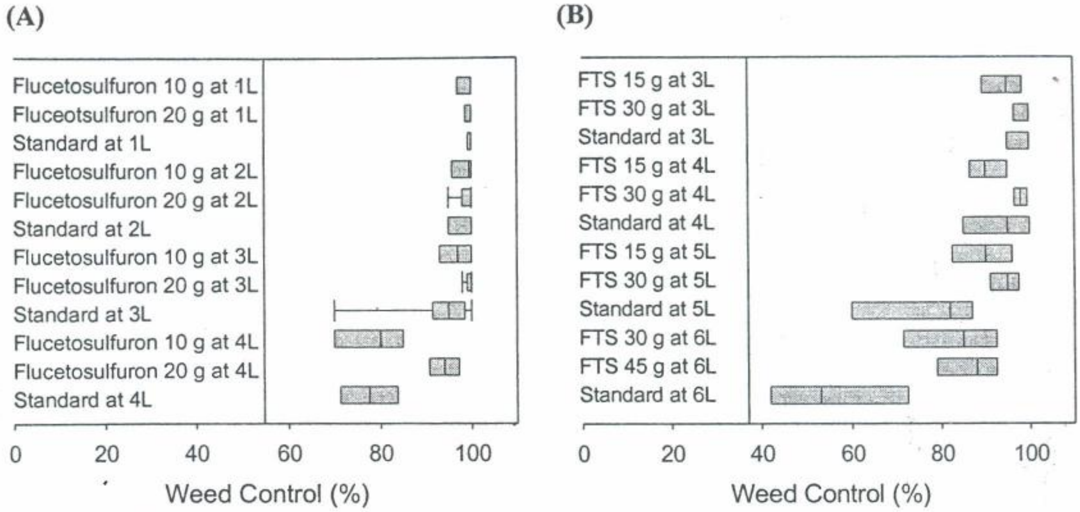


Figure 2. Field performance of flucetosulfuron in controlling *E. crus-galli* by soil application (A) and foliar application (B) at different growth stages in Korea in 2000~2004.

**Field performance in Japan**

During last four years, many field trials have also been conducted in various paddy conditions in Japan. In the case of soil application, flucetosulfuron showed very good efficacy to *E. crus-galli* up to 5 leaf stage in paddy fields (Figure 3A). Flucetosulfuron controlled more than 90% of *E. crus-galli* up to 4 and 5 leaf stage at 20 and 30 g a.i. $ha^{-1}$, respectively. At 20 g a.i $ha^{-1}$, flucetosulfuron controlled effectively *E. crus-galli* up to 4 leaf stage with superiority to commercial standards including pyrminobac mixtures. In particular, the efficacy to *E. crus-galli* at 5 leaf stage was very unique performance of flucetosulfuron. It was unable to compare this efficacy with other commercial standards as there were no products registered to control such a large *E. crus-galli*. In the case of foliar application, flucetosulfuron also showed good efficacy to *E. crus-galli* in paddy fields (Figure 3B). Flucetosulfuron at 20 g a.i. $ha^{-1}$ achieved about 90% control of *E. crus-galli* at 4 leaf stage. When increased the dose to 45 g, the herbicide controlled *E. crus-galli* completely with superiority to commercial standard, bentazone + cyhalofop-ethyl.

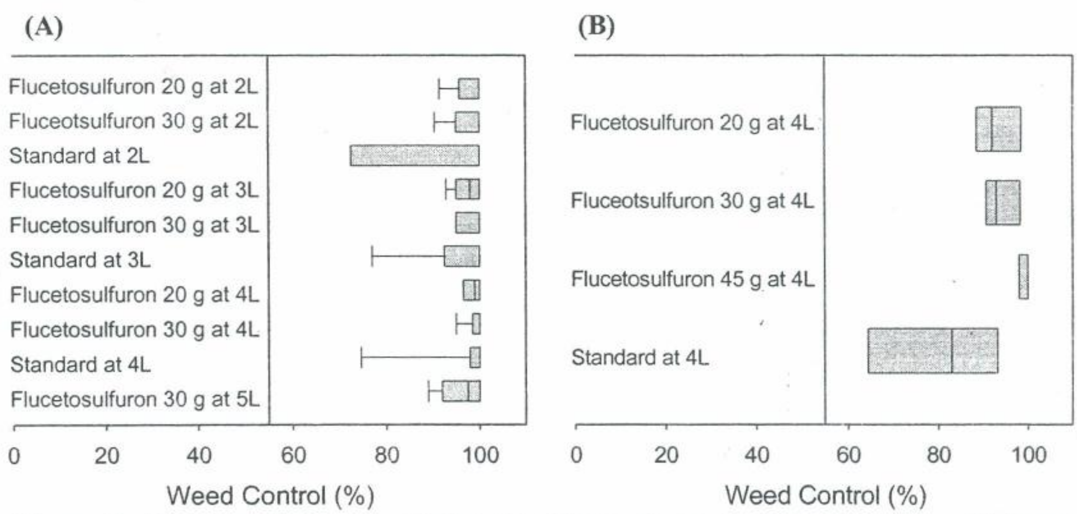


Figure 3. Field performance of flucetosulfuron in controlling *E. crus-galli* by soil application (A) and foliar application (B) at different growth stages in Japan in 2001~2004.

**Field performance in China**

In 2003 and 2004 several field trials have also been conducted in various paddy conditions in China. In the case of soil application, flucetosulfuron at 15~20 g a.i. $ha^{-1}$ showed very good efficacy to *E. crus-galli* up to 3 leaf stage in paddy fields (data not shown). In the case of foliar application, flucetosulfuron showed good efficacy to *E. crus-galli* in paddy fields (Figure 4). Flucetosulfuron at 30 g a.i. $ha^{-1}$ achieved more than 90% control of *E. crus-galli* at 5 leaf stage. By comparison with commercial standard, bensulfuron-methyl + quinclorac, flucetosulfuron performed better in controlling *E. crus-galli*.

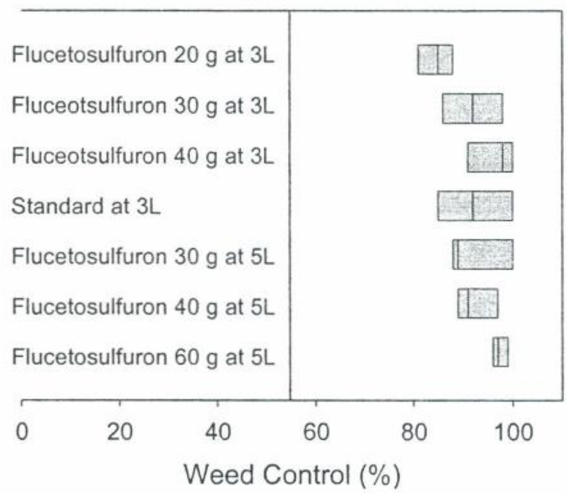


Figure 4. Field performance of flucetosulfuron in controlling *E. crus-galli* by foliar application at different growth stages in China in 2003~2004.

**Field performance in the other countries**

Field trials have also been conducted in Europe, South Asia, and South America to evaluate field performance of flucetosulfuron when applied to foliage of *E. crus-galli* in wet-direct seeded rice cultivation. In Europe, particularly Spain and Italy, flucetosulfuron controlled *E. crus-galli* effectively with equal or superior activity to the commercial standard, cyhalofop-butyl. In Vietnam, when application was made at 14 days after rice seeding, flucetosulfuron at 20 g a.i. $ha^{-1}$ achieved about 98% control of *E. crus-galli* with similar efficacy to cyhalofop-butyl. In Thailand, flucetosulfuron performed a little inferiorly at 20 g a.i. $ha^{-1}$ to bispybibac-sodium, but superiorly at 40 g a.i. $ha^{-1}$ to bispyribac-sodium. In Brazil, flucetosulfuron achieved 90% and 95% control of *E. crus-galli* at 40 and 50 g a.i. $ha^{-1}$, respectively, with superior efficacy to cyhalofop and similar to bispyribac-sodium. In overall, it can be concluded that flucetosulfuron performed better than cyhalofop-butyl and similar to bispyribac-sodium in controlling *E. crus-galli* in rice cultivation in many countries.

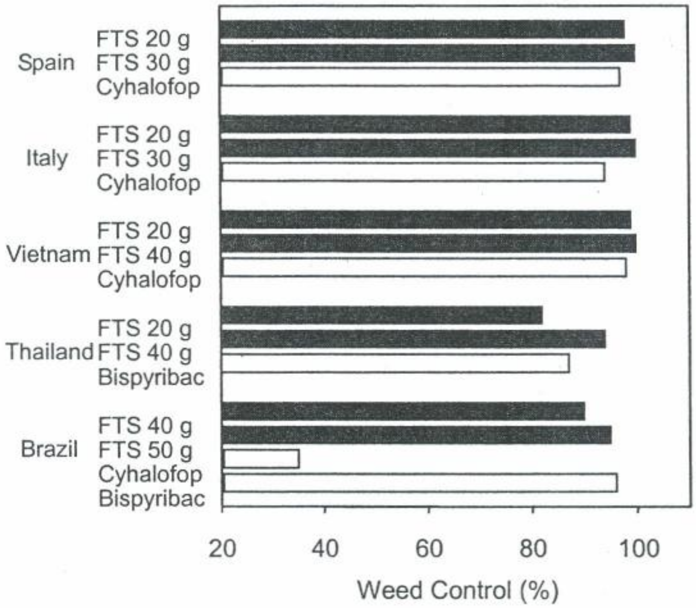


Figure 5. Field performance of flucetosulfuron in controlling *E. crus-galli* by foliar application in Spain, Italy, Vietnam, Thailand and Brazil.

In conclusion, these results show that herbicidal performance of flucetosulfuron in controlling *E. crus-galli* was well demonstrated in various field conditions in many rice-cultivating countries from the North (China) to the South (Brazil). Unlike other herbicides for *E. crus-galli* control, flucetosulfuron controls *E. crus-galli* by both soil and foliar application. Field performance of flucetosulfuron by soil and foliar application and strong activity of controlling *E. crus-galli* up to 5 leaf stage by soil application may thus render flucetosulfuron more flexible and vastly usable for *E. crus-galli* control in various weed control systems.

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**Weed control effect of a new herbicide LGC-42153 in rice fields in China**

X. L. Song, S. Qiang and B. Ma
Weed Research Laboratory Nanjing Agricultural University, 210095, China
wrl@njau.edu.cn

**Abstract:** LGC-42153 is a new sulfonylurea herbicide developed by LG Life Sciences Ltd., Korea. This herbicide can be used to control weeds in both direct seeded and transplanted rice fields. The herbicide has been introduced in China in 2002 to be evaluated for soil and foliar application. The results showed that it could provide excellent control above 90% to barnyard grass (*Echinochloa spp.*), broadleaf and sedge weeds at recommendation rates of 25 to 40 g a.i. $ha^{-1}$ in rice field. It was especially effective to old barnyard grass seedlings with more than seven leaves. And it was safe to rice and the successive crops in field trials.

**Key words**: Control effect, *Echinochloa* spp., flucetosulfuron, LGC-42153, rice, safety.

## INTRODUCTION

Since the mid 1980's, sulfonylurea herbicides such as bensulfuron-methyl and pyazosulfuron-ethyl have been used in paddy fields with diversified cultivation methods. They become the most important herbicides in paddy weed control in the world. They are characterized by high activity and selectivity at extraordinary low application rates, low mammalian toxicity, good safety for crops and environment, convenient application and broad spectrum. And they can control many kinds of annual and perennial weeds such as broadleaf, and sedge weeds (Zhang et al. 2000). However, most of the sulfonylurea herbicides can not provide good control to barnyard grass (*Echinochloa* spp.), one of the most important paddy weeds. They need to be mixed with the other herbicides which could kill barnyard grass such as acetochlor and butachlor so that it could reach one-shot control effect.

LGC-42153, a new sulfonylurea herbicide, has been developed by LG Life Sciences Ltd and Chemical Technique Research Association, Korea jointly (Hwang et al. 2003; Koo et al. 2003; Lee et al. 2003). The popular Chinese, English and trade names are Fubihuanglong, Flucetosulfuron (ISO-proposed) and Hanlesheng respectively. Its molecular weight is 487.3. The molecular formula is $C_{18}H_{21}FN_5O_8S$. LGC-42153 can be used for both soil and foliar application. It has broad spectrum, including gramineous weeds such as barnyard grass, broadleaf weeds and sedges.

The prominent characteristic of LGC-42153 is that it controls barnyard grass very well either by soil or foliar application. In direct seeded rice, LGC-42153 usually was more effective in controlling older *Echinochloa* plants up to 2 –5 leaf stage by spraying, even at 7 leaf-stage in drained plot. In transplanted rice field, LGC-42153 was applied at 5-15 days after transplanting when barnyard grass had 1.5 to 3 leaf stage. Besides it could control other weeds very well. Its persistence was 30-40 days, significantly longer than that of pyrazosulfuron and molinate (Lee et al. 2003). Single application of LGC-42153 could control barnyard grass plants throughout the growing period of rice. And it was safe to rotational crops.

The new type herbicide was authorized in Korea in 2004. Some field experiments were conducted from 2002 for registering in China. The objectives of this study were to evaluate the control effect of this herbicide on barnyard grass and other weeds and determine its safety to rice in China.

## MATERIALS AND METHODS

### Herbicide

10% LGC-42153 WP, supplied by LG Life Sciences Ltd Korea. 53% Mefenacet. Bensulfuron—methyl WP produced by Kexing Pesticide Factory of Nantong, China.

### Rice

Japonica rice variety Yeguang and Wuxianggeng (*Oryza sativa* L.) were used in Nanjing and Nantong, respectively.

### General conditions

Field experiments were conducted in both direct seeded rice and transplanted rice field in Nanjing in 2002 and Nantong in 2003 and 2004. The experimental field was located at Xigou village of Qixia Town of Nanjing on a loam soil with 2.18% organic matter and pH 6.8, While at Xueyao Town of Nantong on a sandy soil with 1.1% organic matter and pH 7.2. In direct seeded rice fields, all rice seeds were soaked in water for 24 h before sowing and was sown at the rate of 200 kg $ha^{-1}$ in Nanjing and Nantong respectively. The fields were flooded at 3-4 leaf stage of the rice seedlings. In transplanted fields, all rice seedlings were transplanted at 4-5 leaf stage. Regular water management practices were used.

### Herbicide application

In the Nanjing experiment, the herbicide was applied at 3-5 leaf stage of weeds on July 4, 2002. In the Nantong experiment, the herbicide was applied at 5-7 leaf stage of barnyard grass on July 15, 2003 and 2004 in both direct seeded (at 45 days after seeding) and transplanted rice field (at 27 days after transplanted). The control herbicide was 25% Quinclorac plus bensulfuron—methyl SC 150 g a.i. $ha^{-1}$ in direct seeded rice in Nanjing, and 53% Mefenacet plus Bensulfuron—methyl WP 318 g a.i. $ha^{-1}$ in transplanting rice in Nanjing and in direct seeded rice and transplanting rice in Nantong. The rate of LGC-42153 was 20, 30, 40, 60 g a.i. $ha^{-1}$ in both direct seeded rice and transplanted rice in Nanjing, 20, 30, 40, 60 g a.i. $ha^{-1}$ in direct seeded rice and 15, 25, 35, 50 g a.i. $ha^{-1}$ in transplanted rice in Nantong, respectively. All herbicides were applied as broadcast sprays at 450 kg $ha^{-1}$ with a hand-pressurized sprayer. All experimental design was a randomized complete block with four replicates per treatment.

### Control effect investigation and data statistics

The control effect was investigated at 20 and 50 days in Nanjing and at 20 and 45 days after application in Nantong in direct seeded rice and in transplanted rice, respectively. Weed densities and fresh weight were determined in five 0.11$m^2$ samplings selected randomly from each plot.
All data analysis was done using SPSS for Windows (SPSS Inc., 2001). The data were analyzed by analysis of variance and treatment means were compared by Duncan's multiple range test at the 5% and 1% level of significance.

## RESULTS AND DISCUSSION

Experimental results in direct seeded rice fields

The results in direct seeded rice of Nanjing and Nantong are shown in Tables 1-3. LGC-42153 provided 73-88% control at 20 g a.i. $ha^{-1}$ and 30 g a.i. $ha^{-1}$ which was similar with that of the control herbicide, while 90-96% control at 40 g a.i. $ha^{-1}$ and 60 g a.i. $ha^{-1}$. To difformed galingale, it provided similar control at 20 g a.i. $ha^{-1}$ with the control herbicide, and significantly better than that of the control herbicide at other higher rates. All rates of LGC-42153 provided good control on broadleaf weeds,. The total control effect increased with the rates of LGC-42153, reaching 90% at 30 g a.i. $ha^{-1}$.

In the Nantong experiment, the results in two years showed that control effects of all experimental rates of LGC-42153 to barnyard grass at 3-7 leaf stage were between 70-100%, significantly higher than that of 53% mefenacet plus bensulfuron-methyl WP with only 10-30% control. There was no difference in control of sheathed monochoria, common ammannia and difformed galingale between recommended rates of LGC-42153 and 53% mefenacet plus bensulfuron-methyl WP. The total control effects of all experimental rates of LGC-42153 were significantly larger than that of 53% Mefenacet plus Bensulfuron—methyl W P.

In the Nanjing experiment, LGC-42153 exhibited relatively good control on all experimental weeds at all treatment rates particularly to barnyard grass and Chinese sprangletop.

Table 1. Control effect of LGC-42153 in direct seeded rice at 20 and 50 days after application. Nanjing, 2002.

| Rate G a..i. $ha^{-1}$ | DFA | Barnyard grass | | Chinese Sprangletop | | Difformed Galingale | | Broadleaf weeds | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | F | D | F | D | F | D | F | D | F |
| LGC 20 | 20 | 79.4Ab | | 73.3ABab | | 82.3Bb | | 84.4 Ab | | 82.5Ab | |
| | 50 | 81.1Ab | 83.3Ab | 75.7Ab | 83.2Ab | 90.9 Aab | 95.4Aab | 90.2 Aab | 93.5Aab | 87.3ABb | 86.4Ab |
| LGC 30 | 20 | 84.4Aab | | 77.9ABa | | 94.3 Aa | | 88.8 Aab | | 87.8Ab | |
| | 50 | 84.1Ab | 88.6Ab | 78.4ABa | 83.5Ab | 94.5Aa | 96.4Aa | 91.8 Aab | 96.0Aa | 89.3ABb | 90.4Aab |
| LGC 40 | 20 | 89.9Aa | | 82.1ABa | | 95.4Aa | | 92.4Aa | | 91.5aa | |
| | 50 | 90.0Aa | 91.4Aa | 82.3Aa | 86.5Aa | 96.1Aa | 96.9 Aa | 92.5Aa | 96.4 Aa | 91.3Aa | 92.5Aa |
| LGC 60 | 20 | 96.0Aa | | 85.3Aa | | 96.3Aa | | 94.7Aa | | 94.4Aa | |
| | 50 | 94.9Aa | 96.1Aa | 86.4Aa | 88.1Aa | 97.2Aa | 97.4 Aa | 95.6Aa | 98.0Aa | 94.7Aa | 96.2Aa |
| 25%Q.B | 20 | 81.0Ab | | 71.1Bb | | 81.7Bb | | 72.2Bb | | 75.1Bc | |
| 150 | 50 | 81.8Ab | 84.6Ab | 76.3Bb | 82.7Ab | 85.5Bb | 88.5Bab | 86.1 Bb | 91.0Ab | 84.4Bb | 86.6Ab |

Note:25%Q. B 150=25% Quinclorac+Bensulfuron—methyl SC, LGC=LGC42153, DFA=days after application
D= Density;;F=Fresh (the same as follow) . Numbers within the same column with different lowercase or uppercase letters are significantly different ($P<0.05$ and $P<0.01$, respectively; Duncan's new multiple range test). Unless otherwise indicated, Numbers with different letters in the following tables are the same meaning.

Table 2. Control effect of LGC-42153 in direct seeded rice at 20 and 45 days after application. Nantong, 2003.

| Rate g a..i.ha$^{-1}$ | DFA | Barnyard | | Sheathed | | Common | | Difformed | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | F | D | F | D | F | D | F | D | F |
| LGC 20 | 20 | 99.8Aa | | 98.1 Aa | | 85.0Bb | | 100Aa | | 98.1 Aa | |
| | 45 | 99.2Aa | 99.9 Aa | 99.4 Aa | 99.4 Aa | 67.8Bbc | 83.3Bb | 100 Aa | 100 Aa | 96.4 Aa | 98.7.Aa |
| LGC 30 | 20 | 99.8 Aa | | 98.1 Aa | | 88.4 Bab | | 100 Aa | | 98.3 Aa | |
| | 45 | 99.6 Aa | 100 Aa | 99.6 Aa | 99.6 Aa | 75.4Bb | 84.0Bb | 100 Aa | 100 Aa | 97.1 Aa | 98.8 Aa |
| LGC 40 | 20 | 99.9 Aa | | 100 Aa | | 93.5Aa | | 100 Aa | | 99.2 Aa | |
| | 45 | 100 Aa | 100Aa | 100 Aa | 100Aa | 77.3Bb | 92.8Aa | 100 Aa | 100 Aa | 98.2 Aa | 99.4 Aa |
| LGC 60 | 20 | 100 Aa | | 99.2 Aa | | 100Aa | | 100 Aa | | 99.9 Aa | |
| | 45 | 100 Aa | 100Aa | 100 Aa | 100Aa | 95.3Aa | 98.2Aa | 100 Aa | 100 Aa | 99.6 | 99.9 Aa |
| 53%M.B | 20 | 30.6Bb | | 87.2Bb | | 69.0Cc | | 100 Aa | | 33.3Bb | |
| 318 | 45 | 0.0Bb | 0 Bb | 100 Aa | 100A a | 100Aa | 100Aa | 100 Aa | 100 Aa | 53. 5 Bb | 50.5 Bb |

Note: 53% M.B. WP = Mefenacet+ Bensulfuron-methyl WP

Table 3. Control effect of LGC-42153 in direct seeded rice at 20 and 45 days after application. Nantong, 2004.

| Rate g a.i. ha$^{-1}$ | DFA | Barnyard grass | | Sheathed Monochoria | | Common Ammannia | | Difformed Galingale | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D | F | D | F | D | F | D | F | D | F |
| LGC 20 | 20 | 70. 7Dd | | 100Aa | | 58.4Cc | | 100 | | 75. 2Cc | |
| | 45 | 86. 1Bb | 95.5Aa | 99.6Aa | 99.7Aa | 69.8Cc | 93.5Ab | 100Aa | 100Aa | 83.4Bb | 95.6Aa |
| LGC 30 | 20 | 88.5Cc | | 100Aa | | 83.2Bb | | 96.1Aa | | 87.3 Bb | |
| | 45 | 94. 7Aa | 98.7Aa | 100Aa | 100Aa | 84.6Bb | 97.7Aa | 100 Aa | 100Aa | 92. 5Aa | 98.2Aa |
| LGC 40 | 20 | 91. 8Bb | | 100Aa | | 84.3Bb | | 100Aa | | 92. 2 Aa | |
| | 45 | 95. 3Aa | 98.9Aa | 100Aa | 100Aa | 91. 3Aa | 97.7Aa | 100 Aa | 100Aa | 94. 0Aa | 98.3Aa |
| LGC 60 | 20 | 97. 3Aa | | 100Aa | | 91. 6Aa | | 100Aa | | 94. 9Aa | |
| | 45 | 96. 9Aa | 99.3Aa | 100Aa | 100Aa | 93. 7Aa | 99.1Aa | 100 Aa | 100Aa | 96. 1Aa | 99.2 a |
| 53%M.B | 20 | 18. 5Cc | | 85. 7Bb | | 80. 3Bb | | 33Bb. 5 | | 57. 7Cc | |
| 318 | 45 | 11. 2Cc | 0Bb | 88. 7Bb | 95.7Aa | 94. 9Aa | 99.2Aa | 90. 5Aa | 96.4Aa | 63. 8Dd | 52.8Bb |

From the above results, LGC-42153 could provide good control effect to gramineous weeds, sedge and broadleaved weeds in direct seeded rice in China.

Experimental results in transplanted rice fields

The results in transplanted rice in Nanjing and Nantong are shown in Tables 4-6. In Nanjing, LGC-42153 at 20g a.i. ha$^{-1}$ and 30 g a.i. ha$^{-1}$ provided similar control effect to barnyard grass, chinese sprangletop, difformed galingale and broadleaf weeds with 53% mefenacet plus bensulfuron—methyl WP 318 g a.i. ha$^{-1}$. LGC-42153 at 40 g a.i. ha$^{-1}$ and 60 g a.i. ha$^{-1}$ had significantly better control than the standard herbicide. The results demonstrated that LGC-42153 at the recommended rate could control most of weeds including barnyard grass, difformed galingale and broadleaf weeds in transplanted rice fields.

Table 4. Control effect of LGC-42153 in transplanted rice at 20 and 50 days after application. Nanjing, 2002.

| Rate g a..i. ha$^{-1}$ | DFA | Barnyard grass D | Barnyard grass F | Chinese Sprangletop D | Chinese Sprangletop F | Difformed Galingale D | Difformed Galingale F | Broadleaf weeds D | Broadleaf weeds F | Total D | Total F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LGC 20 | 20 | 78.0Cc | | 71.9 Bb | | 78.8Cc | | 75.3Dd | | 75.6Dd | |
| | 50 | 83.0Bb | 83.1Bb | 78.6Abc | 75.4Ab | 91.1Aa | 90.9Aa | 86.1Cc | 91.6Aa | 85.5Cc | 85.2Bc |
| LGC 30 | 20 | 83.8Bb | | 76.9 ABa | | 84.8Bb | | 81.8Cc | | 81.8Cc | |
| | 50 | 85.7ABa | 89.9Ab | 78.3 Abc | 81.2Aa | 91.81Aa | 92.1 Aa | 86.2BCc | 93.1Aa | 85.6 Cc | 89.6Bc |
| LGC 40 | 20 | 86.9Ab | | 80.6Aa | | 85.3Bb | | 87.6Bb | | 86.7Bb | |
| | 50 | 90.9Aa | 93.6Aa | 81.2Aab | 83.7Aa | 94.41Aa | 95.3 Aa | 90.5ABb | 95.2Aa | 89.9Bb | 92.5ABb |
| LGC 60 | 20 | 93.7Aa | | 84.6Aa | | 91.0Aa | | 91.2Aa | | 91.3Aa | |
| | 50 | 91.9Aa | 95.2Aa | 81.7Aa | 85.0Aa | 95.4Aa | 96.6 Aa | 94.1Aa | 97.9Aa | 92.9Aa | 94.4Aa |
| 53%M.B | 20 | 79.1Cc | | 75.9Bb | | 76.3Cc | | 77.3Dd | | 77.3Dd | |
| 318 | 50 | 82.9 Bb | 86.5Bb | 77.7Ac | 82.8Aa | 79.6Bb | 88.2Ab | 85.4Cc | 92.0Ab | 83.7Cd | 87.9Cd |

Table 5. Control effect of LGC-42153 in transplanted rice at 20 and 45 days after application. Nantong, 2003.

| Rate g a..i. ha$^{-1}$ | DFA | Barnyard grass D | Barnyard grass F | Sheathed Monochoria D | Sheathed Monochoria F | Common Ammannia D | Common Ammannia F | Difformed Galingale D | Difformed Galingale F | Total D | Total F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LGC 15 | 20 | 27.3Dd | | 63.9Bb | | 80.9Bb | | 100Aa | | 35.5Dd | |
| | 45 | 47.8Cc | 63.7Bb | 81.8Bb | 78.9Bb | 87.8Bb | 96.1Ab | 100Aa | 100 Aa | 55.2Cc | 67.3Bb |
| LGC 25 | 20 | 54.5Cc | | 94.2Aa | | 81.4 Bb | | 100 Aa | | 61.4Cc | |
| | 45 | 79.4Bb | 90.8Aa | 99.3Aa | 99.9Aa | 92.1Ab | 98.2Aa | 100 Aa | 100 Aa | 83Bb | 92.1Aa |
| LGC 35 | 20 | 73.0Bb | | 94.8Aa | | 89.0 Bb | | 100 Aa | | 74.4Bb | |
| | 45 | 92.8Aa | 97.9Aa | 100Aa | 100AA | 95.5Aa | 98.9Aa | 100 Aa | 100 Aa | 93.3Aa | 97.9Aa |
| LGC 50 | 20 | 84.5Aa | | 100Aa | | 95.2Aa | | 100 Aa | | 86.9Aa | |
| | 45 | 93.8Aa | 98.6Aa | 100Aa | 100Aa | 100Aa | 100Aa | 100 Aa | 100 Aa | 94.8Aa | 98.8Aa |
| 53%M.B318 | 20 | 0.0Ee | | 64.7Bb | | 83.3 Bb | | 77.6Bb | | 6.9Ee | |
| | 45 | 19.8Dd | 38.4Dd | 97.6Aa | 99.7Aa | 97.6Aa | 99.3Aa | 100 Aa | 100 Aa | 31.3Cc | 44.3Cc |

Table 6. Control effect of LGC-42153 in transplanted rice at 20 and 45 days after application. Nantong, 2004.

| Rate g a..i. ha$^{-1}$ | DFA | Barnyard grass D | Barnyard grass F | Sheathed Monochoria D | Sheathed Monochoria F | Common Ammannia D | Common Ammannia F | Difformed Galingale D | Difformed Galingale F | Total D | Total F |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LGC 15 | 20 | 61.4Cc | | 64.3CDd | | 35.9Cc | | 98.5 Aa | | 64.7Cc | |
| | 45 | 41.8Cc | 76.4Bb | 71.2Bb | 90.9Ab | 59.8Cc | 82.8Ab | 97.4 Aa | 99.1 Aa | 52.1 Cd | 79.4Bb |
| LGC 25 | 20 | 66.4 Cc | | 74.8BCc | | 55.9Bb | | 95.7 Aa | | 70.1Cc | |
| | 45 | 70.2Bb | 91.5Aa | 79.3 Bb | 93.4Aa | 73.1Bb | 86.7 Ab | 100 | 100 Aa | 70.4 Cc | 91.3Aa |
| LGC 35 | 20 | 78.9 Bb | | 81.5Bb | | 81.9Aa | | 100 Aa | | 85.1Bb | |
| | 45 | 78.9Ab | 91.9Aa | 90.8 Aa | 97.8Aa | 85.8Aa | 94.1 Aa | 100 Aa | 100 Aa | 85.7 | 95Aa |
| LGC 50 | 20 | 90.6Aa | | 92.7Aa | | 85.8Aa | | 100Aa | | 92.4Aa | |
| | 45 | 85.4Aa | 94.6Aa | 93.1 Aa | 98.6Aa | 88.9Aa | 96.5 Aa | 100 Aa | 100 Aa | 88.6 Bb | 96.4Aa |
| 53%M.B318 | 20 | 27.1Dd | | 68.3Dd | | 67.7Bb | | 94.5Aa | | 50.6De | |
| | 45 | 0Dd | 0Cc | 72.4 Bb | 89.1Ab | 80.4Ab | 93.4Aa | 94.4Aa | 98.9Aa | 13.3Dd | 30.6Cc |

The results of experiments in Nantong were consistent in two years. It showed that the control effect of LGC-42153 on barnyard grass increased with the rates. The fresh weight control effect was more than 65% and 90% at 15 g a.i. $ha^{-1}$ and other higher rates respectively, significantly higher than 53% mefenacet plus bensulfuron-methyl WP 318 g a.i. $ha^{-1}$. To sedge and broadleaf weeds, control effects were similar.

From the above results, LGC-42153 could provide good control effect on gramineous weeds, sedge and broadleaved weeds in transplanted rice in China. Considering that treatment was done 25 days later after rice was transplanted, the herbicide provided satisfactory control effects at the rate of 25 g a.i. $ha^{-1}$ similar to what was observed when it was applied 5-10 days after transplanting.

Rice safety and grain yields influenced by herbicide

No injury was observed on treated rice plants in all treatments. This showed that the experimental rates of LGC42153 are safe to direct seeded and transplanted rice. Total rice yield results as influenced by LGC-42153 treatments in direct seeded and transplanted rice in Nanjing and Nantong are shown in Table 7. The results showed that all rice yields with all herbicide treatments were significantly greater than the untreated control and were similar with the control herbicide and manual control. Rice yields increased above 50% both in direct seeded and transplanted rice in Nanjing, 8-20% in direct seeded and transplanted rice in Nantong. These results showed that LGC42153 could significantly increase rice yields.

Table 7. Total grain yield as influenced by LGC-42153 treatments in direct seeded and transplanting rice in Nanjing and Nantong.

| Field | | Total grain yield(kg $ha^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Direct seeded rice | | LGC 20(15) | LGC 30 (25) | LGC 40 (35) | LGC 60 (50) | control heribicide | Manual control | Untreated control |
| | in Nanjing, 2002 | 4967.4Aa | 5441.3Aa | 5160.1Aa | 4802.6Aab | 4466.9A ab | 4314.5Aab | 2237.9Bc |
| | in Nantong , 2003 | 6312.5 Aab | 6305.3Aab | 6586.3 Aa | 6195 Aab | 5740.0A b | 6465 A a | 2822.5Bc |
| | in Nantong, 2004 | 7792 Ab | 8020.0Aab | 8144.0 Aa | 8278.0Aa | 7657.0 Ab | 8238Aa | 5769 Bc |
| Transplanting rice | in Nanjing, 2002 | 6515Ab | 6337.0Ab | 6883Aa | 6719.0Aab | 6810.0Aa | 7546 Ab | 2874Bc |
| | in Nantong, 2003 | 8408.8 Aa | 8335.0 Aa | 8387.5 Aa | 8403.8Aa | 8271.4 Aa | 8328.8Aa | 7686.3Bb |
| | in Nantong, 2004 | 7482 .0Aab | 7927.0 Aa | 8186.0Aa | 8040.0 Aa | 7491.0 Aab | 7999Aa | 6734Bb |

Note: LGC 20(15)=LGC 42153 20 g (a..i.) $ha^{-1}$ in direct seeded rice of Nanjing and Nantong, in transplanting rice of Nanjing. LGC 42153 15 g (a..i.) $ha^{-1}$ in transplanting rice of Nantong. (the rest may be deduced by analogy). Numbers within the same row with different lowercase or uppercase letters are significantly different ($P<0.05$ and $P<0.01$, respectively; Duncan's new multiple range test).

## CONCLUSION

LGC-42153 not only has high activity on, but can also control many annual and perennial paddy field weeds, such as barnyard grass (*Echinochloa crusgalli*(L.) Beauv., *E. crusgalli* var. *mitis*, *E. caudate* Roshev., *E. hispidula* (Retz.) Nees), broadleaf weeds, such as old world arrowhead (*Sagittaria trifolia* L.), common ammannia (*Ammania arenaria* H.B.K., *A. bacifera* L., *A. multiflora* Roxb.), white flowered mazus (*Mazus japonicus* (Thunb.) O Kuntze), Indian rotala (*Rotala indica* (Willd.) Koehne), procumbent false- pimpernel (*Lindernia procumbens* (Krock.) Philcox),prostrate false pimpernel (*Lindernia procumbens* (Kroch.) Philcox), Korsakow monochoria (*Monochoria korsakowii* Regel et Maack), sheathed monochoria (*Monochoria vaginalis* (Burm. F.) Presl ex Kunth), brittle falsepimpernel (*Lindernia crustacea* (L.) ), chara (*Chara fragilis* Desv.), little najad

(*Najas minor* All.), and sedges such as difformed galingale (*Cyperus difformis* L.), late juncellus (*Juncellus serotinus* (Rottb.) C.B. Clarke), flatstalk bulrush (*Scirpus planiculmis* Fr. Schmidt), common bulrush (*Scripus triqueter* L.), rushlike bulrush (*Scripus juncoides* Roxb.), needle spikesedge (*Eleocharis yokoscensis* (Franch. Et Sav.) Tang et Wang), grass–like fimbris (*Fimbristylis miliacea* (L.) Vahl). Therefore, LGC-42153 could be applied as one-shot herbicide at the recommendation rate of 25-40 g a.i. $ha^{-1}$ and 20-35 g a.i. $ha^{-1}$ in both direct seeded and transplanting rice fields in China respectively.

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# Effect of sowing methods and herbicides in direct drilled rice (*Oryza sativa*)

R.K.Singh* and D. Singh
Department of Agronomy, Banaras Hindu University, Varanasi – 221005, India
rks1660bhu@yahoo.com

**Abstract:** A field study was conducted at Agricultural Research Farm of Banaras Hindu University in monsoon season of 2002 and 2003. Rice seeds were drilled on raised beds and flat beds. Cyhalofopbutyl at 120g $ha^{-1}$ POST, butachlor at 1000 g $ha^{-1}$ PRE, butachlor at 850g $ha^{-1}$ PRE followed by propanil at 850 g $ha^{-1}$ POST and butachlor at 850 $gha^{-1}$ PRE followed by 2,4-D at 500 g $ha^{-1}$ POST were applied in both the methods of sowing. The major weeds observed in present study were *Echnochloa colona* (L) Link, *Echinochloa crusgalli* (L) Beauv, *Eleusine indica* (L) Gaerth, and *Cyperus rotundus* L among narrow leaved. The major broad leaved weeds were *Commelina benghalensis* L, *Euphorbia hirta* L. and *Eclipta alba* (L) Harsk. Rice seeded on beds recorded less weeds density and dry weight and produced higher crop growth and grain yield. Butachlor at 850 g $ha^{-1}$ PRE followed by 2,4-D 500g $ha^{-1}$ POST was most effective in controlling narrow and broad leaved weeds. This herbicidal sequence had maximum crop growth, yield attributes and rice grain yield.

**Key Words**: Rice, method of sowing, herbicides, weeds control

## INTRODUCTION

Weeds are one of the most important biological constraints limiting upland (dry land) rice (*Oryza sativa*) production (Singh and Singh, 1983; Arraudeau and Harahap, 1988). Aerobic soil conditions and dry tillage practices, besides alternate wetting and drying encourages. Continuous germination and growth of weeds in crop life cycle. Losses in grain yield varies between 50-91% (Pillai et al. 1976; Singh and Singh, 1983).

In eastern Indo-Gangetic plains of India, direct seeded rice cultivation is characterized by land preparation at optimum moisture followed by broadcasting and /or drilling of rice seeds on flat beds. Pre-emergence herbicides supplemented with 1-2 manual or mechanical weeding is commonly practiced to control weeds. In seasons of high rainfall, inter row cultivation become impractical and ineffective on medium and heavy soils (Moody and Mukhopadhyay, 1982).

Raised beds are widely used in developed countries but have been introduced recently in rice-wheat sequence of Eastern Indo-Gangetic plains (IGPs) of India. Permanent raised bed offer producer a multitude of benefits such as reduced tillage, increased opportunity for crop diversification, mechanical weeding, relay cropping and intercropping and water saving (Connor et al. 2003; Tuong, 2003).

There are many studies on weed management in dry land rice seeding on flat beds, but limited research work has been done in rice sown on raised beds. Thus, the objectives of this study were (i) Whether differences in weeds growth exist among two methods (raised and flat beds) of sowing and (ii) to evaluate the effect of herbicides alone and in sequence on weeds and crop yield.

## MATERIALS AND METHODS

This study was conducted in upland fields at Banaras Hindu University, Varanasi, India in the monsoon seasons of 2002 and 2003. Soil properties of the experimental field at start of first experiment were 0.42% organic carbon, 49.57% sand, 28.81% silt and 21.62% clay and pH 7.4.

The experiment was laid out in a split plot design with methods of sowing in main plot and herbicides with weed free and weedy check as subplots, each with four replications. After land preparation at optimum moisture, raised beds were made with 'DWR' bed planter. The spacing between two furrows was 70 cm and width of beds was 60 cm. Three row of rice variety NDR-97 was seeded on each beds. On flat beds, seeds were sown at a 20 cm row to row distance. The seeding rate was 100 kg $ha^{-1}$. Herbicides – butachlor (1.0 kg a.i. $ha^{-1}$.), PRE- cyhalofop-butyl (0.12 kg a.i. $ha^{-1}$). POST (15 DAS), butachlor (0.85 kg a.i. $ha^{-1}$) PRE followed by propanil (0.85 kg a.i. $ha^{-1}$) POST (10 DAS) and 2,4-D (0.50 kg a.i. $ha^{-1}$) POST (10 DAS) were applied with single flat-fan nozzle boom mounted on a hand sprayer.

At 60 DAS weeds were sampled from two 50 by 50 cm. quadrate randomly placed in each plot. Count was made for different weed species in each sample. Weed samples were oven dried at about 70°C and then weighed. Destructive sampling was done from $m^{-1}$ border rows from each side of plots to record shoot biomass. At harvest, number of panicles were counted from 1 $m^{-2}$ sample area of net plot. Thirty panicles were threshed to count grains per panicle. The grain yield was obtained from 18-$m^{-2}$ sampling area in each plot.

## RESULTS AND DISCUSSION

### Effect of sowing methods and herbicides on weed density and biomass, rice growth and yield

Dominant weed species occurring in the experimental field were *Echinochloa colona*, *Echinochloa crus-galli*, *Cyperus rotundus* and *Cyperus difformis*. Several literature (De Datta, 1980 and Smith et al. 1983) confirm dominance of these weed species in direct dry seeded rice.

Table 1. Effect of sowing methods and herbicides on weeds density at 60 Days after sowing (DAS).

| Treatments | Rate kg $ha^{-1}$ | Weeds Density (per $m^{-2}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *E. colona* | | *E. crus-galli* | | *C. rotundus* | | *C. deformis* | |
| | | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 |
| Flat beds | - | 20.8 (4.58) | 17.73 (4.27) | 10.90 (3.38) | 4.74 (2.30) | 20.12 (4.50) | 16.70 (4.20) | 6.80 (2.70) | 6.00 (2.60) |
| Raised beds | - | 7.17 (2.80) | 6.80 (2.70) | 9.30 (3.10) | 3.80 (2.10) | 17.20 (4.20) | 16.60 (4.10) | 5.50 (2.50) | 4.90 (2.30) |
| LSD (p=0.05) | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Cyhalofop butyl (15 DAS) | 0.12 | 19.30 (4.46) | 14.60 (3.88) | 12.30 (3.60) | 6.10 (2.60) | 22.80 (4.80) | 16.00 (4.10) | 7.70 (2.90) | 6.20 (2.60) |
| Butachlor PRE | 1.0 | 15.98 (4.06) | 16.00 (3.81) | 11.80 (3.50) | 4.60 (2.30) | 21.90 (4.70) | 12.30 (3.60) | 6.90 (2.70) | 5.80 (2.50) |
| Butachlor PRE fb propanil (POST) | 0.85+0.85 | 15.58 (4.01) | 12.80 (3.64) | 9.60 (3.28) | 3.60 (2.00) | 20.70 (4.60) | 11.30 (3.40) | 5.10 (2.40) | 4.90 (2.30) |
| Butachlor PRE fb 2,4-D (EE) POST | 0.80+0.50 | 14.00 (3.80) | 11.90 (3.52) | 7.20 (2.80) | 3.00 (1.90) | 18.60 (4.40) | 6.70 (2.70) | 4.10 (2.20) | 3.80 (2.10) |
| Hand weeding | 20 & 40 DAS | 6.29 (2.60) | 1.90 (1.54) | 3.40 (1.97) | 1.90 (1.54) | 5.10 (2.40) | 2.40 (1.70) | 3.00 (1.90) | 1.50 (1.40) |
| Weedy check | - | 22.30 (4.77) | 16.55 (4.13) | 17.80 (4.30) | 7.60 (2.80) | 24.20 (5.0) | 21.30 (4.70) | 12.60 (3.60) | 6.30 (2.60) |
| LSD (p=0.05) | - | 1.19 | 0.73 | NS | NS | 2.0 | 1.1 | NS | NS |

Data in parenthesis ( ) are transformed $(X+0.5)^{1/2}$ values.

An ANOVA showed no interaction effects between sowing methods and herbicides on weeds so the main effect of sowing methods was determined over four herbicides and two checks (hand weeding and weedy check). Results reveal that weed density and biomass were not significantly affected by sowing methods. Raised bed planting had relatively lower weed density and biomass than flat beds (Table -1).

The crop growth traits such as plant height and shoot biomass were significantly higher in raised wed planting compared with flat beds (Table 2). These traits of rice are reported to be inversely correlated with weed-growth (Fofana and Rauber, 2000; Ni et al. 2000; Gibson et al. 2003).

There were significant differences in weed biomass due to herbicide application over weedy check. Sequential application of butachlor followed by (fb) 2,4-D was significantly superior to other herbicide treatments. Similar trend was observed with butachlor fb propanil over butachlor PRE and cyhalofop-butyl POST.

Table 2. Effect of sowing methods and herbicides on plant height, shoot biomass of crop and weed biomass at 60 DAS

| Treatments | Rate kg $ha^{-1}$ | Plant height (cm) | | Shoot biomass (g $m^{-1}$) | | Weed biomass (g $m^{-2}$) | |
|---|---|---|---|---|---|---|---|
| | | 2002 | 2003 | 2002 | 2003 | 2002 | 2003 |
| Flat beds | - | 51.00 | 55.00 | 49.40 | 54.00 | 43.80 | 25.76 |
| Raised beds | - | 56.00 | 60.00 | 49.40 | 60.20 | 39.27 | 23.10 |
| LSD (p=0.05) | NS | 2.10 | 2.40 | 53.30 | 4.10 | NS | NS |
| Cyhalofop butyl (15 DAS) | 0.12 | 47.00 | 50.60 | 3.50 | 33.20 | 47.83 | 28.46 |
| Butachlor PRE | 1.0 | 48.50 | 53.40 | 39.70 | 48.40 | 42.91 | 22.78 |
| Butachlor PRE fb propanil (POST) | 0.85+0.85 | 53.50 | 57.80 | 47.60 | 56.80 | 40.44 | 20.70 |
| Butachlor PRE fb 2,4-D (EE) POST | 0.80+ 0.50 | 59.60 | 63.40 | 50.80 | 67.50 | 38.37 | 19.31 |
| Hand weeding | 20 & 40 DAS | 59.90 | 61.90 | 63.00 | 75.00 | 19.98 | 12.49 |
| Weedy check | - | 46.40 | 47.20 | 71.90 | 36.90 | 50.90 | 31.76 |
| LSD (p=0.05) | - | 3.10 | 3.80 | 34.80 | 5.10 | 2.08 | 2.10 |

Rice grain yield and yield traits were significantly affected by showing methods and herbicide treatments (Table 3). Rice sown on raised beds produced significantly higher grain yield than flat beds. This was due to increased number of panicles, grain $panicle^{-1}$ and 1000 grain weight. The significant difference in grain number in crop on raised beds might be due to production of high shoot biomass and its translocation for grain formation.

Sequential application of butachlor PRE followed by 2,4-D or propanil POST had significantly more yield than butachlor PRE and cyhalofop butyl as POST. These results confirm the findings of Singh and Ram, 1990 and Kalia and Bindra 1996 who reported significant decrease in weed biomass with corresponding increase in rice yield due to combined use of butachlor with propanil or 2,4-D EE over single application of herbicides.

Table 3. Effect of showing methods and herbicides on yield traits and grain yield (two year pooled)

| Treatments | Rate kg ha$^{-1}$ | Panicles m$^{-1}$ | No. of grains panicle$^{-1}$ | Weight of 1000 grains (g) | Grain yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| Flat beds | - | 390.00 | 43.80 | 19.40 | 3330 |
| Raised beds | - | 397.00 | 45.90 | 20.00 | 3700 |
| LSD (p=0.05) | NS | NS | 1.05 | NS | 3800 |
| Cyhalofop butyl (15 DAS) | 0.12 | 373.00 | 42.10 | 19.60 | 3000 |
| Butachlor PRE | 1.0 | 385.60 | 43.80 | 19.70 | 3600 |
| Butachlor PRE fb propanil (POST) | 0.85+0.85 | 390.00 | 48.90 | 19.30 | 3900 |
| Butachlor PRE fb 2,4-D (EE) POST | 0.80+0.50 | 395.00 | 49.90 | 20.10 | 4100 |
| Hand weeding | 20 & 40 DAS | 430.00 | 51.10 | 19.00 | 4300 |
| Weedy check | - | 310.00 | 33.00 | 20.40 | 2028 |
| LSD (p=0.05) | - | 24.00 | 1.40 | NS | 105.0 |

This study indicated that agronomic management such as raised bed sowing can improve rice cultivar competitiveness against weeds. Sequential application of herbicide can be an effective tool to manage weeds in upland rice.

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# Evaluation of pyrazosulfuron-ethyl 10 WP for weed management in transplanted paddy (*Oryza sativa*)

Ch. Sreedhar [1], G. Shankar [2] and M.S. Mithyantha [3]
Rallis Research Centre, Rallis India Limited, 21 & 22, Peenya Industrial Area, Phase II, Bangalore - 560 058, Karnataka, India
chemuturi.sreedhar@rallis.co.in; gshankar@rallis.co.in; msmithyantha@rallis.co.in

**Abstract:** The study was conducted during the years 2001-2002 and 2002 -2003 to evaluate the usefulness of pyrazosulfuron-ethyl 10WP for weed management in transplanted paddy. Pyrazosulfuron-ethyl 10WP at 20, 25, 30, 35 and 40 g a.i. $ha^{-1}$ was evaluated for its bio-efficacy against broad spectrum of weeds and safety to crop. Standards for comparison were butachlor 50EC at 1250 g a.i. $ha^{-1}$ and pretilachlor 50EC at 500 g a.i. $ha^{-1}$. Weed flora in the experimental plots comprised of the grasses *Echinochloa crus-galli* and *E. colonum,* the sedges, *Cyperus difformis, C. iria, Scirpus maritimus,* and the broadleaf weeds, *Eclipta prostrata, Monochoria vaginalis, Commelina benghalensis, Marsilea quadrifolia* and *Ludwigia parviflora.* Pre-emergence soil surface spray of pyrazosulfuron-ethyl 10WP at 30, 35 and 40 g a.i. $ha^{-1}$ within 3 days of transplanting led to higher per cent control of weeds and lowest dry weight of weeds in observations recorded at 30, 45 days after transplanting and at harvest. Grain and straw yield under these treatments were the highest and were comparable to the standards.

**Key words**: Broad spectrum weed control, pyrazosulfuron-ethyl 10WP, transplanted paddy.

## INTRODUCTION

Rice is the major cereal crop of India and it is grown in almost all states under varied ecosystems. Weed flora in the transplanted paddy fields in different rice growing tracts of India consist of grasses such as *Echinochloa crus-galli, E. glaberescens*; *E. colonum, Leptochloa chinensis, Rottboellia exaltata* and broad leaf weeds, *Ammania baccifera, Eclipta prostrata, Marsilea quadrifolia, Ludwigia parviflora, Monochoria vaginalis*, *Rotala indica*, *Sphenoclea zeylanica* and sedges such as *Cyperus difformis, C. iria, C. rotundus, Fimbristylis miliacea* and *Scirpus maritimus.*( Dhiman and Singh 2004; Ghosh and Ganguly 1993; Govindra Singh et al. 2004; Kurmi and Das 1993; Ray 1973; Sahu and Bhattacharya 1964; Shekhar et al. 2004 and Singh and Rao 1970).

Severe infestation of weeds is one of the main factors responsible for the low productivity of rice. Weed competition depends upon weed species, density of crop and weed, soil moisture, nutrients availability and period of competition. In view of this, weeds must be controlled within the critical period of weed competition to prevent crop losses from weeds. If the weeds are not kept under check during the critical period, it has been reported to lead to crop loss of 30 to 80 per cent (Brar et al. 1997; Chaudhary et al. 1995; Gautam and Mishra 1995; Jena and Mishra 1992; Moorthy and Manna 1989; Paradkar et al. 1997; Pillai and Rao 1974; Saikia and Purshothamam 1996; Singh and Bhan 1986; and Raju and Reddy 1995). Hence, proper weed management practices are essential to obtain better yields in transplanted paddy.

Several pre-emergence herbicides such as anilofos, butachlor, oxadiargyl, oxadiazon, Pendimethalin, 2,4-D and pretilachlor alone or supplemented with hand weeding have been found to be useful for weed management in transplanted paddy (Mishra et al. 1988; Moorthy and Manna 1993; Mishra, 1996; Paradkar et al. 1997; Porwal 1999). For many years, butachlor and pretilachlor have been the most widely used herbicides because of their broad spectrum activity. However, there is a need for alternative herbicides with lower dosages, as continual use of the same herbicide(s) with the same mode of action may lead to shift in weed flora and evolution of resistance in weeds (De Datta 1977; Govindra Singh et al. 2004; Ho and Zuki 1988; Kandasamy and Sankaran 1995; Kim, 1983; Moss and Rubin 1993). Of late, low dosage, high efficacy herbicides to control broader spectrum of weeds have been found promising (Kurchania et al. 2000; Moorthy 2002). Hence, to give farmers a wider choice of affordable and effective herbicides, there is a need to develop environmental eco-friendly molecules of newer chemistries with lower dosages and with different mode of action. In view of this, the present studies were carried out to evaluate the usefulness of pyrazosulfuron-ethyl 10WP for broad-spectrum control of weeds.

## MATERIALS AND METHODS

An experiment each was conducted in the State of Karnataka during Kharif season (June to November) of 2001-2002 and 2002-2003. The trials were laid out in randomized block design with three replications. Twenty-five day old seedlings of rice cv. IR-64 were transplanted at a distance of 20cm x 15cm during both the years in sandy clay loam soils with pH of 7.8 on July 15 and July 10 of the respective year. 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 (DAT). The paddy crop was raised as per recommended package of practices. The treatments consisted of pyrazosulfuron-ethyl 10WP at 20, 25, 30, 35 and 40 g a.i. $ha^{-1}$, besides weed free and weedy check treatments. Standards for purposes of comparison were stand-alone application of butachlor at 1250 g a.i. $ha^{-1}$, and pretilachlor 50EC at 500 g a.i. $ha^{-1}$. Plot size was 10 m x 10 m. Water in the experimental plots was drained out before imposing treatments. Herbicide treatments were applied as pre-emergence blanket soil surface spray three days after transplanting using a knapsack sprayer fitted with flat fan nozzle and a spray volume of 600 L $ha^{-1}$. Water was impounded in the experimental plots one day after treatment.

Phytotoxicity scoring was done at five and 10 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 ). Number of hills per sq m and height of plants were recorded at 30 and 60 DAT. The per cent degree of weed control was visually assessed at 30, 45 DAT and at harvest, on zero to 100 scale, by two observers independently. The average values were transformed to arcsine values and were subjected to analysis of variance. Number of weeds occurring within 1.0 $m^2$ quadrat in each plot was recorded at random at 30, 45 DAT and at harvest. The data on number of weeds were subjected to square-root transformation using $\sqrt{n+1}$. 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. Observations on yield and its attributes were recorded at harvest.

## RESULTS AND DISCUSSION

### Phytotoxicity

Plants in the plots treated with pyrazosulfuron-ethyl 10WP at all the rates tried did not exhibit any symptom of phytotoxicity in terms of leaf injury, vein clearing, wilting, necrosis, epinasty and hyponasty symptoms in plants.

### Weed flora

*Echinochloa crus-galli, E. colonum, Monochoria vaginalis, Marsilea quadriifolia, Commelina benghalensis, Ludwigia parviflora, Sphenoclea zeylanica, Eclipta prostrata, Cyperus difformis, C. iria, Scirpus maritimus* and *Fimbristylis miliacea* were the weeds noticed in the weedy check plots at 30, 45 and 60 DAT.

### Weed control

Treatments of pyrazosulfuron-ethyl at 30, 35 and 40 g a.i. $ha^{-1}$ gave 95% control of the broadleaf weeds, *Eclipta prostrata, Ludwigia parviflora, Monochoria vaginalis, Marsilea quadrifolia, commelina benghalensis* (Fig.1). These treatments gave about 90% control of the sedges like *Cyperus difformis, Cyperus iria, Scirpus maritimus* and *Fimbristylis miliacea*. At this infestation level of grasses, the treatments of pyrazosulfuron-ethyl at 30, 35 and 40 g a.i. $ha^{-1}$ gave 80-85% control

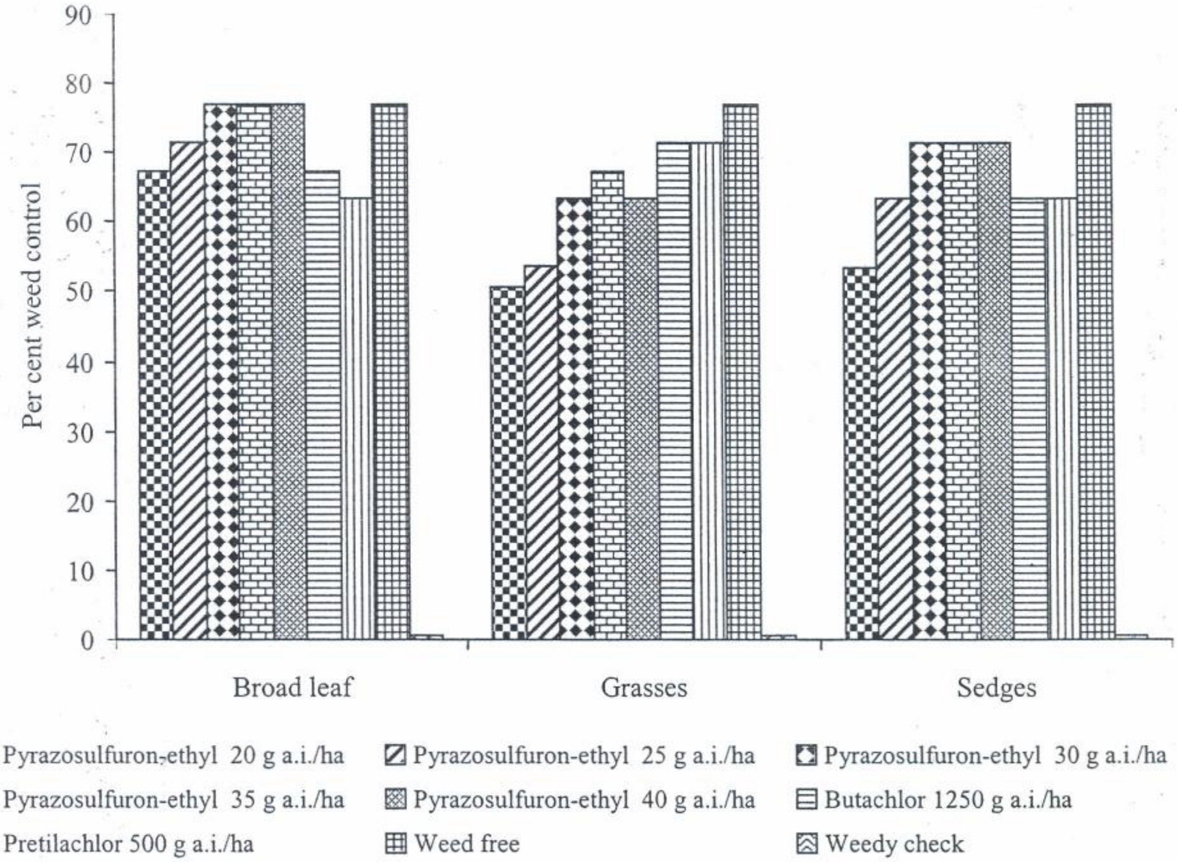


Figure 1. Influence of treatments on per cent weed control
(mean of 2001-2002 &2002-2003)

Effect on weeds

The weedy check treatment recorded the highest number of weeds per unit area. Among the herbicide treatments, pyrazosulfuron-ethyl at 20 and 25 g a.i. $ha^{-1}$ recorded the highest number of weeds and these treatments were significantly inferior to pyrazosulfuron-ethyl at the higher rates and the standard treatments. However, pyrazosulfuron-ethyl at 30, 35 and 40 g a.i. $ha^{-1}$ recorded significantly the lowest number of weeds and these treatments were comparable to that of the standard treatments of butachlor, pretilachlor at the recommended dosage and weed free plots (Table 1). Dry weight of weeds exhibited the inverse trend of results (Table 1).

Table 1. Influence of treatments on biometric parameters.

| Treatments | Dose (g a.i. $ha^{-1}$) | No. of weeds $m^{-2}$* | | | No. of weeds $m^{-2}$* | | |
|---|---|---|---|---|---|---|---|
| | | Broad leaf | Grasses | Sedges | Broad leaf | Grasses | Sedges |
| Pyrazosulfuron-ethyl | 20 | 2.5(5.2) | 4.3(18.0) | 2.4(5.2) | 2 | 8.2 | 4.2 |
| Pyrazosulfuron-ethyl | 25 | 2.0(3.0) | 4.1(16.0) | 1.8(2.2) | 1.3 | 6.4 | 2.9 |
| Pyrazosulfuron-ethyl | 30 | 1.6(1.5) | 3.8(14.0) | 1.6(1.5) | 0.1 | 5.6 | 1 |
| Pyrazosulfuron-ethyl | 35 | 1.5(1.2) | 3.4(11.0) | 1.4(1.0) | 0.1 | 4.4 | 0.5 |
| Pyrazosulfuron-ethyl | 40 | 1.4(1.0) | 3.1(9.0) | 1.4(1.0) | 0.1 | 3.6 | 0.6 |
| Butachlor | 1250 | 3.0(8.0) | 1.4(1.0) | 4.0(15.0) | 3.8 | 0.1 | 7.5 |
| Pretilachlor | 500 | 3.3(10.0) | 1.4(1.0) | 3.4(11.0) | 4.6 | 0.1 | 5.2 |
| Weed free | - | 1.4(1.0) | 1.4(1.0) | 1.4(1.0) | 0.1 | 0.1 | 0.1 |
| Weedy check | - | 5.2(27.0) | 4.5(20.0) | 9.1(83.8) | 10.8 | 14.8 | 42.8 |
| SEm ± | | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.4 |
| CD at 5% | - | 0.4 | 0.3 | 0.6 | 0.6 | 0.7 | 1.2 |

*Mean of 2001-2002 & 2002-2003; Observations recorded at 45 days after transplanting; figures in parentheses indicate the absolute values.

## Yield

Pyrazosulfuron-ethyl at all the rates tried produced significantly more panicles $m^{-2}$, grains $panicle^{-1}$ and hence higher grain yield over the weedy check treatment during both the years (Table 2). However, pyrazosulfuron-ethyl at 20 and 25 g a.i. $ha^{-1}$ recorded the lowest grain and straw yield and these treatments were significantly inferior pyrazosulfuron-ethyl at the higher rates tried. Pyrazosulfuron-ethyl at 30, 35 and 40 g a.i. $ha^{-1}$ recorded significantly higher grain yield due to higher values of the yield attributes as a result of better weed control. These treatments were comparable to the standard treatments of butachlor and pretilachlor at the recommended dosages.

Thus, the results clearly established that pyrazosulfuron-ethyl at 30, 35 and 40 g a.i. $ha^{-1}$ was comparable to the standard treatments of butachlor and pretilachlor for control of broad spectrum of weeds in transplanted paddy with favorable implications on grain and straw yields.

Table 2. Effect of treatments on yield attributes of transplanted paddy.

| Treatments | Dose (g a.i. $ha^{-1}$) | Grain yield (kg $ha^{-1}$) | | Straw yield (kg $ha^{-1}$) | | Panicles $m^{-2}$ | | Grains $panicle^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2001 | 2002 | 2001 | 2002 | 2001 | 2002 | 2001 | 2002 |
| Pyrazosulfuron-ethyl 10WP | 20 | 3980 | 4010 | 6050 | 6817 | 296 | 300 | 54 | 55 |
| Pyrazosulfuron-ethyl 10WP | 25 | 4280 | 4320 | 6579 | 7344 | 339 | 350 | 56 | 59 |
| Pyrazosulfuron-ethyl 10WP | 30 | 4700 | 4920 | 7085 | 8630 | 390 | 410 | 71 | 75 |
| Pyrazosulfuron-ethyl 10WP | 35 | 4775 | 4980 | 7168 | 8700 | 395 | 400 | 70 | 77 |
| Pyrazosulfuron-ethyl 10WP | 40 | 4805 | 5100 | 7207 | 8800 | 410 | 415 | 74 | 78 |
| Butachlor 50EC | 1250 | 4790 | 5085 | 7185 | 8730 | 400 | 410 | 71 | 75 |
| Pretilachlor 50EC | 500 | 4750 | 5018 | 7125 | 8630 | 390 | 400 | 70 | 74 |
| Weed free | - | 4865 | 5160 | 7297 | 8862 | 415 | 430 | 75 | 80 |
| Weedy check | - | 1829 | 1978 | 2650 | 3580 | 179 | 190 | 29 | 32 |
| SEm ± | | 64 | 117 | 76 | 125 | 4 | 5 | 1 | 1 |
| CD at 5% | | 184 | 351 | 228 | 376 | 26 | 32 | 7 | 7 |

Mean of 2001-2002 & 2002-2003

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# Herbicidal efficacy of sodium chlorate granules to buried seeds of *Leptochloa chinensis* (L.) Nees and some Poaceae species

Ken-ichi Sudo[1], Akihiro Ushio[1], Mitsuhiro Adachi[2], Hisao Katahashi[3] and Fumio Ogawa[2]
[1]Hyogo Prefectural Agricultural Technology Center, Kasai, Hyogo, Japan, 679-0198
ken@hyogo.email.ne.jp
[2]Japan Carlit Co., Ltd., Kanda Izumi-cho, Chiyoda-ku, Tokyo, Japan, 101-0024
[3]AgroPartners Corporation, Miyahara, Yodogawa-ku, Osaka, Japan, 532-0003

**Abstract:** The herbicidal efficacy of sodium chlorate granules to *L. chinensis* seeds was investigated. In paddy fields and paddy levees, soil surface was treated with sodium chlorate granule 50 kg ai ha$^{-1}$, 100 kg ai ha$^{-1}$ and 200 kg ai ha$^{-1}$ respectively on December 9, 2002. Soil samples containing seeds were collected from the paddy fields and levees 0-3 cm below the ground surface on April 10, 2003. On June 10 and September 22, 2003, each air-dried soil samples were put in 13 cm-diameter plastic cups. When the seedlings have grown in those plastic cups, the number of each species was counted. As the treatment concentration of sodium chlorate granules increased, the number of seedlings of *L. chinensis* growing in the plastic cups remarkably decreased. On paddy levee which with was treated with sodium chlorate 50 kg ai ha$^{-1}$, the number of seedlings growing in the plastic cups was similar to that in untreated soil. Where sodium chlorate was treated 200 kg ai ha$^{-1}$, the number of seedlings growing in the plastic cups remarkably decreased to the same level of that in the paddy fields. For further herbicidal effect examination of sodium chlorate granules on *L. chinensis* seeds, seeds were soaked in 0.3, 0.6, 1.0, 3.0% sodium chlorate aqueous solutions under the same condition with lighting and 20°C temperature. The seeds soaked in sodium chlorate more than 0.6% solution didn't germinate. These results suggest that the soil surface treatment with sodium chlorate granules helped inhibit the germination of buried seeds of *L. chinensis*.

**Key words:** Buried seeds, *Leptochloa chinensis*, sodium chlorate, soil surface treatment

## INTRODUCTION

*Leptochloa chinensis* L. is a noxious annual weed commonly seen in paddy fields and wet upland fields (for instance, paddy levees) of southwestern Japan. *L. chinensis* produced huge amount of seeds (Kasahara 1974), therefore its seedlings distribute in a large quantity. Although we have to use a foliar treatment herbicide to control this weed, there is a great risk of crop injury. We reported that post-harvest soil surface treatment with granules was effective to reduce a biomass of *Paspalum distichum* (Matsuo et al. 1985, Sudo 2003). In the same report, we sodium chlorate granules restrain buried seeds in paddy fields from germinating. Therefore, the present study was conducted to evaluate the herbicidal efficiency of soil surface treatment with sodium chlorate granules on buried seeds of *L. chinensis* and some Poaceae species (*Alopecrus aequalis* var. *amurensis* and *Poa annua*).

## MATERIALS AND METHODS

### Experiment 1

The first field experiment was conducted in 2001 at Hyogo Prefectural Agricultural Technology Center (HPATC), Hyogo, Japan. The soil texture was loam. The field was severely infested with short-awned foxtail (*Alopecrus aequalis* Sobol. var. *amurensis* Ohwi) in winter. After harvesting of rice on September 20, 2001, rice straws were completely removed from the experimental plots. On October 5, 2001, sodium chlorate granule at 50 kg ai ha$^{-1}$ (SCG-50), 100 kg ai ha$^{-1}$ (SCG-100) and 200 kg ai ha$^{-1}$ (SCG-200) was treated on the soil surface of each paddy field respectively. Replications were conducted in two other plots (5m × 5m) for each concentration. The field had grown no winter crop until April 2002. On April 10, 2002, 2kg soil samples containing seeds were collected at random from 0-3 cm below the ground surface in each plot. All soil samples were air-dried, smashed and riddled using a 3 mm-mesh sieve. 100g riddled soil was put in a leaky plastic cup (13cm diameter × 10cm depth) on November 10, 2002. All the cups were placed in a rainproof house and watered properly. When the seedlings grew in the plastic cups, the number of each species was counted. Replications were conducted in four other cups for each soil sample.

**Experiment 2**

The second experiment was conducted the next winter 2002 to 2003 at the same Center. The dominant weed of this experimental paddy fields and paddy levees was red sprangletop (*Leptochloa chinensis* (L.) NEES). After harvesting of rice, sodium chlorate granules were treated to the soil surface in paddy fields and paddy levees on December 9, 2002. Treatment quantity was the same as experiment 1. Each treatment plot had two replicate plots (paddy fields: 5m × 5m, paddy levees: 0.6m × 5m). On April 10, 2003, soil samples were collected using the same method as experiment 1, and were put in plastic cups. The experiment included two replications and conducted twice (on June 10 and September 22, 2003).

**Experiment 3**

*L. chinensis* seeds were collected from field populations in HPATC on October 2003. Seeds were air-dried and ripe ones were selected out using a stereoscopic microscope, and then stored at the room temperature. Approximately 100 seeds were soaked in 5°C water for 10 days and were placed on the filter paper in a plastic cup (5cm diameter × 5cm depth). 20ml sodium chlorate aqueous solutions (0.3, 0.6, 1.0, 3.0 %) was added into each plastic cup. Distilled water was used as the control. All the cups were placed in a lighted chamber at 20°C. After 30 days, the number of germinating seeds was counted. Replications were conducted in five other cups for each concentration.

## RESULTS AND DISCUSSION

**The efficacy of sodium chlorate to buried seeds of *L. chinensis***

Figure 1 shows the herbicidal effect of different concentrations of sodium chlorate granules on buried seeds of *L. chinensis* in paddy fields and levees. The number of growing seedlings of *L. chinensis* in the control of paddy fields was 298 $cup^{-1}$. The number of growing seedlings of SCG-50, SCG-100 and SCG-200 was 80, 47 and 12 $cup^{-1}$ respectively, indicating the growth of seedlings was more significantly restrained as the quantity of sodium chlorate increased. On paddy levees, the number of growing seedlings in the SCG-50 cup was similar to that in the untreated soil. Besides, a standard deviation in SCG-50 was large; therefore, there was no significant difference in numbers among SCG-50, SCG-100 and control plots. In the SCG-200 cup, the number of growing seedlings was remarkably decreased to the same level of that in paddy fields.

Seeds of *L. chinensis* hardly germinate by ordinary germination treatments. In the present study, seeds were soaked in 5°C water for 10 days before the germinating test (Matsuo et al. 1985). During the test, seeds were soaked in distilled water or sodium chlorate solutions in a lighted chamber at 20°C. The germination ratio of *L. chinensis* seeds in sodium chlorate solutions was significantly lower. The seeds soaked in 0.6% sodium chlorate solution or higher didn't germinate (Fig. 2).

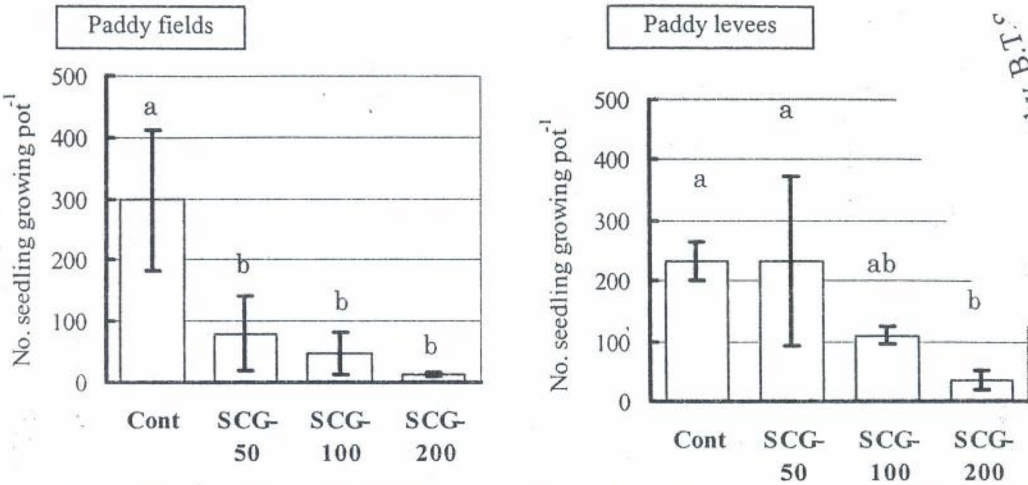


Fig. 1. Effect of different concentrations of sodium chlorate granules on the number of seedling of *L. chinensis* growing in paddy fields and levees. Bars with the same letter are not significantly different at 5% level, as determined by Fisher's protected least significant difference test. Bars represent ± standard deviation.

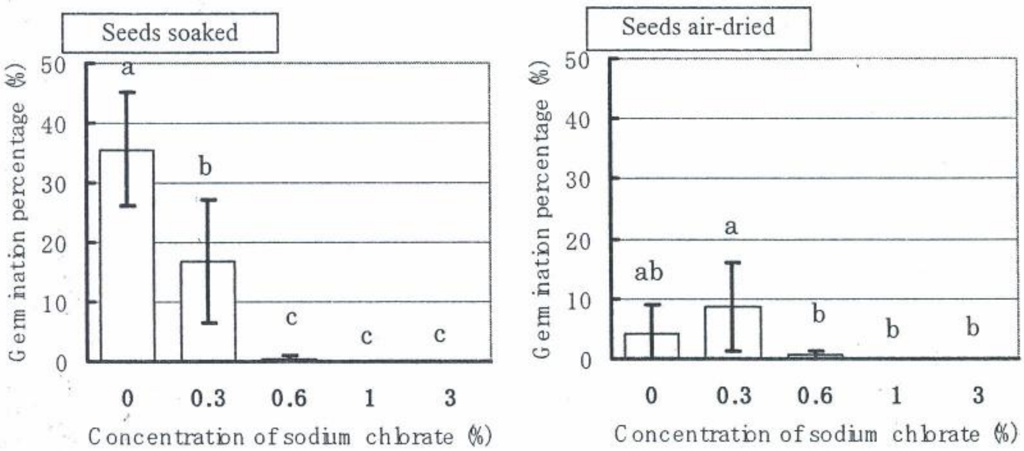


Fig. 2. Effect of different rates of sodium chlorate solution on the germination of (left) seeds soaked in water (right) that air-dried and of *L. chinensis*. Bars with the same letter within each graph are insignificantly different at 5% level, as determined by Fisher's protected least significant difference test. Bars represent ± standard deviation.

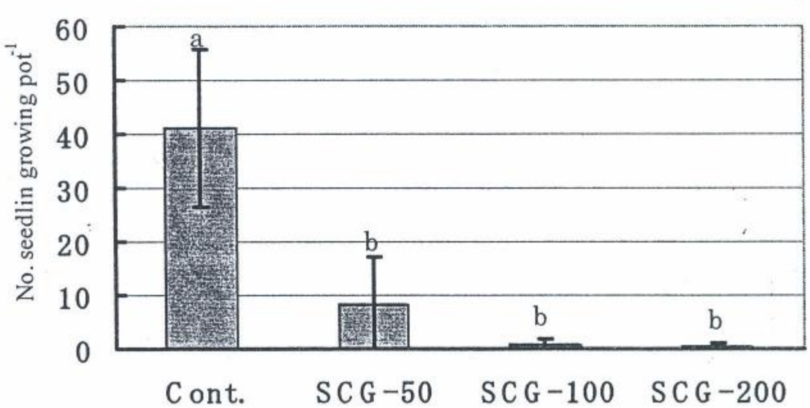


**Fig. 3.** Effect of different concentrations of sodium chlorate granules on the number of *A. aequalis* var. *amurensis* seedlings. Bars with the same letter are not significantly different at 5% level, as determined by Fisher's protected least significant difference test. Bars represent ± standard deviation.

**he effect of sodium chlorate on buried seeds of *A. aequalis* var. *amurensis* and *P. annua***

igure 3 shows the herbicidal effect of different concentrations of sodium chlorate granules on ıried seeds of *A. aequalis* var. *amurensis*. The number of seedlings growing in the control plastic ıp was 41. In contrast, the number in the SCG-50 cup was less than 25% of that of control. In CG-100 and SCG-200, the number was lower than 1. It was obvious that the number of seedlings ecreased as the quantity of sodium chlorate granules increased.

here was remarkable effect of sodium chlorate on buried seeds of annual bluegrass (*Poa annua* L.), ecause no growing seedlings of *Poa annua* was observed at SCG-50, SCG-100 and SCG-200 on pril 10, when the soil samples was collected (Fig. 4).

he results of the experiments indicate that the soil surface treatment with sodium chlorate granules effective to restrain the germination of buried seeds of *L. chinensis*, *A. aequalis* var. *amurensis* ıd *Poa annua* in paddy fields and paddy levees. This inhibitory mechanism of sodium chlorate anules has yet to be seen whether it served as herbicide or it induced the secondary dormancy of e weed. Therefore the soil surface treatment applied in this study will possibly become a new erbicide application method of sodium chlorate granules.

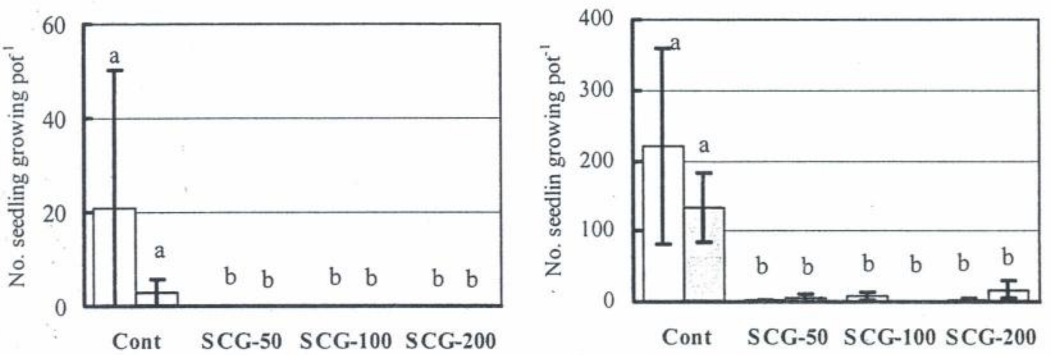


Fig. 4. Effect of different rates of sodium chlorate granules on the number of growing seedlings of *A. aequalis* var. *amurensis* ☐ and *P. annua* ▨ in (left) Experiment 1 and (right) Experiment 2. Bars with the same letter same species within each graph are not significantly different at 5% level, as determined by Fisher's protected least significant test. Bars represent ± standard deviation.

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**Minimum tillage method using Gramoxone in maize growing on slope soil at North West region - Viet Nam.**

Dao Xuan Cuong*[1]; Nguyen Truong Thanh[2]; Ha Dinh Tuan[3]; Le Huy Hoang[3].
[1]Syngenta Vietnam Ltd
[2]National Plant Protection Institute
[3]Viet Nam Agricultural Science & Technology Institute

**Abstract:** Maize is an important crop to Vietnamese especially to Minority living in hilling and mountain. With more than 180 thousand ha of maize, the Northern West region becomes one of main area for maize growing in Viet Nam. However, almost maize grown here is on slope soil with one crop per year, in rainy season, only. The traditional method such as hoeing, drying weed & crop residue collected then firing them before planting maize shows a backward in culturing. This results low yield harvested, waste time in growing and facing big problem of erosion.

In addition this is also one of reason causing calamities such as forest fire, flooding with high water. In order to improve maize planting method in this area, Syngenta Viet Nam in collaboration with the National Plant Protection Institute and Viet Nam Agricultural Science & Technology Institute has carried out an experiment using Gramoxone 20 SL (a non- select contact herbicide with ai is paraquat) as an element in minimum tillage method of maize growing on slope soil to reduce erosion. The experiment is conducted since 2002 at Hoa Binh and Yen Bai provinces where rainfall is as high as 1700 mm yearly. Gramoxone 20 SL with 2 applications, the first is 2 days before planting maize at dosage 4 L $ha^{-1}$ applied directly to growing weed, and the second one is one month later at dosage 2 L $ha^{-1}$ applied as inter row of maize. The traditional method of farmer is arranged as control in the experiment. Result of the experiment shows much benefit in comparison to the traditional method:

- Reduce soil erosion of 34 % during rainy season.
- Shorten growing time by more than 20 days per season.
- Save manpower cost of weeding VND 750,000 $ha^{-1}$.
- Preventing forest fire contributing to environmental protection.

**Key words:** Minimum tillage, maize, erosion reducing, Gramoxone.

## INTRODUCTION

With 180,000 ha of maize grown yearly, the North West becomes an important area of maize production in Viet Nam. Typical features of Maize cultivated here are: gown in steep slope soil of 15 – 25 o; concentrated in rainy season June – September with high annual rainfall of 1700 mm; planting method is backward with hand weeding, weed burning, manual tillage and stick soil for sowing. All these make maize production facing big problem of strong erosion, soil degradation, production cost increased and yield decreased and calamity such as forest fire, flood.

Application of appropriate technologies of cultivation in slope soil here not only reduced considerably soil erosion but gradually improved soil fertility to create good condition for maize growing (Thai Phien, 1999). Some countries now a day have developed a new method called No-tillage or Minimum-tillage Technique for maize planting. WestLafayetta (2002) reports that 4.6 million of ha of maize in Midwest of American is planted using either no-tillage or strip tillage. This method is largely adapted because of its great advantages. Researchers have documented benefits come from no-tillage technique including reducing erosion, improving soil fertility, preserving natural resources (West Lafayetta, 2003). In order to provide farmers a better method for weed control in maize grown on slope soil, to reduce hard work, erosion and avoid negative impact to the environment, Gramoxone 20 SL – a non selective, contact herbicide has been used to study in minimum tillage method for maize grown on slope soil. This study has been conducted by Syngenta Vietnam in collaboration with The National Plant Protection Institute (NPPI) and The Northern Agriculture Research Centre (Viet Nam Agricutural Science & Technology Institute) at Hoa Binh and Yen Bai provinces, since 2002 up to now.

## MATERIALS AND METHODS

The study is conducted by both experimental types of replicated small plot and non replicated large plot. Gramoxone 20 SL, active ingredient Paraquat is a non selective and contact herbicide. It has been manufactured by ICI England, now Syngenta Switzerland and used in more than 120 countries, worldwide. The famous property of this product is broad spectrum with fast action and rain fastness. It can control all parts of weed above soil surface within 1-2 days after application. Hence it is a contact herbicide so it is very safe to maize when applied as pre-planting or inter row for weed control.

Maize variety of Bioseed 9681is planted on slope soil 20-25o in rainy season (July - September). The minimum tillage method is implemented with first application of Gramoxone 20 SL with dosage 4 L $ha^{-1}$ directly to growing weed 2 days before planting and the second one applied as inter row at 30 days after planting with the dosage 2 L $ha^{-1}$. The conventional farmer practice is arranged in the study for comparison on time, manpower & cost saving for weed control and erosion. 2 x additional fertilizer is applied.

Efficacy of weed control by Gramoxone 20 SL is assessed visually at 1 - 2 days after application. Time & manpower spent for hand weeding in Farmer practice is calculated based on actual working of producer.

To compare soil erosion between 2 planting methods, cement soil traps of dimension 2m x 0.75m x 0.5m are designed at the bottom of hilling to collect erosion soil from whole plot of $150m^2$ appropriately.

Erosion soil collected in the traps are weighed and analyzed monthly on N, $P_2O_5$, $K_2O$. Soil from the plots are sampled at random in surface layer 0-25cm depth 2 days before planting and every month after planting for analysis on organic matter, N, $P_2O_5$, $K_2O$ at Laboratory of Soil & Fertilizer Research Institute. Total nutrient lost by erosion is calculated based on multiply the amount of erosion soil by nutrient percent of trapped soil in each month. Yield is actually harvested from whole plot and air dried up to 15%.

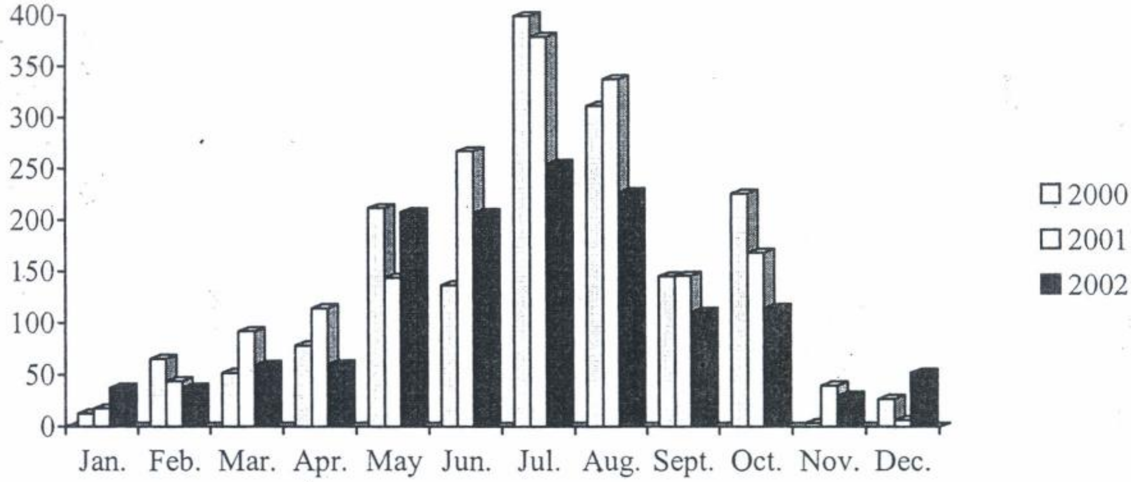
Analysis method on soil nutrient is followed method of FAO – ISRIC (1987 – 1995) and standard method of the National Institute for Soil and Fertilizer – NISF 1998 as follows:

- pH $H_2O$ by pH meter using distilled water – soil solution 1: 25
- OC% by method of Wakley – Black - Total N % by method of Kjeldahl
- Total $P_2O_5$ % by Spectrophotometer - Total $K_2O$ % by Flamephotometer - Available
- $P_2O_5$ by method of Oniani
- Available $K_2O$: by method of Bray II with Flamephotometer and Filter glass K 768mm
- CEC: Soil sample is impacted by $NH_4OAc$ (Amon axetat) at pH = 7 then wash by KCl (Kali clorua).

## RESULTS AND DISCUSSION

Table 1: Monthly rainfall in West North Vietnam

| Year | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2000 | 12.4 | 62.5 | 51.8 | 78.1 | 211.8 | 136.7 | 399.3 | 312.1 | 145.6 | 226.4 | 3.7 | 26.9 |
| 2001 | 17.4 | 43.7 | 92.0 | 114.3 | 144.0 | 267.6 | 379.2 | 388.0 | 146.2 | 168.8 | 39.8 | 6.4 |
| 2002 | 37.3 | 37.4 | 59.4 | 59.9 | 208.0 | 207.3 | 255.2 | 227.6 | 111.0 | 114.9 | 30.0 | 52.2 |




Maize planted in slope soil closely depends on rainfed water. However, figure collected from metorological station at Yen bai province (table 1) shows uneven distribution on rainfall between months in the year. High rainfall time concentrated during June to September is also the time for starting maize grown in North West region. All activities in planting maize at early season together with high rainfall strongly enhance erosion in this area.

Table 2 : Major weeds & efficacy in controlling them by GMX (pre-planting)

| *No* | *Weed species* | *Level infestation* | *Height of weed before application (cm)* | *Efficacy (%)* | |
|---|---|---|---|---|---|
| | | | | *1 DAA* | *2 DAA* |
| 1 | *Ageratum conizoides* | +++ | 78 | 75.1 | 90.2 |
| 2 | *Mimosa indica* | +++ | 53 | 81.5 | 95.8 |
| 3 | *Borreria atlata.* | +++ | 43 | 79.8 | 100 |
| 4 | *Commelina communis* | ++ | 31 | 78.5 | 96.0 |
| 5 | *Bidens pilosa* | + | 26 | 90.8 | 100 |
| 6 | *Crotalaria mucronate* | + | 68 | 92.3 | 100 |
| 7 | *Allmania albida* | + | 91 | 84.7 | 100 |
| 8 | *Eleusine indica* | + | 12 | 86.4 | 100 |
| 9 | *Eragrotis sp* | + | 27 | 100 | 100 |
| 10 | *Leptochloa chinensis* | + | 31 | 100 | 100 |
| 11 | *Saccha rum sp* | + | 187 | 75.0 | 98.8 |
| 12 | *Streblus sp* | + | - | Wilting | 50 |

Note : *DAA: Day after application.* *+++ Heavy infestation*
*Dosage: 4L $ha^{-1}$* *++ Moderate infestation*
*+ Light infestation*

Data in Table 1 shows that there are 12 major weeds damaging maize planted here. Some of them grow very fast (*Ageratum conizoides* 78 cm, *Saccha rum sp* 187cm) and some are difficult to be controlled by other non-selective herbicide such as *Mimosa pudica, Mentha arvensis* (Hoang Anh Cung 1980).At dosage 4 L $ha^{-1}$ applied pre-planting directly to growing weed, Gramoxone shows a fast action in killing weeds. Within 1-2 days from application a high efficacy of 75 – 100% has been obtained to almost weed species. This allows farmers to plant maize earlier right after application 1-2 days and creates possibility for farmers to plant next crop (soybean or upland rice) in optimal condition. Whilst in Farmer practice (Table 4) this time takes 31-37 days. That means farmers have to delay planting time for about one month. This hard work and time consumed results higher production cost of maize when compared to minimum tillage method. Besides the saving of manpower & planting time, the broad spectrum and fast action of Gramoxone in killing weed (Jeff Auu 2001) also allows farmers to plant maize quite actively, not depend on weather condition. And it is more important that this technique contributes in preventing forest fire because weed burning – a main factor causing this calamity has been eliminated.

Table 3 : Major weeds and efficacy in controlling them by GMX (inter row)

| No | Weed species | Level of infestation | Height of weed before application (cm) | Eficacy (%) 2 DAA |
|---|---|---|---|---|
| 1 | *Ageratum conizoides* | +++ | 15 | 99.8 |
| 2 | *Mimosa indica* | +++ | 12 | 99.8 |
| 3 | *Rottbolia exaltala* | ++ | 38 | 89.4 |
| 4 | *Leptochloa chinensis* | ++ | 21 | 91.3 |
| 5 | *Paspalum conjugatum* | + | 26 | 93.9 |
| 6 | *Paspalum sp.* | + | 22 | 100 |
| 7 | *Bidens pilosa* | + | 16 | 100 |
| 8 | *Crotalaria mucronate* | + | 18 | 94.0 |
| 9 | *Allmania albida* | + | 25 | 94.6 |
| 10 | *Eleusine indica* | + | 18 | 80.3 |
| 11 | *Borreria atlata.* | + | 16 | 91.5 |
| 12 | *Streblus sp* | + | - | Dried leaf only |

*Note :* - *DAA : Day after application* - *Dosage: Gramoxone 2 L $ha^{-1}$*

*+++ Heavy infestation*
*++ Moderate infestation*
*+ Light infestation*

Data from this table confirms good performance of Gramoxone even at low rate 2 L $ha^{-1}$. At two days after application as inter-row almost weeds infesting maize is controlled 80-100%. This is once again to show quick effectiveness of Gramoxone in controlling weeds. Time spent for Gramoxone application is just only 3-4 manpower days while farmer have to spend 16-18 manpower days for hand weeding. Out of the hard work as mentioned, doing hand weeding at this growth stage of maize faces some other disadvantages:

- In convenient for farmer working inside high and dense foliage of maize fields.
- Long time in hand weeding allows weeds still alive and having time to compete with maize on nutrient before to be killed.
- Some parts of plant (root, basal stem) may be mechanically injured during hoeing that creates entry points for bacteria or fungi come inside crop causing infestation.

These problems will be avoided once the minimum tillage method using Gramoxone is adopted by farmer.

Table 4: Comparison on manpower for weeding between Farmer Practice and Minimum Tillage method.

| Planting method | Time spent for weeding (manpower day $ha^{-1}$) | | | | | | | Total (manpower day $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | Cutting weed | Drying weed | Burning weed | Removing ash | Hand weeding 30 DAP | GMX appl. Pre-planting | GMX appl. Inter-row 30 DAP | |
| Farmer practice | 18-22 | 2-3 | 2-4 | 7-8 | 16-18 | 0 | 0 | 45-55 |
| Minimim Tillage | 1-2 | 0 | 0 | 0 | 0 | 3-4 | 3-4 | 7-10 |
| Saving | 14 | 3 | 2.5 | 4 | 9 | -3.5 | -3.5 | 50 |

Saving on time & manpower for weeding 50 manpower day $ha^{-1}$ as given in table 4 allows farmer to plant maize quite actively at early rainy season, not depend on either weather condition or available farm worker to contract. This also creates chance for farmer to plant next crop (soybean or upland rice) in optimum condition (in high rainfall month). Beside that another benefit recognized in Minimum Tillage method as seen in table 4 that the new cultural of minimum tillage method has contributed in preventing forest fire because of no more work of weed burning is implemented..

Table 5 : Soil erosion and nutrients lost

| Tillage methods | Erosion soil (kg $ha^{-1}$) | | | Total soil lost (kg $ha^{-1}$) | Total nutrients lost (kg $ha^{-1}$) | | | Yield (ton $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | July | Aug | Sept | | N | $P_2O_5$ | $K_2O$ | |
| Farmer practice | 4250 | 2950 | 2090 | 9290 | 17.8 | 8.9 | 20.1 | 4.21a |
| GXM minimum tillage | 2215 | 2060 | 1856 | 6131 | 11.1 | 6.2 | 12.4 | 4.27a |
| Reduced by GMX-minimum tillage: | | | | | | | | |
| Quantity (kg $ha^{-1}$) | 2035 | 890 | 234 | 3159 | 6.70 | 2.1 | 7.8 | - |
| Percent (%) | 47 | 30 | 11.2 | 34 | 37.6 | 22.2 | 38.6 | - |
| Rainfall (mm) | 345 | 295 | 145 | 785 | - | - | - | - |
| Soil covered by maize (%) | 25 | 95 | 85 | - | - | - | - | - |

Erosion on slope soil is influenced by different factors: Slope degree, rainfall, surface residue, soil texture, tillage method....Among that surface residue plays an important role to reduce erosion by minimizing rain drop splash and run-off velocity (Adam 1966, Myer et al 1970). Percentage of plant residue on soil surface increased as tillage intensive decreased (Kladivko et al 1986). Data in Table 5 shows total amount of soil erosion in minimum tillage is considerably less than in Farmer practice, 6131kg $ha^{-1}$ v s 9290 kg $ha^{-1}$ respectively. It is only 66% of Farmer practice or in other word, minimum tillage method of maize planting reduces 34% soil erosion compared to Farmer practice. The reason for that is in minimum tillage, Gramoxone is applied for weed control. This product only kills the parts of weed above ground to prevent their competition with maize on nutrient but their roots and other parts below ground is intact. Soil texture is not loosen so the cultivated layer is not disturbed resulting less erosion happened. In addition to this, weeds after to be killed their biomass remain in the soil as surface residue. It limits run-off of rain water to reduce erosion.

Contrary to that in Farmer practice, weed is cut, dried, burnt and ash is removed out then soil is hoed or plowed for planting maize. All these works impact strongly to soil structure making the cultivated layer loosen so that when rain coming, this layer, without surface residue is easily moved down leading stronger erosion compared to minimum tillage method.

Within maize growing season, erosion soil in July is more than in August & September. It's amount is 4250 kg, 2950 kg, 2090 kg per ha respectively in Farmer practice. The reason for this might due to in July when maize plant is still small (at vegetation growing stage) soil covered by maize is less, only 25% in comparison to 95% in August and 85% in September. In addition to this rainfall in this month is highest 345mm compared to 295mm and 145mm in August & September respectively. With both factors, higher rainfall & less coverage together with loosen soil surface at early season making soil erosion is occurred strongest in July compared to the rest months of the season. A similar situation is also observed in Minimum till method.

When comparisons on erosion situation in August and September, we find that amount of soil eroded in August is bigger than in September in both Farmer practice & Minimum tillage. This is due to higher rainfall 295mm in August v s 145mm in September. However, the different amount of erosion soil erosion between two these months in Farmer practice, 860 kg $ha^{-1}$ is much more than that one in Minimum tillage 204 kg $ha^{-1}$ only. The main reason here is because in August farmer has to do one more hand weeding by hoeing that as mentioned above makes soil surface loosen so it is strongly impacted by rain than in Minimum tillage where just Gramoxone 2L $ha^{-1}$ applied as inter-row for weed control.

The data in Table 5 also shows that total nutrients lost during season by erosion in Farmer practice is higher than in Minimum tillage, 17.8 kg N, 8.9 kg $P_2O_5$, 20.1 kg $K_2O$ v s 11.1 kg N, 6.2 kg $P_2O_5$, 12.4 kg $K_2O$ per ha respectively. Among key elements of nutrient, potassium lost is biggest 7.8 kg $ha^{-1}$, then come to Nitrogen 6.7 kg $ha^{-1}$ and least is Phosphorus 2.1 kg $ha^{-1}$. Though amount of total nutrient lost in Farmer practice is more than in Minimum tillage, the actual yield of maize harvested is not significantly different between two methods, 4.21 ton $ha^{-1}$ in Farmer practice and 4.27 ton $ha^{-1}$ in Minimum tillage. This may be due to high additional fertilizer applied can compensate the nutrient lost and satisfy the crop demand. Another reason may be due to our study has just been conducted for 3 seasons, the soil degradation is not big enough to affect the yield but the lower one from the Farmer practice might be foreseen in near future. In that situation in order to maintain an equal yield to the Minimum tillage, more fertilizer must be applied in the Farmer practice. That means yield may be the same but production cost is higher in Farmer practice.

Table 6 : Situation of soil fertility in plot.

| Soil quality indicators | 2 days before planting | | July | | August | | September | |
|---|---|---|---|---|---|---|---|---|
| | GMX minimum tillage | Farmer practice | GMX min. till | Farmer prac | GMX min. till | Farmer prac. | GMX min. till | Farmer prac. |
| pH $H_2O$ | 4.44 | 4.31 | 4.89 | 4.59 | 4.90 | 4.46 | 4.50 | 4.40 |
| Total OC (%) | 1.42 | 1.51 | 1.42 | 1.41 | 1.45 | 1.29 | 2.01 | 1.65 |
| Total N (%) | 0.13 | 0.11 | 0.15 | 0.16 | 0.17 | 0.14 | 0.15 | 0.11 |
| Total $P_2O_5$ (%) | 0.08 | 0.07 | 0.07 | 0.08 | 0.08 | 0.07 | 0.10 | 0.06 |
| Total $K_2O$ (%) | 0.15 | 0.14 | 0.12 | 0.12 | 0.13 | 0.11 | 0.14 | 0.08 |
| Available $P_2O_5$ mg $100g^{-1}$ | 3.28 | 3.57 | 3.59 | 3.16 | 2.88 | 2.45 | 17.40 | 12.36 |
| Available $K_2O$ mg $100g^{-1}$ | 7.53 | 8.12 | 8.75 | 8.56 | 15.06 | 14.06 | 18.60 | 13.22 |
| CEC (LdL $100g^{-1}$) | 6.52 | 6.92 | 6.21 | 6.52 | 6.20 | 6.08 | 6.92 | 5.52 |

Soil fertility contributes significantly to high yield of maize thus farmers have to apply fertilizer every season to compensate the nutrient lost by erosion and crop & weed uptake. In comparison between two methods the data in Table 6 shows that not clear difference on soil quality indicators found in July & August as well as before planting. But in September these data are significantly different. This may due to high additional fertilizer is applied concentrate in 2 first months during vegetation growth and reproduction stages of maize. In September at maturity stage no more fertilizer needed to apply in both methods while the erosion in Farmer practice is more than in Minimum tillage (Table 5). Beside that, in Minimum tillage after inter-row application of Gramoxone, died weed remains in soil, their biomass residue has contributed to improve soil fertility.

**Method of Minimum tillage using Grmoxone for Maize growing on slope soil:**

1. Application of Gramoxone 4L $ha^{-1}$ 2 days before planting when rainy season starts.
2. Planting maize 2 days after application
3. Application of Gramoxone 2 l $ha^{-1}$ 30 days after planting (inter-row)
4. Other cultivated work is followed as usual

## CONCLUSION

Using Gramoxone in Minimum tillage method for maize grown in slope soil brings more benefits compared to conventional Farmer practice:

- Save time & manpower in weeding: 21-33 days, 50 manpower days.
- Create chance for maize and next crop growing in optimal duration.
- Reduce erosion 34 % and improving soil fertility
- Contribute in preventing forest fire and in protecting the environment
- Obtain high Economical effectiveness in saving 750,000 VND $ha^{-1}$ compared to farmer practice.

With the mentioned benefits the Minimum tillage method should be largely promoted for farmer to adopt in planting maize on slope soil in North West Vietnam.

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# Broadleaf weed control in maize and soybean with postemergence herbicide fluthiacet-methyl 5%EC

Gao Xingxiang[1], Li Mei[1], Ruan Qingyou[2], Zhang Jianhua[2], Hou Hengjun[2]
[1]Institute of Plant Protection Shandong Academy of Agricultural Sciences, Jinan 250100, China
[2] Station for Plant Protection of Rencheng District, Jining, Shandong, China

**Abstract:** Field experiments were conducted in maize and soybean to determine the efficacy of fluthiacet-methyl 5%EC in 2004 in Shandong province China. The result showed that the grass *Amaranthus retroflexus* L. and *Physalis minima* L. could be effectively controlled by fluthiacet-methyl at the dosage of 3.75~15 g ai $ha^{-1}$, after spraying 30 days, 89.3%~100% in plants effect and 94.7%~99.9% in weight effect weed gained. Relatively good efficacy was achieved with broadleaf weed such as *Portulaca oleracea* L. and *Acalypha australis*. Fluthiacet-methyl did not injure maize, but injured soybean leaves after spraying 3 days, 7 days later the new leaves grew normally, and yield increases were obtained following control of weeds in maize and soybean.

**Key words:** Fluthiacet-methyl, maize, soybean, safety, weed control

## INTRODUCTION

Fluthiacet-methyl is a postemergence contact-type herbicide that provides excellent control of velvetleaf, but also has activity on some other broadleaves. It was registered for soybeans in 1997 and corn in 1998 at use rates of 1.5 to 2.25 oz/A. Fluthiacet-methyl was shown to be practically non-toxic to birds, practically non-toxic to small mammals, practically non-toxic to bees and other beneficial insects and its action will most likely be tank mixed with other products or be sold as a premix to increase the weed spectrum. Now more and more researchers are interesting in studying this herbicide in its clean process, synthesis, and so on. In order to check the effect on weeds in soybean and maize and its safety to plants, we carried out these experiments in Shandong province of China.

## MATERIALS AND METHODS

### Environmental conditions

Both field experiments were all conducted in sandy loam soil in maize and soybean with the main species of weeds *Amaranthus retroflexus* L., *Physalis minima* L., *Portulaca oleracea* L. and *Acalypha australis* on 28th June 2004 at jining, Shandong province China. The spraying day was sunshine, no wind and the mean temperature was 26.7□ and rainfall was 324.5mm in whole experiment period.

### Design and arrangement

The six treatments tested were: fluthiacet-methyl 5%EC 3.75, 7.5, 11.25 and 15 g a.i. $ha^{-1}$ (offered by Shenyang Chemistry Institute), control herbicide lactofen 24%EC 72ga.i. $ha^{-1}$ (bromoxynil octanoate 25% EC 375 g a.i. $ha^{-1}$ in maize) and unsprayed control. There were 4 replications in each treatment. The area of each treatment was $20m^2$, and ranged randomly, the solution were sprayed at 3-4 leaves stage of maize and 1-5 leaves stage of weeds at 600kg $hm^{-2}$.

Toxicity of the herbicides was surveyed by observing the leaves of maize, soybean and recording the symptoms and classification to confirm the safety to maize after spraying 1, 3, 7, 15 and 30 days respectively. The yield of maize was gained to determine the effect on maize production of the trialed herbicides.

Weeds plants were surveyed after spraying 15 and 30 days respectively. 4 points were sampled in each plot and each survey area was $0.25m^2$. The weight of weeds was surveyed after spraying 30 days.

All date were analysed by Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Effects to plants

In experimental period, Fluthiacet-methyl did not injure maize, but injured soybean leaves after spraying 3 days, 7 days later the new leaves grew normally.

In plant yield, broadleaf weed decreased the number and moist weight by Fluthiacet-methyl, but there were a lot of narrowleaf weed, so the effects of Fluthiacet-methyl to harvest were not significant. It increased plant harvest by 5.4, 6.2, 6.9% and 8.1% in soybean (Table 1) and by 2.1,5.6,4.3 and 5.6% in maize(Table 2).

Table 1. Effect of Fluthiacet-methyl 5%EC to harvest of soybean

| Treatment | Plant $hm^{-2}$ | Seed in one ear | Weight (g/100seeds) | Yield ($kg\ m^{-2}$) | Than CK±(%) |
|---|---|---|---|---|---|
| Fluthiacet-methyl 5%EC3.75ga.i. $ha^{-1}$ | 151500 | 117.1 | 15.1 | 178.6 | 5.4 |
| Fluthiacet-methyl 5%EC 7.5ga.i. $ha^{-1}$ | 152400 | 118 | 15 | 179.8 | 6.2 |
| Fluthiacet-methyl 5%EC 11.25ga.i. $ha^{-1}$ | 153000 | 117.6 | 15.1 | 181.1 | 6.9 |
| Fluthiacet-methyl 5%EC 15ga.i. $ha^{-1}$ | 152700 | 118.3 | 15.2 | 183.1 | 8.1 |
| lactofen 24%EC 72 ga.i. $ha^{-1}$ | 152700 | 118.1 | 15.1 | 181.5 | 7.2 |
| Control | 148650 | 113.2 | 15.1 | 169.4 | - |

Table 2. Effect of Fluthiacet-methyl 5%EC to harvest of maize

| Treatment | Plant $hm^{-2}$ | Seed in one ear | Weight (g/100seeds) | Yield ($kg\ m^{-2}$) | Than CK±(%) |
|---|---|---|---|---|---|
| Fluthiacet-methyl 5%EC3.75ga.i. $ha^{-1}$ | 61500 | 341.9 | 28.1 | 393.9 | 2.1 |
| Fluthiacet-methyl 5%EC 7.5ga.i. $ha^{-1}$ | 61500 | 351.4 | 28.3 | 407.7 | 5.6 |
| Fluthiacet-methyl 5%EC 11.25ga.i. $ha^{-1}$ | 61500 | 348.1 | 28.2 | 402.5 | 4.3 |
| Fluthiacet-methyl 5%EC 15ga.i. $ha^{-1}$ | 61500 | 352.5 | 28.2 | 407.6 | 5.6 |
| lactofen 24%EC 72 ga.i. $ha^{-1}$ | 61500 | 347.1 | 27.8 | 395.6 | 2.5 |
| Control | 61500 | 342.3 | 27.5 | 385.9 | - |

### Effects on weeds control

Fluthiacet-methyl 5%EC could controll the grass *Amaranthus retroflexus* L. and *Physalis minima* L. effectively at the dosage of 3.75~15 g a.i. $ha^{-1}$. It had common plant effect to *Acalypha australis*., but had good weight effect in maize or soybean.

In soybean (Table 3) after spraying 15 days, the plant effects of Fluthiacet-methyl 5%EC 3.75, 7.5, 11.25,15 ga.i. $ha^{-1}$ to *Amaranthus retroflexus* L. and *Physalis minima* L. were between 90.2% and 98.8%. Relatively poor efficacy to *Acalypha australis*. was achieved between 45.3% and 65.6%. The total broadleaf plant effects were 81.5%、84.4%、83.3% and 90.6%, respectively, and control herbicide was 94.6%.

After spraying 30 days, the plant effects of four dosages to broadleaf weed were 88.1%, 95.0%, 94.3% and 98.1%, respectively. Immense weight effects were achieved by 98.9%, 99.9%, 99.8% and 99.9%, respectively.

Table 3. Effect of Fluthiacet-methy l 5% EC on broadleaf weed in soybean

| Period | Treatments | Plant effect (%) | | | | | | | Weight effect (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Physalis minima* L. | *Acalypha australis* L. | *Portulaca oleracea* L. | *Amaranthus retroflexus* L. | Total | | | Total | | |
| Sprayed 15 days | Fluthiacet-methyl 5% EC 3.75ga.i. $ha^{-1}$ | 92.7 | 45.3 | 71.4 | 93.3 | 81.5 | c | B | | | |
| | Fluthiacet-methyl 5% EC 7.5ga.i. $ha^{-1}$ | 92.7 | 51.6 | 71.4 | 95.7 | 84.4 | bc | AB | | | |
| | Fluthiacet-methyl 5% EC 11.25ga.i. $ha^{-1}$ | 90.2 | 59.4 | 85.7 | 90.9 | 83.3 | c | B | | | |
| | Fluthiacet-methyl 5% EC 15ga.i. $ha^{-1}$ | 95.1 | 65.6 | 100 | 98.8 | 90.6 | ab | AB | | | |
| | lactofen 24% EC 72 ga.i. $ha^{-1}$ | 100 | 79.7 | 100 | 98.8 | 94.6 | a | A | | | |
| | Control | - | - | - | - | - | | | | | |
| Sprayed 30 days | Fluthiacet-methyl 5% EC 3.75ga.i. $ha^{-1}$ | 100 | 64.1 | - | 95.5 | 88.1 | a | A | 98.9 | b | A |
| | Fluthiacet-methyl 5% EC 7.5ga.i. $ha^{-1}$ | 100 | 79.5 | - | 100 | 95.0 | a | A | 99.9 | a | A |
| | Fluthiacet-methyl 5% EC 11.25ga.i. $ha^{-1}$ | 100 | 79.5 | - | 99.1 | 94.3 | a | A | 99.8 | ab | A |
| | Fluthiacet-methyl 5% EC 15ga.i. $ha^{-1}$ | 100 | 92.3 | - | 100 | 98.1 | a | A | 99.9 | a | A |
| | lactofen 24%EC 72 ga.i. $ha^{-1}$ | 100 | 84.6 | - | 98.2 | 95.0 | a | A | 99.9 | a | A |
| | Control | - | - | - | - | - | | | - | | |

*The values were significantly different from the values with different letters by Duncan's multiple range test.

Table 4. Effect of Fluthiacet-methyl 5%EC on broadleaf weed in maize

| Period | treatments | Plant effect (%) *Physalis minima* L. | *Acalypha australis* L. | *Portulaca oleracea* L. | *Amaranthus retroflexus* L. | Total | | | Weight effect (%) Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sprayed 15 days | Fluthiacet-methyl 5% EC 3.75ga.i. $ha^{-1}$ | 92.7 | 45.3 | 71.4 | 93.3 | 81.5 | c | B | | | |
| | Fluthiacet-methyl 5% EC 7.5ga.i. $ha^{-1}$ | 92.7 | 51.6 | 71.4 | 95.7 | 84.4 | bc | AB | | | |
| | Fluthiacet-methyl 5% EC 11.25ga.i. $ha^{-1}$ | 90.2 | 59.4 | 85.7 | 90.9 | 83.3 | c | B | | | |
| | Fluthiacet-methyl 5% EC 15ga.i. $ha^{-1}$ | 95.1 | 65.6 | 100 | 98.8 | 90.6 | ab | AB | | | |
| | lactofen 24% EC 72 ga.i. $ha^{-1}$ | 100 | 79.7 | 100 | 98.8 | 94.6 | a | A | | | |
| | Control | - | - | - | - | - | | | | | |
| Sprayed 30 days | Fluthiacet-methyl 5% EC 3.75 ga.i. $ha^{-1}$ | 100 | 64.1 | - | 95.5 | 88.1 | a | A | 98.9 | b | A |
| | Fluthiacet-methyl 5% EC 7.5 ga.i. $ha^{-1}$ | 100 | 79.5 | - | 100 | 95.0 | a | A | 99.9 | a | A |
| | Fluthiacet-methyl 5% EC 11.25 ga.i. $ha^{-1}$ | 100 | 79.5 | - | 99.1 | 94.3 | a | A | 99.8 | ab | A |
| | Fluthiacet-methyl 5% EC 15 ga.i. $ha^{-1}$ | 100 | 92.3 | - | 100 | 98.1 | a | A | 99.9 | a | A |
| | lactofen 24% EC 72 ga.i. $ha^{-1}$ | 100 | 84.6 | - | 98.2 | 95.0 | a | A | 99.9 | a | A |
| | Control | - | - | - | - | - | | | | - | |

In maize (Table 4)after spraying 15 days, 91.3%~100% to *Amaranthus retroflexus* L. and 73.3%~100% to *Acalypha australis*., *Portulaca oleracea* L. were gained of Fluthiacet-methyl 5%EC on plant effect The total plant effects to broadleaf weed were 88.1%, 91.0%, 92.2% and 95.1%, respectively.

After spraying 30 days, the total plant and weight effects to broadleaf weed were 85.3%~93.6% and 94.7%~98.3%.

**Path analysis**

Based on the results of experiments in maize and soybean, we say that Fluthiacet-methyl 5%EC was an excellent postmergence herbicide although it had injury on soybean in early period. The grass *Amaranthus retroflexus* L. and *Physalis minima* L. which had 2~5 leaves period could be effectively controlled by fluthiacet-methyl 5%EC at the dosage of 3.75~15 g ai $ha^{-1}$, and relatively good efficacy was achieved with *Portulaca oleracea* L. and *Acalypha australis*. Fluthiacet-methyl 5%EC can be tank mixed with other products which had better effect on narrowleaf weed to increase the weed spectrum.

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# Evaluation of tank mixtures of metribuzin and pendimethalin for weed management in soybean (*Glycine max*) in India

Ch. Sreedhar [1], G. Shankar [2] and M.S. Mithyantha [3]
Rallis Research Centre, Rallis India Limited, 21 & 22, Peenya Industrial Area, Phase II, Bangalore - 560 058, Karnataka, India
[1] chemuturi.sreedhar@rallis.co.in
[2] gshankar@rallis.co.in
[3] msmithyantha@rallis.co.in

**Abstract:** Experiments were conducted in the field during the years 2001-2002 and 2002-2003 to evaluate the usefulness of various application rates of metribuzin 70WP singly and as tank mix with pendimethalin 30E at 500, 750 and 1000 g a.i. $ha^{-1}$ for weed management in soybean. The treatments were imposed as pre-emergence blanket soil surface spray. Stand alone application of metribuzin 70WP up to 525 g a.i. $ha^{-1}$ was safe to soybean. A progressive increase in per cent control of weeds and decrease in dry weight of weeds was noticed with increased dosage of pendimethalin 30E as a tank mix with metribuzin 70WP at 250 g a.i. $ha^{-1}$. Results indicate that the treatments of tank mixture of metribuzin 70WP and pendimethalin 30E at 250+750 and 250+1000 g a.i. $ha^{-1}$were on par with that of stand alone application of metribuzin 70WPat 525 g a.i. $ha^{-1}$ and pendimethalin 30E at 1000 g a.i. $ha^{-1}$ up to 30 days after sowing. However, these tank mixtures were significantly superior to those of stand alone application treatments up to 60 days after sowing.

**Key words**: Bio-efficacy, broadspectrum control of weeds, metribuzin 70WP, pendimethalin, tank mixtures.

## INTRODUCTION

Soybean [*Glycine max* (L.) Merrill.] is one of the important kharif oil seed crops of India. Weed competition is considered a major constraint in soybean cultivation (Bhan et al. 1974). Soybean being initially a slow growing crop and continuous rainfall during this period further aggravates the situation, making the conditions congenial for severe infestation of broad spectrum of weeds. The weeds must be controlled within the critical period of first 30 days of sowing to prevent crop losses. Weeds not kept under check during the critical period may lead to crop loss of 20 to 85%, depending on intensity of infestation, duration of competition and density of crop (Bhan 1974; Bhan, 1975; Dharm et al. 1992; Govindra Singh et al. 2004; Hammerton 1974; Knezeric et al.2003; Kurchania et al. 2001; Kurumawanshi et al. 1995; Moody 1973; Singh and Singh 1987; Tiwari and Kurchania 1990; Zhang ZePu 2003). In soybean fields, the major weed flora are *Cyanotis* spp., *Cyperus rotundus, Commelina benghalensis, Cynodon dactylon, Dactyloctenium aegyptium, Dinebra retroflexa, Echinochloa colona, Eleusine indica, Euphorbia geniculata, Euphorbia hirta, Portulaca oleracea, Parthenium hysterophorous, Trianthema monogyna and Xanthium strumarium.* The conventional method of weed control is time consuming, expensive and laborious. Moreover, rainfall during the cropping period and unworkable soil conditions do not permit, manual weeding during critical crop growth stages. Therefore, chemical weed control is the only alternative to increase the yield of soybean.

At present, pendimethalin, fluchloralin, metolachlor and alachlor are being recommended for chemical weed control in soybean as pre-emergence application or as pre-plant incorporation (Bhalla et al. 1998; Dubey 1998; Jain et al. 1998; Singh and Bhan 1997). There are reports that continuous use of these herbicides resulted in a shift in weed flora. Therefore, there is a need to develop alternate herbicides which may provide broad spectrum control of weeds when applied alone or in combination. In view of this, present investigation was carried out to study the bio-efficacy of tank mixtures of metribuzin and pendimethalin over the existing herbicides for control of mixed population of weeds.

## MATERIALS AND METHODS

Field investigations were carried out for two years in the State of Karnataka during the Kharif season (June to November) of 2001-2002 and 2002-2003 in a field with medium black soil. Soybean variety var. JS335 was sown in a finely prepared seedbed by drill sowing on June 05, 2001 and June15, 2002 respectively in the two years. Sowing was done in such a manner that row spacing of 30 cm and hill distance of 10 cm was maintained using a seed rate of 75 kg seed $ha^{-1}$. The recommended doses of N: $P_2O_5$ at 20:60 and $K_2O$ at 20kg per hectare was applied at the time of sowing.

Treatments of tank mixtures of metribuzin and pendimethalin at 250+500, 250+750 and 250+1000 g a.i. $ha^{-1}$ were compared with stand alone application of metribuzin at 425 g, 525 g a.i. $ha^{-1}$, pendimethalin at 1000 g a.i. $ha^{-1}$, weed free treatment and weedy check plots. The plot size was 10 m x 10 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 backpack sprayer fitted with flat fan nozzle and a spray volume of 750 L $ha^{-1}$. A light irrigation was given to the experimental plots one day after imposing the treatments.

Visual phytotoxicity of herbicide treatments on crop were recorded at 5 and 10 DAS 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). Plant population per unit area and height of plants were recorded at 30 and 45 DAS. Number of weeds per unit area and dry weight of weeds were recorded at 30, 45 and 60 DAS within 1.0 m x 1.0 m quadrat at three places at random in each plot. The data on number of weeds were subjected to square-root transformation using $\sqrt{n+1}$. Dry weight of weeds under each treatment was recorded after drying the weeds in an oven at 70°C until constant weight was achieved. Grain and stover yield was recorded at harvest.

## RESULTS AND DISCUSSION

Phytotoxicity

Plants in the plots treated with tank mixtures of metribuzin and pendimethalin at all the rates tried and stand alone application of metribuzin or pendimethalin did not exhibit any symptom of phytotoxicity in terms of leaf injury, vein clearing, wilting, necrosis, epinasty and hyponasty symptoms in plants. The data on plant population and height of plants (Table 1) further showed that the tank mixtures of metribuzin and pendimethalin at all the rates tried did not exhibit any phytotoxicity on the crop.

Plant population

Number of plants per unit area was not influenced by the treatments at both observations (Table 1).

Height of Plants

Height of plants did not exhibit any significant difference among the herbicide treatments at observations recorded at 30 DAS (Table 1). However, height of plants at 45 DAS under the treatments of tank mixture of metribuzin and pendimethalin at 250+500 g a.i. $ha^{-1}$ and stand alone application of metribuzin at 425 g a.i. $ha^{-1}$ were significantly lower.

Table 1. Effect of treatments on biometric parameters.

| Treatments | Dose (g a.i. $ha^{-1}$) | No. of plants $m^{-2}$* 30 DAT | 45 DAT | Height of plants (cms) * 30 DAT | 45 DAT |
|---|---|---|---|---|---|
| Metribuzin+ Pendimethalin | 250 + 500 | 63.6 | 65.3 | 23.2 | 35.6 |
| Metribuzin+ Pendimethalin | 250 + 750 | 63 | 62.9 | 24.2 | 40.5 |
| Metribuzin+ Pendimethalin | 250 + 1000 | 62.1 | 62 | 24 | 40.9 |
| Metribuzin | 425 | 63.9 | 63 | 23.9 | 34.5 |
| Metribuzin | 525 | 64.0 | 63.3 | 23 | 41.6 |
| Pendimethalin | 1000 | 62.9 | 63.1 | 24 | 41.0 |
| Weed free | - | 64.6 | 64.0 | 24.9 | 41.2 |
| Weedy check | - | 61.2 | 58.3 | 15.4 | 25.3 |
| SEm ± | | | | 0.5 | 0.5 |
| CD at 5% | | NS | NS | 1.7 | 1.5 |

* Mean of 2001 –2002 & 2002-2003

Weed flora

The weedy check plots were infested with broadleaf weeds like *Commelina benghalensis, Euphorbia geniculata, E. hirta, Portulaca oleracea, Parthenium hysterophorous* and *Xanthium strumarium,* the grassy weeds were *Echinochloa colonum, Cynodon dactylon* and *Dinebra retroflexa* and the sedge *Cyperus rotundus.*

Effect on weeds

Weedy check treament recorded the highest number of weeds and dry weight of weeds per unit area at all the observations (Fig.1 and 2). Stand alone application of metribuzin at 425 g a.i. $ha^{-1}$ and tank mixture of metribuzin and pendimethalin at 250+500 g a.i.$ha^{-1}$ led to significant reduction in the number of weeds and dry weight of weeds. Treatments of tank mixtures of metribuzin and pendimethalin at 250+750 and 250+1000 g a.i. $ha^{-1}$ recorded the lowest number and dry weight of weeds and these treatments were comparable to that of the stand alone application of metribuzin at 525 g a.i. $ha^{-1}$, pendimethalin at 1000 g a.i. $ha^{-1}$ and weed free plots at observations recorded at 30 DAS. However, the treatments of tank mixtures of metribuzin and pendimethalin at 250+750 and 250+1000 g a.i. $ha^{-1}$ were significantly superior to that of the standard treatments of sole application of metribuzin and pendimethalin at 45 and 60 DAS.

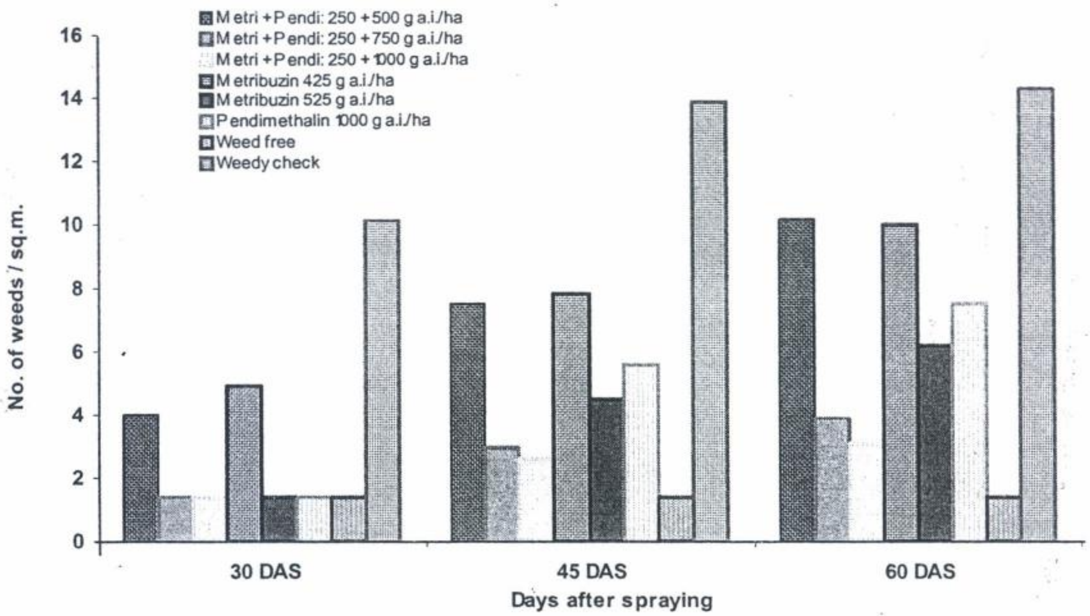


Figure1. Influence of treatments on weed density (mean of 2001-2002 & 2002-2003).

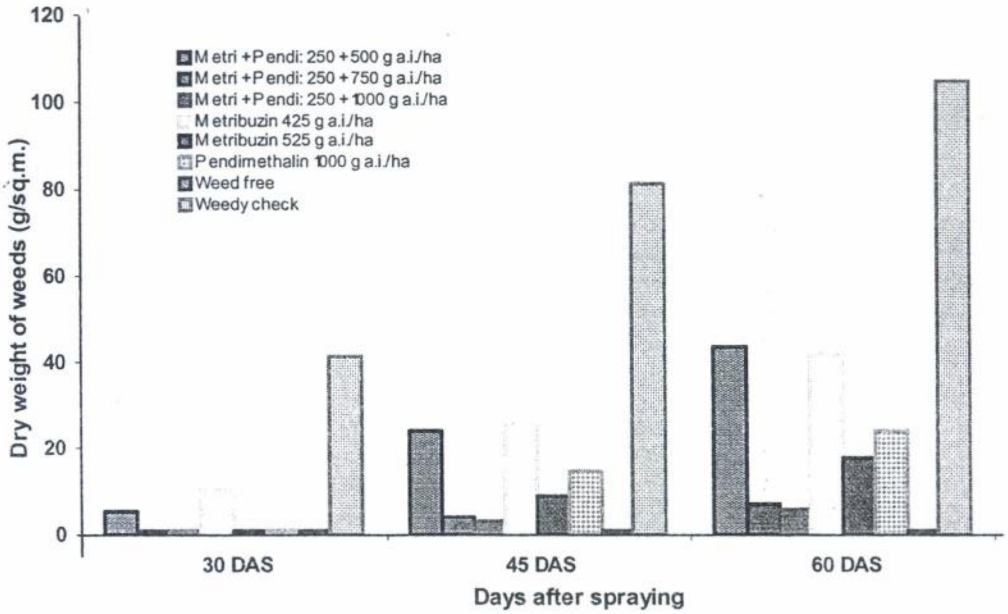


Figure2. Influence of treatments on dry weight of weeds (mean of 2001-2002 & 2002-2003).

Yield

Tank mixtures of metribuzin and pendimethalin at250+750 and 250+1000 g a.i. $ha^{-1}$ recorded the highest number of pods $plant^{-1}$ there by leading to higher grain and stover yield (Table 2). These treatments were significantly superior to that of the standard treatments of stand alone application of metribuzin and pendimethalin at the recommended rates (Table 2). Among the herbicide treatments, tank mixture of metribuzin and pendimethalin at 250+500 and stand alone application of metribuzin at 425 g a.i. $ha^{-1}$ recorded the lowest number of pods $plant^{-1}$, grain and stover yield. The reduction in yield and its attributes were attributed due to weed competition (Bajpai et al. 1973; Malik and Lal 1973). Weedy check treatment recorded the lowest grain and stover yield.

Table 2. Effect of treatments on yield attributes of soybean.

| Treatments | Dose (g a.i. $ha^{-1}$) | No. of pods $plant^{-1}$ | | Grain yield (kg $ha^{-1}$) | | Stover yield (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | 2001 | 2002 | 2001 | 2002 | 2001 | 2002 |
| Metribuzin + Pendimethalin | 250+500 | 14.3 | 17 | 1410 | 1500 | 1833 | 1950 |
| Metribuzin + Pendimethalin | 250+750 | 27.1 | 29 | 1985 | 2080 | 2780 | 2804 |
| Metribuzin + Pendimethalin | 250+1000 | 28 | 29.6 | 2010 | 2150 | 2813 | 2810 |
| Metribuzin | 425 | 15.2 | 17.3 | 1490 | 1520 | 1937 | 1976 |
| Metribuzin | 525 | 26.2 | 28.5 | 1740 | 1800 | 2460 | 2403 |
| Pendimethalin | 1000 | 25.2 | 29.1 | 1720 | 1790 | 2420 | 2390 |
| Weed free | - | 28.9 | 30.1 | 2024 | 2190 | 2831 | 2847 |
| Weedy check | - | 102 | 11.2 | 930 | 1050 | 1209 | 1365 |
| SEm ± | | 1.3 | 1.3 | 73 | 86 | 104 | 114 |
| CD at 5% | | 4 | 4 | 223 | 263 | 315 | 347 |

Mean of 2001 –2002 & 2002-2003


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# Herbicidal and eco-safety weed management in soybean in the entisol of West Bengal during wet season

P. Patra* and R. Haque
Professor, Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya,
Mohanpur 741252, Nadia, West Bengal, India

**Abstract:** A field experiment on weed control in soybean was conducted in Gangetic alluvial soil (entisol) during wet seasons of 2001 and 2002. The treatments consisted of fluchloralin, pendimethalin, oxadiazon and metolachlor at 0.5 kg a.i. $ha^{-1}$, and oxyfluorfen at 0.2 kg a.i. $ha^{1}$, as pre-emergence, alone or in combination with hand weeding (HW) at 30 days after sowing (DAS), spading at 30 DAS, HW at 30 DAS or 30 and 45 DAS, paddy straw mulch at 5.0 t $ha^{-1}$, narrow row spacing (22.5 cm) and unweeded control. Respective weed mortality (%) and weed control efficiency (%) values at 90 DAS were high in twice HW (94 and 95), HW at 30 DAS (91 and 83), pendimethalin + HW (85 and 88), metolachlor + H.W. (84 and 81), mulching (81 and 89) and narrow row spacing (72 and 81). Nodule number and nodule dry weight per plant at 45 DAS were higher in eco-safety weed control treatments than in herbicides. Respective total shoot growth (g $m^{-2}$) at 90 DAS and seed yield (t $ha^{-1}$) of soybean in eco-safely weed control treatments e. g., narrow row spacing (461 and 2.35) and mulching (442 and 2.21), and that of combination of pendimethalin + H.W. (500 and 2.40) were as good as that of recommended practice of twice H.W. (561 and 2.49). Net returns in narrow row spacing (Rs. 19710 $ha^{-1}$) and mulching (Rs. 18230 $ha^{-1}$) were slightly lower than twice H.W. (Rs. 21560 $ha^{-1}$) and pendimethalin + H.W. (Rs. 20484 $ha^{-1}$).

**Key words**: Eco-safety, mulching, weed mortality, weed control efficiency, net return.

## INTRODUCTION

Soybean is grown in India mainly as a rainfed wet season crop. At the early stage of crop growth, the problem of weed competition is acute during the wet season of this area. The efficacy of different herbicides in soybean crop has been studied (Dubey et al. 1997; Patra 1999; Singh et al. 2003; Ralli et al. 2003). But at present, special emphasis is being given on eco-safety methods of weed control. In the present investigation, some eco-safety mechanical and cultural methods were compared with chemical methods of weed control in soybean.

## MATERIALS AND METHODS

The field experiment on weed control in soybean (*Glycine max* L. Merrill) was conducted during wet season of 2001 and 2002 at the Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India, situated at $22.93^0$N latitude and $88.53^0$E longitude and 9.75 m above mean sea level, on the *entisol,* in randomised block design. The treatments consisted of herbicides, *viz.*, fluchloralin, pendimethalin, oxadiazon and metolachlor at 0.5 kg a.i. $ha^{-1}$ and oxyfluorfen at 0.2 kg a.i. $ha^{-1}$ as pre-emergence, alone or in combination with Hand Weeding (HW) at 30 days after sowing (DAS), eco-safety practices *viz.*, spading at 30 DAS, HW once at 30 DAS, HW twice at 30 and 45 DAS, soybean sown at narrow row spacing of 22.5 cm and paddy straw mulching at 5 t $ha^{-1}$ along with unweeded control. Soybean cv. Pb-1 (115 days) was sown normally at 45 cm apart rows at the first week of July with uniform basal dose of fertilizer at 20: 60: 40 kg N: $P_2O_5$ : $K_2O$ $ha^{-1}$ in the form of urea, single super phosphate and muriate of potash, respectively.

## RESULTS AND DISCUSSION

### Effect on weed population

In the experimental field, grass and sedge weeds were dominant over broadleaved ones (Table 1). Weeding twice was the most effective treatment in reducing weed population (at 90 DAS). Among the herbicides, pendimethalin + HW gave the lowest weed population, which was statistically at par with hand weeding twice. Fluchloralin + HW and metolachlor + HW were similar to pendimethalin + HW. Combination of herbicides with hand weeding showed better control of weeds than herbicides alone. Spading and weeding once recorded higher weed population than weeding twice. Weed mortality was the highest in twice hand weeding, followed by pendimethalin + HW, fluchloralin + H.W., metolachlor + H.W., weeding once and spading (Table 1).

Table 1. Effect of different weed control treatments on weed population (no. $m^{-2}$), weed dry weight (g $m^{-2}$) and dry weight of soybean shoot (g $m^{-2}$) at 90 DAS.

| Treatment | Weed population | | | | | Weed dry weight | | | | | Soybean shoot |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grass | Sedge | Broad-leaved | Total | Weed mortality (%) | Grass | Sedge | Broad-leaved | Total | Weed control efficiency (%) | |
| Fluchloralin | 82 | 30 | 6 | 118 | 70.9 | 34 | 5.3 | 2 | 41.3 | 70.4 | 360.6 |
| Fluchloralin + Weeding at 30 DAS | 37 | 17 | 10 | 64 | 84.2 | 16 | 3 | 3 | 22 | 84.2 | 497.3 |
| Pendimethalin | 112 | 38 | 3 | 153 | 62.1 | 44 | 11.6 | 1.2 | 56.8 | 59.2 | 393.3 |
| Pendimethalin + Weeding at 30 DAS | 40 | 12 | 8 | 60 | 85.3 | 6.6 | 5.3 | 4.7 | 16.6 | 88.1 | 500 |
| Oxadiazon | 144 | 51 | 4 | 199 | 50.7 | 70.6 | 8 | 2.2 | 80.8 | 42 | 248 |
| Oxadiazon + Weeding at 30 DAS | 117 | 46 | 4 | 167 | 58.8 | 45.3 | 12 | 1.3 | 58.6 | 57.9 | 288.6 |
| Metolachlor | 171 | 23 | 4 | 198 | 51.1 | 50.6 | 19.3 | 0.8 | 70.7 | 49.3 | 346.6 |
| Metolachlor + Weeding at 30 DAS | 48 | 13 | 3 | 64 | 84.2 | 12 | 13.3 | 0.6 | 25.9 | 81.4 | 402.6 |
| Oxyfluoren | 214 | 16 | 2 | 232 | 42.6 | 66.6 | 12.6 | 1.7 | 80.9 | 41.9 | 317.3 |
| Oxyfluorfen + Weeding at 30 DAS | 78 | 34 | 4 | 116 | 71.4 | 26.6 | 7.3 | 1.4 | 35.3 | 74.7 | 352 |
| Spading at 30 DAS | 30 | 20 | 17 | 67 | 83.4 | 42.6 | 6 | 2.7 | 51.3 | 63.2 | 395.3 |
| Weeding once at 30 DAS | 36 | 17 | 11 | 64 | 84.3 | 13.3 | 7 | 3.3 | 23.6 | 83.1 | 442.6 |
| Weeding twice at 30 & 45 DAS | 10 | 6 | 8 | 24 | 94.1 | 3 | 3.2 | 1 | 7.2 | 94.8 | 561 |
| Narrow row spacing (22.5 cm) | 93 | 17 | 4 | 114 | 71.9 | 20 | 6.6 | 0.2 | 26.8 | 80.8 | 491.3 |
| Mulching (Paddy straw @ 5.0 t $ha^{-1}$) | 52 | 13 | 13 | 78 | 80.8 | 6.2 | 7.3 | 2.1 | 15.6 | 88.8 | 442.8 |
| Unweed control | 345 | 49 | 10 | 404 | | 108 | 21.6 | 9.7 | 139.3 | | 232 |
| **C.D. (at 5%)** | 46 | 12 | 6.2 | 39 | | 10.2 | 7.6 | 0.8 | 11.3 | | 61 |

Effect on nodulation (45 DAS)

### Effect on dry weight of weed

All the weed control treatments reduced the grass and total weed growth significantly over unweeded control (Table 1). Hand weeding twice recorded the lowest dry weight of weed, which was statistically similar to mulching and pendimethalin + HW, in respect of grass, sedge and total weed dry weights. Control of all types of weeds with pendimethalin was similarly reported by Kalpana and Velayutham (2004). Straw mulch reduced the growth of weed, similar to the finding of

Nimje (1996). Narrow row spacing recorded a reduced growth of weed similar to weeding once. Weed control efficiency was the highest with twice hand weeding, followed by mulching and pendimethalin + HW (Table 1). High weed control efficiencies of narrow row spacing and mulching were similarly reported by Nimje (1996).

Effect on dry matter accumulation in soybean

At 90 DAS, shoot growth of soybean in the plots of oxadiazon, alone, or in combination with hand weeding, were at par with unweeded control (Table 1). Growth of soybean in oxyfluorfen-treated plots were also low. This result was due to some toxic effect of these herbicides on plants. In all other treatments, soybean growth augmented significantly over weedy check. Hand weeding twice recorded the maximum growth of soybean shoot. Shoot growth in plot hand weeded twice was appreciably higher than other treatments, followed by pendimethalin + HW, fluchloralin + HW, narrow row spacing and mulching.

Nodule number per plant of soybean was significantly higher in solely hand weeded plots (once or twice), lower in the plots of chemical treatments, narrow row spacing and spading, and similar in the plots of mulching, as compared to unweeded control (Table 2). The dry weight of nodule per plant was high in plots hand weeded once or twice and mulched plots. It was low in all other plots, although the differences from unweeded control were not always significant. Thus herbicides, spading and excess population showed decreasing effect on nodule. Reduction in nodulation with herbicides was similarly reported by Ralli et al. (2003).

Effect on yield components

Toxic effect of oxadiazon and oxyfluorfen reduced the plant population of soybean (Table 2). Narrow row spacing obviously increased the plant population, and other treatments did not show any difference among themselves, in this respect. Twice hand weeding recorded the highest number of filled pod per plant while the weedy check had the lowest. Fluchloralin, pendimethalin and oxadiazon applied alone, and narrow row spacing were not superior to weedy check, in this respect. Other treatments augmented the pod number significantly over weedy check, and were at par with twice hand weeding. Number of seeds per pod did not vary significantly among the treatments. The highest test weight was observed in twice hand weeding, which was at par with single hand weeding, mulching, pendimethalin and metolachlor treated plots, but appreciably higher than other treatments.

Effect on seed yield

The highest seed yield was produced in twice hand weeding treatment, followed by pendimethalin + HW and narrow row spacing (Table 2). The latter two treatments were not significantly superior to fluchloralin + HW, mulching and metolachlor + HW Other workers also reported efficacy of pendimethalin (Dubey et al. 1997; Ralli et al. 2003; Kalpana and Velayuthum 2004), fluchloralin (Nimje 1996; Patra 1999) and metolachor (Dubey et al. 1997). Thus, eco-safety methods of weed control, like narrow row spacing and mulching were as productive as the efficient combinations of herbicides and hand weeding, and sometimes close to twice hand weeding. Yield increase with narrow row spacing and mulching corroborated the finding of Nimje (1996). Oxadiazon and oxyfluorfen treatments, due to toxic effect on crops, reduced the productivity over that of weedy check. Other treatments augmented the productivity of soybean over unweeded control, but were inferior to the earlier treatments. Per cent increase in yield over weedy check was highest with twice hand weeding (76), followed by pendimethalin + HW (70), narrow row spacing (66), fluchloralin + HW (62), mulching (56) and metolacholor + HW (56). Oxyfluorfen and oxadiazon reduced the yield by 5 to 32% over that of control.

Effect on net return and benefit- cost ratio

The highest net return was observed in twice hand weeding treatment, followed by pendimethalin + HW, narrow row spacing, fluchloralin + HW and mulching. Very low return was obtained from oxadiazon and oxyfluorfen treatments, even lower than the unweeded check. The highest benefit–cost ratio was recorded in pendimethalin + hand weeding, followed by narrow row spacing and pendimethalin alone.

The results indicate that pre-emergence application of pendimethalin at 0.5 kg a.i. $ha^{-1}$ + hand weeding at 30 DAS is highly efficient in controlling weeds, and increasing soybean yield and remuneration. Hand weeding twice at 30 and 45 DAS is the most productive, remunerative and ecologically safe, but costly also. But it is practicable where labor is available and farmers can afford the high cost associated with it. Narrow row spacing (22.5 cm) and straw mulching at 5 t $ha^{-1}$ are slightly less productive and remunerative than twice hand weeding and pendimethalin + HW, but are ecologically safe.

Table 2. Effect of different weed control treatments on nodulation at 45 DAS, yield components, seed yield of soybean, net return and benefit-cost ratio.

| Treatment | Nodulation $plant^{-1}$ | | Yield components | | | | Seed yield | | Net return | Benefit cost ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Dry weight (g) | Plant population | No. of filled pod $plant^{-1}$ | No. of seed $pod^{-1}$ | Test Weight (g/100 seeds) | Kg $ha^{-1}$ | % increase (+ve) or decrease (-ve) | ($Rs.ha^{-1}$) | |
| Fluchloralin | 5.7 | 0.037 | 35 | 76.5 | 2.2 | 10 | 2047 | 44.8 | 16107 | 1.1 |
| Fluchloralin + Weeding at 30 DAS | 4.9 | 0.029 | 36 | 92.9 | 2.4 | 9.6 | 2287 | 61.8 | 18447 | 1.16 |
| Pendimethalin | 3.4 | 0.028 | 35 | 81.7 | 2.2 | 10.3 | 2126 | 50.4 | 17634 | 1.24 |
| Pendimethalin + Weeding at 30 DAS | 3.3 | 0.032 | 35 | 97.5 | 2.4 | 10.3 | 2400 | 69.8 | 20484 | 1.32 |
| Oxadiazon | 4.5 | 0.029 | 13 | 83.6 | 2.2 | 9.6 | 956 | -32.3 | 515 | -- |
| Oxadiazon + Weeding at 30 DAS | 4.9 | 0.035 | 14 | 95.3 | 2.2 | 9.3 | 1133 | -19.8 | 1350 | 0.08 |
| Metolachlor | 5.4 | 0.044 | 35 | 86.9 | 2.1 | 10 | 1919 | 35.8 | 14610 | 1.03 |
| Metolachlor + Weeding at 30 DAS | 5.5 | 0.034 | 36 | 94.2 | 2.2 | 10.1 | 2207 | 56.1 | 17670 | 1.14 |
| Oxyfluoren | 3.4 | 0.033 | 19 | 89.9 | 2.3 | 9.6 | 1101 | -22 | 2436 | 0.17 |
| Oxyfluorfen + Weeding at 30 DAS | 4.1 | 0.026 | 18 | 97.6 | 2.5 | 8.8 | 1341 | -5 | 4776 | 0.31 |
| Spading at 30 DAS | 5 | 0.041 | 35 | 80.2 | 2.4 | 9.1 | 1815 | 28.4 | 13350 | 0.96 |
| Weeding once at 30 DAS | 9.4 | 0.074 | 35 | 90.5 | 2.1 | 10.6 | 2057 | 45.5 | 16350 | 1.12 |
| Weeding twice at 30 & 45 DAS | 9 | 0.076 | 36 | 102.5 | 2.6 | 10.8 | 2487 | 76.0 | 21560 | 1.15 |
| Narrow row spacing (22.5 cm) | 3.8 | 0.027 | 53 | 66.6 | 2.5 | 9.3 | 2347 | 65.8 | 19710 | 1.28 |
| Mulching (Paddy straw @ 5.0 t $ha^{-1}$) | 6.8 | 0.07 | 35 | 96.5 | 2.3 | 10.5 | 2210 | 56 | 18230 | 1.1 |
| Unweed control | 7 | 0.048 | 33 | 67.6 | 2.2 | 9.5 | 1413 | | 7950 | 0.6 |
| C.D. (at 5%) | 0.9 | 0.019 | 13 | 17 | NS | 0.9 | 190 | | | |

NS = Not Significant

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# Studies on the selectivity of isoproturon and metsulfuron-methyl against barley (*Hordeum vulgare* L.)

Rajvir Sharma[1], Feibo[2], Wu[2], Gouping Zhang[2]
[1]Division of Agronomy, IARI, New Delhi-110012, India
rajvirsharma@rediffmail.com or drrajvir@yahoo.com
[2]Department of Agronomy, College of Agriculture and Bio-technology
Hua Jia Chi Campus, Zhejiang University, Hangzhou, China

**Abstract**: The present study was conducted in pots to evaluate/ screen the selectivity of new herbicides viz: isoproturon (recommended for the control of grass weeds) and metsulfuron-methyl (recommended for the control of broad leaved weeds) at various doses and at different time of application against barley (*Hordeum vulgare* L.) at the Research Farm of Zhejiang University, Hangzhou, in China during year 2003. The plastic pots (30cm X 10 cm) with a small hole at bottom were filled with clay loam soil Thirteen treatments comprising two herbicides namely isoproturon (469, 938 and 1407 g a.i. $ha^{-1}$) and metsulfuron-methyl (3,6, and 9 g a.i. $ha^{-1}$) as pre-emergence and post-emergence application along with untreated plants were laid out in completely randomized block design. The height of barley plant was significantly affected with pre-emergence application of both herbicides. The tallest plants (37.67 cm) at 35 days after sowing were found in untreated pots which however were statistically at par with post-emergence application of metsulfuron-methyl at 3 and 6 g a.i. $ha^{-1}$ and isoproturon at 938 g and 1407 g a.i. $ha^{-1}$. SPAD value of functional leave known to indicate chlorophyll content and nitrogen concentration was significantly lower due to pre-emergence application of both herbicides except their lowest concentration as compared to their post-emergence application except isoproturon at 1407 g and metsulfuron-methyl at 9 g a.i. $ha^{-1}$. Leaf and tiller development was also significantly lower due to pre-emergence application of both herbicidal treatments. Pre and post-emergence application of metsulfuron-methyl at 3 and 6 g a.i. $ha^{-1}$ and post-emergence application of isoproturon at 469 and 938 g a. i. $ha^{-1}$ did not significantly affect the growth of barley plant. Proline content is a measure of degree of stress under which plant exist due to herbicide application. Proline content was significantly affected due to herbicide treatments and increased significantly with corresponding increase in the levels of their concentration. Proline content was comparatively higher in those plants which were treated with post-emergence application. Enzymatic activities of superoxide dimutase (SOD),peroxidase (POD) and malondialdehyde (MDA) were estimated after 35 days after sowing. SOD activity was significantly higher under herbicide treatments and increased with increasing level of their doses. The highest value of SOD (107.81 n mol $g^{-1}$ FW) activity was recorded in those barley plants which were treated with pre-emergence application of isoproturon at 1407 g a.i. $ha^{-1}$ closely followed by pre-emergence application of metsulfuron-methyl @ 9 g a.i. $ha^{-1}$. Whereas the lowest activity (48.56 n mole $g^{-1}$ FW) of superoxide dimutase was recorded with post-emergenet treatment of metsulfuron-methyl at 6 g a.i $ha^{-1}$. Peroxidases (POD) activity was also significantly influenced by herbicide treatments. But the trend was opposite to SOD activity. Malondialdehyde (MDA) content of the barley plant was also varied significantly due to application of herbicide treatment. The lowest value (5.82 n mol $g^{-1}$ FW) of the content was recorded in untreated barley plant which was however at par statistically with values observed in post-emergent treatment of isoproturon (469 and 938 g a.i. $ha^{-1}$) and metsulfuron-methyl (3 and 6 g a.i. $ha^{-1}$) treated plants. However, no specific increasing trend in MDA content was recorded with corresponding increase in the level of herbicide application. From this study it may be concluded that post-emergence application of both herbicides (isoproturon @ 469,938 g a.i. $ha^{-1}$ and metsulfuron-methyl @ 3, 6 and 9 g $ha^{-1}$) in barley for controlling complex weed flora would be more selective than their pre-emergence application at the respective doses.

**Key words**: Herbicides, selectivity, isoproturon, metsulfuron-methyl, barley, China

## INTRODUCTION

Barley is one of the important commercial crops of China. It has been extensively used for making various beverages like malting quality for making Beer in addition to its uses as fodder for cattle and as additive to some food product for human consumption as a rich source of vitamins for health maintenance. So its cultivation is gaining much popularity amongst farmers as cash crop. Barley productivity is low (4500 kg $ha^{-1}$) as against its potential of 6000-7000 kg $ha^{-1}$. Frequent rains coupled with liberal use of manures promote luxuriant growth of weeds and cause the damage of barley yield to the tune of 20.86-34.30 % as reported by Mu et al. (2000) in China and 10-38 % by Balyan and Malik (1994) in India. Farmers control weeds either manually or by the use of herbicides effectively. Manual weeding is not always possible as it is time consuming, labour and cost intensive. Under such condition, use of herbicides is warranted to realize yield potential of barley crop. Isoproturon and metsulfuron-methyl are reported effective in controlling complex weed flora including grassy and broad leaved weeds respectively in barley crop (Chauhan et al., 1999). Herbicides being chemical and toxic, are environmentally hazardous and known to generate oxidative stress in crop plants (Hendry, 1994). But information about their effect at different levels on barley growth particularly at physiological and bio-chemical level is scanty and meager. Hence the present investigation was taken up to optimize the dose and time of herbicide application in barley by studying the effect of herbicides on growth of crop and their physiological and bio-chemical responses in term of oxidative stress to applied herbicides.

## MATERIALS AND METHODS

The pot experiment was conducted under the green house condition at Zhejiang University, Huajia pond campus, Hangzhou (31.5 N), China during the winter season of 2002-2003. The plastic pots (30 cm X 10 cm) with a small hole at bottom were filled with clay loam soil l having organic carbon content of 1.65%, total soil nitrogen 0.085 %, available phosphorus 38.9 mg $kg^{-1}$ and available potash 30.59 mg $kg^{-1}$ and left for a week to settle down the soil properly. Twenty seeds of Barley cultivar ZAU-3 were sown in each pots on $7^{th}$ march, 2003. The experiment was laid out with following treatments in completely randomized block design with three replications (Table-1). Herbicides were sprayed on pots similarly to field conditions with one litre capacity sprayer using a spray volume of 600 litre $ha^{-1}$ (40 litre $mu^{-1)}$. Treatments consisted of different levels of isoproturon (469, 938, and 1407 g a. i. $ha^{-1}$) and metsulfuron-methyl (3, 6 and 9 g a. i. $ha^{-1}$) applied as pre-emergence and post-emergence along with control. Pre-emergence treatments of both herbicides were applied at one day after sowing whereas post-emergence were applied at 25 days after sowing (DAS). Observations of plant height(cm) (25 and 35 days after sowing),leaf and tiller development (35 days after sowing) and chlorophyll content through SPAD value of fully expanded functional leave were recorded to assess the herbicides effect on plant growth. Level of proline content in plant to be considered important to identify its condition under or above the stress due to several biotic and abiotic conditions was determined at 35 DAS by methods described by Zhang (1992). For the extraction and assay of enzymes (SOD- superoxide dimutase, POD-peroxide and MDA-malondialdehyde), primary leaves were homogenized with 0.05 M potassium phosphate buffer (pH 7.8) in pre-cooled morter and grind the sample with pestle at 4 C. Grinded and filtered material through four musclin clothes layers was centrifuged at 4 C for 20 minutes at 10000 rpm in a cooling centrifuge. The supernatant was used for the assay of superoxide dimutase (SOD), peroxidase (POD) and malondialdehyde (MDA) according to the method described by Chance and Maehly (1955), Bcauchamp and Fridovich (1971) and Zhang (1992).

Table 1 Detail of treatments combinations.

| Treatments | Dose ($g$ a. i. $ha^{-1}$) | Time of application |
|---|---|---|
| Control | -- | Pre-emergence |
| Isoproturon | 469 | -----do---- |
| Isoproturon | 938 | -----do---- |
| Isoproturon | 1407 | -----do--- |
| Metsulfuron-methyl | 3 | -------do---- |
| Metsulfuron-methyl | 6 | -------do---- |
| Metsulfuron-methyl | 9 | ------do--- |
| Isoproturon | 469 | Post-emergence |
| Isoproturon | 938 | ------do---- |
| Isoproturon | 1407 | -------do---- |
| Metsulfuron-methyl | 3 | -------do---- |
| Metsulfuron-methyl | 6 | -------do---- |
| Metsulfuron-methyl | 9 | --------do---- |

Pre-emergence = 1 days after sowing of crop
Post-emergence = 25 days after sowing of crop

## RESULTS AND DISCUSSION

### Effect of Herbicides on Barley Growth:

**Plant Height**: There was highly significant effect of the pre-emergence herbicide treatments on plant height (cm) with successive increase of their concentration. Significantly lower height was recorded under the influence of pre-emergence treatment of isoproturon @ 938 and 1407 g a. i. $ha^{-1}$ and of metsulfuron-methyl @ 9 g a. i. $ha^{-1}$ at 25 DAS. However the difference in plant height in rest of treatments was not found statistically significant as it was recorded before post-emergence treatments (Table 2). While at 35 DAS, height was significantly lower due to pre and post-emergence application of isoproturon at 1407 g a. i. $ha^{-1}$ and metsulfuron-methyl at 9 g a. i. $ha^{-1}$ which however were at par with each other. The tallest plants (36.67 cm) were found in untreated pots, which were at par with pre and post-emergence application of metsulfuron-methyl at 3 and 6 g a. i. $ha^{-1}$ and also with pre-emergence of isoproturon at 469 g a. i. $ha^{-1}$ and post-emergence of isoproturon at 938 and 1407 g a. i. $ha^{-1}$.

**Leaf Development**: Leaf development was expressed in term of their numbers per plant. Number of leaves per plant were differed significantly due to influence of herbicidal treatments. The highest number of green leaves (12.20 $plant^{-1}$) were recorded in untreated pot which, however was at par with pre and post-emergence application of metsulfuron-methyl at 3 and 6 g a. i. $ha^{-1}$ and post-emergence treatment of isoproturon at 469 and 938 g a. i. $ha^{-1}$. The lowest number of leaves (5.89 $plant^{-1}$) was observed in pre-emergence treatment of isoproturon at its highest level (1407 g a. i. $ha^{-1}$), which was however at par with its post-emergence application. Data presented in Table 2 revealed the significant difference in the pre and post-emergence treatments of isoproturon but metsulfuron-methyl did not affect the number of leaves with the change in its time of application.

**Tillers Development**: Yield is the resultant of the tillers development. The highest number of tillers (4.22) per plant were noted in untreated pot at 35 DAS which were at par the values recorded in pre-emergence treatment of metsulfuron-methyl at 3 and 6 g a. i. $ha^{-1}$ and post-emergence of isoproturon at 469 and 938 g a. i. $ha^{-1}$. While the lowest number (1.53) of tillers were registered in pre-emergence treatment of isoproturon @ 1407 g a. i. $ha^{-1}$ which was closely followed by its post-emergence application (1.55 tillers) at the same concentration (Table 2). Isoproturon caused overall significant reduction in SPAD value, leaf development and tillers development with pre-emergence application as compared to metsulfuron-methyl, irrespective of their doses and time of application in the present investigation.

**Effect on SPAD value**: SPAD values of functional leaves are reported to have positive correlation with chlorophyll content and nitrogen concentration (Feibo, et al., 1998).Significantly lower SPAD value were recorded due to pre-emergence treatments of both herbicides at all levels at 25 DAS. The lowest SPAD value (25.98) was recorded in pre-emergence isoproturon treated pots at 1407 g a. i. $ha^{-1}$, which was however differed significantly from the rest of the treatments. At 35 DAS, pre and post-emergence herbicide treatments caused significant reduction in SPAD value of barley crop. The highest SPAD value (39.22) was registered under control (untreated) which was statistically at par with pre-emergence application of the lowest concentration of isoproturon (469 g a. i. $ha^{-1}$) and metsulfuron-methyl (3 g a. i. $ha^{-1}$) and post-emergence of metsulfuron-methyl only at 3 and 6 g a. i. $ha^{-1}$. Post emergence application of isoproturon @ 1407 g a. i. $ha^{-1}$ observed the lowest SPAD value (23.29) among the herbicide treatments but was statistically similar with post-emergence treatment of metsulfuron-methyl @ 9 g a.i. $ha^{-1}$(Table 2).

Table 2. Effect of herbicide treatments on growth of barley plant

| Treatments | Dose (g a.i $ha^{-1}$) | Plant Height (cm) At 25 DAS | Plant Height (cm) At 35 DAS | Number of leaves (No. $plant^{-1}$) | Number of tillers (No. $plant^{-1}$) | SPAD value At 25 DAS | SPAD value At 35 DAS |
|---|---|---|---|---|---|---|---|
| Control | -- | 22.88 ab | 36.67 a | 12.20 a | 4.22 a | 38.13a | 39.22 a |
| Isoproturon | 469 | 20.33 abc | 32.16 ab | 9.77 bc | 2.43 d | 33.67c | 35.91abc |
| Isoproturon | 938 | 19.55 bc | 30.11 bcde | 9.55 bcd | 2.30 de | 29.90 d | 31.70 cde |
| Isoproturon | 1407 | 15.50 d | 23.78 f | 5.89 e | 1.53 e | 25.98 e | 29.42 ef |
| Metsulfuron-methyl | 3 | 21.66 abc | 35.33 ab | 12.33 a | 3.42 abc | 35.28 abc | 35.55 abc |
| Metsulfuron-methyl | 6 | 20.88 abc | 31.55 abcde | 11.11 abc | 3.44 abc | 34.40 bc | 34.81 bcd |
| Metsulfuron-methyl | 9 | 17.75 cd | 27.00 def | 9.00 cd | 2.66 cd | 29.57 d | 33.96 bcd |
| Isoproturon | 469 | 22.94 ab | 33.22 ab | 10.22 abc | 3.67 a | 33.97 bc | 31.75 cde |
| Isoproturon | 938 | 23.30 ab | 32.44 ab | 10.05 abc | 3.56 ab | 36.30 abc | 30.61 de |
| Isoproturon | 1407 | 23.54 a | 27.33 cdef | 7.34 de | 1.55 e | 36.87 ab | 23.29 g |
| Metsulfuron-methyl | 3 | 23.97 a | 35.12 ab | 11.33 ab | 3.78 a | 37.63 a | 37.14 ab |
| Metsulfuron-methyl | 6 | 22.59 ab | 33.11 ab | 11.33 abc | 4.11 a | 36.80 abc | 36.39 ab |
| Metsulfuron-methyl | 9 | 23.80 a | 26.39 ef | 9.77 bc | 2.78 bcd | 36.83 ab | 26.02 fg |

Means with the same letters are not significantly different

### Effect of herbicides on Assay of enzymatic activites in Barley plants:

**Proline content**: Proline content (Table 3) was significantly affected due to herbixcide treatments and increased significantly with corresponding increase in the levels of their concentrations. Untreated plants recorded the lowest proline content (29.07 ug • $g^{-1}$ FW) amongst all the treatments which however were at par with the rest of treatments except pre-emergence treatment of metsulfuron-methyl at 6 and 9 g and post-emergence treatment at 9 g a. i. $ha^{-1}$ and post-emergence treatment of isoproturon at 1407 g a. i. $ha^{-1}$. Pre-emergence application of isoproturon at 469 g a. i. $ha^{-1}$ caused the lowest proline content accumulation (30.40) which, however was at par with all rest of the treatments except pre-emergence treatment of metsulfuron-methyl at 6 and 9 g a. i. $ha^{-1}$ and post-emergence treatment at 9 g and post-emergence treatment of isoproturon at 1407 g a. i. $ha^{-1}$.

Application of isoproturon at 1 DAS did not bring any significant increase in proline content even at its highest concentration except its post-emergence treatment at 1407 g a. i. $ha^{-1}$.

Table 3. Effect of herbicide treatments on proline content and enzymatic activities in barley plants at 35 DAS.

| Treatments | Dose (g a.i.$ha^{-1}$) | Proline content (ug. $g^{-1}$) | SOD activity (nmol $g^{-1}$FW) | POD activity (nmol $g^{-1}$FW) | MDA activity (nmol $g^{-1}$FW) |
|---|---|---|---|---|---|
| Control | -- | 29.07 e | 50.99 e | 29.33 de | 5.82 e |
| Isoproturon | 469 | 30.40 e | 55.34 de | 90.25 a | 8.73 abc |
| Isoproturon | 938 | 32.14 de | 79.72 b | 47.70 cde | 7.83 cde |
| Isoproturon | 1407 | 38.38 cde | 107.22 a | 32.32 de | 10.81 a |
| Metsulfuron-methyl | 3 | 48.67 bcde | 74.81 bcd | 72.66 ab | 8.46 bcd |
| Metsulfuron-methyl | 6 | 61.07 abcd | 79.84 b | 51.18 cd | 8.11 cd |
| Metsulfuron-methyl | 9 | 76.59 ab | 92.74 ab | 52.09 cd | 10.69 ab |
| Isoproturon | 469 | 31.76 e | 55.65 de | 56.04 bc | 6.79 cde |
| Isoproturon | 938 | 54.14 bcde | 56.71 cde | 50.95 cd | 6.39 de |
| Isoproturon | 1407 | 61.98 abc | 75.38 bc | 42.43 cde | 8.72 abc |
| Metsulfuron-methyl | 3 | 31.18 e | 49.69 e | 79.08 a | 7.61 cde |
| Metsulfuron-methyl | 6 | 45.15 cde | 48.56 e | 44.78 cde | 6.72 cde |
| Metsulfuron- methyl | 9 | 84.30 a | 53.58 e | 42.49 cde | 8.46 bcd |

FW: Fresh weight
Means with the same letters are not significantly different

**Superoxide dimutase (SOD) Activity**: The results for antioxidant enzymes presented in Table 3 revealed that activity of SOD enzyme was significantly induced with increasing levels of their concentration.The highest SOD activity (107.22 n mol $g^{-1}$ FW) was recorded with the application of pre-emergence treatment of isoproturon at 1407 g a. i. $ha^{-1}$ closely followed by the pre-emergence application of metsulfuron-methyl at 9 g a. i. $ha^{-1}$. Whereas the lowest activity (48.56) was induced with post-emergence treatment of metsulfuron-methyl at 6 g a. i. $ha^{-1}$ which was however at par with rest of treatments except pre-emergence application of isoproturon at 938 and 1407 g a. i. ha-1 and of metsulfuron-methyl at all doses and post-emergence treatment of isoproturon at 1407 g a.i. $ha^{-1}$.

**Peroxidases Activity (POD)**: Peroxidases activity in barley plants (Table 3) was significantly affected under the influence of herbicide application and increased significantly over untreated plants but decreased with subsequent increase in their levels of application. The lowest value of POD activity (29.33 n mol g-1 FW) was observed in untreated plants which was found at par with rest of treatments except pre-emergence of isoproturon (469 g a. i. $ha^{-1}$) and metsulfuron-methyl (3,6 and 9 g a. i. ha-1) and post-emergence of isoproturon at 469 and 938 g and metsulfuron-methyl at 3 g a. i. ha-1. While the highest POD activity (90.25 n mol g-1FW) was induced with pre-emergence application of the lowest level of isoproturon (469g) which was followed by pre and post-emergence application of metsulfuron-methyl at 3 g a. i. ha-1

**Malondialdehyde (MDA) content**: MDA content of the plants was significantly varied due the application of herbicide treatments(Table 3). The lowest value (5.82 n mol $g^{-1}$FW) of the content was recorded in untreated plants which was however at par statistically with the values observed in post-emergence of isoproturon (469 and 938 g) and metsulfuron-methyl (3 and 6 g a.i. $ha^{-1}$) treated plants. In contrast, the highest (10.81 n mol $g^{-1}$FW) accumulation of MDA content was registered with pre-emergence application of isoproturon at 1407 g a. i. ha-1 closely followed by pre-emergence treatment of metsulfuron-methyl (9g) and isoproturon (469g) and post-emergence treatment of isoproturon at 1407 g a. i. $ha^{-1}$. However no specific increase trend in MDA content was recorded with corresponding increase in the level of herbicide application.


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# Tolerance of different wild oats biotypes to different oat killers under field conditions

G. Hassan
Department of Weed Science, NWFP Agricultural University, Peshawar 25130, Pakistan
hassanpk_2000pk@yahoo.com

**Abstract:** A field experiment was conducted at Malakandher Research Farm, NWFP Agricultural University, Peshawar, Pakistan during Rabi 2003-04 to investigate the tolerance of different wild oats biotypes to herbicides. The experiment was laid out in randomized complete block design under a split-plot arrangement with four replications. The experiment comprised of four wild oat biotypes assigned to the main plots, while 4 herbicides (Aim, Puma super, Affinity and Weedy check) were kept in sub-plots. The Ghaznavi-98 variety of wheat in a sub-plot size of 5 m x 1 m was planted in the 2nd week of November 2003. The data were recorded on wheat density $m^{-2}$, wild oats density $m^{-2}$, wheat leaf area ($cm^2$) $plant^{-1}$, wild oats leaf area ($cm^2$) $plant^{-1}$, wheat plant height at maturity (cm), wild oats plant height at maturity (cm), wheat spike length (cm), number of grains $spike^{-1}$, weight of grains $spike^{-1}$, 1000 grains weight (g), biological yield (t $ha^{-1}$), and grain yield (t $ha^{-1}$). The statistical analyses of data exhibited non-significant differences for tolerance of biotypes to herbicides in all the morphological and agronomic traits, while interaction of biotypes with herbicides was only significant for wheat plant height. Response to herbicides varied significantly for all the morphological and agronomic traits except wheat density, wheat spike length, 1000 grain weight and biological yield. The density of wild oats was the highest in weedy check. For the wild oats density all the herbicides performed equally, statistically significant from the weedy check. As a consequence of phytotoxic effect on weeds, the herbicides increased grain yield. All the herbicides had comparable yields and out-yielded the weedy check. From the findings it is concluded that wild oats present in the areas from where the biotypes were collected can be killed by equal doses of tested herbicides all over the areas and the herbicide Affinity is recommended for controlling weeds in wheat crop.

**Key words**: Biotypes, herbicides, wild oats, yield, yield components.

## INTRODUCTION

At the national level the area under wheat cultivation is 8.034 million ha, with a production of 19.183 million t. The area consists of about 7.001 million ha irrigated and 1.033 million ha of un-irrigated land. In NWFP, the area under wheat cultivation is about 0.732 million ha. One third of this area in NWFP is irrigated, while two third is rainfed giving a total production of 1.064 million t at the rate of 1454 kg $ha^{-1}$ (Anonymous 2003). One of the prime reasons for the increased yield is the introduction of very effective herbicides. But in Pakistan wheat yield (kg $ha^{-1}$) is unfortunately very low and actual farm yield is about 30-35% of the potential yield. Weed interference is one of the important but less noticed constraints, contributing towards low yield of wheat in Pakistan.

Seventy-five percent (75%) of the total population resides in rural areas. Our population is increasing at one of the fastest rates in the world. Our food production should fulfill the food requirements of our population. It is impossible to increase the area horizontally because the cultivated area is already squeezing. Vertical increase is possible because of having enough room to get the potential yield of even the existing varieties of wheat. Weed competition is the only constraint for the wheat yield because insects and diseases are not significant problems. Smuts and rusts are controlled due to incorporation of resistance genes in wheat cultivars. Thus the only pests left are the weeds, which are impeding our efforts in harvesting potential yields.

Weeds reduce crop yield, deteriorate the quality of farm produce and hence reduce the market value of wheat. Weed management increases the cost of production and, thus, is necessary to device such methods which could reduce not only the cost of production but also save time and labor. Among the weedy check methods, the chemical weedy check is one of the recent origins, which is being emphasized, in modern agriculture (Taj et al. 1986).

It has been estimated that crop losses due to weed competition throughout the world as a whole are greater than those resulting from the combined effect of insect pests and diseases. Weeds may encourage the development of fungal diseases, provide shelter for pests of all kinds and act as host plants for parasitic nematodes. There are thus, several reasons for entirely eliminating weeds from the crop environment. As a matter of fact, with the rising costs of labor and power, the use of herbicides will be the only acceptable method of weed control in the future. In situations where there is heavy infestation of weeds there is a need to develop a package of weed management technology, helpful to minimize the weed competition losses in our country. An effective weed management system is a basic requirement and major component of management in the production system (Young et al. 1996 and Norris 1982).

Morikawa (1989) studied 14 dwarf accessions adapted as a weed to barley and wheat fields in eastern Asia were collected in Japan and the Korea Republic. Crosses between the accessions and the cultivated oat Kanota suggested that in 7 accessions, dwarfness was controlled by a dominant gene and in 3 by a recessive gene. Two accessions showed digenic inheritance and accession 288 showed polygenic inheritance, while dwarfness in 342 was controlled by an incompletely dominant gene. Dwarfness was controlled by major genes, except in 288. The dwarfing gene in 812 was allelic to that in 169 but not to that in 153.

Darmency and Thomas (1994), in an experiment initiated in 1987, studied the progress of 3 wild oat genotypes, one of which (phenotype 1) required vernalization, in over 3 years in wheat followed by barley and then rape. In every case a marked fall was observed in the proportion of phenotype 1 in the population.

Regnier and Bakelana (1995) determines the effects of cultivated oats *(Avena sativa)* cv. Ogle and Pennvda planting pattern on early canopy shape and growth of cultivated oats and wild oats *(A. fatua)*, in part to test the assumption of radial plant canopy expansion on which previous theoretical models of crop-weed interference models have been based. Cultivated oat DW and leaf area at crop flowering (64 DAE) also decreased with increasing rectangularity of crop planting pattern. Reductions in cultivated oats growth in rectangular patterns were associated with earlier intraspecific interference and delayed crop canopy closure in rectangular compared to equidistant patterns. Wild oats leaf area and tiller number 64 DAE decreased with more equidistant crop planting patterns, consistent with reduced canopy area 31 DAE and earlier crop canopy closure in equidistant patterns.

Seefeldt et al. (1996) reported that Diclofop-resistant *Avena fatua* in winter wheat fields was identified during 1990-91 in the Willamette Valley of Oregon, USA. Multiple applications of diclofop over a 10-year period provided selection pressure for resistance in these fields. These biotypes differed widely in morphological traits, which suggested they originated independently. Large differences existed in the levels of resistance to diclofop and the patterns of cross-resistance to other aryloxyphenoxypropionate and cyclohexane dione herbicides.

Koscelny et al. (1997) conducted field trials in SW Oklahoma during the 1993-94 and 1994-95 winter growing seasons and found that diclofop at 840 g a.i. $ha^{-1}$, fenoxaprop at 90 g a.i. $ha^{-1}$ and imazamethabenz at 530 g a.i. $ha^{-1}$, when autumn-applied, controlled wild oats by 96, 99 and 95%, and increased wheat grain yields by 26, 29 and 24%, respectively. These herbicides Weedy controlled wild oat over a wider range of growth stages than current labels indicate. The same treatments applied in March were less effective for wild oat Weedy check and did not increase wheat yield.

Korniak and Frey (1996) presented the frequency of occurrence of four varieties of *Avena fatua* in north-eastern Poland. They concluded that frequency of particular varieties had changed noticeably after 12 years. In 1981, var. *fatua* was the most frequent and represented 29.6% of the total. By 1993, this percentage had fallen to 12.2. An even greater change was noted for var. *intermedia* (Lestib.) Lej. & Court. In 1981, its frequency amounted to 28.4%, and in 1993 it was only 5.5%. At the same time there was an over two-fold increase in var. *glabrata* Peterm. (from 22.4 to 48.8%) and one almost as great in var. *vilis* (Wallr.) Hausskn. (from 19.6 to 33.5%).

Kiec 1995 studied variability in *Avena fatua* in Poland during 1986-89 and 10 varieties were identified: vars. *fatua*, *glabrata*, *intermedia* and *vilis*, and 6 others new to Poland. The varieties were separated on the basis of plant height, panicle length, kernel pubescence and colour, callus pubescence, and 1000-seed weight. *A. fatua* var. *fatua* and varieties with long hairs were the most numerous in the survey. Varieties with short callus hairs were generally taller, and had longer panicles and higher 1000-seed weights.

Salarzai et al. (1999) studied the effect of different herbicides for the control of weeds in wheat crop. They concluded that herbicides significantly affected the weed population, weed biological yield and various yield components. The highest grain yield (5030 kg ha$^{-1}$) was obtained in plots treated with Banvel-M as compared to weedy check yield of 4195 kg ha$^{-1}$.

Fayed et al. (1998) reported that Flamprop-M-isopropyl, fenoxaprop-ethyl, tralkoxydim and diclofop-methyl were applied 30 or 60 days after sowing wheat *(Triticum aestivum)* cv. Sakha 69 in field experiments in El-Nubaria, Egypt between 1994 and 1996. The effect on *A. fatua* density was assessed 30 days after spraying and wheat yield components were calculated. Hand weeding, tralkoxydim, diclofop, flamprop-M and fenoxaprop significantly decreased the number of *A. fatua* plants m$^{-2}$ (79.4, 76.7, 81.6, 78.2 and 78.5%, respectively) compared to the unweeded treatment. Early application (30 days after sowing) of chemicals more effectively reduced *A. fatua* density and increased wheat yield components. Tralkoxydim application 30 days after sowing was recommended for maximum grain yield.

Spandl et al. (1997) evaluated rates and application timing of post emergence herbicides for wild oat (*Avena fatua*) weedy check in spring wheat and barley at Crookston, Minnesota, from 1994 to 1996. Diclofop, imazamethabenz, and fenoxaprop + MCPA + thifensulfuron + tribenuron were applied to one- to three-leaf wild oat; and difenzoquat, imazamethabenz, fenoxaprop + MCPA + thifensulfuron + tribenuron, and fenoxaprop + 2,4-D + MCPA were applied to four- to five-leaf wild oat at half, three-quarters and 1X rates. Wild oat weedy check with half rates generally was less than that with three-quarter rates, which was lower than or similar to that with 1X rates. Wild oat biomass was often reduced less with half rates than 1X rates. However, reducing herbicide rates generally did not influence grain yields or net economic return. Grain yields and net economic return were generally greater in herbicide-treated plots than in the untreated weedy check.

Santin et al. (1997) carried out field study during 1995-97 in Spain in a period of heavy precipitation, moderate temperatures and short tillering periods for wheat (cultivars Pane 247 and Anza) and *Avena sterilis*. Similar positive responses to N fertilization (50-250 kg ha$^{-1}$) were found in both species. The traditional tall wheat cv. Pane 247 was more competitive than the semi-dwarf cv. Anza in decreasing the number of panicles and spikelets of *A. sterilis* with increased N doses. Competition for light between wheat and *A. sterilis* was evident.

Korniak and Frey (1996) reported the frequency of occurrence of four varieties of *Avena fatua* in north-eastern Poland. In 1993, samples consisting of 100 *A. fatua* panicles were collected randomly from single fields (each of about 1 ha) of spring cereals, located in 20 different villages. In all, 2000 panicles were collected, representing 20 populations of the species, and the percentage of each botanical variety was determined. Similar studies in the same 20 localities had been conducted previously, in 1981. The frequency of particular varieties had changed noticeably after 12 years. In 1981, var. *fatua* was the most frequent and represented 29.6% of the total. By 1993, this percentage had fallen to 12.2. An even greater change was noted for var. *intermedia* (Lestib.) Lej. & Court. In 1981, its frequency amounted to 28.4%, and in 1993 it was only 5.5%. At the same time there was an over two-fold increase in var. *glabrata* Peterm. (from 22.4 to 48.8%) and one almost as great in var. *vilis* (Wallr.) Hausskn. (from 19.6 to 33.5%).

Stevenson et al. (2000) deciphered data collected from 14 sites in Saskatchewan, Canada, to investigate the influence of weed Weedy check method (cultural versus herbicides) and N and P fertilizers on crop yield of autumn rye, spring wheat, and barley, and the presence and number of weed species. Cultural weed Weedy check included 25% greater crop seeding rate, preseeding tillage closer to the time of seeding, and fertilizer N banding in closer proximity to the seed. Four weed species (wild oat (*Avena fatua*), lambsquarters (*Chenopodium album*), wild buckwheat (*Polygonum convolvulus* [*Fallopia convolvulus*]), and field pennycress (*Thlaspi arvense*) occurred more frequently in plots with cultural weed weedy check compared with herbicide weed weedy check for all cereal crops.

Weaver et al. (1994) investigated an eco-physiological simulation model of competition between *A. fatua* and winter wheat cv. Avalon for light was parameterized and tested. The model simulates growth of each species, in kg DM $ha^{-1}$ $d^{-1}$, from sowing to maturity as a function of irradiance, temperature and various species characteristics. The canopy height of each species, and the density and time of emergence of *A. fatua* were systematically varied while all other parameters were left unchanged. Accurate simulations of growth in mixtures depended upon an accurate description of the canopy height of each species throughout the growing season. Model predictions of winter wheat yield losses in relation to *A. fatua* density and time of emergence showed good agreement with previously published data.

Ibrahim et al. (1995) conducted four pot experiments in 1992-94 at Giza, Egypt examined wild oats/wheat competition. Wheat cv. Sakha 69 was grown with wild oats at wheat + wild oats numbers $pot^{-1}$ of 6 + 6, 6 + 5, 6 + 4, 6 + 3, 6 + 2 or 6 + 1. At these densities, grain yield $plant^{-1}$ was decreased by 58, 54, 48, 43, 16 and 7% in 1992/93, and by 54, 38, 22, 38, 16 and 7% in 1993/94, respectively, compared with wheat grown alone. In the 2nd experiment, crop weed competition was markedly decreased when emergence of wild oats was delayed by 3 weeks. In the 3rd experiment wheat and wild oats achieved 100% germination 9 and 15 d after sowing, respectively. In the 4th experiment, wheat and wild oats were sown on 1, 15 or 30 November or 15 December. The optimum sowing date for wheat was 30 November while the growth of wild oats was most vigorous when sown on 1 November.

Pedreros (2001) conducted two experiments, through additive design, to evaluate the effect of increasing densities of wild oats (*A. fatua*) and Italian ryegrass (*L. multiflorum*), separately, in winter and spring production of wheat (*Triticum aestivum*) at two locations in Chile. Wild oat densities between 0 to 16 plants $m^{-2}$ in the Andean foothills, and 0 to 24 plants $m^{-2}$ in the irrigated valley. Every additional wild oat plant $m^{-2}$ reduced wheat yield by approximately 100 kg $ha^{-1}$. Wild oat densities of 3 plants $m^{-2}$ reduced wheat yield by 4.5% in the irrigated valley, and 3.5% in the Andean foothills, respectively. With the losses projected by the hyperbolic model, the economic threshold varied between 3 and 4.4 wild oat plants $m^{-2}$ , and 27 and 36 Italian ryegrass plants $m^{-2}$ depending on the agroecological area and weed Weedy check cost.

Gonzalez and Santin (2001) concluded that wheat (*Triticum aestivum*) grew with the infesting weed wild oat (*Avena sterilis* subsp. sterilis) in semi-arid conditions of Toledo province, Spain during 1995-97. Both species were affected by the drought conditions, although drought caused more damage to the growth and seed production of *A. sterilis* than of wheat. Both species benefited from nitrogen fertilization, but much more so in the year when water was not a limiting factor. In comparison with the semi-dwarf cultivar (Anza), the tall wheat cultivar (Pane 247) is competitively superior to *A. sterilis*. This competitive superiority was related more to the height of the wheat plant and supposed competition for light than to tiller production. The competition affected the production of *A. sterilis* panicles and spikelets. In addition, the height and competitive ability of the tall wheat cultivar increased more than that of the semi-dwarf cultivar with increasing nitrogen doses.

Major weeds that infested the experiment apart from the planted wild oats were *Phalaris minor, Poa annua, Cirsium arvense, Convolvulus arvensis, Chenopodium album, Fumaria indica, Carthamus oxycantha, Galium aparine* and *Euphorbia helioscopia.*

In view of the importance of the wild oats problem and the vital importance of food for human beings and the relevance to the national economy, an experiment was conducted to investigate the competition between wheat and wild oats for following objectives.

a. To investigate the tolerance of different wild oats biotype to different herbicides
b. To study the efficacy of different grass killers on the yield and yield components of wheat.
c. To decipher the interaction of biotypes with different grass killers.

## MATERIALS AND METHODS

An experiment was conducted at Malakandher Research Farm, N.W.F.P Agricultural University, Peshawar, Pakistan during winter 2003-04. The experiment was laid out in randomized complete block (RCB) design with a split plot arrangement having four replications. In each replication, there were four main plots. Each main plot consisted of five rows, five meter long spaced at 20 cm. Four wild oats biotypes (D.I.Khan (Black), D.I.Khan (White), Mardan, Peshawar) collected from across the North West Frontier Province, Pakistan were assigned to main plots and herbicides (Aim 50 g $ha^{-1}$, Puma super 1.25 L $ha^{-1}$, Affinity 1.75 kg $ha^{-1}$, weedy check) were kept in the sub-plots. The sub plot size was kept at 5 x 1 $m^2$. The seed of Ghaznavi-98 wheat variety was drilled at the rate of 120 kg $ha^{-1}$ on 13$^{th}$ November 2003. The wild oats biotypes were sown at 30 plants $m^{-2}$ right after wheat sowing. All the recommended cultural practices were carried out uniformly in all the treatments during the course of experiment except the treatments.

During the course of the studies, data were recorded on wheat density $m^{-2}$, wild oats density $m^{-2}$, wheat leaf area ($cm^2$) $plant^{-1}$, weed density $m^{-2}$, wheat plant height at maturity (cm), wheat spike length (cm), number of grains $spike^{-1}$, grain weight $spike^{-1}$ (g), 1000 grain weight (g), biological yield (t $ha^{-1}$) and grain yield (t $ha^{-1}$). The data recorded individually for each parameter were subjected to the ANOVA technique by using MSTATC computer software and the significant means were separated by using Fisher's Protected LSD test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

Data recorded on the density of wheat $m^{-2}$, wild oats density $m^{-2}$, wheat leaf area $plant^{-1}$, wild oats leaf area $plant^{-1}$, wheat plant height at maturity (cm), wild oats plant height at maturity (cm), wheat spike length (cm), number of grains $spike^{-1}$, weight of grains $spike^{-1}$, 1000 grain weight (g), biological yield (t $ha^{-1}$) and grain yield (t $ha^{-1}$) of wheat are presented as under.

Wheat density

Statistical analysis of the data showed that wild oat biotypes and herbicides and their interaction had no significant effect on wheat density $m^{-2}$. The data (Table 1) show that among the wild oat biotypes, maximum wheat density (163.313) was recorded in D.I.Khan (Black) biotype, while minimum (wheat density 138.875) was recorded in D.I.Khan (White). Among the herbicide means, maximum wheat density (166.063) was recorded in weedy check, while minimum wheat density (150.583) was recorded in Aim treatment. For interaction of wild oats biotypes with the herbicides, the maximum wheat density (179.250) was recorded in (D.I. Khan black x weedy check) while minimum wheat density (123.00) was recorded in D.I.Khan white X Puma Super treatments. The data exhibit that the density of competing weeds did not reach a level where the mortality of the neighboring species occurs in accordance with the self thinning rule; hence the differences among the density in different treatments were statistically similar.

Table 1. Wheat density $m^{-2}$ as affected by different wild oat biotypes and herbicides.

| *Avena fatua* | Herbicides | | | | Biotype |
|---|---|---|---|---|---|
| Biotype | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | Means |
| D.I.Khan (B) | 142.75 | 164.5 | 166.75 | 179.25 | 163.313 |
| D.I.Khan(W) | 142.25 | 123 | 129.5 | 160.75 | 138.875 |
| Mardan | 143.25 | 162.25 | 133.25 | 153.75 | 148.125 |
| Peshawar | 144.75 | 161 | 147 | 170.5 | 155.813 |
| Herbicide Means | 143.25 | 152.688 | 144.125 | 166.063 | |

Wild oats density

Statistical analysis of the data showed that wild oat biotypes have non-significant effect while herbicides and their interaction with wild oat biotypes have significant differences on wheat density $m^{-2}$. Table 2 shows that among the wild oats biotypes, maximum wild oat density (20.488) was recorded in D,I.Khan (white) biotype while minimum wild oats density (11.500) was recorded in D.I.Khan (black). Among the herbicides means, maximum wild oat density (29.725) was recorded in weedy check, while the minimum (10.250) was recorded in the treatment subjected to Puma Super. For interaction of wild oat biotypes with the herbicides, the maximum wild oat density (31.500) was recorded in Peshawar x weedy check, while the minimum density (1.75) was recorded in D.I.Khan white x Puma Super (Table 2) The data exhibit that despite the statistical non-significance, there is spread among the biotypes for their density indicating a difference in competitive ability of the biotypes.

Table 2. Wild oats density $m^{-2}$ as affected by different wild oat biotypes and herbicides.

| *Avena fatua* | Herbicides | | | | Biotype |
|---|---|---|---|---|---|
| Biotype | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | Means |
| D.I.Khan(B) | 11.25 | 1.75 | 4.5 | 28.5 | 11.5 |
| D.I.Khan(W) | 12.25 | 20 | 20 | 29.7 | 20.488 |
| Mardan | 18.25 | 16.5 | 8 | 29.2 | 17.988 |
| Peshwar | 18 | 2.75 | 12.25 | 31.5 | 16.125 |
| Herbicide Means | 14.938a | 10.250a | 11.188a | 29.725b | |

Wheat leaf area ($cm^2$) $plant^{-1}$

Statistical analysis of the data showed that for wheat leaf area $plant^{-1}$ biotype, herbicide and their interaction had non-significant effect. The data show that among the wild oat biotypes, maximum wheat leaf area $plant^{-1}$ (25.375) was recorded in Peshawar biotype while minimum wheat leaf area $plant^{-1}$ (22.319) was recorded in D.I.Khan (black). Among the herbicides means, maximum wheat leaf area $plant^{-1}$ (25.364) was recorded in Aim, while minimum wheat leaf area $plant^{-1}$ (23.655) was recorded in Affinity (Table 3). For interaction of wild oat biotypes with the herbicides, the maximum wheat leaf area $plant^{-1}$ (27.622) was recorded in Peshawar x Aim while minimum leaf area (21.458) was recorded in D.I.Khan white X Affinity treatment.

Table 3. Wheat leaf area (cm) as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 23.313 | 23.225 | 21.753 | 20.985 | 22.319 |
| D.I.Khan(W) | 25.722 | 24.713 | 21.458 | 22.347 | 23.56 |
| Mardan | 24.798 | 25.075 | 24.455 | 26.84 | 25.292 |
| Peshawar | 27.622 | 21.895 | 26.955 | 25.028 | 25.375 |
| Herbicide Means | 25.364 | 23.727 | 23.655 | 23.8 | |

Weed density

Statistical analysis of the data showed that herbicides had a significant effect while wild oat biotypes and their interaction had non-significant role on weed density. The data in Table 4 enunciate that among the wild oat biotypes, maximum weed density $m^{-2}$ (52.500) was recorded in Peshawar biotype while minimum weed density $m^{-2}$ (48.625) was recorded in Mardan wild oat biotype. Among the herbicides means maximum (82.063) was recorded in weedy check, while minimum weed density $m^{-2}$ (28.000) was recorded in Affinity treatment. For interaction of wild oat biotypes with the herbicides, the maximum weed density (84.250) was recorded in D.I. Khan black x weedy check and Mardan x weedy check, while minimum weed density $m^{-2}$ (24.750) was recorded in D.I.Khan white X Affinity treatment. Since Affinity is a broad spectrum herbicide, it controlled both grasses and broadleaf weeds and consequently the density was the lowest in this treatment. Similarly Puma super is very effective on grasses, thus it followed Affinity in reducing weed population. Our findings are similar with the work of Koscelny and Peeper (1997), Spandl et al. (1997), Salarzai et al. (1999) and Stevenson et al. (2000) who reported reduced weed density in wheat with the application of different herbicides.

Table 4. Weeds density $m^{-2}$ as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan (B) | 48.5 | 48.25 | 25.75 | 84.25 | 51.688 |
| D.I.Khan(W) | 51 | 44.25 | 24.75 | 80.5 | 50.125 |
| Mardan | 42.75 | 33.5 | 34 | 84.25 | 48.625 |
| Peshawar | 52.75 | 50.5 | 27.5 | 79.25 | 52.5 |
| Herbicide Means | 48.750b | 44.125c | 28.000d | 82.063a | |

Wheat plant height at maturity

Statistical analysis of the data showed that wild oats biotypes had a non-significant, while herbicides and their interaction with wild oats biotypes had significant differences. The data in Table 5 show that among the wild oat biotypes, maximum plant height at maturity (81.813 cm)was recorded in D.I.Khan (white) biotype while minimum plant height at maturity w(81.063 cm) as recorded in D.I.Khan (black) wild oat treated plots. Among the herbicides means, maximum plant height at maturity (83.750 cm) was recorded in Affinity and Puma super (83.063) treated plots, while minimum (78.313 cm) was recorded in weedy check treatment. Aim also failed to surpass height from the weedy check. For interaction of wild oats biotypes with the herbicides, the maximum plant height at maturity (86.500 cm)was recorded in Peshawar x Affinity, although it was statistically comparable with several other interactions, while minimum wheat density (74.750) was recorded in D.I.Khan black X weedy check.

Table 5. Plant height at maturity (cm) as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 82.750a-d | 85.000a-c | 81.750a-d | 74.750e | 81.063 |
| D.I.Khan(W) | 81.750a-d | 80.250cd | 85.500ab | 79.750cd | 81.813 |
| Mardan | 78.000de | 85.500ab | 81.250bcd | 80.500bcd | 81.313 |
| Peshwar | 78.750de | 81.500bcd | 86.500a | 78.250de | 81.250 |
| Herbicide Means | 80.313b | 83.063a | 83.750a | 78.313b | |

Wheat spike length

Statistical analysis of the data showed that wild oat biotypes, herbicides and their interaction with wild oat biotypes had non-significant differences. The data (Table 6) show that among the wild oat biotypes, maximum wheat spike length (8.812 cm) was recorded in D.I.Khan black biotypes while minimum wheat spike length (8.150 cm) was recorded in Mardan wild oats. Among the herbicides means, maximum wheat spike length (8.750 cm) was recorded in Puma Super, while minimum (8.150) was recorded in Affinity treatment. For interaction of wild oat biotypes with the herbicides, the maximum wheat spike length (9.125) was recorded in D.I.Khan white x Puma Super while minimum wheat spike length (7.500) was recorded in Peshawar x Aim treatment.

Table 6. Wheat spike length (cm) as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 9 | 8.925 | 8.9 | 8.425 | 8.812 |
| D.I.Khan(W) | 9.075 | 9.125 | 8.55 | 8.25 | 8.75 |
| Mardan | 8.25 | 8.3 | 8.075 | 7.975 | 8.15 |
| Peshwar | 7.5 | 7.8 | 8.925 | 8.425 | 8.269 |
| Herbicide Means | 8.456 | 8.75 | 8.15 | 8.163 | |

Number of grains spike

Statistical analysis of the data showed that wild oat biotypes and their interaction with wild oats biotypes have a non-significant effect while different herbicides had significant effect on number of grains spike$^{-1}$ (Table 7). The data exhibit that among the wild oat biotypes, maximum grains spike$^{-1}$ (52.194) were recorded in D.I.Khan black biotype while minimum grains spike$^{-1}$ (47.575) were recorded in D.I.Khan white wild oats. Among the herbicides means, maximum grains spike$^{-1}$ (61.713) were counted in Affinity, while minimum grains spike$^{-1}$ (39.412) were witnessed in weedy check treatment. For interaction of wild oat biotypes with the herbicides, the maximum grains spike$^{-1}$ (72.200) was recorded in D.I.Khan white x Affinity while minimum grains spike$^{-1}$ (35.850) was observed in D.I.Khan white x weedy check treatment.

Table 7. Number of grains spike$^{-1}$ as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 52.925 | 46.25 | 72.2 | 37.4 | 52.194 |
| D.I.Khan(W) | 46.35 | 49.4 | 58.7 | 35.85 | 47.575 |
| Mardan | 48.55 | 49.65 | 58.1 | 38.65 | 48.737 |
| Peshwar | 47.4 | 46.25 | 57.85 | 45.75 | 49.312 |
| Herbicide Means | 48.806b | 47.888b | 61.713a | 39.412c | |

Grain weight spike

Statistical analysis of the data showed that wild oat biotypes and their interaction had a non-significant effect while herbicides had significant effect on weight of spike. The data in Table 8 depict that among the wild oat biotypes, maximum grain weight spike$^{-1}$ (1.978) was recorded in Mardan biotypes while minimum grains weight spike$^{-1}$ (1.897) was recorded in D.I.Khan black wild oat treated plots. Among the herbicides means maximum grain weight spike$^{-1}$ (2.405) was recorded in Affinity, while minimum (1.561) was recorded in weedy check treatment. For interaction of wild oat biotypes with the herbicides, the maximum grain weight spike$^{-1}$ (2.495) was recorded in D.I.Khan black x Affinity while minimum grain weight spike$^{-1}$ (1.435) was recorded in D.I.Khan black x weedy check treatment.

Table 8. Grain weight spike$^{-1}$ as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 1.95 | 1.708 | 2.495 | 1.435 | 1.897 |
| D.I.Khan(W) | 1.782 | 1.995 | 2.432 | 1.453 | 1.916 |
| Mardan | 1.972 | 2.037 | 2.272 | 1.63 | 1.978 |
| Peshwar | 1.598 | 1.817 | 2.42 | 1.727 | 1.901 |
| Herbicide Means | 1.826b | 1.899b | 2.405a | 1.561c | |

Grain weight

It is evident from the data (Table 9) that the 1000-grain weight (g) was not significantly affected by wild oat biotype, herbicides and their interaction. The mean data manifest that among the wild oat biotypes, maximum 1000-grain weight (36.291 g) was recorded in Mardan biotype while minimum 1000-grain weight (34.689 g) was recorded in D.I.Khan white wild oat treated plots. Among the herbicides means maximum 1000-grain weight (36.909 g) was recorded in Aim treated plots, while minimum (33.976 g) was recorded in Weedy check treatment. For interaction of wild oat biotypes with the herbicides, the maximum 1000-grain weight (38.318 g) was recorded in Mardan x Affinity while minimum 1000-grain weight (32.650 g) was recorded in D.I.Khan white x weedy check.

Table 9. 1000 grain weight (g) as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 37.156 | 34.482 | 34.41 | 34.175 | 35.056 |
| D.I.Khan(W) | 35.265 | 37.143 | 33.697 | 32.65 | 34.689 |
| Mardan | 34.905 | 36.923 | 38.217 | 35.12 | 36.291 |
| Peshwar | 36.313 | 36.815 | 34.23 | 33.957 | 35.329 |
| Herbicide Means | 35.909 | 36.341 | 35.139 | 33.976 | |

Biological yield

It is evident from (Table 10) that wild oats biotypes, herbicides and their interaction had non-significant differences for biological yield (t $ha^{-1}$). The data reveal that among the wild oat biotypes, maximum biological yield (2.412 t $ha^{-1}$) was recorded in D.I.Khan (white) biotypes while minimum biological yield (2.125 t $ha^{-1}$) was recorded in Peshawar wild oat treated plots. Among the herbicides means, maximum biological yield (2.362 t $ha^{-1}$) was recorded in weedy check, while minimum biological yield (2.225 t $ha^{-1}$) was recorded in Aim treatment. For interaction of wild oat biotypes with the herbicides, the maximum biological yield (2.750 t $ha^{-1}$) was recorded in D.I.Khan black x weedy check while minimum biological yield (1.900 t $ha^{-1}$) was recorded in Peshawar x weedy check and Peshawar x Puma super treatment.

Table 10. Biological yield (t $ha^{-1}$) as affected by different wild oat biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 2.3 | 2.45 | 1.9 | 2.75 | 2.35 |
| D.I.Khan(W) | 2.5 | 2.5 | 2.15 | 2.5 | 2.412 |
| Mardan | 2 | 2.55 | 2.35 | 2.3 | 2.3 |
| Peshwar | 2.1 | 1.9 | 2.6 | 1.9 | 2.125 |
| Herbicide Means | 2.225 | 2.35 | 2.25 | 2.362 | |

Grain yield

It is evident from Table 11 that grain yield (t $ha^{-1}$) showed non-significant response to wild oat biotypes and their interaction with herbicides, while the herbicides significantly affected the determination of grain yield. The data show that among the wild oat biotypes, maximum grain yield (1.522 t $ha^{-1}$) was harvested in D.I.Khan (black) biotype while minimum grain yield (1.274 t $ha^{-1}$) was recorded in Peshawar wild oat plot. Among the herbicides means, maximum grain yield (1.502 t $ha^{-1}$) was recorded in Affinity, while minimum (1.156 t $ha^{-1}$) was recorded in the weedy check (Table 11). For interaction of wild oat biotypes with the herbicides, the maximum grain yield (1.670 t $ha^{-1}$) was recorded in Mardan x Affinity interaction, while minimum grain yield (0.810 t $ha^{-1}$) in D.I.Khan white x weedy check. The yields were generally lesser across all the interactions involving weedy check.

These findings are corroborated with the earlier work of Koscelny and Peeper (1997), Spandl et al. (1997), Salarzai et al. (1999) and Stevensen et al. (2000) who reported an increase in wheat yield due the application of various herbicides and consequently a reduced weed density in wheat.

Table 11. Grain yield (t $ha^{-1}$) as affected by different wild oats biotypes and herbicides.

| *Avena fatua* Biotype | Herbicides | | | | Biotype Means |
|---|---|---|---|---|---|
| | AIM | PUMA SUPER | AFFINITY | WEEDY CHECK | |
| D.I.Khan(B) | 1.48 | 1.49 | 1.554 | 1.564 | 1.522 |
| D.I.Khan(W) | 1.524 | 1.514 | 1.4 | 0.81 | 1.312 |
| Mardan | 1.446 | 1.22 | 1.67 | 1.316 | 1.412 |
| Peshwar | 1.564 | 1.216 | 1.38 | 0.936 | 1.274 |
| Herbicide Means | 1.504a | 1.360ab | 1.502a | 1.156b | |

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# Efficacy of pre and post-emergence herbicides for controlling weeds in chickpea in Pakistan

S.Hashim*[1], K. B. Marwat, I. A. Khan, and Z. Hussain
Department of Weed Science, NWFP Agricultural University, Peshawar-25130, Pakistan
saimahashim@yahoo.com

**Abstract:** To study the efficacy of different herbicides for controlling weeds in chickpea, an experiment was conducted at Agricultural Research Station Ahmad Wala, Karak, NWFP Pakistan during Rabi season 2003–04, using RCB design with four replications. The experiment consisted of seven herbicide treatments, a weedy check, and hand weeding. The herbicides treatments included: s-metolachlor (1.44 kg a.i $ha^{-1}$), metribuzin (0.35 kg a.i $ha^{-1}$) and pendimethalin (0.99 kg a.i $ha^{-1}$) (as pre-emergence); isoproturon (0.85 kg a.i $ha^{-1}$), clodinafop (0.03 kg a.i $ha^{-1}$), fenoxaprop-P-ethyl (0.75 kg a.i $ha^{-1}$) and pendimethalin (0.99 kg a.i $ha^{-1}$) as post emergence. Data were recorded on weed density $m^{-2}$, number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of grains $plant^{-1}$, 1000-grains weight (g) and grains yield (kg $ha^{-1}$). For controlling weeds, hand weeding proved to be the best, giving only 9.17 weeds $m^{-2}$ as compared to 33.67 weeds $m^{-2}$ in weedy check plots. Similarly, the highest grain yield (1147.8 kg $ha^{-1}$) was recorded in hand weeded plots. In herbicidal treatments, pendimethalin as pre-emergence produced the highest yield (1060.3 kg $ha^{-1}$) as compared to weedy check plots (338 kg $ha^{-1}$).

**Key words:** Chickpea, herbicides, and weed control.

## INTRODUCTION

Chickpea (*Cicer arietinum*) is the principal pulse that provides a major source of protein in diet of the predominantly vegetarian population. It is traditionally cultivated in arid sandy areas of NWFP but recently its production has declined as the crop has been displaced by the rapid expansion of irrigated areas and the introduction of modern productive wheat cultivars. Two main categories of chickpea are distinguished, based primarily on seed characteristics: the 'desi' type, having relatively small, angular seeds with rough, usually yellow to dark brown testa; and the 'kabuli' type, which has larger, more rounded and creamed colored seeds. The desi-type constitutes about 85% of annual world production and is confined entirely to the Indian sub-continent, Ethiopia, Mexico and Iran. The kabuli-type comprises only a minor area and production, but accounts entirely for the crops of Europe and the America, except Mexico. Other categories are the 'gulabi' (pea shaped) type of central India and green-seeded desi-type of central and northwestern India. In Pakistan during 2002, chickpea was grown on 963,000 ha with a production of 675,200 t. Punjab and Sindh are leaders in chickpea production (Anonymous 2003).

Chickpea yields in Pakistan are lower than the maximum potentials of the cultivars. The gap could be attributed mainly to weed competition in addition to other production constraints. Since chickpea is traditionally grown on residual soil moisture, therefore, weed competition poses a major problem. Common annual weed species include *Chenopodium album, Asphodelus tenuifolius, Argemone mexicana, Carthamus oxyacantha, Cenchrus ciliaris, Cyperus rotundus, Fumaria* sp., *Polygonum sp., Lathyrus spp., Vicia stiva, Euphorbia* sp., *Cynodon dactylon* and *Cirsium arvense* (Saxena and Yadav 1976).

Hand weeding at 30 and again at 60 days after sowing (DAS) essentially eliminates the adverse effect of weed competition (Saxena 1980). In commercial practice, the cultivation of preceding rainy-season fallows not only helps to capture and conserve moisture but also reduces weed infestations. On black soils, in the wetter areas of central India, "haveli" cultivation (the practice of containing water by bunding in the rainy season) serves similar purposes. Inter-row cultivation by tractor or animal-drawn implements is common and facilitated in North Africa by sowing the crop in very wide rows. When properly used, pre-emergence herbicides such as prometryne, tervutryne, pronamide, cyanazine and methabenz-thiazuron accomplish effective and economic weed control, resulting in chickpea seed yields similar to or only slightly lower than those of weed-free treatments (Sheldrake et al. 1997).

Potential yield losses in chickpea due to weeds range between 22-100% (Saxena and Yadave 1976). Post emergence application of pyradate herbicide gave 97.5% weed control (Skrobakova 1999). Bhalla et al. (1998) reported that herbicide treatment gave 50-64% weed control with increased yield. Weed growth was significantly reduced by the use of herbicides and resulted in increased yield of 50% against the control (Stork 1998). Singh (1998) and Sukhadia et al. (1999) pointed out that weeds reduced productivity in chickpea by up to 36.8% and 41-44%, respectively.

In view of the impottance of the problem, this experiment was designed to investigate the efficacy of different herbicides on weed pressure and consequent effects on various parameters of chickpea crop including yield and yield components

## MATERIALS AND METHODS

Conducted at Ahmadwala Agricultural Research Station Karak, NWFP during 2003-04, the experiment was laid out in randomized complete block (RCB) design with four replications and nine treatments. The size of each plot was 1.8x4 $m^2$. Each treatment had six rows, 30 cm apart. Desi type of chickpea was sown for the experiment. Standard agronomic practices were followed.

Table 1. List of treatments used in experiment

| Treatment | Common Name | Time of application | Rate (kg a.i $ha^{-1}$) |
|---|---|---|---|
| Dual Gold 960 EC | s-metolachlor | Pre-emergence | 1.44 |
| Sencor 70 WP | metribuzin | Pre-emergence | 0.35 |
| Stomp 330 EC | pendimethalin | Pre-emergence | 0.99 |
| Isoproturon 50 WP | isoproturon | Post-emergence | 0.85 |
| Topik 15Wp | clodinafop | Post-emergence | 0.03 |
| Puma Super75EW | fenoxaprop-P-ethyl | Post-emergence | 0.75 |
| Stomp 330 EC | pendimethalin | Post-emergence | 0.99 |
| Hand weeding | --- | --- | --- |
| Weedy check | --- | --- | --- |

Data were recorded on weed density $m^{-2}$, plant height (cm), number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of grains $plant^{-1}$, 1000 grains weight (g) and grains yield (kg $ha^{-1}$). The data collected were subjected to statistical analysis and the treatment means, were separated by least significance difference (LSD) test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

### Weed density

Weed density $m^{-2}$ was significantly ($P<0.05$) affected by the various treatments (Table 2). The weeds noticed in the experiment were *Asphodelus tenuifolius*, *Medicago denticulata*, *Chenopodium album*, *Argemone mexicana*, *Carthamus oxycantha*, *Cenchrus ciliaris*, *Cirsium arvense*, *Polygonum sp.*, *Lathyrus spp.*, *Vicia stiva*, *Convolvulus arvensis*, *Anagallis arvensis*, *Fumaria indica*, *Cynodon dactylon*, *Euphorbia* sp., and *Melilotus perviflora*. Maximum weeds $m^{-2}$ (33.67) were recorded in the weedy check plots and minimum weeds were recorded in hand weeding (9.17 $m^{-2}$) plots. The pre-emergence herbicides also exhibited good control of the weeds of chickpea. The results are in conformity with the works of Singh and Sahu (1996) and Balyan and Malik 1996.

### Plant height at maturity

The effect of the different treatments on plant height was not significant ($P>0.05$) as shown in Table 2. Among all treatments, hand weeded plots produced the tallest plants (50.57 cm) while the weedy check had the lowest plant height (37.63 cm). Thakar et al. (1995) and Iqbal et al. (1991) obtained similar results while working on chemical weed control in chickpea.

### Number of branches plant$^{-1}$

The different herbicides and hand weeding had no significant ($P>0.05$) effect on the number of branches plant$^{-1}$. Comparison of the treatments means reflects that maximum number of branches plant$^{-1}$ (3.8) were recorded in the hand weeded plots, while minimum number of branches (2.25) were recorded in weedy check plots as shown in Table 2. The possible reason for the increased number of branches by hand weeding and herbicide treated plots could be the best control of weeds and consequently increased the maximum utilization of available resources, while the least number of branches plant$^{-1}$ in the weedy check could be attributed to weed competition. Singh and Sahu (1996) had obtained similar results in chickpea experiment.

### Number of pods plant$^{-1}$

The herbicides and hand weeding as weed control measures had insignificant effect ($P>0.05$) on the number of pods plant$^{-1}$. The highest (34.15) number of pods plant$^{-1}$ was recorded in hand weeded plots. It was, however, statistically at par with the herbicidal treatments (Table 2). Lowest number of pods was recorded in weedy check plots (21.08). Weeds affected the number of pods plant$^{-1}$ and reduced chickpea yield (Balyan and Malik 1996). The variability maybe attributed to the effectiveness of weed control methods, which ultimately increased the nutrient availability for the crop (Hosseini 1997) and Singh and Sahu (1996).

### Number of grains plant$^{-1}$

The number of grains plant$^{-1}$ was significantly affected ($P<0.05$) by the different herbicidal treatments and hand weeding. The highest (53.5) number of grains plant$^{-1}$ was obtained from hand weeding plots. However, it was statistically at par with the group of the herbicides as given in Table 2. The lowest number of grains plant$^{-1}$ was recorded in the weedy plots (25). The highest number of grains plant$^{-1}$ in hand weeding plots may be due to its effectiveness on weed control. These results agree with the results obtained by Bhalla et al. (1998).

### Weight of 1000 grains

The herbicides and hand weeding had no significant effect ($P>0.05$) on the weight of 1000 grains. The means for 1000 grains weight showed that the highest (205.0 g) was obtained from hand weeded plots. The lowest 1000 grains weight (172.5 g) was recorded in weedy check plots as exhibited by Table 2. The reason for highest 1000 grains weight in hand weeding plots was largely due to the fact that it showed maximum weed control, thus maximized the available resources for the crop and reduced weeds competition, while the fact for lowest 1000 grains weight is the strong weed competition. The results are in line with that reported by Iqbal et al. (1991) and Singh and Sahu (1996).

Grain yield

The different herbicidal treatments and hand weeding had significant ($P<0.05$) effect on grain yield in chickpea. The data in Table 2 indicate that the highest grain yield of (1147.8 kg $ha^{-1}$) was obtained from hand weeded plots. However, it was statistically at par with the herbicidal treatments. The lowest grain yield was recorded in weedy check plots (388 kg $ha^{-1}$). The highest yield in hand weeding plots was probably due to maximum weed control and, thus, the crop flourished and efficiently utilized all the available resources. All the herbicides equally controlled all the weeds in chickpea; the slight differences in their grain yield might be due to the fact that different treatments were facing competition with different weeds. These results are in conformity with Iqbal et al. (1991).

Table 2. Effect of treatments on different parameters of crop.

| Treatment | Weed density $m^{-2}$ | Plant Height (cm) | Number of branches $plant^{-1}$ | Number of pods $plant^{-1}$ | Number of grains $plant^{-1}$ | 1000 grains weight (g) | Grains yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| Dual Gold 960 EC(s-metolachlor) | 10.12 a | 49.63 | 3.5 | 33.55 | 39.15 ab | 198.8 | 979.8 ab |
| Sencor 70 WP (metribuzin) | 26.55 ab | 44.08 | 3.2 | 27.45 | 29.47 ab | 185.8 | 862.8 ab |
| Stomp 330 EC (PRE) (pendimethalin) | 9.48 a | 50.08 | 3.5 | 34.05 | 43.72 ab | 201.3 | 1060.3 ab |
| Isoproturon 50 WP (Isoproturon) | 18.61 ab | 49.3 | 3.5 | 33.47 | 35.47 ab | 196.3 | 951.8 ab |
| Topik 15 WP (clodinafop) | 24.04 ab | 45.05 | 3.3 | 28.25 | 30.83 ab | 187.5 | 931.0 ab |
| Puma Super 75 EW (fenoxaprop-P-ethyl) | 22.11 ab | 45.52 | 3.4 | 30.97 | 33.80 ab | 195 | 947.0 ab |
| Stomp 330 EC (POE) (pendimethalin) | 26.69 ab | 40.5 | 3.05 | 23.55 | 28.95 ab | 183.8 | 635.8 ab |
| Hand Weeding | 9.17 a | 50.57 | 3.8 | 34.15 | 53.50 a* | 205 | 1147.8 a* |
| Weedy Check | 33.67 c | 37.63 | 2.25 | 21.08 | 25.00 b | 172.5 | 388.0 b |
| LSD at 0.05α level | 21.76 | 13.31 | 1.285 | 14.18 | 25.65 | 29.81 | 875.4 |

Means followed by different letters in the respective column are significantly different at % probability level according to LSD test.

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# Response of weeds over-time to different herbicides in potato

S. Hashim, K. B. Marwat and M. A. Khan
Department of Weed Science
NWFP Agricultural University Peshawar-25130, Pakistan
saimahashim@yahoo.com

**Abstract**: Weed management trial in potato was conducted during fall, 2002 at Peshawar, NWFP, Pakistan to assess the weed pressure as affected by various herbicidal molecules at different time intervals. The treatments used against weeds were pendimethalin, triazine, sulfentrazone, triazine+terbuthylazine, metribuzin and trifluralin at 1.32, 0.75, 0.60, 0.75, 0.42 and 1.20 kg a.i. $ha^{-1}$. Weedy check and hand weeding were also included in the study. Weed data were recorded at four intervals during the crop season in all treatments. The response of weed density over time was cubic in monocot, dicot and total weeds. Lowest weed density in $m^{-2}$ (64.35) was recorded in hand weeding, followed by triazine+ terbuthylazine (68.1) and metribuzin (79.95), whereas maximum weed densities also in $m^{-2}$ (147.9, 146.5, 144.4,134.4 and 111.75) were recorded in sulfentrazone, followed by no-weeding, trifluralin, triazine and trifluralin, respectively.

**Key words**: Herbicides, potato, weed control.

## INTRODUCTION

Potato (*Solanum tuberosum)* is second to maize in terms of the number of producer countries and fourth after wheat, maize and rice in global tonnage. The average composition of potato is about 80% water, 2% protein and 18% starch. As a food, it is one of the cheapest and easily available sources of carbohydrates and proteins and furnishes appreciable amount of vitamin B and C as well as some minerals (Woolfe 1987).

Potato is a popular crop in Pakistan and is grown in 110.5 thousand ha with production of 1868.4 thousand tons, with the average yield of 16.9 t $ha^{-1}$. Weeds are an important factor in the management of all land and water resources, but their effective impact is greatest on agriculture. There is no reliable study of worldwide damage caused by weeds. However, it is widely known that losses caused by weeds exceed the losses from any category of agricultural pests, such as insects, nematodes and diseases. Of the total annual loss of agricultural produce from various pests, weeds account for 45%, insects 30%, diseases 20% and other pests 5% (Rao 2000). Similarly, Marwat (2003) reported that 38% losses occur in potato due to weeds equivalent to annual losses of Rs. 3.9 billion in monitory terms. Several reports address the importance of weed management in potato. Tyla and Tamosiunas (1996) and Hashim et al. (2003) reported that herbicide use decreased the weed density and increased the potato yield. All herbicidal treatments significantly reduced weed density over the control (Khan et al. 1995). Due to improved production technology, high rate of fertilizer dose with frequent irrigations tremendously increases weed growth. The weeds successfully compete with the potato crop for water, nutrients and light and thus reduce the yield and quality of tubers (Singh et al. 1986). As the emergence is very slow, the emerging shoots are liable to have serious competition with weeds from germination up to the harvest of the crop. Weed scientists can improve the use of practices for weed management by improving the knowledge of relevant intervention. Zhukova and Loban (2000) and Gruczek (2000) reported that the use of herbicides increased yield, improved crop quality, and increased profitability in potato.

Keeping this scenario in mind, this study was conducted to see the effect of different herbicidal molecules on weeds of potato and the response of weeds density over-time.

## MATERIALS AND METHODS

Response of weeds over time to different herbicides in potato was studied during fall season of 2002 at NWFP Agricultural University Research Farm, N-W, Pakistan, using randomized complete block design with eight treatments and five replications. The size of the treatment was 5 m long with 3 ridges, as well as row to row distance and plant to plant distance of 75 and 20 cm, respectively. Calculating for this area, 25 potato tubers were planted in each treatment. Different herbicides were applied according to their time of application (Table 1).

Table 1. Herbicides used in the experiment, along with their rate and time of application.

| Treatment/Trade Name | Common Name | Active ingredient (kg ha$^{-1}$) | Time of application |
|---|---|---|---|
| Stomp 330 EC | pendimethalin | 1.32 | Pre-emergence |
| Igran 500 FW | triazine | 0.75 | Pre-emergence |
| Authority 4F | sulfentrazone | 0.6 | Pre-emergence |
| Topogard 500 FW | Igran + terbuthylazine (35:15) | 0.75 | Pre-emergence |
| Sencor 70 WP | metribuzin | 0.42 | Post-emergence |
| Treflan 4 EC | trifluralin | 1.20 | PPI |
| Hand weeding | - | - | - |
| Weedy check | - | - | - |

Data was recorded on monocot weeds m$^{-2}$, dicot weeds m$^{-2}$ and total weeds density m$^{-2}$. Irrigation and other agronomic practices were carried out as per requirement of the crop. To see the impact of herbicides on weed density over-time, regression analyses were done using polynomials, to find out the linear, quadratic and cubic effect, respectively (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

As a whole, the crop was not very healthy as it was attacked by early blight (*Alternaria solani*). However, the infestation was uniform and, therefore, the relative effect of weed control treatments on different parameters was consistent.

### Monocot weeds density

The monocot weeds density over-time was statistically significant, the curve response was cubic (Fig. 1). Highest weeds density of monocot was observed during first measurement, then decreased in the second (29.7 m$^{-2}$), increased in third measurement (44.18 m$^{-2}$) and decreased in the fourth (24.45 m$^{-2}$) measurement. Statistically, weed density of monocot during the first and third observations, 48.08 m$^{-2}$ and 44.18 m$^{-2}$, respectively, was higher but at par compared to the second and fourth observations, 29.70 m$^{-2}$ and 24.45 m$^{-2}$, respectively, which yielded lower and statistically similar (at par) weed density. Sabra (2000) reported that a negative correlation between potato yield and weed population was recorded. Organic matter and other soil constituents were correlated positively with the total and broadleaved weeds. The best model fitting the relation between total weed population and potato yields was exponential.

### Dicot weed density

Different herbicidal treatments had significant effect on weeds density of dicot weeds overtime in potato (Fig. 2). The response of weeds density to treatments was cubic. During the first observation, higher weed density of 99.75 m$^{-2}$ was recorded then decreased to 65.78 m$^{-2}$ during the second observation, again increased during the third (95.48 $m^{-2}$) and then again lowered in fourth observation 41.34 $m^{-2}$. The means during first and third observation and second and fourth observations were statistically similar, respectively. Galeev & Mekheev (2000) reported that herbicide application increased yield by 15-28% and increased starch content in tubers by 0.2-0.4%. No negative effect on potato and no residues in tubers at harvest were detected.

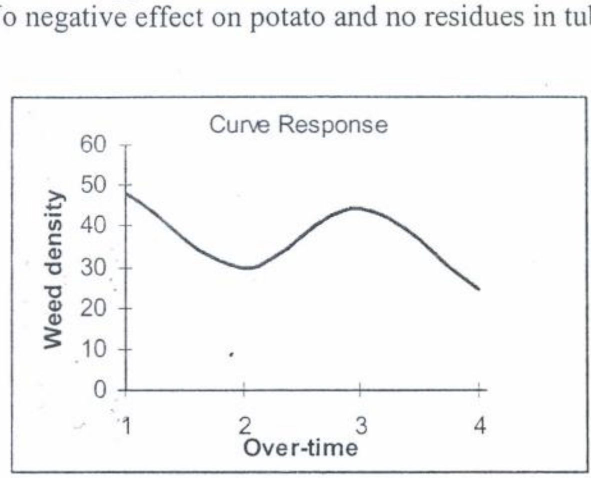


Figure 1. Density of monocot weeds as affected by different herbicides over-time.

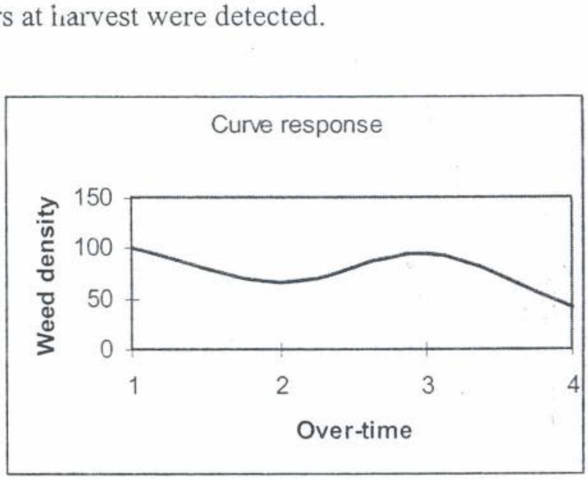


Figure 2. Density of dicot weeds as affected by different herbicides over-time.

Total weed density

As the weed density is the sum of monocot and dicot weeds, the total weeds density over-time was significant and the response of total weeds during four observations at different times during the season was cubic (Fig. 3). The higher number of weeds was recorded in first (147.83 $m^{-2}$) and third (139.65 $m^{-2}$), but lower in the second (95.40 $m^{-2}$) and fourth (65.78 $m^{-2}$) observations. Weed density during first and third as well as second and fourth was statistically similar. A total of 147.9 weeds $m^{-2}$ were found in Authority followed by no-weeding, Stomp, Igran and Treflan having 146.55, 144.45, 137.4 and 111.75 weeds $m^{-2}$, respectively. All these treatments were statistically at par with each other. Lowest weed density was observed in hand-weeding (64.35) followed by Topogard (68.01) and Sencor (79.95), respectively. As the critical weed competition with potato occurs at this stage, the herbicides therefore controlled the weeds at the desirable time. Nedunzhiyan (1998) reported that based on average yield in the two years, the critical period of crop-weed competition was 30-45 days after sweet potato planting.

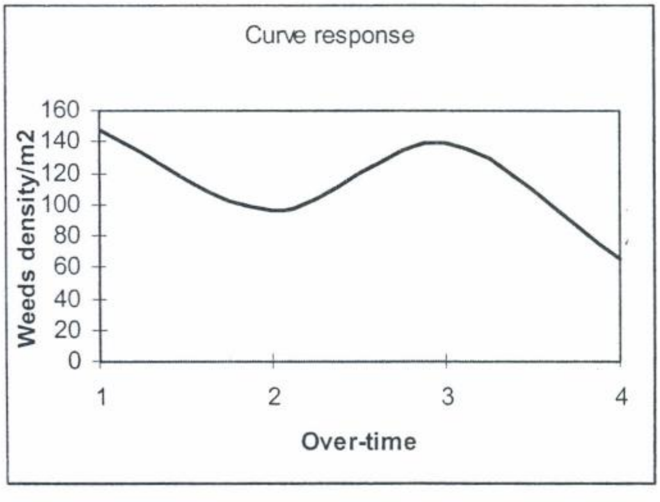


Figure 3. Weed density as affected by different herbicides over-time.

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**Efficacy of different herbicides for weed management in canola in N-W Pakistan**

K. B. Marwat*, Z. Hussain and B. Gul
Department of Weed Science, NWFP Agricultural University,
Peshawar-25130, Pakistan
kbmarwat@psh.paknet.com.pk; kbmarwat@yahoo.com

**Abstract:** To evaluate the effect of different pre and post-emergence herbicides for controlling weeds in rapeseed (variety Oskar), an experiment was conducted at Agriculture Research Station, Mingora during rabi season 2003–04, using randomized complete block (RCB) design with four replications. The experiment was sown in mid-October with nine treatments, viz, seven herbicides, hand weeding, and a weedy check. Each treatment consisted of 4 rows 75 cm apart and 4 m long, giving a total size of 4 m x 3 m. The pre-emergence herbicides included: s-metolachlor (1.92 kg a.i $ha^{-1}$), pendimethalin (1.32 kg ai $ha^{-1}$) and trifluralin (1.2 kg a.i $ha^{-1}$), while the post-emergence herbicides were fenoxaprop-P-ethyl (0.75 kg a.i $ha^{-1}$), clodinafop (0.05 kg a.i $ha^{-}$), oxadiazon (0.36 kg a.i $ha^{-1)}$ and propaquizafop (0.02 kg a.i $ha^{-1}$). Data were recorded on weed density $m^{-2}$, fresh weed biomass (kg $ha^{-1}$), number of branches $plant^{-1}$, number of siliquae $plant^{-1}$, siliqua length (cm), plant height at maturity (cm), number of seeds $siliqua^{-1}$, 1000 seeds weight (g), seed yield (kg $ha^{-1}$) and cost-benefit ratio. Weed density $m^{-2}$, fresh weed biomass and seed yield were significantly affected by the different herbicidal treatments. Pendimethalin treatment exhibited the best performance, with minimum weeds $m^{-2}$ (13.5) and fresh weed biomass (257.9 kg $ha^{-1}$) as compared to weedy check (38 $m^{-2}$) and (806 kg $ha^{-1}$), respectively. Similarly, the highest number of branches $plant^{-1}$ (6.89), number of siliquae $plant^{-1}$ (301), siliqua length (6.8 cm), number of seeds $siliqua^{-1}$ (25.8), 1000 seeds weight (3.99 g), seed yield (1692 kg $ha^{-1}$) and cost-benefit ratio (1:7.47) were recorded in pendimethalin-treated plots as compared to the weedy check.

**Key words**: Canola, herbicides, rapeseed, weed control.

## INTRODUCTION

Rapeseed and canola (*Brassica napus* L.) are the main oil producing crops in Pakistan. They are grown in *rabi* season and have remained the major sources of edible oil in the subcontinent for decades. The Canadian oilseed-breeding programme developed the sweet mustard or canola, which is a high producing type of mustard. It has a better taste as well as highest level of unsaturated fatty acid and thus helps lower blood cholesterol level. It is also a rich source of oil and protein and contains more than 40% oil (Weiss 1983). Canola was introduced recently to our country to increase the domestic edible oil production. It is rapidly replacing the older varieties of rapeseed and mustard.

In Pakistan, the area under rapeseed and mustard cultivation during 2002-03 was 280,000 ha yielding an average of 837 kg $ha^{-1}$. In NWFP, the total area under rapeseed and mustard cultivation was 19,1000 ha with an average production of 447 kg $ha^{-1}$ (Anonymous 2003). The low acreage of rapeseed is partially due to its sowing season that overlaps with the wheat-sowing season. As wheat is the staple food crop, lesser attention is therefore given to oilseed crops. That's why mustard is only grown on almost rain fed and less fertile areas. As a result, not enough edible oil is produced to fulfill the domestic requirement. Edible oil is the second most important import item after petroleum. Canola is a smother crop because of its larger leaves, rapid growth and early canopy closing. Still, weed competition is very critical during the early stand establishment particularly the parasitic weed *Orobanche* sp. (Joel et al. 1995).

Weeds decrease oil production as well as the oil quality. Several methods have been in use for weed control in canola, like hand weeding, cultivation in row cropping, and use of chemicals. But most reliance is made on chemical weed control, as it is comparatively more independent of weather, cheap and saves labor. Pre-emergence herbicides are more effective than post-emergence or manual control methods (Rapparini 1996). Khan et al. (1995) suggested the use of post-emergence plus hand weeding if proper pre-emergence herbicides are unavailable. Keeping in view the importance of the different herbicides for controlling weeds in canola, this experiment was conducted to figure out the most effective, economical and suitable herbicide for the area concerned.

## MATERIALS AND METHODS

In order to study the impact of different herbicides on rapeseed (*Brassica napus* L.) an experiment was sown at Agriculture Research Station, Mingora during 2003-04 on 15$^{th}$ October 2003 using Oskar variety of rapeseed, applying seed rate of 5kg ha$^{-1}$. The experiment was laid out in randomized complete block (RCB) with 9 treatments replicated 4 times. The 9 treatments were Dual Gold 960EC, Stomp 330EC, Treflan 4EC, Puma Super 75EW, Topik 15WP, Ronstar 12L, Agil 100EC, a hand weeding and a weedy check. Each treatment had a size of 4 m x 3 m with 4 rows and row-to-row distance of 75 cm. Details of the treatments used in the experiment are shown in Table 1.

Trifluralin was first incorporated in the soil and the pre-emergence herbicides were applied one day after sowing. The post-emergence herbicides were sprayed at 2-3 leaf stage of the crop after the second application of nitrogen fertilizer.

Data were recorded on weed density m$^{-2}$, fresh weed biomass (kg ha$^{-1}$), number of branches plant$^{-1}$, number of siliquae plant$^{-1}$, siliqua length (cm), plant height at maturity (cm), number of seeds siliqua$^{-1}$, 1000 seeds weight (g), seed yield (kg ha$^{-1}$) and cost-benefit ratio.

Table 1. Detail of treatments used in experiment.

| Treatment | Common name | Application time | Rate (kg a.i ha$^{-1}$) |
|---|---|---|---|
| Dual Gold 960 EC | s-metolachlor | Pre-emergence | 1.92 |
| Stomp 330 EC | pendimethalin | Pre-emergence | 1.32 |
| Treflan 4 EC | trifluralin | Pre-plant Incorporated | 1.20 |
| Puma super 75 EW | fenoxaprop-P-ethyl | Post-emergence | 0.75 |
| Topik 15 WP | clodinafop | Post-emergence | 0.05 |
| Ronstar 12 L | oxadiazon | Post-emergence | 0.36 |
| Agil 100 EC | propaquizafop | Post-emergence | 0.02 |
| Hand weeding | -- | -- | -- |
| Weedy check | -- | -- | -- |

The data recorded were individually subjected to ANOVA by using MSTATC computer software and means were separated by using Fisher's LSD test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

### Weeds density

There was significant ($P<0.05$) effect of different herbicides on weed density. Table 2 shows that the highest weed density (38) was recorded in the weedy check. The lowest weed density (13.5) was recorded in Stomp 330 EC treatment but was statistically at par with the rest of the herbicidal treatments along with hand weeding. This may be due to strong killing effect of Stomp 330 EC on the weeds while weeds in the weedy check were left to grow freely without any disturbance. The difference in weed population in different treatments can be attributed to the fact that some herbicides are more effective for weed control than the other. Similar results have been reported by Tiwari and Kurchania (1993) and Khan et al. (2003). Lolas (1997) also reported that herbicides could effectively control weeds.

### Fresh weed biomass (kg ha$^{-1}$)

Analysis of variance showed that the different herbicidal treatments had significant effect ($P<0.05$) on weed biomass. Table 2 indicates that Stomp 330 EC treated plots had the lowest weed biomass (257.9 kg ha$^{-1}$) but was statistically at par with Treflan 4 EC. The weedy check had the lowest weed biomass (806 kg ha$^{-1}$). The difference in weed biomass among the different treatments could be due to differences in their phytotoxic effect as observed by Tiwari and Kurchania (1993). It is argued that pre-plant-incorporated or pre-emergence herbicide treatments are indispensable, controlling many weeds before they emerge. Weed biomass is reduced through the use of chemicals, which ultimately favours the yield (Lolas 1997).

### Number of branches plant$^{-1}$

The different herbicidal treatments had non-significant effect on the number of branches plant$^{-1}$ as shown in Table 2. The highest number of branches plant$^{-1}$ (6.89) was recorded in plots sprayed with Stomp 330EC, while the lowest (5.69) was in the weedy check. The possible reason for increase in number of branches plant$^{-1}$ by Stomp 330 EC could be the best control of weeds and consequently increased nutrient availability to the crop. The lowest number of branches plant$^{-1}$ in the weedy check could be attributed to weed competition for nutrients, light, moisture and space. The results are in agreement with those reported by Singh et al. (1995). Khan et al. (2003) also stated that application of some herbicides increases number of branches plant$^{-1}$ in rape and mustard.

### Number of siliquae plant$^{-1}$

Analysis of variance indicated that the different herbicidal treatments had non-significant effect on the number of siliquae plant$^{-1}$ (Table 2). The highest number of siliquae plant$^{-1}$ (301) was recorded in Stomp 330EC treated plots, while the lowest (216) was noted in the weedy check. The results are similar to those reported by Thomas (1998) which showed that herbicides efficiently enhance the yield components. Khan *et al.* (1995) reported that application of some herbicides increases the number of siliquae plant$^{-1}$ in rapeseed.

### Siliqual length

Analysis of variance showed that the different treatments had non-significant effect on siliqua length (Table 2). The longest siliqua length (6.8 cm) was recorded in Stomp 330EC treated plots, while the shortest was observed in the weedy check (6.3 cm). The longest siliqua length (6.8 cm) recorded in Stomp 330EC treated plots can be attributed to the best weed management by the chemical treatment, which enabled the flow of nutrients into the crop, thereby enhancing the siliqua length. The shortest siliqua length (6.30 cm) recorded in weedy check could be due to the weeds' competition for nutrients. The result is supported by Raghavan and Hariharan (1991) and Khan *et al.* (2003) who stated that pre-emergence herbicides improve the siliqua length in the rapeseed crop particularly the herbicide Treflan 4EC.

### Plant height at maturity

The effect of the different treatments on plant height was non-significant (Table 3). The weedy check plots had the tallest plants (143.4 cm), while the hand weeding treatment had the shortest plants (127.2 cm). The competition of the rapeseed plants with the weeds in the weedy check plots forced the crop plants to rise higher than their normal heights. All the herbicidal treatments produced almost statistically equal plant height but was comparable with that of weedy check.

Similar results have been reported by Khan *et al.* (2003) who stated that there was non-significant increase in the plant height with the application of some herbicides.

Table 2. Effects of the treatments on different crop parameters.

| Treatments | Weed density $m^{-2}$ | Fresh weed biomass (kg $ha^{-1}$) | Number of branches $plant^{-1}$ | Number of siliquae $plant^{-1}$ | Siliqua length (cm) |
|---|---|---|---|---|---|
| Dual Gold 960 EC (s-metolachlor) | 18.0 ab | 520.8 ab | 6.15 ab | 260 ab | 6.69 ab |
| Stomp 330 EC (pendimethalin) | 13.5 b | 257.9 b | 6.89 a | 301 a | 6.80 a |
| Treflan 4 EC (trifluralin) | 17.8 ab | 296.6 ab | 6.56 ab | 272 ab | 6.77 ab |
| Puma super 75 EW (fenoxaprop-P-ethyl) | 22.3 ab | 653.8 ab | 6.00 ab | 242 ab | 6.44 ab |
| Topik 15 WP (clodinafop) | 21.8 ab | 590.0 ab | 6.00 ab | 247 ab | 6.48 ab |
| Ronstar 12 L (oxadiazon) | 29.3 ab | 682.9 ab | 5.81 ab | 229 ab | 6.42 ab |
| Agil 100 EC (propaquizafop) | 26.5 ab | 654.6 ab | 5.88 ab | 239 ab | 6.43 ab |
| Hand Weeding | 19.0 ab | 540.4 ab | 6.00 ab | 251 ab | 6.62 ab |
| Weedy check | 38.0 a | 806.0 a | 5.69 b | 216 b | 6.30 b |
| LSD at 0.05α level | 21.23 | 522.8 | 1.2 | 85 | 0.52 |

Means followed by different letters in the respective column are significantly different at 5% probability level according to LSD test.

Number of seeds $siliqua^{-1}$

Number of seeds $siliqua^{-1}$ was not significantly affected by various herbicidal treatments. Table 3 shows that the highest number of seeds $siliqua^{-1}$ (25.75) was produced by the Stomp 330EC treatment, while the lowest number of seeds $siliqua^{-1}$ (21.63) was found in the weedy check. The highest number of seeds $siliqua^{-1}$ obtained from Stomp 330EC treatment was perhaps due to its best phytotoxic effect on weeds. On the other hand, the lowest number of seeds $siliqua^{-1}$ in the weedy check plots were probably due to the weed competition against the rapeseed plants which might have greatly reduced the flow of nutrients towards the seeds in the pods. These results are in line with those reported by Yadav et al. (1995) who stated that number of seeds $siliqua^{-1}$ increases with the application of some herbicides.

1000 seeds weight

Analysis of variance showed that herbicides had insignificant effect on 1000-seeds weight (Table 3). Plots treated with Stomp 330 EC gave the highest thousand seeds weight (3.99 g) while the weedy check plots exhibited the lowest thousand seeds weight (3.62 g). The reason for the lowest seed weight in the weedy check plots might be the competition between the weeds against the crop plants. These results are similar to those reported by Yadav et al. (1995) and Khan et al. (2003) who mentioned that seed weight increases with the use of chemical method of weed control.

Seed yield

Analysis of variance depicted that different herbicides had significant ($P<0.05$) effect on seed yield. Table 3 shows the effect of different herbicide treatments on the seed yield. The data indicated that Stomp 330EC treated plots produced the highest seed yield (1692 kg $ha^{-1}$) while the weedy check had the lowest seed yield (1119 kg $ha^{-1}$). However, Stomp 330EC was statistically similar to Treflan

4EC (1666 kg $ha^{-1}$) and statistically at par with the rest of the herbicidal treatments. The best performance of Stomp 330EC can be attributed to the best control of weeds due to weed competition reduction, enabling increased flow of nutrients towards the seeds. The results are supported by Khan et al. (2003) who stated that pre-emergence herbicides significantly increased the seed yield of rapeseed crop. Similar results were obtained by Singh et al. (2000), Yadav et. al. (1995) and Khan et al. (1995). Almost all of them stated that pre-emergence herbicides significantly increase the seed yield by efficiently reducing the weed population to avoid any weed competition in the early critical stages of the crop.

Table 3. Effects of the treatments on Cost-Benefit ratio and some parameters of the crop.

| Treatments | Plant height at maturity (cm) | Number of seeds $siliqua^{-1}$ | 1000 seeds weight (g) | Seed yield (kg $ha^{-1}$) | Cost Benefit Ratio |
|---|---|---|---|---|---|
| Dual Gold 960 EC (s-metolachlor) | 139.4 ab | 25.1 ab | 3.88 ab | 1550 ab | 5.02 |
| Stomp 330 EC (pendimethalin) | 137.9 ab | 25.8 a | 3.99 a | 1692 a | 7.47 |
| Treflan 4 EC (trifluralin) | 136.6 ab | 25.5 ab | 3.92 ab | 1666 a | 6.67 |
| Puma super 75 EW (fenoxaprop-P-ethyl) | 141.1 ab | 24.4 ab | 3.79 ab | 1396 ab | 4.45 |
| Topik 15 WP (clodinafop) | 140.9 ab | 23.4 ab | 3.68 ab | 1400 ab | 3.98 |
| Ronstar 12 L (oxadiazon) | 132.8 ab | 22.2 ab | 3.65 ab | 1275 ab | 1.86 |
| Agil 100 EC (propaquizafop) | 134.8 ab | 23.3 ab | 3.72 ab | 1380 ab | 1.83 |
| Hand Weeding | 127.2 b | 24.9 ab | 3.84 ab | 1535 ab | 1.31 |
| Weedy check | 143.4 a | 21.6 b | 3.62 b | 1119 b | -- |
| LSD at 0.05α level | 16.2 | 4.2 | 0.37 | 543 | -- |

Means followed by different letters in the respective column are significantly different at 5% probability level according to LSD test.

## Cost-Benefit ratio

The cost-benefit ratio was found significant in the different treatments (Table 3). Stomp 330 EC (1: 7.47) produced the highest cost-benefit cost-ratio followed by Treflan 4 EC treatments (1: 6.67). The lowest cost-benefit ratio was recorded in the hand weeding treatment (1: 1.31), which was due to the highest labor cost. These values indicated that all the herbicidal treatments gave optimum cost-benefit ratio as compared to the weedy check. The possible reason for the highest return of herbicides might be their cost and timely weed control as compared to other weeds control methods. Similar work has been done by Khan et al. (1995) and Singh et al. (2000).

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# Efficacy of different herbicides for controlling weeds in onion at higher altitudes

K. B. Marwat*, B. Gul and Z. Hussain
Department of Weed Science, NWFP Agricultural University,
Peshawar-25130, Pakistan
kbmarwat@psh.paknet.com.pk; kbmarwat@yahoo.com

**Abstract:** To study the efficacy of different herbicides for controlling weeds in onion (variety Swat-1), an experiment was conducted at Agriculture Research Station (North), Mingora during rabi 2003–04, using Randomized Complete Block (RCB) design with eight treatments and four replications. The treatments were seven herbicides including pendimethalin (1.32 kg $ha^{-1}$), trifluralin (1.2 kg $ha^{-1}$), s-metolachlor (1.92 kg $ha^{-1}$) used as pre-emergence, while the post-emergence herbicides were 2,4-D (1.13 kg $ha^{-1}$), bromoxynil + MCPA (1.3 kg $ha^{-1}$), clodinafop (0.05 kg $ha^{-1}$) and terbutryn + triasulfuron (0.3 kg a.i $ha^{-1}$) and a weedy check. The effect of all these herbicides were studied on weed kill percentage, fresh weed biomass (kg $ha^{-1}$), size of onion bulbs (ml), onion bulbs $m^{-2}$, plant height (cm), onion diameter (cm), onion yield (kg $ha^{-1}$), and cost-benefit ratio. The parameters significantly affected by the different herbicides were weed kill percentage, size of onion bulbs (ml), onion diameter (cm) and onion yield (kg $ha^{-1}$). The highest weed kill percentage (88.6 %), size of onion bulbs (78.25 ml), onion diameter (5.49 cm) and onion yield (29950 kg $ha^{-1}$) were recorded in pendimethalin as compared to weedy check (00.0 %), (47.75 ml), (4.06 cm) and (13700 kg $ha^{-1}$), respectively. The cost-benefit ratio was also highest (1:29.81) in pendimethalin followed by s-metolachlor (1:19.32), trifluralin (1:17.05) while the lowest was in terbutryn + triasulfuron (1:3.90). Pendimethalin performed well in the parameters that were not significantly affected by the different treatments. It is concluded that the performance of pendimethalin was the best among all the herbicidal treatments followed by s-metolachlor, therefore, pendimethalin is recommended at 1.32 kg a.i $ha^{-1}$ for significantly reducing the weeds population and enhancing the bulb yield in onions.

**Key words**: Herbicide, onion, and weed control.

## INTRODUCTION

Onion (*Allium cepa* L.) belonging to family *Alliaceae* is one of the important vegetable crops not only in Pakistan but also all over the world. It is one of the oldest vegetables mentioned in the Bible as well as in the Holy Quran. It is a condiment crop and consumed as fresh in salads or added in cooking dishes as spice. Apart from furnishing nutrition, it also provides relishing flavors to our diets. Research has suggested that onions in the diet may play a part in preventing heart diseases by reducing blood cholesterol level and triglycerides (Bakhsh and Khan 1990). Onion bulb is rich in phosphorus, calcium and carbohydrates. Onion has diuretic properties and is beneficial to the digestive tract. It is good for the eyes and acts as a heart stimulant and is useful as anti-rheumatic remedies (Shanmugavelu 1990).

Onion can be grown on all classes of soils, i. e., sandy loam to clay loams but clays need lightening with humus application. Onion plant is sensitive to high acidity and produce maximum yield over a fairly narrow range of soil reactions i. e. pH 5.8-6.5 on sandy loam soil. Good yields of onion are produced on muck soils (organic in nature, rich in nitrogen and have high water holding capacity) over a wide range of soil reactions than on mineral soils (Haliburton 1956). Onion is highly sensitive to temperature and photoperiod. Bulb formation is favored by relatively high temperature and longer photoperiod (Thompson and Kelley 1957).

In Pakistan onion was grown on area of 108 thousands hectares in 2002-2003, with a total production of 1.42 million tons at an average yield of 13.2 t ha$^{-1}$. In NWFP, the total production was 193,600 t with an average of 19.3 t ha$^{-1}$ in 10,000 ha (Anonymous, 2003).

The onion yield in Pakistan is lower than the potential yield of the cultivars. This gap could be attributed mainly to weed competition, because onion has poor canopy structure to compete with weeds. At young stage the onion leaves are very small and cannot shade the ground even in advanced stages of growth of the crop. The period from emergence to 4 weeks later is the most critical in the direct seeded onion (Ghafoor 2000; Shadbolt and Holm 1956). In transplanted onion the critical stage is from the time of transplantation up to nine weeks and yield reduction estimates ranged between 4.45 and 70.5% (Garcia et al. 1994).

In Pakistan, weeds are mostly managed manually costing about Rs. 1000 ha$^{-1}$. In contrast, hand weeding costs in USA have been reported to the tune of $9259 ha$^{-1}$, 5-7 times more expensive than using herbicides alone or in combinations (Bannaon *et al.* 1988). Good selective and economical weed control was obtained with the use of herbicides (Suso et al. 1993). Yield increased as in the herbicidal treatments, 8.89 ha$^{-1}$ to 37.92 t ha$^{-1}$, compared with the yield in mechanical cultivation + hoeing treatments 5.49 ha$^{-1}$ to 12.49 t ha$^{-1}$ (Halmagean et al. 1993). Srivastava et al. (1986) obtained significantly higher yield than the weedy check using herbicides and twice of the manual hand weeding in the onion crop.

Keeping in view the importance of different herbicides for controlling weeds in onion, the present experiment was carried out with the following objectives:

1. To evaluate different herbicides for controlling weeds in onion.
2. To figure out the effect of different herbicides on the yield of onion.
3. To find out the most effective and economical herbicide for weed control in onion under the agro-climatic condition of Swat
4. To quantify the phytotoxicity of herbicides if any on the crop.

## MATERIALS AND METHODS

In order to study the effects of different herbicides on weed control in onion, an experiment was conducted at Agriculture Research Station, Mingora. The variety Swat-1 was transplanted during rabi 2003–04. The experiment was laid out in randomized complete block (RCB) design with four replications. Each replication consisted of eight treatments. Each treatment consisted of 5 rows with row-row distance of 20 cm and plant-plant distance of 8 cm, respectively. The detail of the treatments during the study is shown in Table 1.

Table 1. Treatments used in the experiment.

| Treatment | Common name | Time of application | Rate (kg ai ha$^{-1}$) |
|---|---|---|---|
| Stomp 330 EC | pendimethalin | Pre-transplantation | 1.32 |
| Dual Gold 960 EC | s-metolachlor | Pre-transplantation | 1.92 |
| Treflan 4 EC | trifluralin | Pre-transplantation | 1.20 |
| 2,4-D 70 SL | 2,4-D | Post-transplantation | 1.13 |
| Buctril M 40 EC | bromoxynil + MCPA | Post-transplantation | 1.30 |
| Topik 15 WP | clodinafop | Post-transplantation | 0.05 |
| Logran Extra 64 WG | terbutryn + triasulfuron | Post-transplantation | 0.30 |
| Weedy check | --- | --- | --- |

Data were recorded on weed kill percentage, fresh weed biomass (kg $ha^{-1}$), onion bulbs $m^{-2}$, size of onion bulbs (water displaced) (ml), plant height (cm), onion diameter (cm), onion yield (kg $ha^{-1}$) and cost-benefit ratio (CBR).

The data recorded for each trait were individually subjected to the ANOVA Technique by using MSTATC computer software and means were separated by using Fisher's LSD test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

The data recorded on weed kill percentage, fresh weed biomass, onion count $m^{-2}$, plant height, size of onion bulbs, onion diameter, onion yield and cost-benefit ratio in onion variety Swat-1 at Agricultural Research Station, Mingora were statistically analyzed and the results are presented and discussed as under:

### Weed kill percentage

Statistical analysis showed a significant ($P<0.05$) effect of different herbicides on weed kill percentage (Table 2). The weed species infesting the experiment were *Echinochloa crusgalli*, *Paspalum* sp., *Digitaria sanguinalis*, *Chenopodium album*, *Coronopus didymus*, *Synapis arvense*, *Fumaria indica*, *Ranunculus* sp., *Polygonum* sp., *Setaria* sp., *Rumex* sp., *Poa annua*, *Sisymbrium irrio, Amaranthus viridis*, *Elusine indica*, *Cuscuta* sp., *Leptochloa* sp. and *Alternenthra* sp. The highest weed kill percentage (88.6 %) was recorded in plots treated with Stomp 330 EC as pre-emergence, followed by Dual Gold 960 EC (85.0 %), Treflan 4 EC (76.7 %) and Topik 15 WP (76.7 %). The lowest weed kill percentage (00.0 %) was recorded in weedy check plots, and Logran Extra 64 WG (19.4 %) treated plots (Table 2). The variability in weed kill percentage in different treatments can be attributed to the fact that pre-emergence herbicides are more effective for weed control than broad leaf killer herbicides, i.e., Logran Extra and Buctril M 40 EC. These results are similar to those reported by Orkwor et al. (1983) who stated that herbicides applied prior to transplanting gave excellent weed control for at least 12 weeks and resulted in maximum onion yield comparable with those applied with herbicides after transplanting.

### Fresh weeds biomass

The different herbicidal treatments had non-significant effect on weed biomass. Table 2 indicates that the lowest weed biomass (2666 kg $ha^{-1}$) was found in Stomp 330 EC treated plots. However, it was statistically at par with Dual Gold 960 EC (2916 kg $ha^{-1}$), Treflan 4 EC (3166 kg $ha^{-1}$), 2,4-D 70 SL (3874 kg $ha^{-1}$), Buctril M 40 EC (3791 kg $ha^{-1}$), Topik 15 WP (3691 kg $ha^{-1}$) and Logran Extra 64 WG (4208 kg $ha^{-1}$). This might be attributed to the fact that the data for fresh weeds biomass were taken at the end of the season, where almost all of the weeds were present. By this time the persistence/effect of pre-emergence herbicides has been minimized, while, the post-emergence herbicides were selective and only controlled either grassy or broadleaf weeds. As a result, the tolerant or resistant species flourished well. So at the end of the season maximum weeds were present in all treatments and were not significantly different from the weedy check in terms of weed biomass. These results are in line with those reported by Malik et al. (1981) and Sinha and Ratohore (1993).

### Onion bulbs

Onion bulb count was not significantly affected by the different herbicidal treatments (Table 2). As shown in Table 4, the highest bulb count was recorded in Stomp 330EC (49.97) treated plots, while the lowest (39.95) was observed in the weedy check. The variability can be attributed to the fact that although the presence of weeds in certain treatments affected the size of the bulb, it did not prevent the transplants to establish. Thus, approximately similar number of bulbs was obtained from the different treatments. These results agree with the findings of Sarivastava et al. (1986).

## Size of onion bulbs

The size of onion bulb was significantly ($P<0.05$) affected by the different herbicidal treatments (Table 2). The largest bulb size was recorded in Stomp 330EC (78.25 ml). However, it was statistically at par with Dual Gold (70.0 ml), Treflan 4 EC (67.50 ml) and Topik 15 WP (63.75 ml). The smallest bulb size (50.0 ml) was observed in Logran Extra and the weedy check (50.0 ml). The largest size in different treatments is due to the effectiveness of different herbicides, which controlled weeds and ultimately increased the nutrient availability for the crop. The treatments were kept weed-free for the maximum time and, hence, produced larger bulbs. These results are in line with the results reported by Keeling et al. (1990).

Table 2. Treatment effect on different parameters.

| Treatments | Weeds kill percentage | Fresh weeds biomass (kg ha$^{-1}$) | Onion count m$^{-2}$ | Size of onion bulbs (ml) |
|---|---|---|---|---|
| Stomp 330 EC (pendimethalin) | 88.60 a* | 2666 b | 49.97 | 78.25 a* |
| Dual Gold 960 EC (s-metolachlor) | 84.96 ab | 2916 b | 48.58 | 70.00 ab |
| Treflan 4 EC (trifloralin) | 76.69 ab | 3166 ab | 48.77 | 67.50 abc |
| 2,4-D 70 SL (2,4-D) | 67.22 bc | 3874 ab | 46.13 | 51.50 bc |
| Buctril M 40 EC (bromoxynil + MCPA) | 52.57 c | 3791 ab | 46.13 | 53.75 bc |
| Topik 15 WP (Clodinafop) | 76.68 ab | 3791 ab | 42.08 | 63.75 abc |
| Logran Extra 64 WG (terbutryn + triasulfuron) | 19.44 d | 4208 ab | 44.63 | 50.00 c |
| Weedy check | 00.00 e | 4875 a | 39.95 | 47.75 c |
| LSD value at 0.05$\alpha$ | 18.61 | 1740 | 10.39 | 19.79 |

*Means followed by different letters in the respective column are significantly different at 5% probability level according to LSD test.

## Plant height

The effect of different herbicides on plant height was non-significant. All the treatments were statistically similar to each other. This may be attributed to the fact that in case of weed-free conditions, the plants developed to full size without any stress conditions or competition with weeds for nutrients, space and light. In case of weed infestation, however, similar results have been reported by Markovic (1983) who stated that there was no significant effect of herbicides on plant height.

## Onion diameter

The effect of different herbicides on onion diameter was significant ($P<0.05$) as shown in Table 3. The largest onion diameter was recorded in the Stomp 330EC treated plots (5.24 cm), but it was statistically similar with Dual Gold 960 EC (5.085), Treflan 4 EC (4.965), Topik 15 WP (4.727) and Buctril M 40 EC (4.463). The smallest bulb diameter was noted in the weedy check (4.057 cm), followed by Logran Extra 64 WG (4.27). The possible reason for increase in onion diameter by Stomp 330EC, Dual Gold 960 EC and Treflan 4EC could be the best contról of weeds and consequently increased nutrient availability to the crop while the minimum bulb diameter in the weedy check was due to weed competition for nutrients, light, moisture and space. These results are in conformity with Gill et al. (1982) and Manjunath et al. (1989) who reported that weed infestations highly reduced crop vigor, leaf production, and bulb diameter and consequently bulb yield in onion crop.

## Onion yield

The different herbicides had significant effect ($P<0.05$) on onion yield (Table 3). Stomp 330EC treated plots (29950 kg ha$^{-1}$) and Dual Gold (29400 kg ha$^{-1}$) produced the highest yield, but were statistically at par with Treflan 4 EC (27620 kg ha$^{-1}$) and Topik 15 WP (24320 kg ha$^{-1}$), while the lowest bulb yield was recorded in the weedy check (13700 kg $ha^{-1}$) and Logran Extra 64 WG (16920 kg $ha^{-1}$). Pre-emergence herbicides as a whole produced better results. This could be due to the fact that pre-emergence herbicides were more effective than the post-emergence herbicides. Pre-emergence herbicides controlled the weeds throughout the critical stage of the onion, resulting in increased availability of nutrients to the crop. These results are in line with those reported by Singh et al. (1992) and Halmagean et al. (1993).

Cost-Benefit Ratio

Table 3 shows that the highest cost-benefit ratios were recorded in plots treated with Stomp 330 EC (1: 29.81), Dual Gold 960 EC (1: 19.32), and Treflan 4 EC treated plots (1: 17.05). The lowest cost-benefit ratio was recorded in Logran Extra 64 WG treated plots (1: 3.90) but it is still acceptable. These values indicated that all the herbicidal treatments gave optimum cost-benefit ratio as compared to the weedy check. The possible reason for the highest return of herbicides might be their cost and timely weed control as compared to other weed control methods. Similar results have been reported by Warade et al. (1995) and Saikia et al. (1997).

Table 3. Effect of the herbicide treatments on the different parameters.

| Treatments | Plant height (cm) | Onion diameter (cm) | Onion yield (kg $ha^{-1}$) | Cost-Benefit Ratio (CBR) |
|---|---|---|---|---|
| Stomp 330 EC (pendimethalin) | 59.7 | 5.49 a* | 29950 a* | 29.81 |
| Dual Gold 960 EC (s-metolachlor) | 56.1 | 5.08 ab | 29400 a | 19.32 |
| Treflan 4 EC (trifloralin) | 54.25 | 4.96 ab | 27620 ab | 17.05 |
| 2,4-D 70 SL (2,4-D) | 55.05 | 4.32 bc | 18700 cde | 8.88 |
| Buctril M 40 EC (bromoxynil + MCPA) | 55.8 | 4.46 abc | 21520 bcd | 7.67 |
| Topik 15 WP (Clodinafop) | 55.8 | 4.73 abc | 24320 abc | 9.32 |
| Logran Extra 64 WG (terbutryn + triasulfuron) | 54.85 | 4.28 bc | 16920 de | 3.9 |
| Weedy check | 59.65 | 4.06 c | 13700 e | ----- |
| LSD value at 0.05α | 8.171 | 0.891 | 6558 | |

*Means followed by different letters in the respective column are significantly different at 5% probability level according to LSD test.

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# Herbicidal bioefficacy, phytotoxicity and post-harvest shelf life in onion

T.K.Maity[1*], D.T.Bhutia[1], R.K.Ghosh[2] and J.Chakraborty[3]
[1]Department of Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India ,741252.
drtapanmaity@yahoo.co.uk/prof_tapanmaity@rediffmail.com
[2] Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India, 741252.
[3]Indofil Chemicals Company, Kolkata, West Bengal, India, 700017.

**Abstract:** Weed competition in onion is a global problem and losses due to weeds have been reported as high as 70-75%. The crop has very poor capability to compete with weeds due to its short stature, non-branching habit, sparse foliage, shallow root system, and slow initial growth. Use of liberal doses of fertilizers and more frequent irrigation favor more weed growth. Very close planting of the crop and its shallow root system also make mechanical weeding quite ineffective, seedlings sustain injury. The study was conducted at the University Research Farm, Kalyani, Nadia during 2002-2003. It aimed to evaluate the comparative bio-efficacy of different treatments on weeds with lower doses of herbicides and their effects on various physiological parameters, bulb yield and its post harvest shelf life. The treatments in the study were: Check/Control, Hand Weeding (HW), Weed Free, Oxyfluorfen (100g a.i.), Pendimethalin (750 g a.i.), Agil (100 g a.i.), Oxyfluorfen (100 g a.i + HW), Agil (100 g a.i. + HW), and Oxyfluorfen (100 g a.i. + Agil 100 g a.i.) The most commonly observed weeds were *Cynodon dactylon, Cyperus rotundus, Eleusine indica, Echinochloa colonum, E. crusgalli* (among narrow-leaved) and *Ageratum conyzoides, Physalis minima, Portulaca oleracea* and *Euphorbia hirta* (among broad-leaved). Oxyfluorfen 100 g a.i. + HW treatment significantly reduced the weed population and biomass at harvest (33.33 $m^{-2}$ & 11.39 g $m^{-2}$) over the control of (130.00 $m^{-2}$ & 33.36 g $m^{-2}$). The highest bulb yield was obtained with weed free treatment (11.91t $ha^{-1}$) followed by Oxyfluorfen 100 g a.i. + HW (10.77 t $ha^{-1}$), which was *at par* with hand weeded treatment (10.47 t $ha^{-1}$). The season long, crop weed-competition (control) reduced bulb yield by 37.61%. The post-harvest shelf life of the bulbs revealed no sign of sprouting and rotting after three months of storage. The weight loss of the bulbs was recorded maximum in weed-free treatment followed by Pendimethalin and Oxyfluorfen + HW for the same period.

**Keywords:** Bioefficacy, biomass, herbicide, phytotoxicity, post harvest shelf life, weed population

## INTRODUCTION

Onion is by far the most important vegetable crop grown in India and is consumed by common masses all year round. The increasing demand of onion for domestic and export markets have encouraged farmers to grow the crop on larger scale. However, its production and productivity in India are very low compared to many other countries. Farmers are not aware of improved varieties, suitable production technologies, pest management and post harvest management. Among the management problems, weeds pose a serious threat to onion cultivation. Onion has very poor capability to compete with weeds due to its short stature, non-branching habit, sparse foliage shallow, root system, and slow growth in the initial growth stage. Moreover, use of liberal doses of fertilizers and more frequent irrigations favour more weed growth. Very close planting of the crop and its shallow root system also make mechanical weeding quite ineffective, as seedlings sustain injury. Crop growth can also be suppressed even if the weeds are present for only two weeks. Thus, this investigation was undertaken to the study the effect of herbicides integrated with manual weeding on onion.

## MATERIALS AND METHODS

The experiment was conducted at University Research Farm, Kalyani of Bidhan Chandra Krishi Viswavidyalaya from December 2002 to April 2003 in a sandy loam soil in randomized block design with three replications. The treatment combinations were weedy check/control, hand weeding (HW) at 25 and 45 days after planting (DAT), weed free, Oxyfluorfen (100 g a.i. $ha^{-1}$) as post emergence (POE) at 25 DAT, Pendimethalin (750 g a.i. $ha^{-1}$) as pre-planting (PP) at 2 days before transplanting (DBT), Agil (100 g a.i. $ha^{-}$) as pre-emergence(PE) at 2 DAT, Oxyfluorfen + HW at 45 DAT, Agil + HW at 25 DAT and Oxyfluorfen + Agil. Seedlings of onion cv. Sukhsagar, a popular cultivar in this agro-climatic zone, were transplanted on 23rd December, 2002 in a plot size of 4 m × 2.2 m each with its normal package of practices. The recommended fertilizer dose was 125:60:100 kg of NPK $ha^{-1}$. The crop was harvested on 20th April, 2003. Observations were made on types of weed flora, weed populations and dry matter taken on 50 and 75 DAT and harvesting, weed control efficiency (WEC), weed index (WI), yield attributes and yield as well as post-harvest shelf life of the bulbs of onion.

## RESULTS AND DISCUSSION

The most commonly noticed weeds were *Ageratum conyzoides* L., *Amaranthus viridis* L., *Argemone mexicana* L., *Portulaca oleracea* L., *Euphorbia hirta* L., and *Physalis minima* L. (among broad-leaved weeds) and *Cyperus rotundus* L., *Digitaria sanguinalis* (L.) Scop. *Echinochloa crusgali* (L.) Beauv. *E. colonum* L., and *Eleusine indica* (L.) Gaertn. (among narrow-leaved weeds). The total weed population kept on increasing as the crop matured (Table 1). The best result in relation to lowest number of weeds (33.33 nos.) was recorded with oxyfluorfen + HW at 45 DAT treatment followed by HW at 25 and 45 DAT (35.33). Reduced dry matter accumulation by weeds was observed in different weed control treatments. Oxyfluorfen + HW at 45 DAT gave the lowest dry weight at all stages of observation followed by twice HW at 25 and 45 DAT. Among the three sole herbicides applied in this experiment, Pendimethalin showed its superiority in these respects. Lower weed population and dry weight in the integrated treatment of oxyfluorfen + (HW) at 45 DAT may be attributed to initial suppression of weed growth which resulted in less crop-weed competition as reflected in lower weed population, dry weight and weed control efficiency. Similar results have been reported by Singh et al. (1986) and Nadagouda et al. (1996). The ability of Pendimethalin to suppress the growth of all types of weed flora is due to its higher persistence in the soil other sole herbicides applied in this study. This result is in conformity with the findings of Al-Kothayari and Hassan (1999).

The weed-free treatment exhibited maximum values of all yield attributes indicating its superiority over other treatments (Table 2). Higher values for length, diameter and weight of bulb in this treatment may be attributed to the season-long weed-free situation resulting in better availability of light, nutrients, moisture and space for the growth of the crop. Pendimethalin was found more effective than Oxyfluorfen, Agil or the combination of both because it was able to control all types of weed flora more efficiently than the other two herbicides. The treatment oxyfluorfen + (HW) at 45 DAT recorded the next best results in these relation which is mainly because of suppression of initial weed growth, leading to less crop–weed competition. This treatment was followed closely by HW at 25 and 45DAT. All these attributes in the control had lower values due to highest weed competition, resulting in unfavourable growing condition for the crop. Similar to yield attributes, yield was influenced significantly by different treatments. Highest bulb yield (11.91t $ha^{-1}$) was recorded in the weed-free treatment followed by oxyfluorfen + HW at 45 DAT (10.77 t $ha^{-1}$) and HW at 25 and 45 DAT (10.47 t $ha^{-1}$). The weed-free treatment the highest yield mainly because of utilization of resources in better way. Initial suppression of weed growth by oxyfluorfen + HW at 45 DAT and HW at 25 & 45 DAT was reflected in the increased yields in these treatments, which could be supported by weed index as well.

Table 1. Effect of different treatments on total population and dry weight of weeds and weed control efficiency (WCE).

| Treatment | Total population (nos. $m^{-2}$) | | | Dry weight ( $g\ m^{-2}$ ) | | | WCE (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50 DAT | 75 DAT | Harvest | 50 DAT | 75 DAT | Harvest | 50 DAT | 75 DAT | Harvest |
| Control | 96 | 118.66 | 130 | 29.8 | 56.04 | 66.36 | - | - | - |
| HW at 25 & 45 DAT | 11.33 | 28.66 | 35.33 | 0.19 | 6.76 | 12.16 | 99.36 | 88.14 | 81.67 |
| Weed Free | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - |
| Oxyfluorfen @100g a.i. $ha^{-1}$ | 50.66 | 66.66 | 86 | 16.2 | 34.47 | 42.43 | 45.63 | 39.56 | 36.6 |
| Pendimethalin @750 g a.i.$ha^{-1}$ | 20 | 31.33 | 44 | 4.55 | 10.75 | 15.91 | 84.73 | 81.15 | 76.02 |
| Agil @100g a.i. $ha^{-1}$ | 46.66 | 59.33 | 71.33 | 10.17 | 25.34 | 33.11 | 65.87 | 55.79 | 50.1 |
| Oxyfluorfen @100 g a.i.$ha^{-1}$ + HW at 45 DAT | 12.66 | 24 | 33.33 | 0.16 | 6.56 | 11.39 | 99.46 | 88.49 | 82.83 |
| Agil@100 g a.i. $ha^{-1}$ + HW at 25 DAT | 22.67 | 39.33 | 50.66 | 5.13 | 13.96 | 19.88 | 82.78 | 75.31 | 70.04 |
| Oxyfluorfen @100 g a.i. $ha^{-1}$ + Agil @100 g a.i. $ha^{-1}$ | 36 | 72 | 93.33 | 9.84 | 27.45 | 36.03 | 66.97 | 51.01 | 45.7 |
| S.Em. (±) | 2.194 | 2.539 | 2.285 | 0.669 | 0.782 | 0.985 | | | |
| C.D. at 5% | 6.579 | 7.611 | 6.851 | 2.004 | 2.346 | 2.954 | | | |
| CV | 11.555 | 8.993 | 6.548 | 13.702 | 6.72 | 6.471 | | | |

Table 2. Effect of different treatments on length, diameter and weight of bulb, total yield of bulb and weed index in onion.

| Treatment | Bulb | | | Bulb Yield (t $ha^{-1}$) | Weed Index (WI) (%) |
|---|---|---|---|---|---|
| | Length (cm) | Diameter (cm) | Weight (g) | | |
| Control | 30.3 | 28.98 | 21.3 | 7.43 | 37.165 |
| HW at 25 & 45 DAT | 36.98 | 36.78 | 30.03 | 10.47 | 12.09 |
| Weed Free | 38.96 | 39.25 | 34.5 | 11.91 | |
| Oxyfluorfen (100g a.i. $ha^{-1}$) | 33.11 | 31.81 | 23 | 8.19 | 31.234 |
| Pendimethalin (750 g a.i.$ha^{-1}$) | 35.81 | 35.66 | 28.16 | 9.73 | 18.304 |
| Agil (100g a.i. $ha^{-1}$) | 33.96 | 31.86 | 24.66 | 8.53 | 28.295 |
| Oxyfluorfen (100 g a.i.$ha^{-1}$) + HW at 45 DAT | 37.03 | 37.17 | 31.16 | 10.77 | 9.572 |
| Agil (100 g a.i. $ha^{-1)}$ + HW at 25 DAT | 35.16 | 33.22 | 27.9 | 9.64 | 19.059 |
| Oxyfluorfen @100 g a.i. $ha^{-1}$ + Agil @100 g a.i. $ha^{-1}$ | 33.56 | 31.6 | 23.83 | 8.24 | 30.814 |
| S.Em. (±) | 1.116 | 1.009 | 0.818 | 0.237 | |
| C.D. at 5% | 3.345 | 3.025 | 2.453 | 0.709 | |
| CV | 5.515 | 5.067 | 2.21 | 4.345 | |

Table 3. Effect on post harvest shelf life of bulb

| Treatment | Weight of Bulb (g) | | | Physiological weight loss (%) | |
|---|---|---|---|---|---|
| | Harvest | 2nd Month | 3rd Month | 2nd Month | 3rd Month |
| Control | 605 | 575 | 560 | 4.96 | 7.43 |
| HW at 25 & 45 DAT | 912 | 870 | 845 | 4.6 | 7.34 |
| Weed Free | 1065 | 1008 | 970 | 5.35 | 8.92 |
| Oxyfluorfen (100g a.i. $ha^{-1}$) | 720 | 683 | 665 | 5.13 | 7.63 |
| Pendimethalin (750 g a.i.$ha^{-1}$) | 855 | 812 | 780 | 5.02 | 8.77 |
| Agil (100g a.i. $ha^{-1}$) | 750 | 718 | 700 | 4.26 | 6.66 |
| Oxyfluorfen (100 g a.i.$ha^{-1}$) + HW at 45 DAT | 930 | 878 | 850 | 5.59 | 8.6 |
| Agil (100 g a.i. $ha^{-1)}$ + HW at 25 DAT | 807 | 772 | 755 | 4.33 | 6.44 |
| Oxyfluorfen @100 g a.i. $ha^{-1}$ + Agil @100 g a.i. $ha^{-1}$ | 706 | 670 | 645 | 5.09 | 8.64 |
| S.d. | 131.65 | 123.83 | 117.92 | | |
| Mean | 816.66 | 775.22 | 752.22 | 4.92 | 7.82 |

This result confirms the findings of Singh et al. (1998) and Ved Prakash et al. 2000). The effectiveness of pendimethalin may be attributed to higher persistence of the herbicide in the soil, thereby suppressing the weed flora for longer duration, resulting in less crop-weed competition. For this reason, higher bulb yield was obtained.

The data presented in Table 3 revealed that the weight of the bulbs considerably decreased during storing. There were also no signs of rotting and sprouting during storage for three months under ambient conditions. The average physiological weight loss in the second month 4.92%. Maximum weight losses were observed in oxyfluorfen + HW at 45 DAT (5.59%) followed by Weed Free treatment (5.35%). In the following month (3rd month), the average weight loss was 7.94% with the maximum weight loss in weed-free (8.92%) followed by pendimethalin (8.77%).

Physiological weight loss in onion was due to respiration of the bulbs. Although onion has a low respiration rate, maximum weight loss in the weed-free treatment was due to a faster rate of respiration than other treatments, as bigger bulb size had faster respiration rate.

It may be concluded that the tedious, cumbersome, costly and non-availability of labours at critical crop-weed competition stage in the hand weeding treatment may be replaced by Integrated Weed Management (IWM) system, by application of oxyfluorfen 100g a.i.$ha^{-1}$ as POE in the initial stage and one hand weeding at 45 DAT as it recorded statistically similar performance.

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# Behavior of oxadiazon in soil and its effect on turfgrass establishment, growth, and development

P. C. Bhowmik
Department of Plant, Soil, and Insect Sciences, University of Massachusetts - Amherst, MA 01003-7245
pbhowmik@psis.umass.edu

**Abstract:** Oxadiazon, 3-[2,4-dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2(3H)-one, is a herbicide with both pre- and post-emergence activity that is registered internationally for weed control in *Oryza sativa*, *Gossypium hirsutum*, *Arachis hypogaea*, vegetables, and tree fruits. It has also been registered in the United States for pre-emergence control of weeds in turfgrasses and ornamentals. Oxadiazon strongly adsorbs to the surface of soil organic matter, and is largely immobile, and remains in the top 5 cm of the soil profile. It is relatively stable in the soil and produces metabolites that do not accumulate. Degradation is primarily due to aerobic microbial activity, with degradation taking longer under extremely dry or flooded conditions. Up to 55% of soil applied oxadiazon can be strongly adsorbed and rendered biologically inactive within 20 weeks of application. Oxadiazon causes rapid necrosis and photo-bleaching of plant tissues. Visible plant symptoms are similar to those produced by p-nitro-substituted diphenyl ether herbicides. Germinating seedlings are affected as they emerge through the oxadiazon containing soil layer. Oxadiazon in the granular formulation is safely used for pre-emergence weed control on a wide variety of established warm and cool season turfgrasses. Injury that may occur is transitory in nature, consisting of leaf yellowing in cool season grasses and delayed green-up in warm season grasses. Oxadiazon does not affect turfgrass root, rhizome or stolon growth, and does not decrease sod strength, or affect the establishment of turfgrass from sod, plugs or sprigs. Germination of turfgrass seeds can be reduced when sufficient oxadiazon residues are present. In most cases establishment from seed can be made in oxadiazon treated soil, if seeding is delayed until 4 months after application.

**Key words**: Microbial degradation, photodecomposition, sod strength, soil adsorption, turfgrass establishment

## INTRODUCTION

Oxadiazon is a herbicide with both pre- and post-emergence activity. It is registered internationally for weed control in crops such as *Oryza sativa* L., *Gossypium hirsutum* L., *Arachis hypogaea* L., vegetables, and tree fruits (Anonymous 2002). Since 1978, oxadiazon (Chipco Ronstar) has been registered in the United States for pre-emergence control of weeds in turfgrass and ornamentals (Bingham et al. 1995). This document provides a comprehensive review of the behavior and persistence of oxadiazon in soil. It also highlights the effects of oxadiazon on turfgrass establishment, growth, and development.

## SOIL BEHAVIOR AND PERSISTENCE

### Photodecomposition

Photolysis is a recognized pathway for the decomposition of pesticides in the environment. Oxadiazon dissolved in water was found to degrade in the presence of natural daylight or artificial light produced by mercury vapor arc lighting, and had a half-life of approximately 25 minutes with nearly complete degradation occurring within 20 h of exposure (Laurent and Chabassol 1976). Artificial light degraded oxadiazon more rapidly than daylight but both light sources induced similar transformations of the parent compound. Ultra violet light was primarily responsible for photolysis. Oxadiazon does not undergo appreciable photodecomposition when applied to the soil (Laurent and Chabassol 1976). Under field conditions, 80% of oxadiazon can be photo-degraded within 8 months of application but under turfgrass conditions, degradation due to photolysis may be significantly slower since most of the oxadiazon is bound up in the soil and thatch, and is not exposed to light. Thatch layer consists of undecomposed or partially decomposed layer of organic matter situated above the soil surface.

Adsorption characteristics

Soil adsorption of oxadiazon is due primarily to its hydrophobic association with the soil organic matter surface (Carringer et al. 1975). Compounds of low water solubility such as oxadiazon (0.7 ppm) are preferentially adsorbed to hydrophobic components of organic matter (polymers comprised of certain alcohols, proteins, carboxyls, carbohydrates, and saturated and unsaturated cyclic and heterocyclic ring structures) and removed from solution. Degree of adsorption is directly related to soil organic matter content (Ambrosi et al. 1977). Oxadiazon is not adsorbed to the soil clay fraction, and adsorption is not affected by the cation exchange capacity of the soil.

Oxadiazon is rapidly adsorbed in the soil with significant adsorption occurring during the first half hour after application (Amrosi and Desmoras 1973). Initial adsorption is not affected by soil type, but only continues in soils with organic matter contents greater than 1% (Ambrosi et al. 1977). In high organic matter soils, adsorption levels may not reach a dynamic equilibrium with soil organic matter for several weeks. The majority of the oxadiazon is adsorbed to the fulvic acid fraction, with lesser amounts being adsorbed to the humic acid and humin fractions.

Soil adsorption plays a critical role in the herbicidal activity of oxadiazon. The proportion of oxadiazon soil residues detected by bio-assay compared to the residues detected by gas chromatograph (GC) analysis declines from an average of 100% immediately after application to 55% at 20 weeks post application (Barrett and Lavy 1984). Since greater than 95% of oxadiazon in the soil can be extracted and identified by GC analysis (Guardigli 1987), adsorption and deactivation must be considered when determining the biological availability of oxadiazon in a soil. A large reservoir of biologically inactive oxadiazon may remain in a soil long after herbicidal activity is lost.

Leaching characteristics

Oxadiazon is virtually immobile in soil. Guardigli (1971) found oxadiazon to be strongly adsorbed in the first 5 cm of soils with an acidic pH and would not leach even after exposure to 63.5 ha-cm of simulated rainfall. In soils with pH values greater than 7, leaching of oxadiazon down to 15 cm would occur to a limited extent with 63.5 cm of rainfall.

In a leaching study, radio-labeled oxadiazon was applied to the surface of 4 different soils (loamy sand, sandy loam, silt loam, and clay loam). Water was percolated through the soil columns, and 95% of the applied herbicide was recovered in the first 5 cm of the soil profile (Ambrosi and Desmoras 1976). In another study conducted by Ambrosi and Helling (1977), oxadiazon was applied to 5 soils (ranging in pH from 5.1 to 7.7 and organic matter content from 0.14% to 4.68%) that were aged for 6 months under aerobic conditions, and then leached with water. TLC auto-radiography showed oxadiazon to be largely immobile with only trace amounts discovered in the soil profile.

A long-term field study was conducted by Chemlawn Inc. to investigate the leaching potential and degradation of oxadiazon (Guyton et al. 1985). Oxadiazon was applied to a *Festuca arundinacea*

Schreb. turfgrass growing on a sandy loam soil treated with oxadiazon at rates of 4.5 to 5.6 kg $ha^{-1}$ on an annual basis from 1977 to 1984. Greater than 90% of the oxadiazon residue remained in the top 2.5 cm of the soil. Only when 5.5 kg $ha^{-1}$ of oxadiazon was applied for 8 consecutive years, residues were found to a depth of 15 cm and this fraction comprised only 3% of the total oxadiazon residue.

Metabolism and degradation in soil

Oxadiazon is stable in soil and produces metabolites that do not accumulate. Stability is much greater in flooded and sterile soils, which indicates that degradation is primarily due to aerobic microbial activity (Ambrosi and Desmoras 1976; Ambrosi and Hellig 1977). Bacterial species believed to decompose oxadiazon include: *Alcaligenes eutrophus*, *Psuedomonas putida*, *Rhodococcus rubropertinctus*, and *R. rhodochrous* (Finkelshtein et al. 1984). Oxadiazon does not inhibit growth of other soil bacteria. Fungi and actinomycetes do not appear to use oxadiazon as a metabolic substrate.

Major metabolites of oxadiazon include a carboxylic acid, a phenol, and a dealkylated derivative and are not believed to be herbicidally active (Ambrosi et al. 1977). Due to oxadiazon's low water solubility, degradation products occur at very low concentrations with none exceeding 0.06 ppm. The percentage of metabolites never exceeds 10% of the parent oxadiazon fraction in the soil.

Soil dissipation of oxadiazon has been investigated in many soil types. Ambrosi and Desmoras (1973) reported half-lives of 150 days in the laboratory and 180 days in field trials in a silt loam soil. Barrett and Lavy (1984) found 59% of applied oxadiazon remaining after 20 weeks in a Crowley Silt Loam (Typic Albaqualfs). Finkelshtein et al (1984) reported that oxadiazon degraded more slowly in a podzolic soil when flooded than when held at field moisture capacity. After one year of incubation 27% of the applied oxadiazon remained in the moist soil, while 57% was recovered from the flooded soil. Average half-life of oxadiazon is documented as six months by Prost (1985). Chakraborty et al (1999) reported that dissipation of oxadiazon in Kalyani alluvial soil in India followed first-order kinetics and $DT_{50}$ values ranged from 44 to 45 days.

## EFFECTS ON TURFGRASS ESTABLISHMENT, GROWTH AND DEVELOPMENT

Mode of action

Oxadiazon is absorbed through the aerial parts (shoots and leaves) and is minimally absorbed by roots either with pre- or post-emergence application (Petrinko 1971). Plant tolerance to foliar application of oxadiazon is related to the rate of herbicide absorption through the leaf tissue (Archireddy et al. 1984). Leaf-absorbed oxadiazon is translocated mainly to the shoot, with minimal translocation to the root. If oxadiazon is absorbed by roots, it does not translocate throughout the plant (Ambrosi and Desmoras 1973; Bingham et al. 1980).

Oxadiazon causes rapid necrosis and photo-bleaching of plant tissues (Duke et al. 1989). Visible effects are much like those caused by p-nitro-substituted diphenyl ether herbicides such as aciflourfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid. Light is essential for its herbicidal activity, with damage being characterized by rapid light dependent membrane disruption that can be detected within one hour of application (Ambrosi and Desmoras 1973; Duke et al. 1989; Nissan 1975). The first ultrastructural damage evident is breakage of the tonoplast and plasmalemma. Duke et al. (1989) postulates that oxadiazon inhibits conversion of photophyrin IX (a photodynamic dye capable of herbicidal activity) to Mg-photophyrin, causing rapid membrane peroxidation. Temperature has little effect on herbicidal activity. Porphyrin synthesis inhibitors such as gabaculine and dioxyheptanoic acid almost completely prevent the herbicidal activity of oxadiazon.

Effects on shoot growth

Oxadiazon injury to young grass leaves appears initially as a bleaching of leaf tips followed by death of plant crowns (Hurto and Turgeon 1979). Damage to emerging shoots is greater when seedlings emerge through an oxadiazon containing thatch layer. Thatch layer consists of undecomposed or partially decomposed layer of organic matter situated above the soil surface. Thatch does not bind oxadiazon as tightly as soil, and more of the applied herbicide is available to the seedling.

Small seeded grass species are more sensitive to oxadiazon injury than large seeded grasses (Nissan 1975). Resistance decreases in the following order: *Zea mays* L., (threshold soil concentration 1 ppm) > *Triticum aestivum* L., *Sorghum bicolor* Moench., *Oryza sativa* (0.3 ppm) > *Dactylis glomerata* L., *Eragrostis curvula* L., *Phleum pratense* L., *Lolium multiflorum* Lam., *Festuca arundinacea*, *Digitaria ascendens* (H.B.K.)Henr., *Poa annua* L., (0.1 ppm) > *Echinochloa crus-galli* (L.) Beauv., and *Agrostis palustris* L..

Oxadiazon is safely used in the granular form on a wide variety of warm- and cool-season turfgrasses including: *Lolium perenne*, *Poa pratensis*, *Festuca arundinacea*, *Agrostis palustris*, *Cynodon dactylon* [L.] Pers., *Zoysia japonica* Steud., *Stenotaphrum secundatum* (Walt.) Kuntze.. Temporary injury that may occur consists of leaf yellowing in cool season grasses and a delay in green-up of warm season grasses (Johnson 1976; Johnson 1978; Johnson 1986). Johnson (1983) reported oxadiazon injury to 'Tifway' *C. dactylon* grown under putting green management. Turfgrass injuries included delayed spring green-up, turfgrass discoloration (reddening), and stand thinning. Root growth was not significantly affected.

In a putting green study, Bhowmik et al. (1995) found no significant differences in tiller number, clipping dry weight, root length or root weight between oxadiazon treatment and the untreated control plot, when turfgrass plugs taken 3, 6, and 9 weeks after treatment from the *A. palustris* putting green and grown for 8 weeks in sand culture pots in the greenhouse. Bentgrass grown under putting green management is more tolerant to oxadiazon than *C. dactylon* (Johnson 1986). *A. palustris* stands were not thinned, but temporary discoloration did occur. The injury was within acceptable limits.

Effects on root, stolon and rhizome growth

Oxadiazon has little effect on root growth in either warm or cool season turfgrass species. In a study investigating effects of pre-emergence herbicides on rooting of *Eremochloa ophiuroids* [munro] Hack., *Festuca arundinacea*, *Poa pratensis*, *C. dactylon*, and *Z. japonica*, Lewis et al. (1988) reported that Oxadiazon-treated sod had root strengths comparable to untreated sod. Bhowmik (1996) reported that root strength of one-year old *P. pratensis* sod-treated with oxadiazon at 4.5 kg $ha^{-1}$ was similar to that of untreated turfgrass. Bhowmik et al. (1995) also reported no injury or thinning of *A. palustris* after three consecutive yearly application of oxadiazon at 2.2 kg $ha^{-1}$ on a putting green. Similar results were reported for *C. dactylon* by Bignham and Hall 1985, Dernoeden et al (1988), and Johnson (1980); for *Poa pratensis* by Christians (1982), Jagschitz (1986a; 1986b), Bhowmik (1996), and Bhowmik et al. (1993a; 1993b); and for *Agrostis palustris* by Dernoeden et al. (1988) and Bhowmik et al. (1995).

Differences in responses of cultivars to oxadiazon have been reported for *P. pratensis* and *C. dactylon* (Johnson (1980) and Christians (1982). Christians (1982) found that 'Newport' and 'Baron'

*P. pratensis* cultivars' root and rhizome weights decreased by oxadiazon, while the cultivars 'Park' and 'Enmundi' were not injured. Root growth of 'Ormond' *C. dactylon* decreased by oxadiazon, while 'Tifway', 'Tifgreen', and 'Tifdwarf' were unaffected.

Effects on turfgrass establishment from seed

Effects of oxadiazon on seedling grasses are related to herbicide placement and concentration. When oxadiazon was mixed with the soil and placed at different depths (Barrett and Lavy 1984), greater injury occurred to *F. arundinacea* seedlings when the chemical was placed nearer the soil surface. Comparable injury was caused by oxadiazon applied either at 2.0 kg $ha^{-1}$ in the top 1.2 cm of soil or at 16 kg $ha^{-1}$ applied at a depth of 3.2 to 11.5 cm (application rate was calculated based on information provided in the materials and methods by Barrett and Lavy (1984).

Application of 4 kg $ha^{-1}$ in the 1.2 - 3.2 cm zone resulted in the same amount of injury as 16 kg $ha^{-1}$ applied throughout the soil to a depth of 11.5 cm. Analysis of the soil profile for oxadiazon at 45 days after application indicated minimal movement of oxadiazon below the level of application. Lower percentages of oxadiazon remained where applications were made to the upper portion of the soil profile.

Fall over-seeding of established cool season turfgrass is a standard cultural practice used to fill in bare areas and improve stand quality. Fall over-seeding of warm season grasses with *L. perenne* provides color during the dormant period of warm season grass. A considerable number of studies have been conducted investigating effects of spring application of preemergence herbicides on fall over-seeding of turfgrass.

Nash and Dernoeden (1982) showed that acceptable *L. perenne* cover could be obtained with seedings made 10 weeks following a 2.2 kg $ha^{-1}$ application of oxadiazon. At 4.5 kg $ha^{-1}$, acceptable *L. perenne* cover was obtained on seedings made at least 20 weeks after herbicide application. Verticutting prior to seeding at 5, 10, and 15 weeks following herbicide application enhanced *L. perenne* germination. Bingham and Schmidt (1983) found that oxadiazon at 2.2 and 3.4 kg $ha^{-1}$ could be applied 2 months prior to *L. perenne* seeding without affecting establishment. Bhowmik and Prostak (1998) reported excellent stand density of *L. perenne* from reseeding 4, 6 and 8 weeks after oxadiazon (2G) treatment. There were no differences in stands from 2.2 or 4.4 kg $ha^{-1}$ rates of oxadiazon. *L. perenne* stands were over 80% 4 WAT, and over 90% 6 and 8 WAT.

Other cool season grasses are less tolerant of oxadiazon residues than *L. perenne*. Relative tolerance of evaluated cool season turfgrasses to oxadiazon soil residues is: *L. perenne* > *Festuca rubra* > *Poa pratensis* > *Agrostis palustris* (Worrad 1988). *Agrostis palustris* is extremely sensitive to oxadiazon residues during establishment. When planted 5 months after oxadiazon application (3.0 kg $ha^{-1}$) *Agrostis palustris* cover was reduced by 28%. No establishment occurred for 4 months following a 6.0 kg $ha^{-1}$ application at 5 months after treatment cover was reduced by 67%.

In a study investigating the effect to herbicides on F. *rubra* and *P. pratensis* renovation seedings, Jagschitz (1986a and 1986b) reported that 3.4 kg $ha^{-1}$ of oxadiazon reduced germination of *Festuca rubra* when seeded less than 22 weeks after herbicide application. *P. pratensis* germination was reduced by 32% (compared to the untreated control) when seeded 22 weeks after oxadiazon application. Earlier plantings were more severely affected (84% reduction for the 15 week post application seeding).

Establishment from sprigs

*C. dactylon* is normally established from sprigs during an active growing period in spring and early summer. Annual grass weeds compete with *C. dactylon* sprigs and can reduce the rate of establishment. Weed control is critical during this period, and oxadiazon is commonly used during *C. dactylon* establishment.

In an evaluation of herbicides for use during 'Tifway' *C. dactylon* establishment, oxadiazon (2G) applied at 3.4 kg $ha^{-1}$ in a single application or 1.7 kg $ha^{-1}$ applied twice did not injure *C. dactylon* (Johnson (1975). Similar results were reported by Bingham and Hall (1985) when several herbicides were evaluated for use during establishment of 'Vamont', 'Midiron', and 'Tifway' *C. dactylon* cultivars. Oxadiazon (2G) at rates of up to 4.5 kg $ha^{-1}$ did not retard ground cover development of the three cultivars studied. Overall root growth and cold temperature tolerance of the cultivars were not affected by oxadiazon treatment.

Establishment from sod

Sodded turfgrass is normally established in whole area coverage or from plugs or strips, and control of annual weeds is critical in the development of a healthy, vigorous turf. Oxadiazon is commonly used in these establishment practices and has been shown to provide excellent weed control without causing turf injury (Bingham et al. 1995).

In a study evaluating effects of pre-emergence herbicides on four *C. dactylon* cultivars, Kenna and Dunfield (1986) found that 9.0 kg $ha^{-1}$ of oxadiazon did not adversely affect the percent field plug survival, percent ground cover, or root quality of 'Tifgreen', 'Sunturf', 'U-3', or common *C. dactylon* cultivars. Prodiamine, pendimethalin, and benefin all caused significant injury to the cultivars for parameters evaluated in the study. Oxadiazon at 3.4 kg $ha^{-1}$ did not adversely affect root weight, root length, percent cover, or rate of establishment of 'Belair' and 'Meyer' *Z. japonica* (Fry et al. 1986). The number of stolons and rate of stolon branching were greater in the oxadiazon treatments than the untreated control.

Oxadiazon is safe for use in establishment of *P. pratensis* and *Z. japonica* from plugs. With machine planting of 'A-20' *Poa pratensis* plugs the rate of establishment and the control of *P. annua* was the best in plots treated with oxadiazon (2.2 kg $ha^{-1}$) (Solon and Turgeon 1975). Bhowmik (1996) reported rooting strength of *P. pratensis* in a field study that common pre-emergence herbicides at recommended rate for 2 to 3 years did not cause root pruning or did not adversely affect the overall growth and quality of *P. pratensis*..

Oxadiazon has been shown to be safe for use prior to laying *P. pratensis* sod. Oxadiazon at rates of 3.4 to 4.6 kg $ha^{-1}$ applied between 1 and 18 weeks before sod laying did not effect the rooting, root strength, or turf vigor of *P. pratensis* (Jagchitz 1980; Jagchitz 1986b). Oxadiazon did not effect rooting of P. *pratensis* when it was applied to the sod 5 weeks prior to cutting (Shearman et al. 1979).

Oxadiazon can be safely used for pre-emergence weed control on established turfgrasses and during turfgrass establishment from sod, plugs, and sprigs. Oxadiazon does not affect turfgrass root, shoot, stolon, or rhizome growth and does not affect sod strength. Results of research over the past 25 years show that oxadiazon is largely immobile in soil. Downward movement of oxadiazon is related to its adsorption to fine clay and humic particles that travel downward via soil macropores. Dissipation of herbicidal activity is due to aerobic microbial degradation and strong soil adsorption.

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# Effects of plant height and water availability on response of Quilpie mesquite (*Prosopis velutina)* to a selection of herbicides applied by the basal stem method

E.C. Sparkes[1] and D.J. Midmore[2]
[1]Alan Fletcher Research Station, Department of Natural Resources and Mines
PO Box 36, Sherwood, Queensland 4075, Australia
[2]School of Biological and Environmental Sciences, Central Queensland University,
Rockhampton, Queensland 4702, Australia
earl.sparkes@nrm.qld.gov.au; d.midmore@cqu.edu.au

**Abstract**: Quilpie mesquite (*Prosopis velutina* Woot.) is an invasive, prickly woody weed that is believed to have been introduced into southwest Queensland in the 1930s. Although it produces palatable pods and provides shade for grazing stock, it develops dense thickets, reducing pasture growth. Field results using triclopyr-based formulations with basal stem application showed inconsistencies that appeared to be related to rainfall patterns. In March 1995, we tested the efficacy of selected herbicides using dieseline as the basal stem herbicide carrier. Herbicide treatments were set out adjacent to the south boundary of Comongin Station Quilpie, Queensland. Plants with root system in a predominately dry condition after spraying were more susceptible to the applied herbicides than plants with greater access to soil moisture, particularly within one month of herbicide application. At the second assessment in November 1995, a distinct pattern of plant degradation occurred because of herbicide-induced plant degeneration, which is linked to water availability. Herbicide efficacy showed significant responses to the effect of water availability ($P < 0.01$), with a significant interaction between water access and herbicide treatment ($P < 0.01$). High soil moisture on the day of application promotes herbicide efficacy, provided follow-up rain did not occur. The higher strength dicamba and triclopyr formulations showed the most effectiveness irrespective of plant height ($< 1.5$ m or $\geq 1.5$ m) or water availability. Smaller plants were more susceptible to applied herbicides. The recovery response of triclopyr + picloram treated plants when close to ponding depressions was possibly because of the buffering capacity provided by the larger biomass of mesquite bordering channels, enabling detoxification of the lower dose formulations.

**Key words**: Control, basal-stem, herbicide, *Prosopis*, mesquite, soil-moisture.

## INTRODUCTION

Quilpie mesquite (*Prosopis velutina*) is a persistent woody weed mostly restricted to Quilpie Shire in Queensland, but with extended ranges in New South Wales and Western Australia. The botanical features of mesquite favour survival in dry conditions. The *P. velutina* treated in this experiment ranged from plants 2-3 m tall.

The capacity of *P. velutina* to tolerate herbicides if plenty of water is available after herbicide application is linked to the topography and soil type in the target area. The channel country around Quilpie has sandy red clay loam, which is quickly waterlogged (National Wilderness Red Index 1994). So gradual are the gradients of the land there that floodwaters slow down, spread out, and break up into hundreds of small braided channels (Short and Blair 2000). When the channels cease to flow, many small ponds are left to sustain near plant life.

This experiment was conducted to find an alternative to the high cost of control afforded by triclopyr ester, and, *a posteriori*, to find out if soil water availability after herbicide application affects herbicide efficacy. Triclopyr ester is the main oil-based basal stem treatment for controlling Quilpie mesquite.

## MATERIALS AND METHODS

### Location and site

The trial occurred near Quilpie, Queensland (DNRM map ref: SG55-9 Quilpie [1975] BL 3869) on grazing land on the southern boundary fence line of the Comongin property.

Within the experimental area, Quilpie mesquite (*Prosopis velutina*) grew in semi-arid open grazing channelled clay loam verging on the flood plain adjacent to the Bulloo River (26.514797$^0$S; 144.34747$^0$E). The dominant canopy species were Coolabah trees (*Eucalyptus coolabah*. Blakely and Jacobs), Silver-leaf ironbark (*E. melanophloia* F. Muell), Beefwood (*Grevillea striata*, R. Br.), and Gidgee (*Acacia cambagei*, R.T. Baker) (nomenclature follows Anderson (1993)). Species to 4 m tall included Silver cassia (*Senna artemisioides* (D.C.) Randell), Budda (*Eremophila mitchellii* Benth.) and Gooramurra (*Eremophila bignoniiflora* Benth.) F. Muell) (Lazarides and Hince 1993).

### Design

The experiment incorporated three factors influencing *P. velutina* growth, namely herbicide treatments, water accessibility, and plant size. The herbicide treatments were set out in a balanced fully replicated trial, with randomised plots repeated in each of three blocking groups.

Because each plot was ~ 4 ha with up to 1000 plants within each datum area incorporating water holding channels, the further blocking factor, water availability was introduced to the experiment *a posteriori*. Environmental scientists adopt similar approaches where pollution studies have indicated the presence of the "key indicator" in an initially not noticed pattern (Thomson and Seber 1996). Water availability was coded as 1: dry soil profile (>5 m away from ponded areas or channels); 2: moderate amount of water being available to treated plants (>3 and ≤ 5 m away from ponded areas or channels) and 3: for plants subjected to inundation or ponding adjacent to root zones following rain events (≤ 3 m from a flood channel). Assessment occurred on at least 30 randomly selected plants from within each datum area within the selected plots.

Experimentalists applied the herbicides in March 1995 and assessed over 1-year only, because of a control strategy that involved further treatment immediately following the last assessment. Weather data showed above-average rainfall for 1995.

### Equipment

Croplands Swismex Lagus Jal hand-pumped knapsack sprayers of 8 litres capacity were used for this experiment. They were fitted with 1 m spraying wands and 2 mm adjustable nozzles. Sprayers were pressurised to 1000 kPa. For best results, the sprayer nozzle was adjusted to deliver a narrow, low-pressurised cone-shaped jet. The dieseline mixture was sprayed lightly but evenly on the stems up to 50 cm from the ground for small plants and up to 1 m above the branching point for older plants above three metres high. This was on some occasions ~ 2 m above-ground level but more commonly <1.5 m from the ground.

Heights of targeted mesquite plants were measured before treatment application using a Herga and Co clinometer - UK manufacture No. DMS-3. Plants < 1.5 m were considered as small and plants ≥ 1.5 m were considered large.

### Analysis

The data were analysed with Systat 9 software, using repeat measure multivariate analysis of variance (Wilkinson and Coward 1994) as outlined by Sparkes and Panetta (1997) with three factors influencing *P. velutina* growth, i.e., Treatment, plant size (Plants < 1.5 m or ≥ 1.5 m), and water availability. A graphical presentation was used to reflect differences between the treatments and size of the treated plants (Figure 1) and the affects of the habitat through water availability (Figure 1). Moderation of post-hoc growth affecting factor comparisons occurred using Tukey adjustment (Wilkinson and Coward 1994). Dunnett's test was used in a two-sided one-way test to decide whether the independent herbicide treatments were significantly different from the control.

## RESULTS AND DISCUSSION

All herbicide treatments were significantly different from the control, and access to water through the root zone had a significant effect on rating plant response to herbicide ($P<0.001$). The affect of the plant size on the overall response through the assessments was highly significant (P<0.001).

The interaction effect (Tabachnick and Fidell 2001) of water access*treatment was significant ($P<0.001$). However, judging by the size of the $F$ ratio, access to water was a significant factor in determining the treatment response. Where the root system remained in a predominately dry condition (Fig. 2 - low water access), plants were distinctly more susceptible to the applied herbicides. The interaction between herbicide treatment and plant size ($P<0.001$) was highly significant. The data shows the herbicide treatments were significantly more effective on smaller plants.

Treatment 1, the highest rate of dicamba amine, provided 100% kill in the dry soil, 60% kill in the marginally moist soil and 50% in wet soil. Comparatively, treatment 4, triclopyr ester, provided 54% kill in the dry soil, 57% kill in the marginally moist soil, and 40% in wet soil. Both treatments displayed the greatest margin in herbicide performance between efficacious treatments and treatments 5-15. Higher strength triclopyr ester treated plants growing in dry soil were often dead to the ground, but not the roots, compared with other treatments.

Of the treatments that incorporated dicamba or triclopyr alone (Treatments 1 and 4), the two higher strength formulations showed greater efficacy irrespective of plant size or water availability and did not differ significantly between themselves ($P = 0.95$).

However, lower strength mixtures of these herbicides often had poor efficacy (Figure 1 and 2). Treatments 6, 7 and 8 performed poorly on larger plants where water availability was high after herbicide application. Treatment 6, the lowest strength triclopyr butoxyethyl ester, was significantly less effective than treatment 7, the mixture of the strongest strength of triclopyr butoxyethyl ester + picloram isooctyl ester (P<0.01). The triclopyr butoxyethyl ester + picloram isooctyl ester was released commercially many years after the release of triclopyr ester of similar strength and with a higher relative cost. However, the detection of residual compounds like picloram may prevent successful application for graziers looking for certified organic beef registration.

Treatments 8 – 15 (Figure 1) showed comparatively poor efficacy. Treatments 8 and 9, medium and low concentration of triclopyr + picloram esters, targeted on *P. velutina* and growing under high-water availability, were less efficacious at the second assessment compared those growing in low water availability areas and subjected to the highest concentration of triclopyr + picloram ester (treatment 7) ($P< 0.01$). Thus the impact of the dosage response and amount of soil moisture following spraying, projects a response trend for this formulation (Figure 1). Where the root system remained in a predominately drier condition (Fig. 2 - low water access) plants were distinctively more susceptible to the applied herbicides. Treatments 12 to 15, various 2,4-D formulations, showed poor efficacy (Figure 1). Water accessibility is the "key indicator" that was observed to induce the pattern of reshooting of sprayed plants (Figure 1). The rainfall pattern for 1995 (Figure 1) provided conditions that were ideal for herbicide application with a dry March preceded by a supplementary rain in January and February. The unexpected high volume rain incidents in May caused flooding of channels and catalyzed the reshooting of mesquite treated with triclopyr-based products.

*P. glandulosa* with its root system in a predominately drier condition after application were more susceptible to the applied herbicides (Bovey 1998), within one-month post-herbicide application. High soil moisture on the day of application promotes herbicide efficacy ((Sparkes 2003). Herbicide treated plants, particularly those subjected to triclopyr + picloram ester that had continued access to water, were able to recover.

Table 1. Herbicide treatments applied using the basal stem application technique.

| Treatment | Herbicide | Product a.i. g $L^{-1}$ | Mls product 5 $L^{-1}$ | Spray solution a.i. g $L^{-1}$ |
|---|---|---|---|---|
| 1 | Dicamba® oil soluble amine | 400 | 125 * | 10 |
| 2 | Dicamba® oil soluble amine | 400 | 100 * | 8 |
| 3 | Dicamba® oil soluble amine | 400 | 75 | 6 |
| 4 | Garlon 600® | 600 | 83.33 * | 10 |
| 5 | Garlon 600® | 600 | 55.55 * | 6.67 |
| 6 | Garlon 600® | 600 | 41.66 | 5 |
| 7 | Access® (triclopyr ester) | 240 | 111 * | 5.34 |
| | (picloram ester) | 120 | | 2.67 |
| 8 | Access® (triclopyr ester) | 240 | 84 * | 4.03 |
| | (picloram ester) | 120 | | 2.02 |
| 9 | Access® (triclopyr ester) | 240 | 56 | 2.69 |
| | (picloram ester) | 120 | | 1.34 |
| 10 | Grazon® DS (triclopyr ester) | 300 | 166 | 9.96 |
| | (picloram amine) | 100 | | 3.32 |
| 11 | Grazon® DS (triclopyr ester) | 300 | 106 | 6.36 |
| | (picloram amine) | 100 | | 2.12 |
| 12 | Grazon® DS (triclopyr ester) | 300 | 84 | 5.04 |
| | (picloram amine) | 100 | | 1.68 |
| 13 | 2,4-D | 500 | 333 | 33.3 |
| 14 | 2,4-D | 500 | 212 | 21.2 |
| 15 | 2,4-D | 500 | 166 | 16.6 |
| 16 | Control | - | - | - |

* Common dilutions present in standard basal stem formulations with dieseline carrier.


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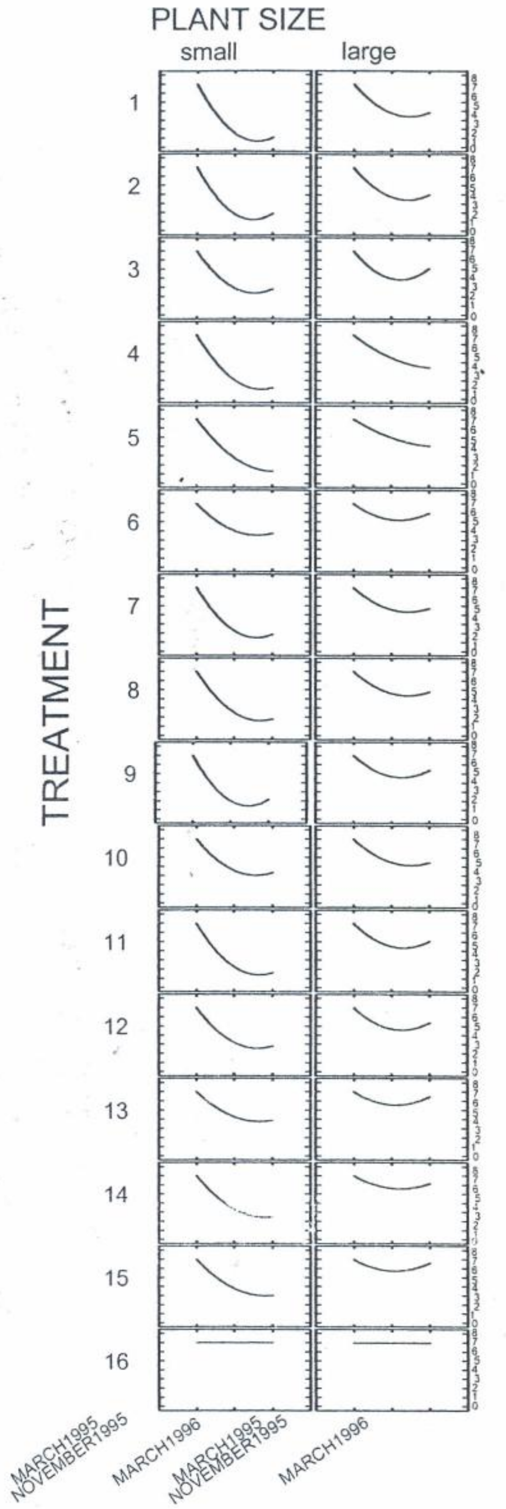


Figure 1 Herbicide treatment responses aligned with plant size. Size division small < 1.5 metres and large ≥ 1.5 m. Two assessments were carried out following a first assessment at herbicide application. Treatment effect ratings were rating 7 = no damage; rating 6 = leaves yellowing; 5 = leaf death; 4 = stem death from tip < 20cm.; 3 = stem death from tip >20 cm.; 2 = total stem death; 1 = death of plant.

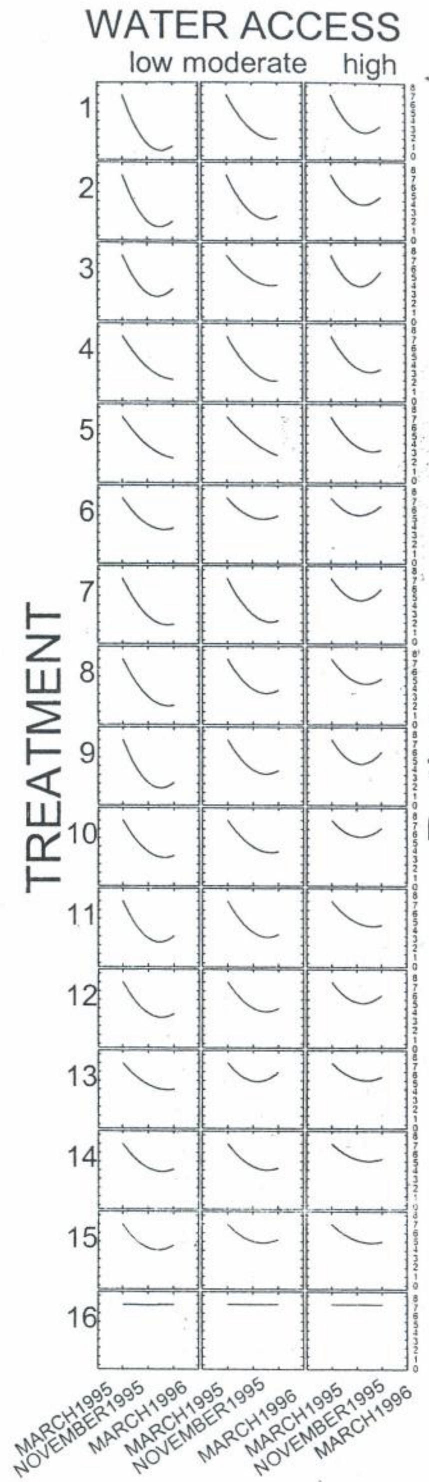


Figure 1 Effect of herbicide treatments. Assessments were made twice following application. Assessment times and response ratings are as for

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**Evaluation of newer molecules for chemical weed control in tea (*Camellia* spp. L.) plantations in South India**

Ch. Sreedhar[1], G. Shankar[2] and M.S. Mithyantha[3]
Rallis Research Centre, Rallis India Limited, 21 & 22, Peenya Industrial Area,
Phase II, Bangalore - 560 058, Karnataka, India
[1]chemuturi.sreedhar@rallis.co.in; [2]gshankar@rallis.co.in; [3]msmithyantha@rallis.co.in

**Abstract:** Among the agronomic practices that influence production, productivity and quality parameters of tea, weed management has attained significant importance in view of the near exclusive dependence on herbicides for chemical weed control. Experiments were conducted in the field during the years 2000-2001 and 2002-2003 at Koppa Estate, Karnataka, to evaluate the usefulness of glyphosate 41 SL at 720 g a.i. $ha^{-1}$ as tank mix either with metsulfuron-methyl 25 WP at 4 g a.i. $ha^{-1}$ or carfentrazone-ethyl 40 WP at 20, 25 and 30 g a.i. $ha^{-1}$ respectively. Paraquat dichloride 24 WSC was also evaluated at 360 and 540 g a.i. $ha^{-1}$ as tank mix individually with metsulfuron-methyl 25WP at 4 g a.i. $ha^{-1}$ and carfentrazone-ethyl 40WP at 20, 25 and 30 g a.i. $ha^{-1}$ respectively for broad spectrum control of weeds. Glyphosate 41SL as tank mix with metsulfuron-methyl 25WP at 720+4 g a.i. $ha^{-1}$ and glyphosate 41SL +carfentrazone-ethyl 40WP at 720+30 g a.i. $ha^{-1}$ gave effective control of resistant weeds like *Ageratum conyzoides, Cynodon dactylon, Crassocephalum crepidioides, Mitracarpus villosus, Spermacoce ocymoides and Spermacoce latifolia* compared to stand alone application of glyphosate 41SL at 720 g a.i. $ha^{-1}$. A progressive increase in per cent control of weeds and decrease in dry weight of weeds per unit area was noticed with increased dosage of carfentrazone-ethyl 40WP. Paraquat dichloride 24WSC in combination with Carfentrazone 40WP at 30 g a.i. $ha^{-1}$ gave effective control of these weeds than with metsulfuron methyl 25WP at 4 g a.i. $ha^{-1}$.

**Key words:** Additives, broad spectrum, tank mixtures, weed control, weed flora.

## INTRODUCTION

Tea (*Camellia spp.* L.) is grown in about 0.4 million ha in India. Tea in south India is located at altitudes ranging from 500 to 2200 m above the mean sea level in the hilly tracts of Western Ghats of India.

Tea is grown in a wide range of soil types that are chiefly acidic in reactions; tea soils are latosols in south India (Barua 1989). The tea growing districts of south India vary from each other in total rainfall, rainfall pattern, soil type, texture, altitude and climatic conditions. It is also well established fact that such conditions favour luxuriant growth of mixed population of weeds and even the age of tea fields from pruning has been found to influence weed flora (Devei et al. 1992; Roberts and Margaret 1980).

Weeds pose a serious problem in young tea plants in new clearings, pruned fields, mature tea fields and vacant patches in mature tea fields. The need for weed management persists till a complete ground cover is achieved by the spread of tea bushes (Sharma 1976).

Chemical weed control in tea has been found to be more efficient and cheaper than manual weeding (Onsando 1989; Rahman 1974; Rahman 1975). Both pre-emergence and post-emergence herbicides are being recommended for weed control in tea (Chakravartee 1994; Kotoky and Barbora 1993). The most popular recommendations that are being practiced in south India are tank mixtures of glyphosate, 2,4-D sodium salt and non-ionic wetting agent for control of mixed population of weeds, dominated by broadleaf weeds. Combination of glyphosate, kaolin along with the non-ionic wetting agent is recommended for mixed population of weeds predominated by grasses (Chakravartee and Barbora 1993; Satyanarayana et al. 1992; 1993; Sharma 1977; 1978; Sharma et al.1980).

However, there are reports that some of the broadleaf weeds have developed resistance to the above said combinations. In this context, molecules of newer chemistries with lower dosages have been evaluated as tank mix with glyphosate and paraquat dichloride respectively for achieving broader spectrum of weed control.

## MATERIALS AND METHODS

Experiments were carried out during the year 2000-2001 and 2002-2003 at Koppa Estate, Koppa, Karnataka, situated at an altitude of around 1100 m above mean sea level. Mature tea fields in their first year from pruning were selected for the study. There were 11 and 15 treatments and each treatment was replicated three times. The trials were laid out in randomized block design and the plot size was 10 m x 10 m. The treatments consisted of glyphosate 41 SL at 720 g a.i. $ha^{-1}$ as tank mix either with metsulfuron-methyl 25 WP at 4 g a.i. $ha^{-1}$ or carfentrazone-ethyl 40 WP at 20, 25 and 30 g a.i. $ha^{-1}$ respectively. Paraquat dichloride 24 WSC was also evaluated at 360 and 540 g a.i. $ha^{-1}$ as tank mix individually with metsulfuron-methyl 25 WP at 4 g a.i. $ha^{-1}$ and carfentrazone-ethyl 40 WP at 20, 25 and 30 g a.i. $ha^{-1}$, respectively, for the control of mixed population of weeds.

The standards for the purpose of comparison were stand alone application of glyphosate 41 SL at 720 g a.i. $ha^{-1}$, paraquat dichloride 24 WSC at 360 and 540 g a.i. $ha^{-1}$, metsulfuron-methyl 25 WP at 4 g a.i. $ha^{-1}$, carfentrazone-ethyl 25WP at 20, 25 and 30 g a.i. $ha^{-1}$, tank mixture of glyphosate 41 SL and 2,4-D sodium salt 80 WP at 720 +1120 g a.i. $ha^{-1}$ in combination with non-ionic wetting agent at 500 ml $ha^{-1}$ and the untreated control.

Herbicide treatments were applied as post-emergence blanket foliar spray directed towards the weeds at the four to five leaf stage. The treatments were imposed through a low pressure developing knapsack sprayer fitted with WFN 040 nozzle, at a pressure of 1.00 kg $cm^{-2}$. The volume of spray fluid used was equivalent to 450 L $ha^{-1}$.

The average values were transformed to arc sine values and were subjected to analysis of variance. Number of weeds occurring within 1.0 $m^2$ quadrat in each plot was recorded at random at 15, 30, 45 and 60 days after imposing the treatments. The data on number of weeds were subjected to square-root transformation using $\sqrt{n+1}$. Dry weight of weeds in each treatment was recorded after drying the weeds in an oven at $70^0$ C until constant weight was achieved.

## RESULTS AND DISCUSSION

Weed flora

At the time of imposing the treatments, all the plots were predominantly infested with broadleaf weeds such as: *Ageratum conyzoides, Bidens biternata, Conyza ambigua, C. leucantha, Crassocephalum crepidioides, Drymaria diandra, Emilia sonchifolia, Eupatorium odoratum, Mimosa pudica, Oxalis corniculata, Polygonum chinensis, Polygonum nepalense, Spermacoce ocymoides, Spermacoce latifolia, Ischaemum rugosum, Mitracarpus verticillatus, Scoparia dulcis* and the grasses such as *Cynodon dactylon, Digitaria adscendens, Paspalum conjugatum and Panicum repens.*

Weed Control

Biometric observations

The untreated control recorded the highest number of weeds per unit area and was significantly inferior to that of herbicide treated plots (Table 1). There was significant reduction in number of weeds per unit area in the treatments of stand alone application of carfentrazone-ethyl at 20, 25 and 30 g a.i. $ha^{-1}$, metsulfuron-methyl at 4 g a.i. $ha^{-1}$ and glyphosate at 720 g a.i. $ha^{-1}$, in that order (Table 1). Treatment of tank mixture of glyphosate and metsulfuron-methyl at 720+4 g a.i. $ha^{-1}$ recorded significantly lower number of weeds and was superior to that of the standard treatments at all the observations recorded. There was a significant reduction in number of weeds with increase in dosage of carfentrazone-ethyl at 20, 25 and 30 g a.i. $ha^{-1}$ as tank mix with glyphosate at 720 g a.i. $ha^{-1}$.

Table 1. Influence of treatments on weed density.

| Treatment | Dosage (g a.i. $ha^{-1}$) | Number of weeds $m^{-2}$ * 7DAT | 15DAT | 30DAT | 45DAT | 60DAT |
|---|---|---|---|---|---|---|
| Glyphosate 41SL + Metsulfuron-methyl 25WP | 720 + 4 | 7.8 (59.8) | 2.0 (3.0) | 1.4 (1.0) | 1.4 (1.0) | 3.6 (11.9) |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720+ 20 | 9.7 (93.0) | 5.7 (31.4) | 5.1 (25.0) | 6.0 (35.0) | 7.5 (55.2) |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720 + 25 | 9.8 (95.0) | 4.7 (21.0) | 4.0 (15.0) | 5.1 (25.0) | 6.7 (43.8) |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720 + 30 | 9.5 (89.2) | 3.6 (11.9) | 3.3 (9.8) | 4.0 (15.0) | 5.9 (33.8) |
| Glyphosate 41SL | 720 | 9.8 (95.0) | 7.8 (59.8) | 6.8 (45.2) | 7.3 (52.2) | 9.3 (85.4) |
| Carfentrazone-ethyl 40WP | 20 | 13.0 (168.0) | 13.0 (168.0) | 13.9 (192.2) | 14.0 (195.0) | 14.0 (195.0) |
| Carfentrazone-ethyl 40WP | 25 | 11.7 (135.8) | 11.0 (121.0) | 11.3 (126.6) | 11.8 (138.2) | 12.2 (147.8) |
| Carfentrazone-ethyl 40WP | 30 | 10.6 (111.3) | 9.6 (91.1) | 9.6 (91.1) | 10.5 (109.2) | 11.0 (120.0) |
| Metsulfuron-methyl 25WP | 4 | 10.2 (103.0) | 9.2 (83.6) | 9.0 (80.0) | 10.3 (105.0) | 10.6 (111.3) |
| Glyphosate41SL+2,4-D Sodium salt+NIWA | 720+1120+ 500 | 9.3 (85.4) | 3.3 (9.8) | 3.0 (8.0) | 3.7 (12.6) | 5.4 (28.1) |
| Untreated control | - | 14.1 (197.8) | 14.1 (197.8) | 14.3 (203.4) | 14.0 (195.0) | 14.0 (195.0) |
| SEm ± | | 0.3 | 0.1 | 0.2 | 0.4 | 0.3 |
| CD at 5% | | 1.1 | 0.6 | 0.7 | 0.6 | 0.5 |

* $\sqrt{n+1}$ transformed values; Mean of 2000-2001 and 2002-2003;
Figures in parenthesis indicate the absolute number of weeds .$m^{-2}$

Treatments of stand alone application of paraquat dichloride, metsulfuron-methyl, carfentrazone-ethyl recorded the highest number of weeds and were significantly superior to all the tank mixture treated plots (Table 2). Treatments of paraquat dichloride at 540 g a.i. $ha^{-1}$ in combination with carfentrazone recorded lowest number of weeds per unit area and were significantly superior to that of paraquat dichloride at 360 g a.i. $ha^{-1}$ (Table 2). These findings highlight that the concentration of paraquat dichloride in the tank mix is important in determining its bio-efficacy. Victor (2003) reported that straight application of carfentrazone-ethyl even at 20 g a.i. $ha^{-1}$ failed to contain broadleaf weeds. Thus, the results of the present study indicate that, the beneficial effect of the combination may be attributed to synergism between the two herbicides (Rao et al. 1977; Yang Zu Nang 1978).

Table 2. Influence of treatments on weed density.

| Treatment | Dosage (g a.i. $ha^{-1}$) | Number of weeds $m^{-2}$ * | | | |
|---|---|---|---|---|---|
| | | 7DAT | 15DAT | 30DAT | 45DAT |
| Paraquat dichloride 24WSC + Metsulfuron-methyl 25WP | 360 + 4 | 6.1 (36.2) | 6.2 (37.4) | 8.5 (71.2) | 11.3 (126.6) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 20 | 5.2 (26.0) | 5.3 (27.0) | 8.0 (63.0) | 10.3 (105.0) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 25 | 4.4 (18.3) | 4.4 (18.3) | 6.5 (41.2) | 9.2 (83.6) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 30 | 3.8 (13.4) | 3.7 (12.6) | 5.7 (31.4) | 8.1 (64.6) |
| Paraquat dichloride 24WSC | 360 | 8.2 (66.2) | 8.6 (72.9) | 9.3 (85.4) | 13.2 (173.2) |
| Paraquat dichloride 24WSC + Metsulfuron-methyl 25WP | 540 + 4 | 4.8 (22.0) | 4.8 (22.0) | 7.8 (59.8) | 10.2 (103.0) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 20 | 4.1 (15.8) | 4.0 (15.0) | 6.7 (43.8) | 8.8 (76.4) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 25 | 3.3 (9.8) | 3.0 (8.0) | 5.5 (29.2) | 7.5 (55.2) |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 30 | 2.4 (4.7) | 2.4 (4.7) | 4.5 (19.2) | 6.5 (41.2) |
| Paraquat dichloride 24WSC | 540 | 6.6 (42.5) | 6.0 (35.0) | 8.8 (76.4) | 11.3 (126.6) |
| Carfentrazone-ethyl 40WP | 20 | 13.6 (183.9) | 13.0 (168.0) | 14.3 (203.4) | 15.5 (239.2) |
| Carfentrazone-ethyl 40WP | 25 | 12.8 (162.8) | 12.0 (143.0) | 12.9 (165.4) | 13.5 (181.2) |
| Carfentrazone-ethyl 40WP | 30 | 10.9 (117.8) | 10.9 (117.8) | 11.1 (122.2) | 12.1 (145.4) |
| Metsulfuron-methyl 25WP | 4 | 11.1 (122.2) | 10.2 (103.0) | 10.0 (99.0) | 11.6 (133.5) |
| Untreated Control | - | 15.1 (227.0) | 16.0 (255.0) | 16.0 (255.0) | 15.8 (248.6) |
| SEm ± | | 0.1 | 0.1 | 0.1 | 0.3 |
| CD at 5% | | 0.6 | 0.5 | 04 .6 | 0.8 |

* $\sqrt{n+1}$ transformed values; Mean of 2000-2001 and 2002-2003

Figures in parenthesis indicate the absolute number of weeds $.m^{-2}$

Dry weight of weeds exhibited similar trend of results as that of number of weeds (Table 3 and 4).

Table 3. Influence of treatments on dry weight of weeds.

| Treatment | Dosage (g a.i. $ha^{-1}$) | Dry Weight of weeds (g $m^{-2}$)* | | | | |
|---|---|---|---|---|---|---|
| | | 7DAT | 15DAT | 30DAT | 45DAT | 60DAT |
| Glyphosate 41SL + Metsulfuron-methyl 25WP | 720 + 4 | 36.0 | 0.9 | 0.7 | 2.6 | 4.8 |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720+ 20 | 62.4 | 14.6 | 7.8 | 10.5 | 24.1 |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720 + 25 | 56.5 | 8.5 | 4.5 | 7.5 | 13.5 |
| Glyphosate 41SL + Carfentrazone-ethyl 40WP | 720 + 30 | 50.2 | 3.6 | 3.0 | 4.5 | 12.0 |
| Glyphosate 41SL | 720 | 80.4 | 41.9 | 33.8 | 35.6 | 46.5 |
| Carfentrazone-ethyl 40WP | 20 | 91.6 | 93.9 | 102.5 | 106.3 | 110.8 |
| Carfentrazone-ethyl 40WP | 25 | 89.5 | 80.6 | 90.7 | 94.2 | 96.4 |
| Carfentrazone-ethyl 40WP | 30 | 86.7 | 62.9 | 42.8 | 59.5 | 62.9 |
| Metsulfuron-methyl 25WP | 4 | 85.2 | 64.2 | 44.0 | 57.7 | 61.0 |
| Glyphosate41SL+2,4-D Sodium salt+NIWA | 720+1120+500 | 49.3 | 3.0 | 2.4 | 3.9 | 10.9 |
| Untreated control | - | 108.8 | 114.7 | 119.5 | 123.9 | 128.7 |
| SEm $\pm$ | | 1.0 | 0.8 | 0.2 | 0.3 | 0.6 |
| CD at 5% | | 3.2 | 2.4 | 0.6 | 0.9 | 2.0 |

* Mean of 2000-2001 and 2002-2003

Table 4. Influence of treatments on dry weight of weeds.

| Treatment | Dosage (g a.i. $ha^{-1}$) | Dry Weight of weeds (g $m^{-2}$)* | | | |
|---|---|---|---|---|---|
| | | 7DAT | 15DAT | 30DAT | 45DAT |
| Paraquat dichloride 24WSC + Metsulfuron-methyl 25WP | 360 + 4 | 16.5 | 17.2 | 39.0 | 56.9 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 20 | 12.1 | 12.1 | 28.3 | 47.0 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 25 | 8.4 | 8.2 | 18.5 | 38.5 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 360 + 30 | 6.0 | 5.6 | 14.1 | 30.2 |
| Paraquat dichloride 24WSC | 360 | 26.2 | 28.7 | 46.2 | 72.1 |
| Paraquat dichloride 24WSC + Metsulfuron-methyl 25WP | 540 + 4 | 9.9 | 9.9 | 29.1 | 40.6 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 20 | 7.0 | 6.7 | 19.7 | 35.1 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 25 | 4.4 | 3.8 | 13.1 | 25.3 |
| Paraquat dichloride 24WSC + Carfentrazone-ethyl 40WP | 540 + 30 | 2.1 | 1.9 | 8.6 | 17.2 |
| Paraquat dichloride 24WSC | 540 | 15.2 | 15.7 | 35.4 | 46.3 |
| Carfentrazone-ethyl 40WP | 20 | 103.1 | 110.6 | 125.9 | 130.6 |
| Carfentrazone-ethyl 40WP | 25 | 66.3 | 60.2 | 60.0 | 66.9 |
| Carfentrazone-ethyl 40WP | 30 | 60.9 | 56.7 | 51.0 | 62.9 |
| Metsulfuron-methyl 25WP | 4 | 61.5 | 57.9 | 51.3 | 63.4 |
| Untreated Control | - | 125.7 | 143.6 | 150.8 | 149.9 |
| SEm $\pm$ | | 0.3 | 0.5 | 0.6 | 1.1 |
| CD at 5% | | 1.2 | 1.5 | 1.9 | 3.2 |

* Mean of 2000-2001 and 2002-2003

Based on the results of the study it is concluded that tank mixtures of glyphosate at 720 g a.i. ha-1 in combination with metsulfuron-methyl at 4 g a.i. ha-1 gave effective control of broad spectrum of weeds in tea plantations. Tank mixture of paraquat dichloride at 540 g a.i. ha-1 with carfentrazone-ethyl at 30 g a.i. ha-1 also gave effective control of weeds.

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# Bio-efficacy of XL 20AG in managing weed flora in tea plantation

S. Maiti, A.Khuntia, Pritam Ghosh, Subrata Ghosh and R.K.Ghosh
Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252 (Nadia), India; rkgbckv@rediffmail.com

**Abstract:** Tea crop plays an vital role towards the Indian economy though its cultivation is restricted in geographical distribution to the tropics between $20^0$N and $20^0$S latitudes. Climate has a profound effect on the persistence of weeds and this competes more in the perennial crop tea at its various stages of crop growth. Weeds affect most of the matured tea plantation more as high nutrient encourages weed growth. Therefore weed management is needed at regular intervals in tea plantation. *Digitaria, Eleusine, Cynodon, Paspalum* and *Echinochloa* among grassy weeds and *Cyperus* and *Kllyinga,* in sedges where as *Scoparia, Borreria, Ludwigia, Oxalis, Commelina* and *Ageratum* among broadleaves are dominant weed flora in the northern tea plantation of West Bengal. Considering the economics an experiment was conducted to manage these weeds during 2002 at the Kamalpur tea Estate of North West Bengal, India with a new formulation of Glyphosate. Results revealed that XL20AG applied @ 60 or 40 ml litre$^{-1}$ of water showed full control of all types of weed flora within three to four weeks after application. The effectively of XL20AG was better than Glyphosate 41% SL applied @ 10 ml litre$^{-1}$. This chemical was checked the resurgence of tubers, stolons or rhizomes of some weeds. There was no phytotoxicity of this chemical to tea plant.

**Key words**: Tea, weed management, herbicide, new formulation.

## INTRODUCTION

Tea, an important plantation crop of India, plays a key role in national economy. Soil and climate has a profound influence proliferation of weeds. The management practice and climatic condition in tea growing areas of West Bengal often create the condition, favourable for growth of the weeds. Weeds cause yield reduction in Tea to the tune of 15-40 % in this region (Samanta and Roy, 2005). Hence, effective control of weeds during the critical period of crop-weed competition results poor growth of weeds and good stands of tea. Thus, for effective and economic weed management, tea needs suitable and effective herbicides to keep the ground weed-free during the critical period. Keeping the above view, a field experiment was conducted to study the efficacy of XL 20 AG on weed control and its phytotoxicity, if any, on tea.

## MATERIALS AND METHODS

The field experiment was conducted at Section 10 of Kamalpur Tea Estate, Darjeeling, West Bengal at $89^0$E longitude and $27^0$N latitude during summer 2002. The experimental soil was clay loam in texture, acidic in nature, high in organic matter content with a pH of 6.1.Temperature begins to rise from May and reaches maximum in July. It starts dropping from middle of October onwards and becomes minimum in January. The average rainfall is 3000 mm per annum of which around 75 % rainfall occurs during June to September. The treatments consisted of XL 20 AG at five different doses (15,20,30,40 and 60ml l$^{-1}$water), Glyphosate 10 ml l$^{-1}$ and untreated control. One metre strip between two tea row was used as plot with a length of hundred metre. Required quantities of herbicides were sprayed with a backpack sprayer with a nozzle WFN 0.040 in a spray volume of 450 lit ha$^{-1}$. The weed samples were collected randomly from each treatment for taking their dry weight by using a quadrate of 1mx1m. from the population of weed samples were used to calculate percentage weed control (population and dry weight basis) as compared to untreated one at 5, 15, 45 and 60 days after spraying (DAS) of the herbicides.

## RESULTS AND DISCUSSION

The predominant weed flora present in the experimental field were *Digitaria sanguinalis, Eleusine indica, Cynodon dactylon, Paspalum scrobiculatum* and *Echinochloa colonum* among grasses and *Cyperus rotundus* and *Kyllinga* sp. in sedges whereas *Scoparia dulsis, Borreria hispida, Ludwigia octovalvis, Oxalis corniculata, Commelina benghalensis* and *Ageratum conyzoides.*

At 5 DAS, all the weeds showed toxicity to weeds. XL 20AG at 60, 40 and 30 ml $l^{-1}$ water and glyphosate41% SL greatly reduced the weed population upto 45 DAS (Table 1). However, some regrowth of weeds took place at 60DAS. In contrast, in the lowest two doses of XL 20AG at 15 and 20 ml $l^{-1}$ water, regrowth took place 15 days earlier as compared to other herbicides used. Table II that XL 20 AG at its all doses and Glyphosate 10 ml $l^{-1}$ drastically reduced the dry weight of weeds at 45 DAS. Among the herbicides, XL 20 AG 60 ml $l^{-1}$ water provided maximum weed control of 98.4% by reducing the dry weight from 24.7 to 0.4 $gm^{-2}$ closely followed by XL 20 AG 40 ml $l^{-1}$ water and Glyphosate. On the contrary, dry weight in untreated control increased from initial 24.6 to 31.8 $gm^{-2}$. However, XL 20 AG provided 95.0 to 98.4% reduction in weed dry weight at 45 DAS depending on the dose. The higher was the dose, the greater was the control of weeds in tea plantation.

Table 1. Effect of treatments on percentage weed control (population basis)

| Treatment | Dose (ml $l^{-1}$water | Percentage weed control (population basis) Days after spraying | | | |
|---|---|---|---|---|---|
| | | 5 | 15 | 45 | 60 |
| XL 20AG | 60 | 93 | 95 | 96 | 92 |
| XL 20AG | 40 | 86 | 94 | 95 | 86 |
| XL 20AG | 30 | 83 | 89 | 93 | 83 |
| XL 20AG | 20 | 79 | 88 | 86 | 81 |
| XL 20AG | 15 | 77 | 86 | 83 | 77 |
| GLY 41% SL | 10 | 89 | 94 | 95 | 88 |
| Weedy check | - | - | - | - | - |

GLY=Glyphosate

Table 2. Effect of treatments on weed dry weight and percentage weed control (dry weight basis) in tea

| Treatment | Dose (ml $l^{-1}$water) | Dry weight of weeds ($gm^{-2}$) | | Percentage weed control (Dry weight basis) |
|---|---|---|---|---|
| | | Initial | 45 Days after spraying | |
| XL 20AG | 60 | 24.7 | 0.4 | 98.4 |
| XL 20AG | 40 | 22.3 | 0.6 | 97.4 |
| XL 20AG | 30 | 24.2 | 0.9 | 96.4 |
| XL 20AG | 20 | 25.4 | 1.0 | 96.2 |
| XL 20AG | 15 | 23.0 | 1.2 | 95.0 |
| GLY 41% SL | 10 | 23.9 | 0.5 | 97.9 |
| Weedy check | - | 24.6 | 31.8 | - |

GLY=Glyphosate

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# Effects of different herbicides on weed control in coconut nurseries in the dry zone of Sri Lanka

S.H.S. Senarathne
Agronomy Division, Coconut Research Institute, Lunuwila, Sri Lanka
shsumith71@yahoo.com and agronomy@cri.lk

**Abstract:** The effect of four different weed management systems on the growth of coconut seedlings was evaluated to determine the most economical and effective method of controlling weeds in coconut nurseries in the low country dry zone of Sri Lanka. The treatments were; hand weeding ($T_1$), application of glyphosate at 1.44 kg ai ha$^{-1}$ ($T_2$), application of Diuron at 3.2 kg ai ha$^{-1}$ ($T_3$) and application of oxyfluorfen at 270 g ai ha$^{-1}$ ($T_4$). All treatments were applied at three monthly intervals. According to the results, $T_2$ and $T_3$ significantly reduced the weed biomass compared with those of other treatments and the emergence of new weed seedlings was very low in Diuron applied plots ($T_3$). The growth of coconut seedlings (in terms of girth) increased significantly ($P<0.05$) in $T_3$ at the end of the nursery period. Treatment with glyphosate at 1.44 kg ai ha$^{-1}$ ($T_2$) resulted in reduced growth of seedlings at the latter part of the experiment. The highest growth rate of coconut seedlings (girth) was observed in $T_3$ plots. The most effective and economically viable herbicide combination was the application of Diuron at 3.2 kg ai ha$^{-1}$ ($T_3$) for controlling weeds in coconut nurseries.

**Key words**: Coconut, diuron, glyphosate, oxyfluorfen, nursery, weed

## INTRODUCTION

Coconut (*Cocos nucifera* L.) is by far the most extensively cultivated major plantation crop in Sri Lanka (Liyanage and Liyanage 1992). High quality coconut seedlings must be used to establish and maintain a healthy and uniform plantation. Therefore, much attention needs to be paid at the nursery stage to ensure production of quality seedlings. Coconut seedlings are raised in nurseries prior to field planting. Proper care and maintenance of seedbed facilitate the selection of early germinating vigorous seedlings. Genetic correlation studies carried out by Liyanage and Abeywardana in 1957 have shown that early germination and seedling vigor is correlated to adult palm characters such as early flowering and high nut and copra yield.

During the nursery growth of seedlings, there must be adequate maintenance if the plants are to be vigorous and transplantable (Peries and Everad 1993). Among the cultural practices, weed management is an important but an expensive agronomic input in a coconut nursery. However, much information on the effect of growth reduction of seedlings due to weed competition in the coconut nurseries is lacking. The most popular weed control method in coconut nurseries is hand weeding but it is labor intensive and time-consuming (Senarathne and Perera 2004). The major weed species present in coconut nurseries in the dry zone of Sri Lanka are *Imperata cylindrica, Cynodon dactylon, Panicum repens, Mimosa pudica, Hedyotis auricularia* and *Hyptis suaveolens*.

Glyphosate (N- (phosphonomethyl)-glycine) is a commonly used herbicide in mature coconut plantations (Senarathne and Perera 2003). It is a non-selective post emergence herbicide, which controls a wide range of monocotyledonous and dicotyledonous annuals, biennials and perennials (Boyall 1998). Diuron (3-(3,4-dichlorophenyl)-1,1dimethylurea) is a urea herbicide used to control a wide variety of annual and perennial broadleaf weeds, grass weeds and mosses (Caroline-Cox 2003). At present, it is widely used in sugarcane and pineapple cultivations in Sri Lanka. Oxyfluorfen (2-chloro-1 (3-ethoxy-4-nitrophenoxy)-4-(trifluormethyl benzene)) is a selective pre-emergence herbicide for weed control in a variety of crops, which controls a wide spectrum of annual broadleaf weeds and a few grasses (Chauhan and Ramakrishanan 1981). Therefore, careful testing of these weedicides is important to find the most economical and effective means of controlling weeds in the coconut nursery.

## MATERIALS AND METHODS

An experiment was carried out in August 2003 to July 2004 in a coconut nursery located at Wilpotha province in the Low Country Dry Zone of North-Western Sri Lanka. The average day temperature of this site was around 30°C. The rainfall during the period of study was uneven with dry spells.

Seed nuts were spaced 45 cm between rows and 15 cm within rows in the nursery bed. The treatments applied following a Randomized Complete Block Design with three replicates include:

- $T_1$. Manual weeding
- $T_2$. Application of glyphosate at 1.44-kg ai $ha^{-1}$ (4l $ha^{-1}$)
- $T_3$. Application of Diuron at 3.2 kg ai $ha^{-1}$
- $T_4$. Application of Oxyflurofen at 0.27 kg ai $ha^{-1}$ (1.25 l $ha^{-1}$)
- $T_5$. Uncontrolled

Each experimental plot was of 2.44 m x 3.04 m in dimension planted with 40 seed nuts. Treatments were made according to a fixed schedule using a hand sprayer on the entire surface of the plots. All treatments were repeated at three month intervals, starting from August 2003 and ending in July 2004. Weed biomass was randomly collected as samples in 0.25 m x 0.25 m quadrant and weighed every month. Plant height and stem girth were measured as growth parameters of coconut seedlings. The seedlings of major weed species that emerged were counted to calculate the weed seedling density.

## RESULTS AND DISCUSSION

Effect of different herbicides on weed biomass

Among the treatments, the lowest weed biomass was produced by $T_2$ (1.44 kg ai $ha^{-1}$ Glyphosate, - continuous application) and $T_3$ (3.2 kg ai $ha^{-1}$ of Diuron) (Figure 1). The other treatments were not so effective in suppressing weeds satisfactorily and interfered with hand weeding. Moreover, $T_2$ and $T_3$ were effective in reducing both monocotyledonous and dicotyledonous weeds. Glyphosate is a widely used, non-selective, foliage applied herbicide known to be highly toxic to *Panicum repens* (Manipura & Somaratne 1974). Diuron is used for both complete vegetation control and selective weed control in certain crops. Although hand weeding treatment suppressed weed growth temporarily, fast re-growth was observed, especially with monocotyledonous weeds. Generally hand weeding causes more damage to dicotyledonous weeds and aerial parts of monocotyledonous weeds and less damage to root systems or underground plant parts such as stolons, bulbs and rhizomes of monocotyledonous weeds. Hence, dicotyledonous weeds are much easier to control by hand weeding. Boyall (1998) showed that when glyphosate was applied, it gets translocated into the underground rhizomes and destroys all viable buds. Therefore, this is the ideal method for controlling monocotyledonous weeds. However, dicotyledonous weeds seeds in the soil germinate with time (Figure 2) and produce new weed population on glyphosate-applied plots.

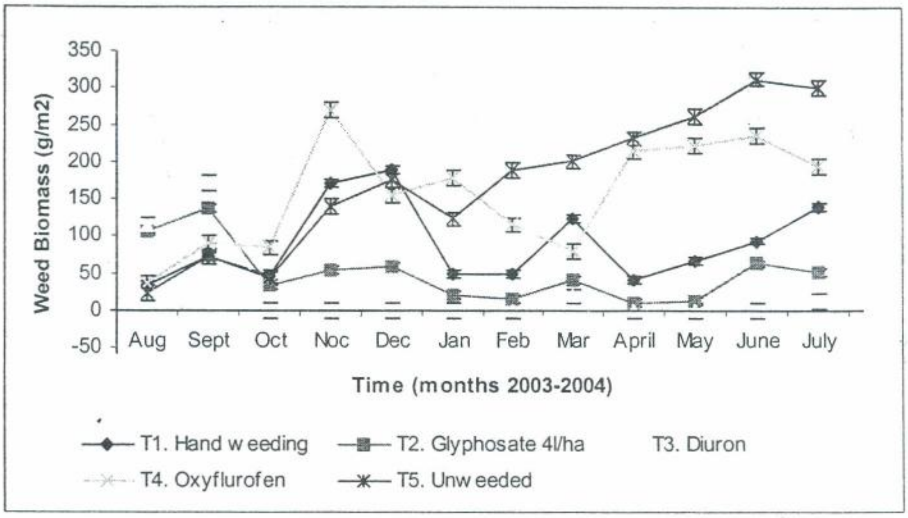


Figure 1. Weed biomass (g $m^{-2}$) as affected by five different weed management methods (from August 2003 to July 2004) in a coconut nursery. Treatments were applied in October 2003, January 2004 and April 2004. Vertical bars indicate $\pm$ SE of the mean.

Diuron, also a selective systemic herbicide, is more effective against broadleaved weeds and grass weeds (Caroline-Cox 2003). Diuron has longer residual activity as very low weight of weed biomass was recorded in $T_3$ plots. It was a very effective herbicide in the control of both types of weeds in coconut nurseries. Emergence of new weed seedlings was very low in diuron-applied plots (Figure 3). All the treatments were applied at three-month intervals but $T_3$ did not need three applications because after two applications weed growth was reduced significantly. Oxyfluorfen was less effective in controlling both types of weeds. Emergence of new weed seedlings, particularly dicotyledonous weeds, was very high in oxyfluorfen applied plots (Figure 4).

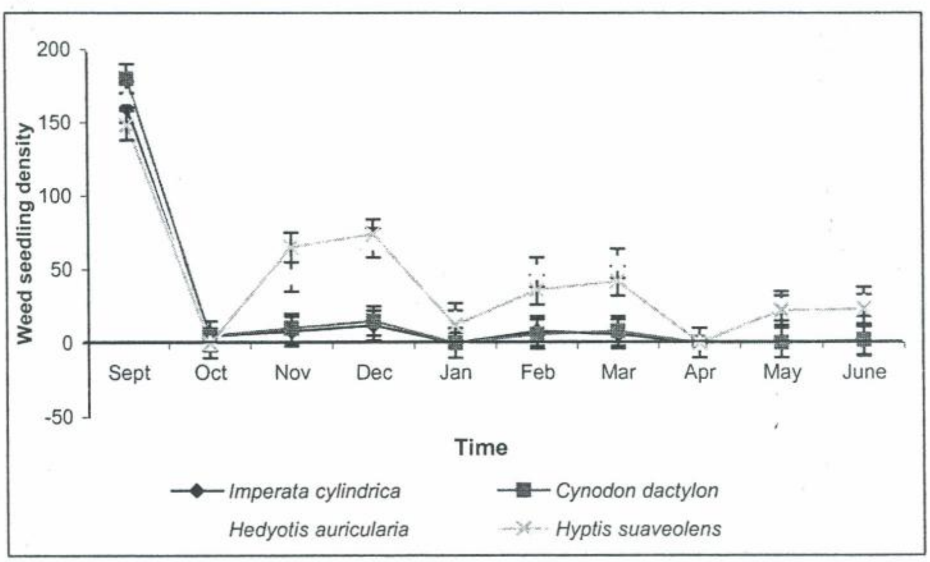


Figure 2. Effect of glyphosate on the emergence of major weed species (Number of seedlings $m^{-2}$) in a coconut nursery. Treatments were applied in October 2003, January 2004 and April 2004. Vertical bars indicate $\pm$ SE of the mean.

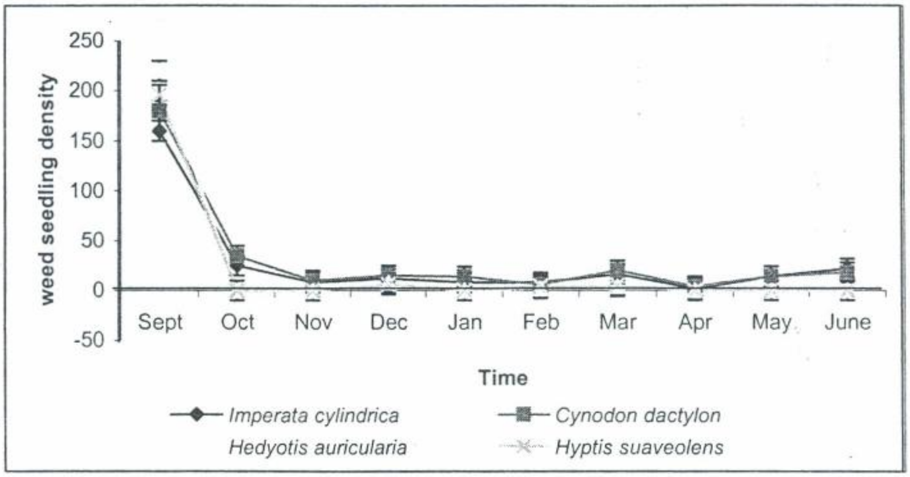


Figure 3. Effect of diuron on major weed species seedling emergence (number of weed seedlings $m^{-2}$) in a coconut nursery. Treatments were applied in October 2003, January 2004 and April 2004. Vertical bars indicate ± SE of the mean.

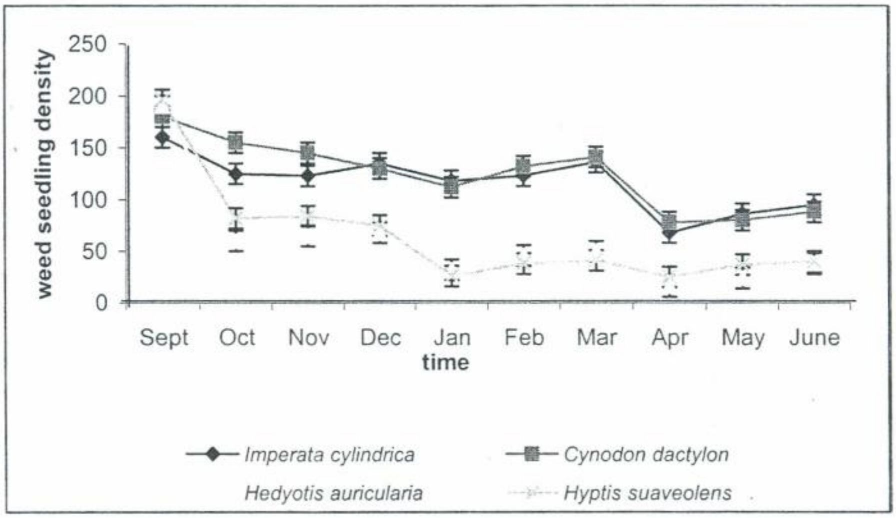


Figure 4. Effect of oxyfluorfen on major weed species seedling emergence (number of weed seedlings $m^{-2}$) in a coconut nursery. Treatments were applied in October 2003, January 2004 and April 2004. Vertical bars indicate ± SE of the mean.

Effect of different herbicides on coconut seedling growth

Application of diuron at 3.2 kg ai ha $^{-1}$ha significantly increased ($P<0.05$) the girth of seedlings compared to the other treatments (Table 1). Seedling height however, significantly increased in the unweeded plots (Table 2). In selecting high quality vigorous seedlings, broad and well-spread leaves, stout stems, and short petioles are considered as desirable characteristics. Partly spreading, narrow leaved, lanky seedlings were produced in unweeded plots due to high competition for light with weeds. Seedling height is a negative character in selecting vigorous coconut seedlings.

Seedling girth started to increase during the latter part of the nursery period This could be attributed to reduced competition for soil moisture and nutrients due to less or no weed growth in plots applied with glyphosate or diuron. The seedling girth in T2 and T3 treatment were highest at the end of the experiment In $T_2$, coconut seedling growth was also reduced and this may be due to glyphosate toxicity on the coconut seedlings. Some yellow colored patches also appeared on the leaves of seedlings in glyphosate-applied plots ($T_2$). It is also important to note that manual control of weeds did not result in any significant increase in growth as compared to unweeded plots. This implies that the competition by weeds for nutrients and moisture has not been effectively suppressed by hand weeding. It is also interesting to note that there was no significant difference among seedlings in herbicide applied plots, hand weeded plots and in $T_4$ treated plots.

Table 1. Effect of different weed control treatments on the seedling girth of coconut seedlings (cm)) at Wilpotha.

| Treatments | Nov 03 | Dec 03 | Jan 04 | Feb 04 | Mar 04 | April 04 | May 04 | June 04 | July 04 | Growth function | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$- Hand weeding | 7.0 | 7.7 | 9.2 | 10.0 | 10.6 | 12.0 | 12.6 | 13.5 | 13.8 | Y=0.89X+6.2 | 0.986 |
| $T_2$- Glyphosate | 8.1 | 8.7 | 10.0 | 11.1 | 11.6 | 13.4 | 14.2 | 15.9 | 16.4 | Y=1.09X+6.6 | 0.990 |
| $T_3$- Diuron | 7.1 | 8.0 | 9.5 | 10.4 | 11.2 | 12.6 | 13.6 | 15.8 | 17.2 | Y=1.23X+5.5 | 0.985 |
| $T_4$- Oxyflurofen | 7.8 | 8.2 | 9.3 | 10.1 | 10.7 | 11.7 | 12.3 | 13.2 | 13.1 | Y=0.73X+7.0 | 0.982 |
| $T_5$- Unweeded | 7.8 | 8.4 | 95 | 10.4 | 10.9 | 12.2 | 12.9 | 13.5 | 14.1 | Y=0.81X+6.9 | 0.998 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ** | ** | | |
| LSD (P=0.05) | | | | | | | | 1.16 | 1.15 | | |
| CV% | | | | | | | | | | | |

* Significantly different at P=0.05; ns, not significantly different at P=0.05. Y= Coconut seedling girth (cm) t = Time (months)

Table 2. Effect of different weed control treatments on the height of coconut seedlings (cm) at Wilpotha.

| Treatments | Nov 03 | Dec 03 | Jan 04 | Feb 04 | Mar 04 | April 04 | May 04 | June 04 | July 04 |
|---|---|---|---|---|---|---|---|---|---|
| $T_1$- Hand weeding | 50.1 | 60.5 | 74.3 | 81.2 | 84.1 | 90.9 | 101.5 | 113.5 | 116.0 |
| $T_2$- Glyphosate | 66.6 | 76.7 | 92.8 | 99.8 | 106.0 | 113.4 | 129.5 | 146.9 | 130.6 |
| $T_3$- Diuron | 48.4 | 63.4 | 80.0 | 80.2 | 85.0 | 94.8 | 109.1 | 122.7 | 127.7 |
| $T_4$-Oxyflurofen | 59.4 | 71.7 | 84.9 | 91.6 | 98.7 | 106.6 | 116.4 | 127.7 | 125.7 |
| $T_5$-Unweeded | 60.8 | 74.0 | 85.4 | 95.1 | 99.4 | 106.6 | 116.7 | 130.5 | 150.0 |
| Significance | ns | ns | ns | ns | ns | ns | ns | ns | ** |
| LSD (P=0.05) | | | | | | | | | 18.5 |
| CV% | | | | | | | | | |

* Significantly different at P=0.05; ns- not significantly different at P=0.05.

In terms of seedling height and girth, the highest growth rate of coconut seedlings was observed in the $T_3$ (Tables 1 and 2). The seedling girth increment was 1.29 cm per month (Table 1).

Cost benefit analysis of different weed control methods

The costs of different weed control methods are given in Table 3. Highest seedling growth was achieved by the application of diuron at 3.2 kg ai $ha^{-1}$ ($T_3$).

Table 3. Cost analysis of different weed control methods.

| Methods of weed control | Number of rounds | Cost per 1000 seedlings per round | Total cost per 1000 seedlings (Rs) |
|---|---|---|---|
| T1- Hand weeding | 4 | 400.00 | 1600.00 |
| T2- Glypho 1.4 kg $ha^{-1}$ | 3 | 46.67 | 140.00 |
| T3- Diuron 3.2kg ai $ha^{-1}$ | 2 | 156.80 | 313.60 |
| T4-Oxyflu 0.27 kg ai $ha^{-1}$ | 3 | 75.00 | 225.00 |

Includes material & labor cost
Average price of commercial product of glyphosate SL Rs 400 /liter,
Average price of commercial product of diuron SL Rs 560 /400g,
Average price of commercial product of oxyfluorfen SL Rs 1200 / 400ml
Average labor wage: SL Rs 200/ manday, US 1$ = Sri Lankan (SL) Rs 95

Application of glyphosate at 1.44 kg ai $ha^{-1}$ and diuron at 3.2 kg ai $ha^{-1}$ produced the lowest weed biomass. However, the most cost-effective method of controlling weeds was to apply diuron at 3.2 kg ai $ha^{-1}$. Hand weeding was not economical as it involved a total cost of SL Rs 1600.00 per 1000 seedlings. $T_4$ was less expensive but not effective in controlling weeds.

It could be concluded that application of diuron at 3.2 kg ai $ha^{-1}$ is the most economical weed control method to produce good quality seedlings in coconut nurseries. Alternatively the application of glyphosate at 1.44 kg ai $ha^{-1}$ can be recommended but with less efficacy.

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# Glyphosate–adjuvant interactions: review of recent experiences

S. D. Sharma[1], N. Chandrasena[2], and M. Singh[3]

[1]CCS Haryana Agricultural University, Regional Research Station, Karnal, India

[2]Ecosystem Restoration, 1, Kawana Court, Bella Vista, NSW 2153, Australia

[3]Citrus Research &Education Center, IFAS, University of Florida, 700 Experimental Station Road, Lake Alfred, FL 33850-2299, USA

nimchan@iinet.net.au

**Abstract:** Glyphosate is the most widely used herbicide in the world. Surfactants are well known to enhance its uptake, translocation and field performance. The interactions between glyphosate formulations and surfactants, however, are not simple and depend on factors which include leaf surface, droplet characteristics, differential wetting of leaves, adjuvant type and the chemical form of the herbicide. Understanding the complexity of these interactions is critical in order to reduce the amount of glyphosate being used worldwide, particularly in environmentally sensitive areas.

In most species, addition of common adjuvants to glyphosate reduces the surface tension of spray solutions and droplet contact angles, leading to increased spray deposition, spray retention, and leaf wetting. There are many examples in the published literature where such adjuvant effects translate directly to increased uptake and enhanced glyphosate activity. There are other instances, however, which show that adjuvant effects on glyphosate treatments are more species specific than previously appreciated; increased spray delivery does not always result in increased control of certain weeds. In this paper, some examples indicate the complex interactions between glyphosate, adjuvants and plant responses. Reasons for lower effectiveness of glyphosate treatments in the field include the hydrophobic nature of waxes, poor droplet spreading, increased drying time and antagonism in the presence of certain adjuvants.

Crop-based oils and methylated seed oils, which also increase leaf wetting, have increased the effectiveness of glyphosate treatments on many difficult-to-control weed species. Results of recent studies are discussed, indicating that the enhanced activity of glyphosate in the presence of oil-based adjuvants possibly involves humectant action or a solubilization effect on epicuticular waxes on the target leaves.

**Key words:** Adjuvants, glyphosate, surfactants.

## INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine is a global herbicide because of its versatility in economically controlling a broad spectrum of weeds under varied agricultural, industrial, amenity and domestic situations (Bayliss 2000). Once applied the leaf surface, glyphosate has several barriers to overcome before it becomes lethal to a plant. These are: (a) epicuticular wax on the cuticle; (b) the cuticle itself; (c) walls of epidermal cells, and (d) plasmalemma of cells. The efficacy of glyphosate depends largely on the success of passing through these barriers, before translocation within a plant to its sub-cellular sites of action.

With the aim of overcoming the cuticular barriers, many studies have examined the formulation factors involved- such as glyphosate concentrations, glyphosate-adjuvant mixes, surfactant and/or adjuvant concentration, spray droplet sizes, surface tension reduction in solutions, and leaf surface characteristics. These have revealed the complex nature of the interactions between different surfactants, surfactant rates, herbicide rates and target species, as major factors influencing the uptake of glyphosate (Liu and Zabkiewicz 1997 2000; Knoche and Bukovac 1993; Kirkwood et al. 2000; Leaper and Holloway 2000).

Despite a vast amount of research, the basis of enhancement of foliar uptake of glyphosate caused by adjuvants is still not well understood. Improved glyphosate efficacy with surfactants has been attributed to increased leaf wettability, droplet contact area and penetration, resulting from reduced surface tension and contact angle of droplets on leaf surfaces. Surfactants may also increase glyphosate uptake by influencing spray droplet size, droplet drying time and overall spray retention on leaf surfaces, and may also increase cuticular permeability and/or stomatal penetration (Kirkwood 1993; 1999).

Our research work has focused on using adjuvants to improve the bio-efficacy of glyphosate, so that its rates can be reduced. This review is based on some laboratory and field research, which demonstrate the complexity of the interactions between different weed species and adjuvants on the efficacy of glyphosate.

## GLYPHOSATE- SURFACTANT INTERACTIONS

### Influence of epicuticular wax

The role of epicuticular wax and the influence of two surfactants on the uptake of glyphosate a.i. were studied using three weed species- teaweed (*Sida spinosa*), velvetleaf (*Abutilon theophrasti*) and sicklepod (*Cassia obtusifolia*) (Sharma and Singh 1999).

These species had different amounts of polar wax (14, 64 and 93%, respectively), as a % of total waxes on adaxial leaf surfaces (25.5, 27.6 and 40.6 $\mu g\ cm^{-2}$), respectively. Glyphosate (Rodeo™) treatments (0.56 kg a.i. $ha^{-1}$ in 188 L $ha^{-1}$ of water) were given to the three weeds, either alone or formulated with surfactants X-77 (non-ionic) and Silvet L-77 (organosilicone). During spray treatments, the 3$^{rd}$ fully expanded leaf was kept covered with aluminium foil, and later, after removing the cover, the adaxial surface of this leaf was treated with $^{14}C$-glyphosate, applied as 5 x 2 µl droplets (10 µl).

Contact Angles (CA) measured correlated with the amount of polar wax on leaf surfaces, being least on teaweed and highest on sicklepod. Surfactants significantly reduced surface tension (ST) and CA of glyphosate droplets on both Teflon slides and test leaves.

Uptake and translocation of glyphosate without surfactant decreased significantly as the % of polar waxes on adaxial leaf surfaces increased in the species (Table 1). However, incorporation of X-77 or L-77 significantly increased the uptake and translocation of $^{14}C$-glyphosate by all species, and this increase was greater with L-77 than with X-77 in all species. The overall effect of increased glyphosate uptake was well correlated with low ST and low CA, increased spreading of droplets in the presence of the surfactants.

Greenhouse efficacy evaluation trials consistently showed significantly higher % control of all species achieved by Rodeo + L-77, which is attributed to super spreading properties and increased surface contact promoted by L-77. However, the species-specific nature of the glyphosate-surfactant interaction was also obvious from the study.

Table 1. Effect of surfactants on uptake and translocation of $^{14}C$-glyphosate and overall control of test weed species with varying amounts of cuticular polar waxes.

| Treatment | Teaweed | Velvetleaf | Sicklepod |
|---|---|---|---|
| | | *Uptake as % of applied | |
| Glyphosate (control) | 28.03 e | 21.80 f | 20.97 f |
| Glyphosate + X-77 | 58.27 b | 38.60 c | 34.23 d |
| Glyphosate + L-77 | 66.43 a | 56.87 b | 56.77 b |
| | | Translocation as % of applied | |
| Glyphosate (control) | 4.48 f | 3.78 g | 3.4 g |
| Glyphosate + X-77 | 9.75 d | 5.87 e | 6.07 e |
| Glyphosate + L-77 | 35.40 a | 20.57 b | 11.17 c |
| | | Control % | |
| Glyphosate (control) | 45.0 cd | 41.3 d | 43.8 cd |
| Glyphosate + X-77 | 55.0 b | 47.5 cd | 66.3 a |
| Glyphosate + L-77 | 68.8 a | 50.0 bc | 66.3 a |

*In all Tables Uptake = $^{14}C$ in the whole plant; Translocation = $^{14}C$ in the whole plant, excluding the treated leaf. Recovery of $^{14}C$ activity: 95%. Values followed by the same letter are not significantly different (Duncan's New Multiple Range Test).

The interaction of plant surface waxes and surfactants on uptake of glyphosate was seen in another study (Sharma et al., 2001), which used Commelina (*Commelina communis*) and Nightshade (*Solanum nigrum*). The leaf surface properties of these two weeds were significantly different. Nightshade's leaf surface had large amounts of non-polar waxes.

Glyphosate (Rodeo™) treatments on the weeds were the same as above, either alone or formulated with Kinetic® (0.25% v/v), which is a blend of polyalkyleneoxide modified poly-dimethyl-siloxane (organosilicone) and non-ionic surfactants. Experimental procedures for $^{14}C$-glyphosate uptake studies were also as same as given previously.

Both uptake and translocation of $^{14}C$-glyphosate were significantly higher in the presence of the surfactant than without surfactant, in both species. However, between the two species, greater uptake and translocation of $^{14}C$-glyphosate was in Nightshade (Table 2).

Table 2. Effect of Kinetic® on $^{14}C$-glyphosate uptake and translocation by Commelina and Nightshade.

| Harvest time (h) | Uptake (% of applied) | | | | Translocation (% of applied) | | | |
|---|---|---|---|---|---|---|---|---|
| | Commelina | | Nightshade | | Commelina | | Nightshade | |
| | - S* | + S | - S | + S | - S | + S | - S | + S |
| 0.25 | 2 | 20 | 6 | 26 | 1 | 2 | 1 | 14 |
| 1 | 6 | 27 | 9 | 35 | 2 | 3 | 2 | 23 |
| 6 | 14 | 35 | 24 | 54 | 2 | 5 | 4 | 37 |
| 24 | 21 | 51 | 51 | 66 | 4 | 6 | 9 | 43 |
| 48 | 26 | 58 | 59 | 68 | 9 | 16 | 17 | 46 |
| LSD (P≤0.05) | 3 | | 2 | | 1 | | 2 | |

*- S: no surfactant; + S: with surfactant.

The increased entry of glyphosate, with or without surfactant into Nightshade is attributed to its relatively smooth leaf surface, which had less polar wax (or greater amounts of non-polar wax). Uptake and translocation results were well correlated with the % control of these two weeds achieved by glyphosate (± surfactant) (data not presented).

Scanning electron micrographs of treated Commelina leaves showed that the application of glyphosate alone disrupted the epicuticular waxes on the adaxial leaf surface, but the waxes remained intact on the cuticle. Treated with the organosilicone surfactant alone, the waxes appeared dissolved, dried and with cracks. Similar damage to cuticles and epidermal tissues caused by surfactants has been reported (Feng et al. 1999). With glyphosate + surfactant, surface waxes were disrupted and dissolved; trichomes were ruptured from the base, and stomata were also disrupted. The surfactant-aided disruption of epicuticular wax on Commelina probably allowed increased penetration of glyphosate active ingredient into the plant.

In both our studies, glyphosate uptake was greater through non-polar cuticles, and glyphosate was repelled by leaf surfaces with more polar (hydrophilic) components. The presence of surfactants allowed such repellence to be overcome. However, there are other studies, which have produced somewhat different results. For instance, Chachalis et al. (2001) reported that lower efficacy of glyphosate was related to the more hydrophobic (non-polar) nature of epicuticular wax of redvine (*Brunnichia ovata*) leaves, compared to those of trumpet creeper (*Campis radicans*), a susceptible species.

The wax mass per unit area (22-37 μg cm$^{-2}$) was similar in the two species regardless of leaf age. However, trumpet creeper was consistently more susceptible to glyphosate than redvine and the CA of droplets was lower on its leaves. Trumpet creeper leaves have dense trichomes and glands and significant surface micro-roughness compared to redvine, with no trichomes or glands. Therefore, it is possible that leaf surface characteristics played a significant role in increased glyphosate uptake by this susceptible species.

Species-specificity of responses

The species-specific nature of the adjuvant-enhanced uptake of glyphosate was observed in another study with hairy beggarticks (*Bidens frondosa*) and guinea grass (*Panicum maximum*), which differed in leaf surface characteristics (Sharma and Singh 2000).

Plants were treated with glyphosate (Rodeo™ 0.56 kg a.i. ha$^{-1}$ in 188 L ha$^{-1}$ of water), alone or formulated with adjuvants: (a) non-ionic surfactant Ortho X-77 (0.25% v/v), a mixture of alkylaryl-polyoxyethylene glycols, free fatty acids and isopropanol; (b) organosilicone Silwet L-77 (0.1% v/v), and (c) Methylated Seed Oil, MSO (1% v/v). Measurement of static ST and CA of a range of concentrations of the three adjuvants mixed with glyphosate indicated that L-77 caused the greatest reduction in ST and CA (Figure 1).

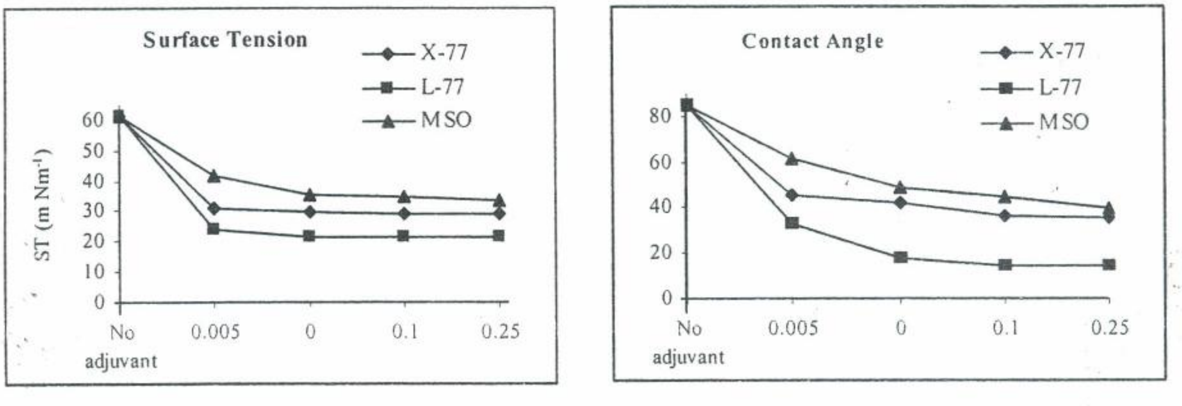


Figure 1. Effect of adjuvant concentrations on (a) Surface tension of glyphosate solutions and (b) Contact angles on a Teflon slide.

In *B. frondosa*, all three adjuvants greatly increased the uptake and translocation of $^{14}$C-glyphosate (Table 3). In the presence of L-77 >50% of the applied $^{14}$C-glyphosate was taken up by *B. frondosa* within 15 minutes. At 6 h, and thereafter, glyphosate uptake was significantly higher with MSO than with X-77.

In *P. maximum*, uptake and translocation of $^{14}$C-glyphosate increased with both X-77 and MSO, but not with L-77. In fact, compared with the glyphosate alone treatment, L-77 had an antagonistic effect on uptake and translocation of glyphosate in this grass species. The efficacy trial confirmed L-77 induced antagonism of glyphosate uptake by *P. maximum*.

Similar antagonism of glyphosate by L-77 in grasses has been reported (Liu and Zabkiewski 1997; Juying and Dastgheib 2001). The common explanation is that the spread of droplets caused by L-77 in the range of 0.1-0.2% is very large, and hence, the dose/leaf area covered could be too low and not conducive to cuticular penetration through diffusion. It is well known that glyphosate uptake is better from concentrated droplets. This explanation is probably true for our study as well. In addition, the concentration of L-77 (0.1%) may not have been sufficient to permit stomatal infiltration in *P. maximum*.

Both of the above effects could be dependent on the nature of the target species foliage. Usually, grass species are more susceptible to glyphosate than broad-leaved weeds; hence, this antagonism by organosilicone surfactants is significant to note.

Table 3. Effect of surfactant types on $^{14}$C-glyphosate uptake and translocation in *Bidens frondosa* and *Panicum maximum*.

| Treatment | *B. frondosa* | | | | *P. maximum* | | | |
|---|---|---|---|---|---|---|---|---|
| | Uptake as % of applied | | | | | | | |
| | 0.25 h | 6 h | 24 h | 48 h | 0.25 h | 6 h | 24 h | 48 h |
| Glyphosate (control) | 6.9 | 25 | 28 | 28 | 2.2 | 14 | 18 | 37 |
| Glyphosate + X-77 | 29.8 | 45 | 50.1 | 49.9 | 3.5 | 16 | 23.5 | 32.6 |
| Glyphosate + L-77 | 52.5 | 77 | 84.6 | 83.9 | 3.6 | 3.1 | 5.1 | 7.5 |
| Glyphosate + MSO | 17.2 | 43 | 61.5 | 62.4 | 2.2 | 12.5 | 23.1 | 36.8 |
| LSD (P = 0.05) | 1.2 | | | | 0.8 | | | |
| | Translocation as % of applied | | | | | | | |
| | 0.25 h | 6 h | 24 h | 48 h | 0.25 h | 6 h | 24 h | 48 h |
| Glyphosate (control) | 2.5 | 12.3 | 13.0 | 14.2 | 0.9 | 4.1 | 15.9 | 18.1 |
| Glyphosate + X-77 | 15.1 | 35.2 | 40.6 | 40.6 | 1.2 | 5.9 | 14.4 | 20.4 |
| Glyphosate + L-77 | 30.1 | 65.4 | 70.4 | 70.3 | 1.3 | 1.9 | 3.0 | 5.2 |
| Glyphosate + MSO | 7.5 | 31.1 | 48.8 | 48.6 | 1.0 | 4.4 | 13.6 | 25.2 |
| LSD (P = 0.05) | 1.4 | | | | 0.5 | | | |
| | % Control | | | | | | | |
| Glyphosate (control) | 36.3 (± 6.3)* | | | | 17.5 (± 2.9) | | | |
| Glyphosate + X-77 | 52.5 (± 11.9) | | | | 30.0 (± 4.1) | | | |
| Glyphosate + L-77 | 94.0 (± 6.2) | | | | 0 (± 0) | | | |
| Glyphosate + MSO | 77.5 (± 9.6) | | | | 58.8 (± 4.8) | | | |

*Numbers in parenthesis are Standard Errors of the Means.

Our study also showed the significantly increased control of both species achieved by glyphosate, assisted by MSO. The increased uptake and translocation of $^{14}C$-glyphosate 6 h after treatment is indicative of either a solubilizing effect on the cuticular waxes, or humectant effect on the droplets, or both. These effects have been recorded by others as well (Wyrill and Burnside 1977) and have been reviewed by Kirkwood (1993; 1999).

Glyphosate-surfactant interactions- some field studies

In Australia, several field studies were conducted during 1999-2000 in a watershed catchment to evaluate the efficacy of surfactants on Biactive® glyphosate for the control of large weed infestations. Only Biactive® glyphosate, which does not contain additional surfactants, was registered in Australia for 'over or near water' applications.

In one trial, weed infestations were large stands of Lantana (*Lantana camara*) and Groundsel bush (*Baccharis halimifolia*) in the Lake Samsonvale catchment in South-East Queensland. Infestations were 2.0-3.5 m tall shrubs with dense foliage. Both species have great potential for regeneration and regrowth. Field plots were $\approx 50$ $m^2$ (Lantana) or $\approx 25$ $m^2$ (Groundsel) and three replicates. Lantana plots were mostly unbroken stands; Groundsel plots were dense clusters of large plants (1-5 plants $m^{-2}$).

Field treatments were glyphosate Biactive® (a) without surfactant (1.8 or 2.7 Kg a.i. $ha^{-1}$), (b) + non-ionic surfactant Agral 90™ (0.05 or 0.1% v/v), or (c) + Pulse™ (0.2% v/v), an organosilicone. Plots were treated with either 10 L $plot^{-1}$ (Lantana) or 5 L $plot^{-1}$ (Groundsel) ($\approx$ carrier volume of 2000 L $ha^{-1}$). The use of slightly higher rates in this field trial was because it was possible that various cations in the lake water sources may antagonize glyphosate. Nalewaja and Matysiak (1991) reported that higher rates could overcome the influence of antagonistic salts in glyphosate spray carriers.

Single applications of Biactive® glyphosate alone were ineffective on the mature stands, as indicated by significant regrowth at 8 WAT. However, the addition of the surfactants to glyphosate significantly increased the control of both species. The increase of glyphosate efficacy caused by both rates of the non-ionic Agral 90 was significantly higher than the enhancement due to the organosilicone surfactant Pulse™.

The organosilicone- Pulse™ increased the efficacy of glyphosate on Lantana, but the influence on Groundsel was much less. The spray containing Pulse™ spread almost immediately on the foliage of both species.

Run-off occurred from leaves of both species with Pulse™ (data not presented), but this run-off from Groundsel leaves was much more marked than from Lantana. This may explain the loss of glyphosate efficacy and the species-specific control response observed.

It is possible that the relative high carrier volume used in the trial, necessitated by the maturity of the stands, may have contributed to loss of activity due to spray run-off from Groundsel. The spread of droplets caused by L-77 may have also caused the glyphosate dose per leaf area to be very low, which would not be conducive to cuticular penetration through diffusion. If anything, these results emphasize the need to be much more mindful of the adjustment required in relation to surfactant concentrations and carrier volume, in using particularly the organosilicones, as has been pointed out (Liu and Zabkiewicz 1997).

Table 4. Glyphosate-surfactant interactions on field control of mature woody weed stand of Lantana and Groundsel.

| Treatment | Lantana | | Groundsel | |
|---|---|---|---|---|
| | Herbicide Rate (Kg a.i. $ha^{-1}$) | | | |
| | 1.8 | 2.7 | 1.8 | 2.7 |
| | % Control (4 WAT)* | | | |
| Glyphosate (control) | 34 | 56 | 25 | 48 |
| Glyphosate + Agral 90 (0.05%) | 60 | 90 | 55 | 90 |
| Glyphosate + Agral 90 (0.1%) | 65 | 95 | 62 | 95 |
| Glyphosate + Pulse (0.2%) | 64 | 88 | 38 | 75 |
| LSD (P = 0.05) | 5.4 | | 6.8 | |
| | % Regrowth (8 WAT)* | | | |
| Glyphosate (control) | 100 | 65 | 100 | 60 |
| Glyphosate + Agral 90 (0.05%) | 35 | 20 | 25 | 10 |
| Glyphosate + Agral 90 (0.1%) | 20 | 0 | 25 | 10 |
| Glyphosate + Pulse (0.2%) | 15 | 0 | 60 | 25 |
| LSD (P = 0.05) | 5.0 | | 8.2 | |

*visual estimation of control (0-100% control)

A second adjuvant-glyphosate efficacy Trial was conducted in Botany Wetlands in Sydney, a nutrient-enriched pond system with deeply entrenched infestations of mature, 2-2.5 m tall *Ludwigia peruviana* (Chandrasena et al 1998). The field Trial was conducted in early summer November 1997. Average air temperature was 25$^{0}$C and relative humidity 55%.

Treatments were glyphosate Biactive® (a) without adjuvant (1.8, 2.7 or 3.6 Kg a.i. $ha^{-1}$), (b) + Agral 90™ (0.05% v/v), (c) + Pulse™ (0.2% v/v), or (d) + Synertrol, a vegetable-oil concentrate containing 832 g $L^{-1}$ of emulsifiable Canola oil. Three replicate plots (25 $m^2$ of 100% *L. peruviana*) per treatment were treated with 5 L $plot^{-1}$ (carrier volume 2000 L $ha^{-1}$).

The results (Fig. 2) indicated the dose-dependent glyphosate control of *L. peruviana* achieved at 4 WAT, and the consistent enhancement of glyphosate efficacy by both Agral and Pulse. Incorporating the vegetable oil- Synertrol also improved the performance of glyphosate on *L. peruviana* significantly. Droplets without adjuvants wetted the leaves poorly and dried on leaf surfaces within 3-5 minutes, whereas the adjuvant-incorporated droplets were moist even after 12-15 minutes (data not presented). Pulse caused some run-off from treated leaves, but run-off was not significant in the other treatments.

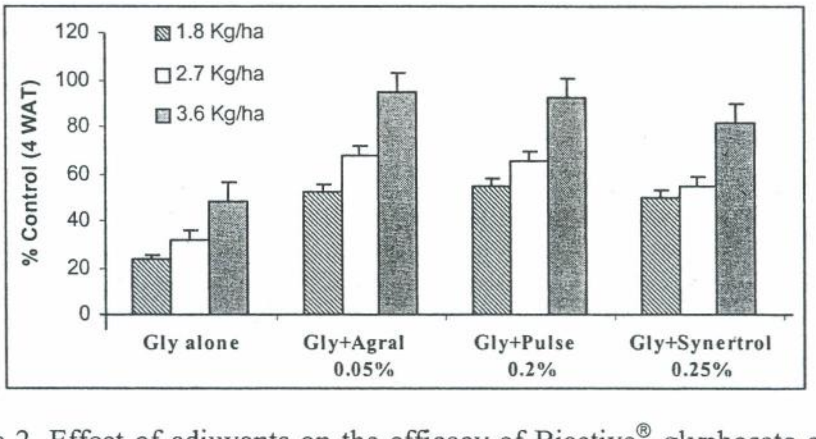


Figure 2. Effect of adjuvants on the efficacy of Biactive® glyphosate on mature stands of *Ludwigia peruviana*.

In both of the field studies reported, the increased efficacy of Biactive® glyphosate caused by adjuvants was obvious and relatively easily explained by the observed improved wetting of target leaves and increased droplet drying times. There was evidence that Agral 90 was consistently better than others in assisting Biactive® glyphosate uptake, and that carrier volume needs to be lower to benefit fully from incorporating an Organosilicone surfactant.
The increase in glyphosate efficacy caused by the Canola oil-based Synertrol is significant, because this biodegradable adjuvant may be more acceptable to be used with Biactive® glyphosate 'in cr near water' under the current conditions in Australia.

Efficient delivery of active ingredient to target sites is a fundamental requirement for herbicide activity. In the case of glyphosate, this can be achieved by influencing the efficiency of spray retention (decreased ST and CA), cuticle penetration and tissue absorption, by using a suitable adjuvant. Selection of a suitable surfactant can reduce the degree of incompatibility between the polar glyphosate and the largely non-polar cuticle.

The herbicide-adjuvant-plant interaction is a complex system. Understanding the different roles of adjuvants in enhancing glyphosate efficacy is essential for optimum utilization of this global herbicide. Glyphosate uptake is very dose dependent, and adjusting the amount of added surfactant to control droplet spread, and hence, applied dosage per leaf, is a simple option for enhancing uptake into any species.

The mechanism of surfactant-induced transfer of glyphosate active ingredient across the cuticle is still not well established and could involve one or more of the following processes: (i) change in solubility relationships and partitioning processes that are favorable to transfer, (ii) decrease in the resistance of the cuticle to diffusion, and (iii) activation of specific polar or non-polar routes through the cuticle. Our studies, among others, have established that there is a linear relationship between properties such as ST and CA, and the uptake of glyphosate. However, many glyphosate efficacy studies, including ours, show that greater herbicidal activity can be produced by slightly higher rates of surfactants.

We believe that surfactants do not simply exert their effects on plant surfaces; they could penetrate through the waxy cuticle into the underlying tissues, thereby assisting the uptake of glyphosate. Other adjuvants, such as vegetable oils, may provide for greater uptake of glyphosate by creating and prolonging conditions conducive to cuticular penetration. The interactions between plant-species, glyphosate and surfactant rates are not simple, and need to be considered when planning weed control programs, so that the ultimate aim of reducing the amount of glyphosate used in the world and costs incurred, can be reduced.

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# A simple technique to evaluate types of herbicide mixtures for pre-registration in Indonesia

S. Tjitrosemito
BIOTROP, P.O. Box 116, Bogor, Indonesia

**Abstract:** A technique to test types of herbicide mixtures was developed using 2,4-D and glyphosate, and their mixtures. Three species were used as test weeds, i.e., *Bidens pilosa, Brachiaria sp.,* and *Cyperus kyllingia*. These weeds were planted in glasses containing soil and warm casting mixture for about 3 weeks before being sprayed with the herbicide treatments, i.e., glyphosate at 0, 72, 144, 216, and 288 g ai $ha^{-1}$ equivalent, 2,4-D at 0, 180, 360, 540, 720 g ai $ha^{-1}$ equivalent, and their mixture at 0, 54, 108, 162, and 216 g ai $ha^{-1}$ equivalent. Harvesting was done at 14 DAT, by cutting fresh parts, collected and placed in labeled paper bags, oven dried, and the dry weight data were recorded. The percentages of damage were calculated from the dry weight. With probit analysis LD50 of 2,4-D, glyphosate, and their mixture were obtained, 486.5, 105.8, and 105.9 g ai $ha^{-1}$, respectively. It was found using ADM analysis that expected $LD_{50}$ of the mixture tested was 143.1 g ai $ha^{-1}$, higher than that obtained from the experiment. It was concluded that the mixture was not antagonistic. The technique was easy and quick to complete, and is appropriate for pre-registration tests.

**Key words**: ADM, antagonistic, herbicide mixture

## INTRODUCTION

The Indonesia Government through the Department of Agriculture (DA) administers management of pesticides (read: herbicides) well to make herbicides available to consumers ( farmers, planters) any time of the year at reasonable prices. The Indonesian Department of Agriculture has been implementing regulations for various pesticides since 1970 through the promulgation of Government Decree No. 7/1973 that directs the management of distribution, storage, and usage of pesticides. The technical statement of the regulation is in accordance with FAO International Code of Conduct on the Distribution and Use of Pesticide (FAO 2003). Only herbicides that comply with the regulations are allowed to be marketed in Indonesia. Those herbicides must be effective, safe to crops, animals, human, and environment, fulfill the technical and administration requirements and utilized only on conditions specified in the labels of herbicide containers. A permanent permit may be issued by the government to an agrochemical company to market a herbicide for 5 years, and at the end of the period it must be tested again to ensure the quality and comply with existing regulations. In this case this pesticide must go through a process to obtain another permanent permit entitling it to be marketed for another 5 years. Otherwise, the herbicide will not be allowed to be utilized in Indonesia. Herbicides like 2,4,5 T and amitrole are prohibited for use in Indonesia. A temporary permit only allows the herbicide to be marketed for one year. It should be evaluated again to get permanent permit for 5 years.

The mounting public pressures to reduce herbicides usage have been taken up by the Government (the DA) well by proposing and implementing a series of regulations aimed at reducing the use of pesticides (including herbicides). The decree No.12 Year 1992 on cropping systems and stature No. 6 Year 1995 on crop protection laid a political foundation that crop protection should be carried out within the framework of Integrated Pest Management (IPM). This idea should be translated into regulations that support the principle and objective of IPM, i.e. to reduce the distribution and usage of herbicide. However, since late 1990s Indonesia has experienced a multi crisis crippling a greater part of economic activities including those on crop productions. The cost of pesticides soared sky high reducing further activities on crop production and making life much more difficult for a greater portion of the population.

To reduce the price of pesticides, the DA issued a new regulation No. 343.1/Kpts/TP.270/7/2001 on the registration of pesticides replacing the old regulation No. 944/Kpts/TP.270/5/1989. In the old regulation an active ingredient of any pesticide is allowed to be registered only under three different formulations. In the new regulation there is no restriction in the registration with regard to the number of formulations developed from an active ingredient. The new regulation opens up more activities that are available for many small local companies. These new companies are competing to sell pesticides; they import generic active ingredients from many sources overseas and formulate their own products, register them at the DA and market them accordingly at lower prices. The consumers mainly farmers and planters are currently enjoying 15-20% lower price than before the regulation was implemented (Untung 2004). This deregulation of pesticide registration and the accompanying ease of getting permit to import generic herbicides facilitate not only lowering of herbicide prices but also increases the consumption of herbicides, or other pesticides (Figure 1).

It is quite obvious that the increase in the number of formulation is much more than that of the active ingredients. At the international level, the addition of new available active ingredients in the past 20 years has been slow if any. The situation of increasing herbicide consumption in Indonesia is also enhanced by the new regulation permitting development of various formulations. The challenge to this development is to ensure that all formulations should be effective, safe for crops, animals, human and environment and comply with the existing regulations. In particular the development of herbicide mixtures consisting of two or more active ingredient and marketed as such should be tested to ensure that the mixture is not antagonistic. The problems of testing have been much discussed (Tjitrosemito and Burhan 1995; Tjitrosemito 2002).

In this paper the author presents an easy method of testing herbicide mixture formulations for pre-registration to be marketed by agrochemical companies by which the mixture can be evaluated and, therefore, to provide a technical support to the DA to develop regulations related to herbicide mixtures based on which the decision to accept or to reject the registration.

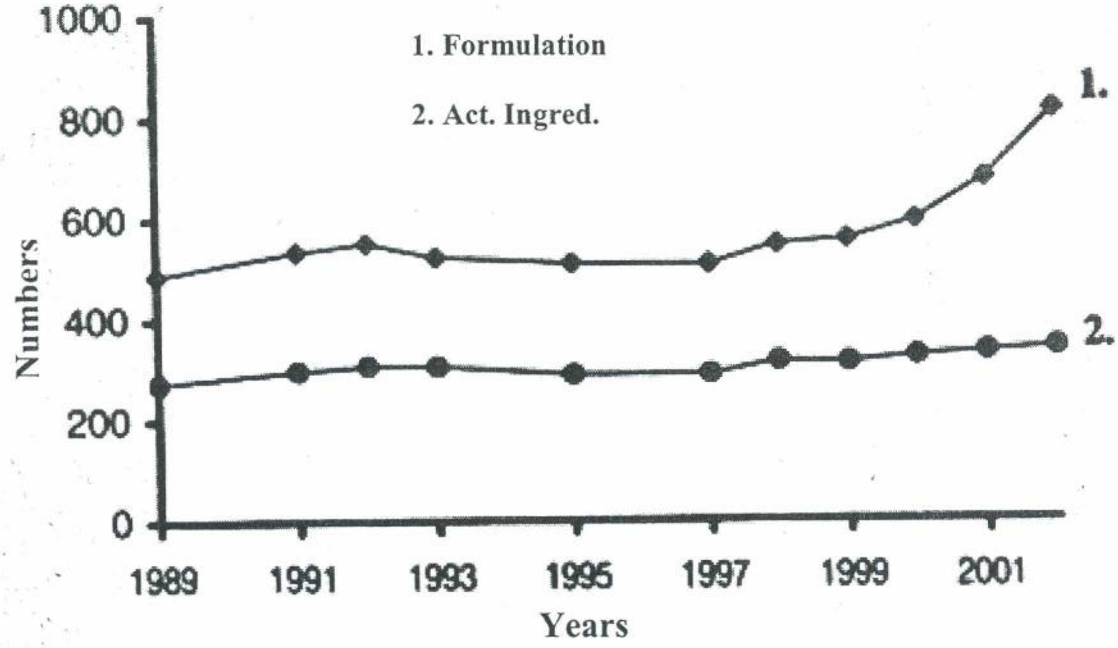


Figure 1. Numbers of pesticide active ingredients and formulations up to 2001.

## MATERIALS AND METHODS

This experiment was carried out at the BIOTROP Greenhouse in Tajur, Bogor, West`Java, Indonesia. Weeds selected for testing were chosen from areas where the mixture will be used. At least three species of weeds were selected representing co-dominant species categorized as broad leaved weeds, grasses, and sedges with one species each. In this work the selected weeds were *Bidens pilosa* (Asteraceae), *Brachiaria* sp. (Graminae), and *Cyperus kylingia* (Cyperaceae). The herbicide mixture consisted of glyphosate and 2,4-D at the ratio of 240/120 active ingredient, with EC formulation.

The weed seeds were soaked in water for 24 h and planted at about 0.5 cm below the soil surface in glasses containing the growth media. The plastic glasses, 9 cm in diameter at the top end, and 12 cm in height, were perforated at the bottom to facilitate water diffusion from underneath to the growth media up. The growth media consisted of a mixture of Tajur soil and warm casting at the ratio of 1:1 (by volume). The soil was cleaned off from any gravel, and organic debris, air dried and passed through a fine screen. The soil and warm casting mixture was oven heated up to 105°C for 24 h to kill any soil pathogens that may interfere with the growth of planted weeds.

Ten seeds of each weed species were planted. These glasses were placed in plastic trays measuring 40 x 30 cm with a side wall of 7 cm. These trays were filled with water to automatically water the glass of growing weeds. The weed seeds emerged 4-5 days later. After seven days weed seedlings were thinned out leaving three uniform plants/glass. The weeds were maintained carefully and at 20 days after thinning they were sprayed with the prescribed herbicide treatments in four replications.

Spraying was done using a knapsack sprayer equipped with pressure gauge and T-jet nozzle, calibrated to deliver an equivalent of 400 L ha$^{-1}$. Weeds were taken from the tray and placed randomly on the ground inside a marked 4 m$^2$ area. Sprayed weeds were collected and placed back in properly labeled new strays.

The three herbicides, glyphosate, 2,4-D, and their mixture (240/120) were applied at the following doses, glyposate at 0, 72, 144, 216,and 288 g ai ha$^{-1}$ equivalent, 2,4-D at 0, 180, 360, 540, 720 g ai ha$^{-1}$ equivalent, and their mixture at 0, 54, 108, 162, and 216 g ai ha$^{-1}$ equivalent. Weed species receiving the same treatments were placed in the same trays, and maintained carefully up to harvest.

Harvesting was carried out 14 days after treatment (DAT) by cutting the fresh part of weeds from all treatments. These were collected and placed in labeled paper bags, oven dried at 80°C for 48 h weighed and the data were recorded accordingly. The percentage of damage was calculated as follows: $D_p = [1-(W_{ft}/W_{fc})\} \times 100\%$, where $D_p$ = percent of damage, $W_{ft}$= dry weight of fresh part under treatments, and $W_{fc}$= dry weight of fresh part from control.

The percentages of damage of the three weed species were averaged and transformed to probit, while the doses were changed to their logarithmic values. The relationship between values of probit and log (dose) were linear, and the linear regression of each herbicides was computed. $LD_{50}$ was estimated by taking the value of probit = 5, which would give a value of log ($LD_{50}$). By converting to antilog the value of $LD_{50}$ was obtained from the logarithmic table. Having found the values of $LD_{50}$ using isobol technique (Tammes 1964) in the framework of Additive Dose Model (Hatzios and Penner 1985) the type of herbicide mixture can be evaluated whether antagonistic or not.

## RESULTS AND DISCUSSION

The data on dry weights (not presented in the paper) were used to calculate the percentage of damage from these three weed species, and averaged to represent a way of evaluating damage to weed situation in the field. The percentage of damage of weeds is presented in Table 1.

The data showed a range that may be too high, but it is typical of data on herbicide effect on more than one weed species. Although theoretically the range would have extended from 5 to 95% damage, the percentages of damage derived from the measurement of dry weight would be difficult to achieve without an extra expense for additional doses to be tested. It will increase the cost of testing for the herbicide. The data on these percentages of damage were then transformed to probit by reading from the probit table, while the doses were transformed to their logarithmic values (Table 2).

Table 1. The percentage of damage of B. *alata, Brachiaria* sp., *C. kyllingia* (average of three weed species) due to various herbicide treatments.

| Herbicides | Doses (g ai ha$^{-1}$) | Replication 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| 2,4-D | 180 | 29.93 | 29.83 | 13.77 | 33.85 |
| | 360 | 44.41 | 42.50 | 22.56 | 39.91 |
| | 540 | 51.65 | 50.32 | 35.47 | 57.83 |
| | 720 | 67.70 | 74.49 | 59.73 | 75.84 |
| Glyphosate | 72 | 30.39 | 42.14 | 41.31 | 30.69 |
| | 144 | 57.77 | 59.97 | 57.67 | 47.39 |
| | 216 | 79.52 | 66.97 | 83.20 | 88.04 |
| | 268 | 90.64 | 1.65 | 94.73 | 96.43 |
| Mixture of | 54 | 19.46 | 20.62 | 28.42 | 43.72 |
| 2, 4-D and | 108 | 37.75 | 34.27 | 37.84 | 61.53 |
| glyphosate | 162 | 54.98 | 59.18 | 57.59 | 75.57 |
| | 216 | 82.33 | 88.27 | 81.76 | 86.32 |

Table 2. The values of probit obtained from the probit table and log (dose) of herbicide concentration used in the testing.

| Herbicides | Log (doses) X | Probit values (Y) 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|---|
| 2,4-D | 2.2553 | 4.4731 | 4.4702 | 3.9106 | 4.5838 | 17.4377 |
| | 2.5563 | 4.8595 | 4.8112 | 4.2448 | 4.7445 | 18.6800 |
| | 2.7324 | 5.0412 | 5.0075 | 4.6286 | .1964 | 19.8737 |
| | 2.8573 | 5.4589 | 5.6585 | 5.452 | 5.6996 | 22.0622 |
| | Sub total | 19.8327 | 19.9474 | 18.0292 | 20.2243 | 78.0336 |
| Glyphosate | 1.8573 | 4.4875 | 4.8020 | 4.7805 | 4.4960 | 18.5661 |
| | 2.1584 | 5.1960 | 5.2518 | 5.1920 | 4.9350 | 20.5748 |
| | 2.3345 | 5.8237 | 5.4345 | 5.9620 | 6.1760 | 23.3962 |
| | 2.4594 | 6.3167 | 6.3820 | 6.6168 | 6.7995 | 26.1150 |
| | Subtotal | 21.8239 | 21.8703 | 22.5514 | 22.4065 | 88.6521 |
| Mixture of | 1.7324 | 4.105 | 4.1798 | 4.4294 | 4.8417 | 17.5914 |
| 2, 4-D and | 2.0334 | 4.6881 | 4.5962 | 4.6897 | 5.2919 | 19.2659 |
| glyphosate | 2.2095 | 5.1254 | 5.2323 | 5.2015 | 5.6915 | 21.2507 |
| | 2.3345 | 5.9268 | 6.1902 | 5.9050 | 6.0945 | 24.1165 |
| | Subtotal | 19.8808 | 20.185 | 20.2256 | 21.9196 | 82.2245 |

The relationship between probit values (Y) and log (doses) (X) theoretically is linear in the form of Y= a + bX. Using statistical calculation the regression obtained and the subsequent value of $LD_{50}$ are presented in Table 3.

Table 3. Regression equations developed from each herbicides and the corresponding $LD_{50}$.

| Herbicides | Regression | $R^2$ | LD 50 (g ai $ha^{-1}$) |
|---|---|---|---|
| 2,4-D | Y = 0.1368 + 1.8098 X | 0.8361274 | 486.5 |
| Glyphosate | Y = -1.1632 + 3.0441 X | 0.9298745 | 105.8 |
| Mixture | Y = 0.1193 + 2.5284 X | 0.8377375 | 105.9 |

A high value of regression coefficient was obtained in all three herbicides tested. This indicates a good prediction of the value of X at a given value of Y. A good fit of the regression line was obtained (Tables 4, 5 and 6).

Table 4. Analysis of variance of regression line for 2,4-D.

| Source of Var. | df | Sum of S | Means of S | Var. Ratio | F Table |
|---|---|---|---|---|---|
| Doses | 3 | 2.9157 | 0.9719 | 14.155** | 5.95 |
| Regression | 1 | 2.4379 | 2.4379 | 35.506** | 9.33 |
| Deviation | 2 | 0.4778 | 0.2389 | 3.479 NS | |
| Error | 12 | 0.8240 | 0.0687 | | |
| Total | 15 | 3.7397 | | | |

Table 5. Analysis of variance of regression line for glyphosate.

| Source of Var. | df | Sum of S | Means of S | Var. Ratio | F Table |
|---|---|---|---|---|---|
| Doses | 3 | 8.1498 | 2.7166 | 55.252** | 5.95 |
| Regression | 1 | 7.5783 | 7.5783 | 154.131** | 9.33 |
| Deviation | 2 | 0.5715 | 0.2858 | 5.812 NS | |
| Error | 12 | 0.5900 | 0.0492 | | |
| Total | 15 | 8.7398 | | | |

With the regression lines derived from the collected data proven to be reasonable to predict the value of $LD_{50}$, the next step was to measure if the mixture was antagonistic or not. (It is required by the regulation that any marketed mixtures of herbicides should not be antagonistic). The model to test the mixture was Additive Dose Model developed by Tammes (1964) and shown in Figure 2.

Table 6. Analysis of variance of regression line for the mixture of 2,4-D and glyphosate.

| Source of Var. | df | Sum of S | Means of S | Var. Ratio | F Table |
|---|---|---|---|---|---|
| Doses | 3 | 5.9032 | 1.9677 | 27.068** | 5.95 |
| Regression | 1 | 5.3648 | 5.3648 | 73.795** | 9.33 |
| Deviation | 2 | 0.5385 | 0.2692 | 3.704NS | |
| Error | 12 | 0.8724 | 0.0727 | | |
| Total | 15 | 6.7756 | | | |

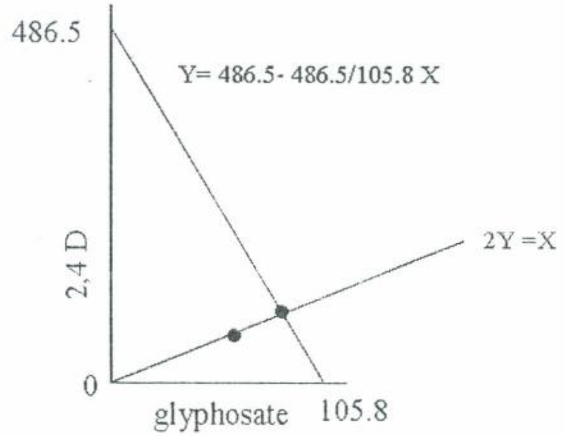


Figure 2. ADM solution to find the expected value of $LD_{50}$ from the mixture tested

The expected $LD_{50}$ value of the mixture tested is the interaction point of lines $Y = 486.5 - 486.5/105.8\ X$ and $2Y = X$ (equation representing the active ingredient ratio of the mixture). The solution of these two equations gives a value of $Y = 47.7$ and the corresponding value of $X = 95.4$. Summing these two concentration components up $(47.7 + 95.4 = 143.1)$ gives an $LD_{50}$ of 143.1 g ai $ha^{-1}$. This value is still higher than the value of $LD_{50}$ of 2,4-D and glyphosate mixture obtained from the actual experiment, i.e. 105.9 h ai $ha^{-1}$ (see Table 3). This result supports the contention that the mixture of glyphosate and 2,4-D in this particular ratio and tested in this work is not antagonistic.

The technique developed is very simple, quick and quite reliable. Using this technique almost everyone will be able to carry out the data calculation. This is important for the Indonesian government where there are more than 300 formulations of herbicides tested annually, making it difficult for the DA staff to do the testing by themselves. The constraint to this test is on the difficulty in obtaining the probit table. Recently, however, the probit table has been available from the internet. This method facilitates herbicide testing by the DA staff and agrochemical companies can obtain results of the test rapidly.

## ACKNOWLEDGEMENT

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# Biological Control

**Two decades of weed biocontrol in the Pacific region**

M. D, Day[1], W.Orapa[2] and R.Muniappan[3]
[1]Alan Fletcher Research Station, PO Box 36, Sherwood, Qld 4075, Australia
Michael.Day@nrm.qld.gov.au
[2]Secretariat of the Pacific Community, Private Mail Bag, Suva, Fiji Islands
[3]College of Agriculture, University of Guam, Mangilao, 96923, USA

**Abstract:** Weed biocontrol projects in the Pacific region over the past 20 years were assessed. Twenty-three biocontrol agents have been released on 10 weeds in 13 of the 22 Pacific Island Countries and Territories. Most of the activities or projects in the south Pacific were the result of donor-funding, particularly ACIAR and AusAID (Australia), GTZ (Germany), CIRAD (France), NZAid (New Zealand) and recently the EU, while island countries and territories such as Guam and Palau, in the northern Pacific benefited from their association with the United States. There have been numerous successes, most notably the control of *Salvinia molesta, Pistia stratiotes, Eichhornia crassipes, Mimosa diplotricha* and *Chromolaena odorata* in one or more countries. In other weed projects such as on *Lantana camara*, success has not eventuated and research is still needed to overcome this weed. While the projects centred on one or two countries initially, expertise and the transfer of agents to other countries continued long after the projects officially concluded. Agents of the above weeds have been moved around, sometimes fortuitously to other countries, with similar success in most cases. In addition to past research, there are a number of new projects, working on weeds such as *Mikania micrantha* and *Coccinia grandis* that are being developed. In this paper, we summarise the projects undertaken in the Pacific region over the past 20 years, provide information on the status of each of the weeds targeted, and outline some potential new biocontrol projects.

**Key words:** Biological control, *Chromolaena odorata*, *Lantana camara*, *Eichhornia crassipes*

## INTRODUCTION

Apart from a few opportunistic introductions in the early 1900s, particularly against *Opuntia* spp. (Cactaceae) (1930s), *Cyperus rotundus* L. (Cyperaceae) (1930s) and *Lantana camara* L. (Verbenaceae) (1911-1930s), active programs in the biocontrol of weeds in the northwestern Pacific have been fairly recent. Biocontrol programs were initiated after World War II and concentrated mostly on *Clidemia hirta* (L.) D. Don. (Melastomataceae), *Xanthium strumarium* L. (Asteraceae), *L. camara* and *C. rotundus*. These programs involved the transfer of natural enemies that had already been released and established in Hawaii or Australia to Micronesia and other countries in the South Pacific (Julien and Griffiths 1998). Waterhouse and Norris (1987) gave a good review of all the weed biocontrol projects until the mid 1980s. However, apart from reviews by Muniappan (1988), Denton *et al.* (1991) and Schreiner (1989) on *L. camara* and Schreiner (1989) on *C. hirta* in Palau, there has not been any review on many of the weed biocontrol programs in the Pacific region. Since the mid 1980s, the number of biocontrol projects has increased considerably, both in terms of countries utilising biocontrol and the number of weeds targeted. This paper gives an account of weed biocontrol programs since the mid 1980s and discusses some future prospects.

### Weed Biocontrol Programs (1985-2005)

*Chromolaena odorata* (L.) R. M. King and H. Robinson (Asteraceae)

*Chromolaena* is a neotropical plant that has become an invasive weed in tropical Africa, Asia, Micronesia, Papua New Guinea and East Timor. It has also established in some areas in northern

Queensland, Australia, where it is the subject of an eradication campaign. *Chromolaena* is mostly a problem of plantation crops, disturbed forests, pastures, and along roadsides. It flowers in November-December in the northern hemisphere and June-July in the southern hemisphere.

Biocontrol of *Chromolaena* first started in 1970 when the moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) and the flower-feeding weevil *Apion brunneonigrum* Beguin-Bellecoq (Coleoptera: Brenthidae) were imported into Malaysia. However, the research expanded in 1984, when a project in Guam supported by US Department of Agriculture began. Of the several natural enemies evaluated for control of *Chromolaena*, *P. pseudoinsulata* and the gall fly, *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) have proven the most effective and have been used widely in the pacific.

*Pareuchaetes pseudoinsulata* was introduced and field established on Guam in 1985. It was subsequently introduced to the Northern Mariana Islands of Rota in 1985, Tinian and Saipan in 1986 (Muniappan *et al.* 2004) and in Yap (Federated States of Micronesia) in 1988 (Muniappan *et al.* 1988) and 2004 (R. Muniappan pers. obser.), where it established. Further introductions conducted in the Federated States of Micronesia on Pohnpei in 1988-90 (Esguerra *et al.* 1991), Kosrae in 1992 (Esguerra 1998) and Chuuk in 2004 (Englberger pers comm.) also resulted in establishment. It was released in Palau in 1989 (Miles *et al.* 1991) and again in 1996-97 (Muniappan pers. obser.) but it did not establish. Releases are being continued but establishment has not been confirmed.

*P. pseudoinsulata* was also introduced into Papua New Guinea in 1998, as part of an ACIAR-funded project. The moth was widely released but only established in Morobe province, despite being released in large numbers (Bofeng *et al.* 2004).

*C. connexa* was imported into Guam in 1998 from Indonesia, with field releases commencing in 2002 and establishment achieved by 2003. Field releases conducted in Palau in 1999 also resulted in establishment. Releases of this fly in Saipan and Rota in 2003 temporarily established and then died out. Another release conducted in Rota in 2005 resulted in establishment. Further releases of the fly in Pohnpei in 2003, Chuuk and Kosrae in 2004 (Englberger 2004) and Yap in 2005 have resulted in field establishment (Muniappan pers. obser.).

*C. connexa* was introduced into Papua New Guinea in 2001, where it quickly established. Since then it has established in 11 provinces, and is spreading and controlling chromolaena in two provinces (Day and Bofeng unpubl. data). With funding from ACIAR, the gall-fly has been introduced into East Timor, but it is too early to confirm establishment (M. Day pers. comm.).

The mite, *Acalitus adoratus* Keifer (Acari: Eriophyidae), has been fortuitously introduced into Micronesia from Asia, but it is not effective. Two agents introduced from Trinidad, *A. brunneonigrum* in 1984 and the stem boring moth, *Mescinia parvula* (Zeller) (Lepidoptera: Pyralidae) in 1984 and 1986, were released in Guam but did not establish. The shoot boring fly, *Melanagromyza eupatoriella* Spencer (Diptera: Agromyzidae), imported from Trinidad to Guam in 1987 was mostly parasitized and no releases were made.

Muniappan *et al.* (2005) have recently reviewed the spread of chromolaena and the efforts to suppress it on a global basis for the last two decades through biocontrol. Six international workshops conducted since 1988 and 16 newsletters produced on this weed and its management, can be retrieved at: http://www.ehs.cdu.edu.au/chromolaena/siamhome.html

*Coccinia grandis* (L.) Voigt (Cucurbitaceae)

Ivy gourd is a recent introduction to the Mariana Islands. It is of East African origin and has been introduced and naturalized in several countries in Asia, tropical Central and South America and the Pacific. However, it has become invasive only in Hawaii, Saipan and Guam. Hawaii has imported three natural enemies from East Africa and successfully suppressed this weed. These three agents have since been introduced to Guam. The leaf mining weevil, *Acythopeus cocciniae* O'Brian and Pakaluk (Coleoptera: Curculionidae), was released in Guam and Saipan in 2003 and has established in both the islands (Muniappan pers. obser.). The petiole galling weevil, *Acythopeus burkhartorum* O'Brian and Pakaluk (Coleoptera: Curculionidae) was released in Guam in 2004 and Saipan in 2005 but field establishment has not been confirmed. Several shipments of a third natural enemy, the stem boring moth, *Melittia oedipus* Oberthor (Lepidoptera: Sessidae), have been imported to Guam quarantine facility since 2001for host specificity studies. However, no field releases of this moth have been made thus far.

*Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae)

Water hyacinth is one of the world's worst water weeds, affecting many tropical countries by blocking waterways, preventing access to fishing grounds, markets and hospitals. Successful biocontrol of water hyacinth has been achieved in many countries since the mid 1970s (Julien and Griffiths 1998). *Neochetina eichhorniae* (Curculionidae) was introduced into Papua New Guinea in 1986 and, along with *N. bruchi* (1993), has controlled water hyacinth in many parts of the country, including the worst infestations along the Sepik River (Julien *et al.* 1999). *N. eichhorniae* has also been introduced into the Solomon Islands (1988) and Vanuatu (2004). However, its status in these countries is unknown.

*Lantana camara* L. (Verbenaceae)

*Lantana* is a weed of both agricultural and natural ecosystems and has been the focus of biocontrol projects worldwide for over 100 years. While there has been some control in some countries, the weed is still a problem in most places where it has naturalised (Day *et al.* 2003). The leaf blotch miner, *Calycomyza lantanae* Frick (Diptera: Agromyzidae), was introduced to Guam in 1992 (Muniappan *et al.* 1992) and Fiji in 1996 from Australia and to Pohnepei from Guam in 1996 (Esguerra *et al.* 1997a), while *C. lantanae* and *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae) have been fortuitously introduced to Chuuk in 2002 (Muniappan and Reddy 2003) and to Saipan and Yap in 2004 (Muniappan pers. obser.). *Lantanophaga pusillidactyla* (Walker) (Lepidoptera: Pterophoridae) and *Epinotia lantana* Busck (Lepidoptera: Tortricidae) were fortuitously introduced to Yap (Muniappan 1989).

The leaf-mining beetle *Octotoma scabripennis* (Chrysomelidae) was introduced into Fiji and the Solomon Islands in 1993and Niue in 1994. It failed to establish in Fiji, while its status is unknown in the latter two countries. Two other agents, *Teleonemia scrupulosa* (Tingidae) and *Uroplata girardi* (Chrysomelidae), were introduced into Niue and the Solomon Islands in the early 1990s. *U. girardi* established and is causing damage in both countries, while *T. scrupulosa* is present in the Solomon Islands and its status is unknown in Niue (Julien & Griffiths 1998). *Charidotis pygmaea* (Chrysomelidae) was introduced into Fiji from Australia in 1995 but failed to establish.

Research in biocontrol continues, with new agents being studied in Australia and South Africa. Some of these include the budmite *Aceria lantanae* (Eriophyidae), the herring-bone miner *Ophiomyia camarae* (Agromyzidae), a root-feeding weevil *Longitarsus* sp. (Chrysomelidae), a petiole galling weevil *Coelocephalapion camarae* (Brentidae) and the rust *Prospodium tuberculatum* (Pucciniaceae) (Day *et al.* 2003).

*Mikania micrantha* Kunth (Asteraceae)

Mile-a-minute is a weed of plantations and food gardens in many countries of the Pacific (Waterhouse and Norris 1987). Only one agent, *Liothrips mikaniae* (Phlaeothripidae), has been released in the Solomons in 1988 and this failed to establish (Julien and Griffiths 1998). However, several other agents currently under investigation elsewhere show promise. The moths *Actinote anteas* and *A. thyla pyryha* (Nymphalidae) are currently being tried in Indonesia, while the rust *Puccinia spegazzinii* (Uredinales) is being tried in India and China. A new project with funding from ACIAR is being developed to introduce these agents into Fiji and PNG.

*Mimosa diplotricha* (=invisa) C. Wright ex Suavalle (Fabaceae)

The psyllid, *Heteropsylla spinulosa* Muddsman, Hodkinson and Hollis (Hemiptera: Psyllidae), was introduced into Australia in 1988. It established and achieved some control along the coastal areas of north Queensland. It was subsequently released in Pohnpei in 1992 and Yap in 1993-1994 (Esguerra *et al.* 1997b), where it established and suppressed the weed. It was also introduced to Palau in 2000, but its status is unknown. Elsewhere, it was released in Samoa in 1988 as part of an ACIAR project, Fiji and PNG in 1993, Cook Islands and the Solomons in 1994 and American Samoa in 1997. It has established in all areas and is exerting control or partial control. Two other agents, *Psigida walkeri*, introduced into Cook Islands in 1994, and *Scamurius* sp., introduced into Samoa in 1988, failed to establish (Julien and Griffiths 1998).

*Pistia stratiotes* L. (Araceae)

Successful biocontrol of water lettuce has been achieved in many countries, particularly those in Africa from introductions of *Neohydronomus affinis* (Curculionidae) from Australia. Following good control in Australia and elsewhere, *N. affinis* was also introduced to PNG in 1985, where it established and has achieved good control at some sites (Julien and Griffiths 1998).

*Salvinia molesta* D.S. Mitchell (Salviniaceae)

Following the success of *Cyrtobagous salviniae* (Curculionidae) in Australia, PNG and many countries in Africa and Asia, the beetle was introduced to Fiji in 1991. The beetle quickly established and has controlled *Salvinia* in a number of locations.

*Sida acuta* Burman f. and *S. rhombifolia* L. (Malvaceae)

The leaf-feeding beetle *Calligrapha pantherina* (Chrysomelidae) was introduced into Australia to control both *Sida* spp. in the Northern Territory. Following its establishment in Australia, it was then introduced to PNG, Fiji and Samoa before1998 and Vanuatu in 2004. Control has been achieved in some areas in PNG and Fiji, while impacts of the agent in Samoa, are still to be determined and it is too early to determine its status in Vanuatu (Julien and Griffiths 1998).

*Xanthium strumarium* L. (Asteraceae)

Noogoora burr is mainly a weed of pastures, where it can impact on production and cause death to the stock. The stem-boring moth *Epiblema strenuana* (Tortricidae) was introduced and established on parthenium, ragweed and noogoora burr in Australia (Julien and Griffiths 1998). Along with other agents that were introduced, it has contributed to the control of noogoora burr and ragweed and the partial control of parthenium. It was introduced twice to PNG to control noogoora burr in 2002 and 2003. However, both attempts failed, with the culture dying out before establishment could be confirmed (Day pers. comm.).

## DISCUSSION

The introduction of successful agents from one country to another is a very cost-effective method of controlling weeds. The control of *Salvinia* and water hyacinth, using agents imported from Australia, highlight the benefits of collaboration within the Pacific region. More recently, biocontrol agents for *Chromolaena* are being moved around Micronesia, then to PNG and now to East Timor. Preliminary studies show that this weed is also being controlled in local areas of some countries or islands. The practice of assisting our neighbours in controlling serious weeds using successful agents should be encouraged in countries where some of the above mentioned weeds occur but no biocontrol agents have been introduced. The challenges ahead lie in targeting weeds where little is known about their controls.

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# Biological control of weeds in Papua New Guinea

Warea Orapa
Plant Protection Service, Land Resources Division
Secretariat of the Pacific Community, Private Mail Bag, Suva, Fiji Islands
WareaO@spc.int

**Abstract:** Biological control (biocontrol) has been the preferred method for invasive alien weed control in Papua New Guinea (PNG) while the use of herbicides is largely restricted to plantation agriculture situations. There have been 15 introductions of biological control agents in less than four decades of classical biological control efforts against weeds. Generally weed biological control has been more successful in terms of establishment of biocontrol agents and their effectiveness in controlling the target weeds than biocontrol against arthropod pests or snails. There are several weeds under some level of biocontrol and these include puncture vine (*Tribulus cistoides*), lantana (*Lantana camara*), salvinia (*Salvinia molesta*), water lettuce (*Pistia stratiotes*), creeping sensitive plant (*Mimosa diplotricha*), water hyacinth (*Eichhornia crassipes*) and two species of *Sida*. A biological control project is continuing for chromolaena (*Chromolaena odorata*). Attempts to introduce a biocontrol agent for Noogoorra burr (*Xanthium strumarium*) were not successful. Plans are being developed to target mile-a-minute weed (*Mikania micrantha*) and Giant Sensitive Plant (*Mimosa pigra*) using biological control. There is a need for research to find suitable natural enemies for other weeds of considerable concern to agriculture, biological diversity and the environment, as the use of other methods of weed control such as herbicides is not feasible for invasive weed management in PNG.

**Key words**: Biological control, Papua New Guinea, weeds

## INTRODUCTION

Many introduced weeds are serious impediments to agriculture and other land use systems in Papua New Guinea (PNG, Fig. 1) but weed management has lagged very much behind arthropod pest management for a variety of reasons. Economic costs, human health, and environmental concerns have continued to play a role in decisions on the use of various weed management strategies. Agriculture in PNG is still dominated by smallholder semi-subsistence farming, which relies heavily on manual labour for weeding and culture methods to suppress weeds. The use of chemical herbicides is almost negligible in smallholder farming situations, largely due to the prohibitive costs of herbicides and reliance on shifting cultivation, which requires little weed control or is due to lack of awareness on the impact of weed competition. National efforts against introduced weeds have concentrated on the use of biological control more than herbicides; the latter are more appropriate and utilised in commercial plantation cropping situations.

Biological control activities in PNG date back to 1927 when unspecified predators were moved from larger islands to the Niningo Atolls in the Manus Islands for the control of coconut scales (*Aspidiotus destructor*) (Wilson 1960). To date, 15 biological control agents have been introduced for weed control compared to 42 species of parasitoids for insect pest control, and four (4) against snail pests during the 77 years of classical biological control history in PNG. However, biocontrol agents introduced for weeds have been more successful compared to biological control efforts against arthropod and mollusc pests (Table 1).


Figure 1. Map of Papua New Guinea

Table 1. A comparison of the effectiveness of weed biological control agents (BCA) liberated as against those released against insect and mollusc pests since 1927 in PNG. Effective agents have provided 'some' to 'full' suppression of the target pest.

| | PEST TYPE | | |
|---|---|---|---|
| | Insects | Weeds | Snails |
| Total number of BCA species liberated | 42 | 15 | 4 |
| Number of liberated BCAs established on target weeds | 24 | 13 | 1 |
| Number of established BCAs effective against target weeds | 15 | 10 | 0 |

The high level of success of biological weed control agents may be attributed to the fact that all projects were 'transfer projects' which, according to Julien (1989), enjoy a high level of success because research on host-specificity and effectiveness of agents initially are done by another country. Most failures of biological control programmes generally occur because of poor environmental suitability of an introduced agent, and possibly also due to poor or unsuitable implementation strategies used by the projects themselves (Julien and Orapa 1999).

This paper reviews the status of all intentional introductions of weed biological control agents, present biocontrol work, and future prospects in biological weed control in PNG.

## TARGETS AND STATUS

Ten introduced invasive weeds have been the targets of biological control programmes in PNG. The following is a review of the status of each of these weeds and their agents. For reference to locations refer to Figure 1. Additional information on the biocontrol agents liberated is given in Table 2.

*Tribulus cistoides* (Zygophyllaceae)

The history of biological control agent introductions against weeds in PNG is short. It dates back to 1966 when the weevil *Microlarinus lareyni* was introduced and released around Port Moresby for the control of puncture vine, *Tribulus cistoides.* A year later another agent *M. lypriformis* was imported to control the same weed (Bourke et. al. 1973; Young 1982). While puncture vine occasionally occurs in the drier areas around Port Moresby, the present status of control by the two *Microlarinus* species is unknown.

*Lantana camara* (Verbenaceae)

Lantana is a very aggressive invasive weed in a number of tropical and subtropical regions including Australia. Lantana threatened to invade disturbed sites, dry open plains and forest reserves. Six biocontrol agents are recorded as introduced and established in PNG against lantana (Day et al. 2004). The hispine beetle *Uroplata girardi*, and the tingid bug *Teleonemia scrupulosa* were released in 1972 and 1973, respectively (Young 1982). Another biological control agent, the lantana seed mining agromyzid fly *(Ophiomyia lantanae)*, was recorded from lantana seeds at Popondetta in September 1973, at Wau near Bulolo in 1975 (Greve and Ismay 1983). Another agromyzid fly (*Calycomyza lantanae*) was reported after 1977 (Julien and Griffiths 1998). However, it is not clear when and how these agents were introduced into PNG. *U. girardi* and *T. scrupulosa* are commonly found where lantana occurs. The weed has not become as troublesome as feared earlier in PNG for unknown reasons. Two reasons may be possible: these few agents have provided effective control or invasive biotypes may be absent and so is not able to compete successfully with the dense tropical vegetation characteristic of most areas in PNG.

*Salvinia molesta* (Salviniaceae)

Successful control of the small floating fern salvinia (*Salvinia molesta*) in the Sepik River of PNG remains as one of the world's most successful textbook cases of classical weed biological control projects. Control was achieved within two and half years of initial release of the biological control agent but only after discovering the important role of nitrogen in the population dynamics of *Cyrtobagous salviniae*, the tiny weevil used as a biological control agent (Room and Thomas 1985; Thomas and Room 1986a). Initially, the biological control agent for salvinia collected from the Amazon basin was incorrectly identified as *Cyrtobagous singularis.* Upon recognising this species' inability to control the species of salvinia found in Australia, new research led to the proper identification of *S. molesta* and the tiny weevil *C. salviniae* was introduced and released in the Sepik River system in 1982 (Thomas and Room 1986a). An estimated 250 km$^2$ of salvinia cover (or 54% of the stationary water surface of the lower half of the Sepik River) was reduced to just 2 km$^2$ (0.5%) within two years of establishment of the weevil (Room and Thomas 1986b). The salvinia invasion had directly affected the daily livelihoods of over 100,000 people and the access to remote river villages by tourists. The successful control of salvinia in the Sepik resulted in the return of normalcy to villagers' livelihoods and the natural ecosystem.

*Pistia stratiotes* (Araceae)

Water lettuce (*P. stratiotes)* is a floating aquatic aroid and thought to be native to Southeast Asia including New Guinea, where it may be attacked by a number of natural enemies. However, increases in the abundance of this plant in a number of locations including the Sepik River led to a decision to introduce the tiny weevil *Neohydronomus affinis* in 1985 (Laup 1986). Without adequate resources to control *P. stratiotes*, successful control has been achieved on the Sepik River as well as on several infestations in Lae and in some dredge ponds at Bulolo, 50 km south of Lae. This author released 100 adult weevils of *N. affinis* on a small infestation near Kimbe (West New Britain) in 1995 and by 1999 there was excellent control.

*Eichhornia crassipes* (Pontederiaceae)

Water hyacinth (*E. crassipes*) was first found growing in gold dredge ponds at Bulolo in June 1962. It attracted attention and within the same year of discovery it was the target of a major eradication effort from 3 out of 55 gold dredge ponds found infested. A combination of manual labour to remove the weed from ponds as well as spraying with 0.2% 2, 4-D ester and burning of uprooted plants with diesel fuel in the hope of eradicating it but re-growth occurred from treated as well as nearby ponds in April 1963 and follow up treatments occurred. In October 1962 water hyacinth was gazetted as a 'notifiable noxious weed; making it obligatory for landholders to notify an inspector of plants of its presence and making it illegal for removal from the Wau-Bulolo area. Despite these measures, the weed persisted and eventually moved to most parts of the country. It was carelessly introduced into the Sepik River in 1986, immediately after the Salvinia problem was brought under control. This rather attractive plant spread rapidly until it started causing serious problems on village livelihoods in the late 1980s and early 1990s.

Biological control against *E. crassipes* began with the introduction and release of the mottled water hyacinth weevil *Neochetina eichhorniae* at Madang in June 1985. The Australian government aid agency AusAID supported a biological control project (1993 - 1998), which resulted in the introduction and release of three additional agents. The related weevil *N. bruchi* was introduced in March 1993. Both weevils are very widely established, often together, and have been responsible for successful control of water hyacinth (Julien and Orapa 2001). The moth *Niphograpta albiguttalis* was introduced in 1994 and released at several lowland and highland water hyacinth infestations. This agent appears to have failed to establish at all release sites. *N. albiguttalis* prefers the bulbous parts of water hyacinth petioles associated with newly invading or less crowded plants such as at the edges of taller water hyacinth stands. It is suspected that lack of suitable plants (bulbous form) at the points of release together with other factors contributed to this failure. A second moth, *Xubida infusellus*, was introduced in 1997 and field released in one high area and four lowland areas between March 1997 and early 1999. *X. infusellus* prefers and feeds inside the tall petioles of water hyacinth plants, ringing and eventually killing the top part of the leaf. It is established only in a highly eutrophic lake invaded by water hyacinth near Port Moresby (Julien and Orapa 2001). Another insect, the mirid bug *Eccritotarsus catarinensis*, was host-tested in Australia but was considered not suitable for importation and release by the PNG government

*Mimosa diplotricha* (Mimosaceae)

The creeping giant sensitive plant (*Mimosa diplotricha)* has occurred as a serious weed in many provinces. Its impact was particularly serious on grazing properties in the Markham and Ramu valleys and several other parts of PNG. In 1991 Ramu Sugar Ltd imported the tiny sap sucking psyllid bug *Heteropsylla spinulosa* from Australia and released this agent on its grazing properties in the Gudsup area and in the Laloki area near Port Moresby. The establishment of *H. spinulosa* and successful control of *M. diplotricha* on the Gudsup properties were reported by Kuniata (1994).

*Heteropsylla spinulosa* has spread naturally on *M. diplotricha* in many areas away from these original release sites including spread up into the cooler Waghi Valley at 1600 metres altitude and in January 2005 was found spreading further west into the Southern Highlands where it is restricted by the absence of the weed. Elsewhere *M. diplotricha* is now less common than *M. pudica* in many places because the psyllid is giving effective control (Orapa, personal observation).

*Chromolaena odorata* (Asteraceae)

Serious concerns on Chromolaena (*Chromolaena odorata)* were first raised in 1992 following quarantine survey along the PNG-Indonesia border when the weed was found increasing near Vanimo in the northwest. Since then additional large infestations of the weed were located in several other areas including New Britain. A biological control project commenced in 1998 with funding support from the Australian Centre for International Agriculture Research (ACIAR), which was already funding a regional programme in Indonesia and the Philippines. The leaf-feeding moth *Pareuchaetes pseudoinsulata* was imported and first released in March 1999 on chromolaena in the Markham Valley, and later in other areas. This moth is established and causes sporadic damage only in the Markham Valley in areas receiving less than 1000mm of rainfall annually. No recoveries have been made elsewhere (Orapa et al. 2001).

In January 2001, the stem galling fly *Cecidochares connexa* was supplied by the Philippine Coconut Authority in Davao, where the agent was awaiting approval for field releases. This gallfly is widely established at all release sites in PNG and spreading (Orapa and Bofeng 2004). At Namatanai (New Ireland) and the Markham Valley, it has already reduced large chromolaena stands 3 years after first release (Bofeng and Day *this proceedings*).

Table 1. Natural enemies of weeds introduced into Papua New Guinea and their status. (Note: Aust. = Australia and ? denotes unknown)

| Target Weed | Biological Control Agent Introduced | Year | Origin of BCAs | Result | References |
|---|---|---|---|---|---|
| *Tribulus cistoides* L. Zygophyllaceae | *Microlarinus lareynii* (Duval) (Coleoptera: Curculionidae) | 1966 | Ex Italy via Hawaii | Not established | Young (1982) |
| Puncture vine | *M. lypriformis* (Wollaston) | 1967 | Ex Italy via Hawaii. | Established and effective. | Bourke et al (1973) |
| *Lantana camara* L. (Verbenaceae) Lantana | *Ophiomyia lantanae* (Froggatt) (Diptera: Agromyzidae) | ? | Mexico. | Fortuitous spread; reported at Oro (1973) and Wau (1975). | Day et al. (2003); |
| | *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) | 1973 | Ex Mexico via Aust. | Established. Effective. | Greve & Ismay (1983); Young (1982) |
| | *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) | 1972 | Ex Brazil via Aust | Established. Impact unknown | |
| *Salvinia molesta* Mitchell (Salviniaceae) Salvinia | *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae) | 1982 | Ex Brazil via Aust. | Established. Effective. On Sepik R. 250 km$^2$ of salvinia reduced to 5 km$^2$ in 2 years. | Room & Thomas (1985); Thomas & Room (1986) |
| *Pistia stratiotes* L. (Araceae) Water lettuce | *Neohydronomus affinis* Hustache (Coleptera: Curculionidae) | 1985 | Brazil via Aust | Established. Effective most sites except in Bulolo. | Laup (1986). W. Orapa, (pers. obs.). |

| | | | | | |
|---|---|---|---|---|---|
| *Mikania micrantha* (Asteraceae) Mile-a-minute | *Liothrips mikaniae* (Priesner) (Thysanoptera: Phlaeothripidae) | 1988 | Ex Trinidad via Malaysia via Solomons. | Colony failed in quarantine. A prospective future target | J.Moxon (pers. comm.) |
| *Mimosa diplotricha* Martius ex Colla (Mimosaceae) Creeping sensitive plant | *Heteropsylla spinulosa* Muddiman, Hodkingson & Hollis (Hemiptera: Psyllidae) | 1993 | Ex Brazil via Aust. | Established. Effective all areas. In trial plots caused up to 98% reduction. | Kuniata (1994) |
| *Eichhornia crassipes* Mart Solms-Laub. (Pontederiaceae) Water hyacinth | *Neochetina eichhorniae* Hustache (Coleoptera: Curculionidae) *Neochetina bruchi* Warner | 1985 | Brazil via Aust. | Established. Effective. 50-90% reduction with *N. bruchi* | Laup (1986) ; Julien & Orapa, (2001) |
| | *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae) | 1993 | Brazil via Aust. | Established. With *N. eichhorniae* providing effective control. | |
| | *Xubida infusellus* (Walker) (Pyralidae) | 1994 | Ex Argentina via Aust. | Not established | |
| | | 1997 | Ex. Argentina via Aust. | Established at one site. Minor impact on weed. | |
| *Sida acuta* L., S. rhombifolia (Malvaceae) | *Calligrapha pantherina* Stål (Col: Chrysomelidae) | 2000 | Ex Mexico via Aust. | Established. Very effective and spreading very rapidly. | |
| *Chromolaena odorata* King & Robinson (Asteraceae) (Chromolaena) | *Pareuchaetes pseudoinsulata* Regos Barros (Lep: Arctiidae) | 1999 | Ex Trinidad via Guam. | Established at one location only. Sporadic impact. | Orapa et. al (2001) ; |
| | *Ceccidochares connexa* Macquart (Dipt: Tephritidae) | 2001 | Ex. Brazil, via Philippines. | Established and already providing control after at some sites. Releases and impact assessment continuing | |

*Sida rhombifolia* and *S. acuta* (Malvaceae)

These two *Sida* species have been serious weeds in PNG and were especially significant in the Markham Valley west of Lae, mainly on pastures but also under cropping situations in the highlands provinces (mainly coffee), roadsides and other disturbed areas in PNG. In the Markham Valley, managed cattle pastures were severely overgrown by *Sida* species during and after the drought induced by the El Nino phenomenon in 1997. At the end of the drought in mid-1998, significant tracts of pasture were rendered useless by *Sida* spp invasion. Efforts such as slashing and ploughing in *Sida* using tractors proved expensive and difficult and could not solve the problem for many farmers including Ramu Sugar Ltd (RSL). RSL had three tractors dedicated to slashing *Sida* all year round on 37,000 hectares of infested ranch lands. The cost of applying 2, 4-D (the preferred herbicide for Sida control) to 2000 ha twice a year for RSL were US$116,000 (1997), US$136,000 (1998), US$98,000 (1999) and US$21,000 in 2000 (L.S. Kuniata, RSL pers comm.). The introduction of the leaf-feeding beetle, *Calligrapha pantherina,* from Darwin Australia in January 2000 reduced these costs as well as the weed problem. Within a few months the beetle rapidly spread and reduced *Sida* stands at six initial release sites and within 12 months the beetle had spread to more than 150 km of the valley and reduced large areas of the weeds. The agent has spread naturally into the highland areas (from Markham Valley) and on New Britain and New Ireland in the Bismarck Archipelago from single releases of a few hundred individuals during 2002 and 2003, respectively.

*Xanthium strumarium* (Asteraceae)

Seasonal increases in the abundance of Noogoorra burr (*Xanthium strumarium*) in the Markham Valley west of Lae is an ongoing problem on large tracts of grazing land. The weed often grows quickly and competes with pastures, while the foliage of the weed is considered toxic to livestock. In 2001 Trukai Industries, a rice company but with significant interests in beef farming, funded a biological control project. The stem-galling moth *Epiblema strenuana* was introduced into a post-entry quarantine facility in Lae early 2002 but this failed when the small starter colony died in quarantine. Two repeated introductions from Queensland Australia were made but these were similarly lost during post-entry quarantine screening. This work was to be one of only three private sector driven and funded biological control efforts in PNG but work has ceased as a result of discontinued interest by the financier who had high expectations of biological control.

## PROSPECTS

A wide range of weeds continue to impact on agriculture and the natural environment affecting food security as well as biodiversity. Biological control, though limited in its usage, will continue to remain as one of the primary tools for addressing specific invasive weed problems in PNG. However, general weed control in plantation agriculture will continue to rely on the use of herbicides such as paraquat, glyphosate and to some degree 2, 4-D. At present, there are a number of weeds that could be potential targets for biological control. Some of the planned biological control activities for PNG in the next few years include the introduction, screening and release of a number of host-specific natural enemies of some target weeds. Plans are being made to introduce again the leaf-mining agromyzid fly *Calycomyza eupatorivora* for chromolaena control at the time of preparation of this paper (M. Day and I. Bofeng, pers comm). An earlier attempt to introduce it from South Africa in 2004 failed when the colony died during long transit.

Large areas of cash cropping and subsistence agriculture in moist lowland areas and the islands are threatened by the spread of mile-a-minute weed (*Mikania micrantha*). *M. micrantha* is already a serious weed of oil palm, cocoa and coconut plantations and small holder food gardens in PNG. In 1988 an attempt was made to introduce the thrip *Liothrips mikaniae* from Malaysia via the Solomon Islands, but this agent was lost in quarantine (J. Moxon pers. comm.). A new regional biological control project is planned by the Secretariat of the Pacific Community (SPC) based in Fiji and to be supported by ACIAR. Potential biocontrol agents already showing promise against *M. micrantha* elsewhere will be introduced and host-tested for suitability in the Pacific Islands including PNG prior to liberation.

Another troublesome weed which has not yet spread but has the potential of being a biological control target is the prickly giant sensitive plant, *Mimosa pigra* (Orapa and Julien 1996). It threatens to invade large areas of lowland provinces and periodically inundated wetlands if not brought under control quickly. An integrated programme with strong emphasis on biological control has been ongoing in the northern Australia and plans are being developed to adopt some of these to address the looming problem in PNG. Other serious weeds of particular concern deserving urgent biological control research include Itch grass (*Rottboellia cochinchinensis),* Johnson grass (*Sorghum halepense*), molasses (*Melinis minutiflora*) and Elephant grass (*Pennisetum purpureum*). Some research on potential agents of *R. coccinchinensis* have been reported (Reeder et al 1996). However, finding introducing biological control agents for grass weeds will not be easy, as close relatives are important traditional food crops. Other important invasive weeds in PNG requiring attention include: spike pepper (*Piper aduncum)*; African tulip (*Spathodea campanulata*); and, merremia

(*Merremia peltata*). These are major environmental weeds with serious consequences on biodiversity, agriculture and livelihoods in PNG and the Pacific region and require management centred on biological control strategy. However, developments in biological control is however constrained by chronic lack of funding, awareness on the importance of invasive weeds management by decision makers and the subsequent lack of capacity to progress in this area of natural resource management.

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## Study on the efficacy of *Setosphaeria rostrata* in controlling *Leptochloa chinensis* in net house and field conditions

H.L.Thi[1] and D.V.Chin[1]
[1]Cuulong Delta Rice Research Institute
Co Do- Can Tho- Vietnam
duongvanchin@hcm.vnn.vn

**Abstract:** Two experiments, one in the net house and another in the field, were conducted at the Cuulong Delta Rice Research Institute, Mekong Delta, Vietnam during Summer-Autumn season of 2004 to find out the efficacy of a fungus *Setosphaeria rostrata* to control *Leptochloa chinensis* in rice. The floating rice husks as low-tech mycoherbicide carrying fungal spores spread the biological agent to the target weed. Results reveal that rice husk formulation has the same weed control efficacy as compared to that of kaolin form. Application one time at 7 days after sowing (DAS) is better than that of twice at 12 DAS and 17 DAS. The dose of $10^{12}$ spores.ha$^{-1}$ is sufficient to control *Leptochloa chinensis* in pot experiment but higher dose at $10^{13}$ spores ha $^{-1}$ is needed to ensure high weed control efficiency. Rice plants suffer phytotoxicity at the rate of $10^{13}$ spores ha$^{-1}$ but they recover very soon and grain yields are not adversely affected significantly. Treatments with weed control efficiency higher than 90% in both pot- and field conditions are: T2 [weed free check] (100%), T4 [*S. rostrata* (Kao.) @$10^{12}$] (99.3%-100%) and T8 [*S. rostrata* (Husk) $10^{13}$ once] (99.3%-100%). Rice yields under all treatments are higher than that of untreated check [T1] statistically except T5 [*S. rostrata* (Husk) @$10^{10}$] (2.19T.ha$^{-1}$) in the field condition. In pot experiment, treatments T4 [*S. rostrata* (Kao.) @$10^{12}$] (5.78T.ha$^{-1}$) and T8 [*S. rostrata* (Husk) $10^{13}$once] (6.03T.ha$^{-1}$) have the same yield with T2 [weed free check] (5.99T.ha$^{-1}$) statistically. Two most promising treatments in the field condition are T7 [*S. rostrata* (Husk) $10^{12}$ once] (4.13T.ha$^{-1}$) and T8 [*S. rostrata* (Husk) $10^{13}$ once] (4.40T.ha$^{-1}$) which have the same yields with weed free check [T2] (4.44T.ha$^{-1}$) statistically.

**Key words:** Floating mycoherbicide, *Setosphaeria rostrata*, *Leptochloa chinensis*

## INTRODUCTION

Rice is the most important crop in Vietnam. Direct seeding is the popular method of crop establishment in the South of Vietnam. From the past, farmers in the North of this country have practised transplanting but nowadays they have shifted partly into direct seeding also. Grasses are major weeds in direct seeded rice with the most important species namely *Echinochloa crus-galli*. However, one more grass species, *Leptochloa chinensis*, has become popular recently. Two main reasons for this succession are: (i) The shortage of water in rice production, particularly in Summer-Autumn season and (ii) The popular use of some herbicides which are weak in controlling *Leptochloa chinensis* such as: pyrazosulfuron-ethyl, bispyribac-sodium, pyribenzoxim... In order to check this trend, an attempt has been made by using fungus as biological agent to control this weed. Results from the collaboration between the Cuulong Delta Rice Resaerch Institute (CLRRI) and the Australian Center for International Agricultural Research (ACIAR) in the 1990's conluded that *Setosphaeria rostrata* is a very promising fungus to control *Leptochloa chinensis* in rice. However, the mass production of this fungus to produce mycoherbicide by high-tech industry with sophisticated fermenters is costly, particularly in the developing countries like Vietnam. In this research, low-tech industry to produce mycoherbides with rice husks has been used. This mycoherbicide was tested in the net house and rice field conditions to explore the possibility of using rice by-product to produce low-cost mycoherbicide for *in -situ* utilization in the future.

## MATERIALS AND METHOD

- Materials: Mycoherbicide in the form of kaolin was produced in advanced and kept in a refrigerator before utilization. PDA medium was used to propagate the spores of *Setosphaeria rostrata*. This mass of spores was washed away, filtered by thick cloth. The solution containing spores was mixed with kaolin powder and moisture was removed by fan during the process of evaporation in room temperature. The spore concentration in this formulation was $10^8$ spores $g^{-1}$. Rice husk mycoherbicide was produced by mass production of spores in the mixture of husk+ rice bran+maize powder (6:2:2). This substratum was sterilized in plastic bags at 120°C within 30 minutes. The sterilized mixture was cultured by *Setosphaeria rostrata* spores and kept for 20 days. After fungal multiplication with large amount of spores, the substrata will be dried by fan. The spore concentration in the husk mycoherbicide was approximately 2 x $10^6$ spores $g^{-1}$.

- Pot experiment: The experiment was conducted in pots with the diameter of 30cm. Four hills of rice var. IR64 was sown apart with the distance of 20cm with 5 pre-germinated seeds each. *Leptochloa chinensis* seedlings at one leaf stage were transplanted into pots with the density of 20 plants per pot in a cross shape with 10 plants each line. The design and treatments in pot experiment are similar with that of field one.

- Field experiment: Rice var.IR64 was sown in line by drum seeder at the rate of 100 kg. $ha^{-1}$. Five kilograms of weed seeds of *Leptochloa chinensis* were broadcasted randomly in the experimental field. Rice was sown on 29 May 2004 and harvested on 29 September 2004. The plot size was 5 $m^2$ (2.24 m x 2.24 m) with the surrounding bunds covered by plastic sheets. Randomized complete block design was used with 4 replications. Twelve treatments include: (i) untreated check, (ii) hand weeding twice at 28 and 35 DAS in the field experiment or weed free check in pot experiment, (iii) spraying kaolin mycoherbicide @$10^{12}$ spores $ha^{-1}$ once at 12 DAS, (iv) spraying kaolin mycoherbicide @$10^{13}$ spores $ha^{-1}$ once at 12 DAS, (v) broadcasting husk mycoherbicide @ $10^{10}$ spores $ha^{-1}$ once at 12 DAS, (vi) broadcasting husk mycoherbicide @ $10^{11}$ spores $ha^{-1}$ once at 12 DAS, (vii) broadcasting husk mycoherbicide @ $10^{12}$ spores.$ha^{-1}$ once at 12 DAS, (viii) broadcasting husk mycoherbicide @ $10^{13}$ spores.$ha^{-1}$ once at 12 DAS, (ix) broadcasting husk mycoherbicide @ $10^{10}$ spores $ha^{-1}$ twice at 12 and 17 DAS, (x) broadcasting husk mycoherbicide @ $10^{11}$ spores $ha^{-1}$ twice at 12 and 17 DAS,(xi) broadcasting husk mycoherbicide @ $10^{12}$ spores $ha^{-1}$ twice at 12 and 17 DAS, (xii) broadcasting husk mycoherbicide @$10^{13}$ spores $ha^{-1}$ twice at 12 and 17 DAS.

Data to be collected were: Number of infected leaves at different stages, dead weed plants at different stages, weed and rice dry weight, rice phytotoxicity, yield components and grain yield of rice. The weed control efficiency in A treatment (%) = [dry weight of weeds under untreated check - dry weight of weeds under A treatment] divided by [dry weight of weeds under untreated check] and multiply with 100.

All data were analysed by IRRISTAT and SAS programs.

## RESULTS AND DISCUSSION

The number of infected leaves of *Leptochloa chinensis* were counted at 3, 7, 14, 21, 35 and 42 days after mycoherbicide application (DAA). Those numbers were calculated in percentage and presented in the table 1.

Table 1: Percentage of infected *Leptochloa chinensis* leaves (%) as affected by treatments at 3, 7, 14, 21, 35 and 42 DAA.

| Treatments | Leaf infection (%) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 7 | 14 | 21 | 35 | 42 |
| T1) Untreated check | 0.0i (*) | 0.0i | 0.0h | 0.0e | 0.0d | 0.0d |
| T2) Weed free check | 0.0i | 0.0i | 0.0h | 0.0e | 0.0d | 0.0d |
| T3) *S.rostrata* (Kao.)@$10^{12}$ | 73.4c | 91.0b | 98.5a | 100.0a | 100.0a | 100.0a |
| T4) *S.rostrata* (Kao.) @ $10^{13}$ | 85.8a | 98.2a | 100.0a | 100.0a | 100.0a | 100.0a |
| T5)*S. rostrata* (Husk) @$10^{10}$ | 33.3f | 29.5f | 22.7e | 14.0c | 3.3d | 0.8cd |
| T6) *S.rostrata* (Husk)@$10^{11}$ | 38.0e | 32.7f | 22.4e | 12.9c | 3.5c | 1.3c |
| T7) *S.rostrata*(Husk)@$10^{12}$ | 56.9d | 77.6d | 89.7c | 100.0a | 100.0a | 100.0a |
| T8)*S.rostrata*(Husk)@$10^{13}$ | 79.8b | 96.0a | 100.0a | 100.0a | 100.0a | 100.0a |
| T9)*S.rostrata*(Husk)@$10^{10}$.twice | 11.8h | 8.5h | 5.7g | 0.0e | 0.0d | 0.0d |
| T10)*S.rostrata*(Husk)@$10^{11}$.twice | 21.9g | 17.2g | 10.6f | 3.4d | 0.7d | 0.0d |
| T11)*S.rostrata*(Husk)@$10^{12}$.twice | 39.8e | 43.5e | 52.8d | 54.9b | 58.0b | 58.3b |
| T12)*S.rostrata*(Husk)@$10^{13}$.twice | 76.6bc | 85.2c | 93.2b | 100.0a | 100.0a | 100.0a |
| | ** | ** | ** | ** | ** | ** |
| CV% | 6.8 | 6.3 | 4.7 | 3.4 | 2.4 | 1.6 |

Remark: (*) Means in the same column followed by the same letter are not significantly different ($P<0.01$) according to Duncan Multiple Range Test.

The data showed that if at 3 DAA the level of infection was high, the incidence of disease tended to increase at later stage. Those were in the cases of treatments T3, T4, T7, T8, and T12. In contrast, the ineffective treatments with lower percentage of infection at 3DAA, the weed *Leptochloa chinensis* tended to recover at later stage resulting in less severity caused by the fungus. Those such treatments were: T5, T6, T9 and T10. The data of typical treatment T5 observed at 3, 7, 14, 21, 35, 42 DAA were 33.3; 29.5; 22.7; 14.0; 3.3 and 0.8% respectively. Low doses of spore concentration at the level of $10^{10}$ and $10^{11}$ spores.ha$^{-1}$ in both methods of application once or twice were not effective in leaf infection. At 42 DAA, the percentage of leaf infection were 0.8; 1.3; 0.0 and 0.0% under treatments of T5, T6, T9 and T10 respectively. Application twice had the lower efficacy than those of once. The comparison between treatments T7 and T9 was the evidence. Under T7, the infected leaf percentages were 56.9; 77.6; 89.7; 100.0; 100.0; 100.0% at 3,7,14, 21, 35 and 42 DAA. All treatments at the rates of $10^{12}$ and $10^{13}$ spores.ha.$^{-1}$ had the same performance of weed control except T11.

+ Phytotoxicity on rice plants:

The phytotoxicity levels caused by mycoherbicides on rice plant var. IR 64 were presented in table 2.

Table 2: Percentage of infected rice leaves (%) as affected by treatments observed at 3,7,14,21 and 35 DAA.

| Treatments | Leaf infection (%) | | | | |
|---|---|---|---|---|---|
| | 3 | 7 | 14 | 21 | 35 |
| T1) Untreated check | 0.0e (*) | 0.0c | 0.0c | 0.0c | 0.0b |
| T2) Weed free check | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T3) *S.rostrata* (Kao.)@$10^{12}$ | 35.2b | 19.1b | 8.1b | 2.1b | 0.0b |
| T4) *S.rostrata* (Kao.)@$10^{13}$ | 55.5a | 31.3a | 14.1a | 6.3a | 1.1a |
| T5) *S.rostrata* (Husk)@$10^{10}$ | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T6) *S.rostrata* (Husk)@$10^{11}$ | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T7) *S.rostrata* (Husk)@$10^{12}$ | 5.4d | 1.6c | 0.0c | 0.0c | 0.0b |
| T8) *S.rostrata* (Husk)@$10^{13}$ | 13.6c | 3.8c | 1.1c | 0.0c | 0.0b |
| T9)*S.rostrata*(Husk)@$10^{10}$twice | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T10)*S.rostrata*(Husk)@$10^{11}$twice | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T11)*S.rostrata*(Husk)@$10^{12}$twice | 0.0e | 0.0c | 0.0c | 0.0c | 0.0b |
| T12)*S.rostrata*(Husk)@$10^{13}$twice | 8.2d | 2.3c | 0.0c | 0.0c | 0.0b |
| | ** | * | ** | ** | ** |
| CV(%) | 35.0 | 64.4 | 15.1 | 12.5 | 35.8 |

Remark:(*) Means in the same column followed by the same letter are not significantly different (P<0.01) according to Duncan Multiple range Test.

The kaolin form [T3, T4] had higher level of phytotoxicity on rice plants as compared to that of husk form. Rice plants tend to recover very soon after application and at 42DAA, rice plants under all treatments were normal except that under treatment T4 with 1.1% leaf infection.

+ Dead plants of *Leptochloa chinensis*:

The data on percentage of Leptochloa chinensis dead plants affected by treatments were counted at 3, 7, 14, 35 and 42 DAA. These data were shown in table 3.

Table 3: Percentage of dead plants of *Leptochloa chinensis* (%) as affected by treatments at 3, 7, 14, 35 and 42 DAA.

| Treatments | Dead plants (%) | | | | |
|---|---|---|---|---|---|
| | 3 | 7 | 14 | 35 | 42 |
| T1)Untreated check | 0.0f | 0.0g | 0.0g | 0.0f | 0.0e |
| T2)Weed free check | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a |
| T3)*S.rostrata*(Kao.)@$10^{12}$ | 76.8b | 85.4b | 92.9b | 98.9ab | 100.0a |
| T4)*S.rostrata*(Kao.)@$10^{13}$ | 94.0a | 99.9a | 100.0a | 100.0a | 100.0a |
| T5)*S.rostrata*(Husk)@$10^{10}$ | 0.0f | 7.3h | 0.0g | 0.0f | 0.0e |
| T6)*S.rostrata*(Husk)@$10^{11}$ | 0.0f | 4.5f | 5.6f | 8.7e | 8.8d |
| T7)*S.rostrata*(Husk)@$10^{12}$ | 45.3e | 77.9d | 75.8d | 90.2c | 91.3b |
| T8)*S.rostrata*(Husk)@$10^{13}$ | 70.0c | 85.4b | 97.8a | 100.0a | 100.0a |
| T9)*S.rostrata*(Husk)@$10^{10}$twice | 0.0f | 0.0g | 0.0g | 0.0f | 0.0e |
| T10)*S.rostrata*(Husk)@$10^{11}$twice | 0.0f | 0.0g | 0.0g | 0.0f | 0.0e |
| T11)*S.rostrata*(Husk)@$10^{12}$twice | 0.9f | 10.4c | 14.9e | 16.1d | 16.1c |
| T12)*S.rostrata*(Husk)@$10^{13}$twice | 63.0d | 79.4c | 85.3c | 97.3b | 99.9a |
| | ** | ** | ** | ** | ** |
| CV (%) | 6.2 | 5.2 | 4.2 | 3.0 | 1.5 |

Remark: (*) Means in the same column followed by the same letter are not significantly different (P<0.01) according to Duncan Multiple Range Test.

The data on percentage of *Leptochloa chinensis* dead plants affected by treatments were counted at 3, 7, 14, 35 and 42 DAA. Data showed that at 3DAA, there were only four treatments which had the weed dead percentage more than 50%. Those were T3 (76.8%), T4 (94.0%), T8 (70.0%) and T12 (63.0%). The level of infection increased over time. Low level of spore concentration ($10^{10}$ and $10^{11}$ spores.ha$^{-1}$) had no effect on weed control. Those were in the case of treatments T5, T6, T9 and T10. Two forms of mycoherbicides either kaolin or rice husk had the same performance. Application twice had lower efficacy than that of once. The highest weed control efficiency were under T3 (100%), T4 (100%) and T8 (100%) followed by T12 (99.9%) and T7 (91.3%).

+Weed and rice dry weight:

The dry weight of *Leptochloa chinensis* and rice var. IR64 at harvest were presented in table 4.

Table 4: Dry weight of weed and rice at harvest (g.pot$^{-1}$) as affected by treatments.

| Treatments | Dry weight (g. pot$^{-1}$) | |
|---|---|---|
| | *Leptochloa chinensis* | Rice |
| T1)Untreated check | 237.0a(*) | 49.5f |
| T2)Weed free check | 0.0d | 252.5bc |
| T3)*S.rostrata*(Kao.)@$10^{12}$ | 0.0d | 252.0bc |
| T4)*S.rostrata*(Kao.)@$10^{13}$ | 0.0d | 267.0a |
| T5)*S.rostrata*(Husk)@$10^{10}$ | 230.5a | 81.3e |
| T6)*S.rostrata*(Husk)@$10^{11}$ | 192.3c | 95.5d |
| T7)*S.rostrata*(Husk)@$10^{12}$ | 15.5d | 250.3bc |
| T8)*S.rostrata*(Husk)@$10^{13}$ | 0.0d | 266.3bc |
| T9)*S.rostrata*(Husk)@$10^{10}$twice | 226.0ab | 59.8f |
| T10)*S.rostrata*(Husk)@$10^{11}$twice | 209.8bc | 81.5e |
| T11)*S.rostrata*(Husk)@$10^{12}$twice | 210.3bc | 88.5de |
| T12)*S.rostrata*(Husk)@$10^{13}$twice | 4.8d | 261.3ab |
| | ** | ** |
| CV(%) | 12.5 | 4.4 |

Remark: (*) Means in the same column followed by the same letter are not significantly different (P<0.01) according to Duncan Multiple Range Test.

There was a negative relationship between weed and rice biomass. High level of weed dry weight, high weed competition resulted in low dry matter accumulation by rice due to weed-crop competition. Dry weight of weed was brought down significantly as compared to untreated check except treatments at low doses of spores (T5 and T9). *Leptochloa chinensis* was completely killed by treatments T3, T4 and T8. The other treatments T7 and T12 had the same level of weed control with those three treatments but some plants of weed remained. In case of biological yield of rice, all treatments caused the increment of rice yield as compared to untreated check except the treatment of low dose applied twice (T9). The highest rice yields were under T4 (267 g. pot$^{-1}$), T8 (266.3 g. pot$^{-1}$) and T12 (261.3 g. pot$^{-1}$). Some other treatments such as T2 (252.5 g. pot$^{-1}$), T3 (252.0 g. pot$^{-1}$) and T7 (250.3 g. pot$^{-1}$) had the same yield statistically but lower than those of T4, T8 and T12.

+ Yield components and grain yield:

The data on yield components and rice grain yield were presented in table 5.

Table 5: Yield components and grain yield (T.ha$^{-1}$) as affected by treatments.

| Treatments | Panicle length (cm) | No. tillers per hill | No. grain per panicle | Unfilled grain percentage | 1000 grain weight | Yield |
|---|---|---|---|---|---|---|
| T1)Untreated check | 20.9e (*) | 6.0d | 14.9h | 71.3a | 17.6f | 2.24h |
| T2)Weed free check | 27.6a | 7.5a | 40.9a | 37.8g | 26.8a | 5.99a |
| T3)*S.rostrata*(Kao.)@$10^{12}$ | 26.8abcd | 6.9abc | 35.9c | 36.0e | 22.7bc | 4.44d |
| T4)*S.rostrata*(Kao.)@$10^{13}$ | 26.0d | 7.1ab | 40.3a | 34.4f | 23.6b | 5.78e |
| T5)*S.rostrata*(Husk)@$10^{10}$ | 26.2cd | 6.2d | 18.3g | 46.7b | 20.9de | 3.59e |
| T6)*S.rostrata*(Husk)@$10^{11}$ | 26.9abcd | 6.4cd | 25.8e | 43.9c | 22.1cd | 4.37d |
| T7)*S.rostrata*(Husk)@$10^{12}$ | 26.9abcd | 7.0ab | 37.2b | 36.7e | 22.7bc | 5.03c |
| T8)*S.rostrata*(Husk)@$10^{13}$ | 27.4ab | 7.0ab | 38.1b | 33.2fg | 23.4bc | 6.03a |
| T9)*S.rostrata*(Husk)@$10^{10}$twice | 26.4bcd | 6.5bcd | 15.7h | 46.9b | 20.4e | 2.68g |
| T10)*S.rostrata*(Husk)@$10^{11}$twice | 26.2cd | 6.6bcd | 21.3f | 45.7b | 21.2de | 3.23f |
| T11)*S.rostrata*(Husk)@$10^{12}$twice | 26.7abcd | 6.6bcd | 31.7d | 41.4d | 21.3de | 4.14d |
| T12)*S.rostrata*(Husk)@$10^{13}$twice | 27.1abc | 6.9abc | 37.7b | 36.2e | 22.6bc | 5.40b |
| | ** | ** | ** | ** | ** | ** |
| CV (%) | 2.7 | 6.3 | 2.9 | 2.0 | 4.2 | 5.58 |

Remark: (*) Means in the same column followed by the same letter are not significantly different (P<0.01) according to Duncan Multiple Range Test.

The variation in panicle length, number of panicle per hill, number of filled grains per panicle, unfilled grain percentage, 1000-grain weight were measured and analysed statistically. All these components would contribute to the final performance of grain yield. Rice yields varied from 2.24 T.ha$^{-1}$ to 6.03T.ha$^{-1}$. Yields under all treatments were increased statistically as compared to untreated check (2.24 T.ha$^{-1}$). The highest yield was under T8 (6.03 T.ha$^{-1}$) which was equal with weed free check T2 statistically (5.99 T.ha$^{-1}$) followed by T12 (5.40 T.ha$^{-1}$). The next reasonable treatments were T4 (5.78 T. ha$^{-1}$) and T7 (5.03 T. ha$^{-1}$) which were equal statistically among themselves. The treatments T3 (4.44 T.ha$^{-1}$), T6 (4.37 T.ha$^{-1}$) and T11 (4.14 T.ha$^{-1}$) had the same yield. Yields of the rest of treatments were very low due to very high competition from *Leptochloa chinensis*.

+ Results in pot and field experiment: Weed dry weight (g.m$^{-2}$), weed control efficiency (%), rice yield (T. ha $^{-1}$) as affected by treatments were presented in table 6.

In case of net house, weed control efficiency reached 100% under T2, T3, T4 and T8. The other treatments which had the weed control efficiency higher than 90% include T7 (93.5%) and T12 (98.0%). Generally, weed control efficiency in the field condition is lower than that of net house. Only one treatment [T2] with weed control efficiency of 100%. The other treatments with high weed control efficiency are T4 (99.3%) and T8 (99.3%). All weed control treatment were superior in terms of rice yield to untreated check significantly except T5 (2.19T ha$^{-1}$) in the field condition. In pot experiment, rice yield under treatment T4 (5.78T ha $^{-1}$), T8 (6.03 T. ha$^{-1}$) were similar with that of weed free check T2 (5.99T ha$^{-1}$) respectively. However, in the field condition, treatment T7 (4.13 T ha$^{-1}$) and T8 (4.40 T.ha$^{-1}$) had the same yield with weed free check T2 (4.44T ha$^{-1}$)

Table 6: Weed dry weight (g.m$^{-2}$), weed control efficiency (%), and rice yield (T.ha$^{-1}$) as affected by treatments in net house and field conditions.

| Treatments | Weed dry weight | | Weed control efficiency | | Yield | |
|---|---|---|---|---|---|---|
| | Net house | Field | Net house | Field | Net house | Field |
| T1)Untreated check | 3,385.7a | 188.5a | 0.0 | 0.0 | 2.24h | 1.68f |
| T2)Weed free check | 0.0d | 0.0c | 100.0 | 100.0 | 5.99a | 4.44a |
| T3)S.rostrata(Kao.)@1012 | 0.0d | 38.3d | 100.0 | 79.7 | 4.44d | 3.67c |
| T4)S.rostrata(Kao.)@1013 | 0.0d | 1.3e | 100.0 | 99.3 | 5.78a | 3.80c |
| T5)S.rostrata(Husk)@1010 | 3,292.9a | 191.2a | 2.7 | -1.4 | 3.59e | 2.19ef |
| T6)S.rostrata(Husk)@1011 | 2,747.1c | 155.2b | 18.9 | 17.7 | 4.37d | 2.90d |
| T7)S.rostrata(Husk)@1012 | 221.4d | 42.6d | 93.5 | 77.4 | 5.03c | 4.13abc |
| T8)S.rostrata(Husk)@1013 | 0.0d | 1.4c | 100.0 | 99.3 | 6.03a | 4.40ab |
| T9)S.rostrata(Husk)@1010twice | 3,228.6ab | 159.4b | 4.6 | 15.4 | 2.68g | 2.57de |
| T10)S.rostrata(Husk)@1011twice | 2,997.1bc | 148.0b | 11.5 | 21.5 | 3.23f | 2.49de |
| T11)S.rostrata(Husk)@1012twice | 3,004.3bc | 116.4c | 11.3 | 38.2 | 4.14d | 2.73de |
| T12)S.rostrata(Husk)@1013twice | 68.6d | 39.7d | 98.0 | 78.9 | 5.40b | 3.84bc |
| | ** | | ** | | ** | ** |
| CV (%) | 12.5 | | 20.5 | | 5.68 | 12.44 |

Remark (*): Means in the same column followed by the same letter are not significantly different (P<0.01) according to Duncan Multiple Range Test.

## CONCLUSION

Rice husks are plenty in the Mekong delta of Vietnam at the rice mills after milling. These materials can be used as fuel during the process of burning and other purposes. However, one attempt has been made to use rice husk as floating substrata to carry fungal spores of *Setosphaeria rostrata* to control *Leptochloa chinensis* in rice fields. Results in this pot experiment showed that it is possible. The combined substrata of rice husk + bran+ maize powder in sterilized condition were used to multiply fungal spores. During the process of application, some spores drop onto weed leaves. The other spores move along rice husks and infect the shoots of weeds. This form of mycoherbicide caused the same infection as compared with kaolin form of mycoherbicide at the same rate. Application one time at the same rate had higher efficacy as compared to that of twice both in pot and field conditions. This technology is promising and can be continued to do research on the field condition in the future.

# The role of golden apple snail, *Pomacea canaliculata* (Lamarck) in paddy weeding in lowland irrigated transplanted rice ecosystems: Experiences from the Philippines

E. C. Martin[1], R. C. Joshi[1], T. Wada[2], A. R. Martin[1], L. S. Sebastian[1]
[1]Department of Agriculture-Philippine Rice Research Institute (DA-PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija, 3119, Philippines
rcjoshi@philrice.gov.ph, joshiraviph@yahoo.com
[2]National Agriculture Research Center for Kyushu Okinawa Region (KONARC), 2421 Suya, Nishigoshi, Kikuchi, Kumamoto 861-1192, Japan
twada@affrc.go.jp

**Abstract:** The golden apple snail (GAS), *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae), is a top pest of rice in many Asian rice farms. The farmers' first line of defense is to use non-specific chemicals to have "instant" kill of GAS, without any concern on the environment, their health, and its effects on non-target organisms. Some organic rice farmers in Korea and Japan, however, do not kill GAS but employ them to feed on aquatic weeds in rice fields and thus save expenses on herbicides. We evaluated the innovation of Korean and Japanese farmers at the PhilRice Central Experimental Station (CES) fields using large-sized fields (each 0.25 ha). We also demonstrated the GAS paddy weeding effects in several farmers' fields in villages of Nueva Ecija, Aurora, and Negros Occidental provinces during the 2003 and 2004 dry and wet seasons. We also provided insights on how to use resident (field) GAS populations for weed control.

**Key words**: Golden apple snail, invasive alien species, lowland irrigated transplanted rice, *Pomacea canaliculata*, weed density, weed management

## INTRODUCTION

The golden apple snail (GAS), *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae), native to Argentina and known for having a high nutritive value as food for human beings and farm animals, was introduced in the Philippines and several Asian countries in the 1980's (Mochida 1987; Joshi et al. 2003; Joshi 2005). Few years after its introduction, rice farmers experienced GAS damage. The damaged young rice seedlings results in poor crop stand and yield losses in fields where shallow water depth is not maintained after transplanting. In addition, the use of various formulations of non-specific molluscicides and misuse of pesticides cause environmental problems (Dancel and Joshi 2000). Six years after its introduction in the Philippines, GAS invaded about 3.6% of the total area planted to rice (Rejesus et al. 1988). Many organic farmers in Japan and Korea use GAS for paddy weeding (Wada et al. 2002) without using herbicides. The relationship between rice farmers and GAS has evolved into a symbiotic one, thus conserving the non-target aquatic faunal diversity. The benefits of utilizing GAS as bio-weeder far exceeds those obtained with the utilization of ducks, carps or shrimps for paddy weeding (Yusa et al. 2003). This paper demonstrates the efficacy of GAS as paddy weeder in the lowland irrigated transplanted rice fields.

## MATERIALS AND METHODS

Large sized field experiments were conducted in the Central Experiment Station (CES) of the Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz, Nueva Ecija, and in farmers fields in Bantug, Muñoz, Nueva Ecija, Dingalan, Aurora and Lacaron, Valladolid, Negros Occidental, Philippines from 2003 to 2004 to demonstrate the weed control efficiency of GAS in lowland irrigated transplanted rice cultivation system and show how to avoid unnecessary spending on synthetic molluscicides and herbicides. The popular inbred rice variety, IR64, was used during 2003 wet and dry seasons and two hybrid rice varieties, Mestizo 1 and Mestizo 3, were used during 2004 dry and wet seasons at PhilRice. Mestizo 1 and PSB Rc82 were used during 2003 and 2004 dry season and wet seasons at Bantug. In Dingalan and Valladolid, IR64 and PSB Rc10 were used respectively during 2004 WS. Weed density and species were observed during the critical early stages of the rice crop.

Seedbed preparation. An area of 400 $m^2$ was prepared for seedbed. The raised seedbed with dimensions of 2 m wide and 10 m long, was prepared for easy draining of water. Carbonized rice hull (CRH) was applied just above the seedbed two days before seeding. With saturated water level and no inorganic fertilizer, the seedlings' leaves were erect and pale, with hard culm, making it difficult for GAS to damage them.

Field preparation and treatments. In 2003 and 2004 cropping, 0.5 ha field was divided into two plots. One plot was applied with Niclosamide 250 EC (synthetic commercial molluscicide) to kill all GAS present in the field prior to transplanting. No molluscicide application was done in the other plot. Both fields were prepared thoroughly and leveled well to maintain shallow depth of water to minimize GAS damage to newly transplanted seedlings. No herbicide was applied in both fields. Twenty-one day-old seedlings were transplanted at 20 cm x 20 cm between hills and rows with 2 seedlings $hill^{-1}$. The area was kept saturated and allowed to dry up slowly to enhance weed emergence. Water was introduced to 2 cm depth at 6-8 days after transplanting (DAT) or when weeds had reached 1-2 leaf stage.

## RESULT AND DISCUSSION

The weed species identified in all plots were grasses (*Echinochloa* spp. and *Leptochloa chinensis*), sedges (*Cyperus* spp. and *Fimbrystylis miliacea*), and broadleaves (*Ludwigia octovalvis,* and *Sphenoclea zeylanica)*. Weed density was recorded in 1.5 $m^{-2}$ at 10 DAT was higher in the plot with GAS than in the plot without GAS during the dry season (DS) 2003. This is because water was not yet introduced in the plots at that time (Fig. 1A). However, after water introduction, weed densities at 30 and 45 DAT from plot with GAS was much lower than in plots without GAS. This implies that re-entry of water is an important factor in paddy weeding. When there is water, snails move around; if there is no water, they simply bury themselves in the soil. No water is added into the field right after transplanting and maintained at saturated condition for several days to enhance weed growth. After the weeds have sprouted and grown to 1-2 leaf stage, water re-entry is done. The snails then come out from hibernation underground to look for food. During the wet season (WS) 2003, higher weed density was recorded in plots without GAS starting from 15 to 45 DAT (Fig. 1B). Higher weed density beginning from 15 until 45 DAT was recorded in plot without GAS during 2004 DS and same trend was noted during WS 2004 (Figure 1A-B). Moreover, higher rice grain yield for both WS and DS of 2003 and 2004 was recorded in plot with GAS than in plot without GAS (Table 1).

Table 1. Rice grain yield at PhilRice-CES, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines, 2003-2004 cropping seasons.

| Year / Season | Variety | Grain yield (t $ha^{-1}$) | |
|---|---|---|---|
| | | With GAS | Without GAS |
| 2003 DS | IR 64 | 7.3 | 5 |
| 2003 WS | IR 64 | 5 | 4.6 |
| 2004 DS | Mestizo 1 | 7.65 | 6.26 |
| 2004 WS | Mestizo 3 | 5.52 | 3.12 |

Paddy weeding experiments were also conducted in farmers' fields in different locations from 2003-2004 wet and dry seasons (Table 2). Results showed that higher weed density was noted in plot without GAS as compared in plot with GAS. Rice grain yield for both wet and dry seasons of 2003-2004 was higher in plot with GAS and subsequently had lower weed seed weight than in plot without GAS, which have lower rice grain yield and higher weed seed weight. This implies that more weed seeds were deposited in plots without GAS thus weed seeds are carried over to the next cropping season. This may result in increased weed density in the field over time. Similar trends were observed for all the farmers' fields in both seasons. These field evaluations provide insights on how to use GAS as paddy weeder in lowland irrigated transplanted rice ecosystems. With this GAS management technique, we are converting a pest in lowland irrigated transplanted rice systems to become an ally of the farmer by altering its feeding behavior. Although land preparation is similar to the recommended practice, it is necessary to level the field well to control water depth as well as the movement of GAS. Shallow water depth alters the feeding behavior and movement of GAS. It is recommended that seedlings must be 21 days old, sturdy and have at least three leaves. In all plots, prior to transplanting, the average resident GAS size was between 15-20 mm, with a density of 10 $m^{-2}$. We recorded a maximum of 5.6% missing hills in our first trial at PhilRice-CES. In all subsequent trials we recorded less than 1% missing hills. This practice is not appropriate for direct-seeded rice as rice and weeds germinate at the same time. It cannot be done on rice field environments where water depth is difficult to maintain. This practice discourages strictly farmers to collect GAS and put them in their rice fields, because if the water depth is less than half the height of the GAS shell, the GAS buries inside the soil. In such a condition GAS cannot feed.

Just like Japanese and Korean farmers who do not spray chemicals to kill GAS in their fields to practice organic farming, Filipino farmers can also reduce the total cost of rice production by managing GAS. This is the first report to demonstrate that paddy weeding by GAS is possible even in the tropical paddy fields.

Table 2. Weed density at farmer's fields in experimental sites in the Philippines, 2003-2004 cropping seasons.

| Year / Season | Village | Variety | Weed density 1.5 $m^{-2}$ | | | | | | | | Rice grain yield ($t\ ha^{-1}$) | | Weed seed weight ($t\ ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | With GAS | | | | Without GAS | | | | | | | |
| | | | DAT | | | | DAT | | | | With GAS | Without GAS | With GAS | Without GAS |
| | | | 15 | 30 | 45 | 60 | 15 | 30 | 45 | 60 | | | | |
| 2003 DS | Bantug | Mestizo 1 | - | 14 | 167 | - | - | 167 | 146 | - | 9.3 | 7.7 | - | - |
| 2003 DS | Bantug | Mestizo 1 | - | 11 | 180 | - | - | 180 | 106 | - | 8.75 | 7.18 | - | - |
| 2004 WS | Bantug | PSB Rc 82 | 0 | 0 | 0 | 0 | 0 | 73 | 37 | 39 | 5.95 | 5.9 | 0 | 0.53 |
| 2004 WS | Bantug | PSB Rc82 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 5.12 | 4.9 | 0 | 0.5 |
| 2004 WS | Bantug | PSB Rc14 | 0 | 0 | 0 | 0 | 132 | 170 | 108 | 65 | 7.21 | 4.67 | 0 | 2.95 |
| 2004 WS | Bantug | PSB Rc82 | 2 | 0 | 0 | 0 | 58 | 100 | 59 | 36 | 4.38 | 4.08 | 0 | 0.83 |
| 2004 WS | Bantug | PSB Rc82 | 0 | 0 | 0 | 0 | 0 | 27 | 26 | 37 | 5.89 | 4.87 | 0 | 2.08 |
| 2004 WS | Caraksacan | IR 64 | 0 | 0 | 0 | 0 | 0 | 16 | 22 | 10 | 8.3 | 6.6 | - | - |
| 2004 WS | Lacaron | PSB Rc-10 | 0 | 0 | 0 | 0 | † | † | † | † | 3.5‡ | 2.9‡ | - | - |

(-) No available data. (†) Weeds not counted because of low density due to flooding, (‡) Affected by rice tungro virus disease.

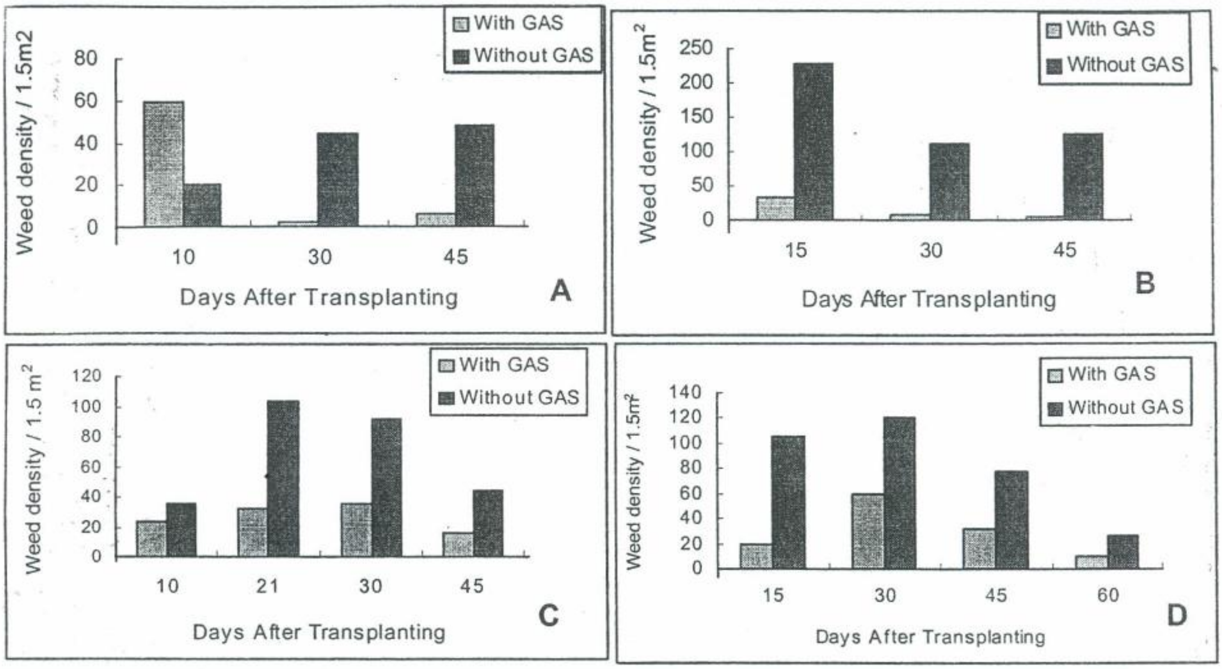


Figure 1. Weed density from 10 to 60 DAT during the 2003 (A&B) and 2004 (C&D), Dry and Wet Seasons, respectively, PhilRice-CES.

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# Integrated bio-control of water hyacinth (*Eichhornia crassipes*) using plant product and insects

R.M. Kathiresan* and I. Gnanavel
Department of Agronomy, Annamalai University, Annamalainagar
Tamilnadu, INDIA – 608 002
rm.kathiresan@sify.com

**Abstract:** Classical bio-control of *Eichhornia crassipes* using insect agents is constrained in many of the tropical watershed environs with interrupted host range due to seasonal water flow and complete drying of water during the hot summer months. Accordingly, the need for reinforcing the classical bio-control approach with sustainable short-term measures has been realized and studies were done to explore the possibility of integrating the plant product of dried leaf powder of *Coleus amboinicus / aromaticus,* that was shown to be extremely allelopathic on water hyacinth, with established insect agents *Neochetina eichhorniae / bruchi.* Earlier studies showed that the plant product was absorbed through the roots causing death of the weed within 5 h of application to the water body, and imparted fresh weight reduction of the weed within nine days. However the dose requirement was high with 20g $L^{-1}$ of water and foliar spray of the plant product was in vain, as the absorption of the plant product was curtailed by the cuticular barrier. The present investigation attempted to integrate the plant product with insect agents *N. eichhorniae / bruchi.* Two different sequences of integration, viz., application of plant product to water body first followed by the release of insect agents on to the weed, and release of the insects first on the weed followed by spraying of the plant product on the weed, were compared. The first sequence of plant product-insects failed to produce additive response as the insects migrated to untreated healthy plants. However, the second one with the sequence of insects-foliar spray of plant product showed additive or synergistic response with efficient control of water hyacinth with in a season. The approach also proved safe in terms of water quality and fish growth. The plant product was also compatible with insect agents without causing any histopathological injuries.

**Key words**: Bio-control, integration, insect agents, plant product, Water hyacinth.

## INTRODUCTION

The aquatic weed water hyacinth, (*Eichhornia crassipes* (Mart.) Solms - Laubach; Pontederiaceaca), is an erect, free floating, stoloniferous and perennial herb. Excessive infestations deleteriously affect water traffic, water quality, and infrastructure for pumping and hydro electricity generation, water use, and bio-diversity. Other problems include water loss due to evapo-transpiration and an increase in the populations of vectors of human and animal diseases.

In Tamilnadu state, India, the Veeranum Lake and its distributaries form the major irrigation source that covers a larger proportion of the rice tract of the state with a command area of 18,000 ha. This lake and its distributaries in recent days have been infested with *E. crassipes.* Frequent mechanical removal of this weed is highly expensive, labor intensive, and time consuming. Chemical herbicides, even though effective are not popular because of their high cost and pollution hazards. Biological control using insect agents, though accepted to be the only sustainable option, does not eliminate the weed and even after its implementation, large quantities of water hyacinth biomass frequently remain in water bodies. Biological control requires a minimum of several years, usually 3 to 5 years, for the insect population to increase to a density where the weed is in substantial decline (Harley et al. 1996). Further, the success of biological control depends on the availability of a continued range of weed host.

In India most of the water bodies are constrained with seasonal water flow and interrupted host range, as weed dries off in hot summer. Accordingly, there are two ways to improve the effectiveness of biological control of water hyacinth, (i) by multiplying the insects in large numbers and releasing these at an inoculation load adequate to eliminate the weed within a single season, but is impracticable (ii) through augmenting the effects of the existing agents by integrating with other short term control methods.

Research at Annamalai University regarding the allelopathic potential of native plants on water hyacinth has shown that dried leaf powder of *Coleus amboinicus / aromaticus* applied @ 25 g $L^{-1}$ to the water body is absorbed by the roots of water hyacinth and kills the weed with in 24 h, imparting a near cent per cent fresh weight reduction with in one week. Further, if introduced on the leaves of weed, the plant product is active at 0.1 g $L^{-1}$ dose (Kathiresan 2000). Based on the above facts, the studies were conducted to explore the possibility of integrating the product of dried leaf powder of *C. amboinicus / aromaticus*, with established insect bio-control agents *Neochetina eichhorniae / bruchi* to achieve control of water hyacinth with in a season.

## MATERIALS AND METHODS

The experiments were conducted at the Department of Agronomy, Faculty of Agriculture, Annamalai University, India. Two different sequences that are possible for integrating the bio-control tools were compared. The first sequence involves application of plant product into the water body with to weaken the weed plant and predispose it to rapid destruction by the insect agents later. The second sequence involves releasing the insect agents first followed by spraying the plant product on the weed canopy with the expectation that the absorption of plant product by the weed canopy could be assisted by the insect damage on the weed foliage.

For studying the efficiency of the first sequence, water hyacinth plants were taken in plastic containers of size 16 x 12 x 7 cm and treated with dried leaf powder of *C. amboinicus / aromaticus* at varying doses viz. 5, 10, 15, 20 and 25 g $l^{-1}$, in two sets. In one set of these plant product treated containers with water hyacinth, insect agents (*N. bruchi / eichhorniae*) were released @ 2 insects $plant^{-1}$ with in a couple of hours later, whereas the other set was kept as such. Another container with water hyacinth was released with the insect agent alone and one more was retained as an untreated control. These treatments were replicated five times and observed for fresh weight reduction at five days interval and reduction in chrolophyll content that were analysed under completely randomized block design for working out least significant difference with 0.05 % probability. The second sequence of a possible integration of both bio-control tools was studied by releasing the insect agents @ 2 $plant^{-1}$ on the water hyacinth plants in plastic containers and spraying the plant product at varying concentrations viz. 25, 20, 15, 10 and 5 per cent aqueous extracts. These treatments were compared with a treatment that accommodated a water hyacinth plant, released with insects alone with out plant product spray as main treatments of a split plot design with five replications. The sub-treatments of this experiment comprised the varying length of time lag or interlude between the release of insect agents and plant product spray viz. 10 Days After Insect Release (DAIR), 20 DAIR and 30 DAIR.

The observations included reduction in fresh weight and chlorophyll content. The migrational behavior of the insects as influenced by the integration was also studied using steel frames with closely knit gauze cloth accommodating two of the plastic containers with water hyacinth. In one of the containers with weeds, the different treatments pertaining to different sequences of integration were taken up and the other container with weeds was left free without any treatment. The movements of insect agents to the untreated plants remaining within the enclosure at stipulated intervals were recorded as the insects that migrated. The impact of these treatments on water quality, insects mortality and histopathology of fish were also studied from a different experiment with treatments comprising, water hyacinth alone, water hyacinth released with insects alone, water hyacinth with insects + 25 % plant product spray at 10 DAIR and another treatment where in the cement tanks of dimension 120 cm x 65 cm were accommodating fishes alone without either the weed or it's bio-control agents. In each treatment tank two fish fingerlings of five different species rohu, mrigal, catla, common carp and grass carp (ten fingerlings in total) were released. The histological studies on insect organs viz., cuticular membrane, fat body, foregut, midgut, hindgut, salivary gland and testis were undertaken to evaluate the impact of the plant product spray on the insect tissues. The standard technique described by Gurr (1959) was followed for histological studies. In all the experiments plant product spray was undertaken by soaking the plant product in water at respective dosages for 24 h and spraying the aqueous extract with required quantity of water to make a spray fluid of 500 L $ha^{-1}$, through a high volume hand compression sprayer fitted with flood jet deflector nozzle.

## RESULTS AND DISCUSSION

Although the study consist several experiments and different observations, few of them alone are discussed here considering space constraint, clarity, and precision. The approach or sequence of treating the water body first with plant product followed by the release of insect agents on the weed host failed to offer any additive or synergistic impact on the weed. The lethality observed on the weed, with a near full reduction in biomass of the weed at 10 DAT in the treatment comprising 25 g $L^{-1}$ of plant product followed by insects, could be solely attributed to the allelopathic injury suffered by the weed due to the plant product. It was more of a reflection on independent allelopathic effect of the plant product on the weed rather than an enhanced activity due to combined or integrated mode of injury by both the insect agents and plant product in other treatment involving lesser doses of plant product, viz., 20, 15 g $L^{-1}$ as well. In these treatments weed lethality and biomass reduction to non-traceable levels were observed over a prolonged duration of exposure (Table 1). The plant product interrupted membrane permeability and caused electrolyte leakage and root dysfunction, thereby leading to weed lethality and biomass reduction (Kathiresan 2000). Lack of additive or synergistic interaction between the plant product and insect agents in controlling the weed, particularly in this sequence of treating the water body first with plant product followed by release of insect agents, was due to migration of insect agents from treated partially dying plants to untreated healthy plants as their preferred choice of feed is not available in plants whose physiology is struck by the plant product, lacking electrolytes. This could be appreciated from higher insect migration of 86.66 % recorded with the plant product at 25 g $L^{-1}$ + insects, on 1 DAT itself.

However, the approach or sequence of releasing the insect agents first on the weed and spraying the plant product on the weed canopy later to imparted an additive or synergistic impact in controlling the weed. The integrated approach comprising the release of insect agents on the weed first followed by the foliar spray of 25 % plant product exhibited a higher degree of inhibition with cent per cent reduction in fresh weight on 60 DAIR (Table 2). By virtue of their feeding behavior, the insects imparted leaf scrapping, thereby removing the cuticular lining and exposing the inner soft parenchymatous tissue beneath. Scrapping enabled better absorption of the plant product sprayed on the foliage and thus, in addition to partially damaging the weed vigor and physical stature, the insects also served as a vehicle or penetrant that help absorption and translocation of the plant product. Further, the plant product did not deter the insects either by anti-feeding or repulsive mode as seen from the 20 % insect migration in treatment that included the plant product spray at the highest concentration tried. Among the length of interlude or time lag between release of insects and spraying of plant product compared, a gap of 10 days proved optimum. This is because at longer interludes of 20 and 30 days, the voracious insect feeding behavior resulted in severe destruction of the histology of the weed, which is essential for absorption and translocation of the plant product. Further once absorbed, the activity of the plant product was greater than the damage by insect feeding. As application of the plant product was delayed by another 10 or 20 days, ultimate control of the weed was also delayed.

Table 1. Impact of the integrated approach of treating water body with the plant product followed by the release of insect agents (percentage reduction in fresh weight of *E.crassipes*).

| Treatments | 1 DAT | 10 DAT | 20 DAT | 30 DAT |
|---|---|---|---|---|
| $T_1$ - Control | 0.01 (0.00) | 0.01 (0.00) | 0.01 (0.00) | 0.01 (0.00) |
| $T_2$ - 5 g plant product | 0.01 (0.00) | 0.01 (0.00) | 0.01 (0.00) | 0.01 (0.00) |
| $T_3$ - 10 g plant product | 13.05 (5.10) | 25.56 (18.61) | 33.80 (30.95) | 38.06 (38.00) |
| $T_4$ - 15 g plant product | 24.20 (16.80) | 50.93 (60.28) | 90.00 (100.00) | 90.00 (100.00) |
| $T_5$ - 20 g plant product | 32.08 (28.20) | 61.34 (77.00) | 90.00 (100.00) | 90.00 (100.00) |
| $T_6$ - 25 g plant product | 38.06 (38.00) | 90.00 (100.00) | 90.00 (100.00) | 90.00 (100.00) |
| $T_7$ - Insects alone | 8.13 (2.00) | 25.84 (19.00) | 33.83 (31.00) | 42.71 (46.00) |
| $T_8$ - 5 g plant product + Insects | 9.53 (2.80) | 27.97 (22.00) | 35.26 (33.33) | 44.46 (49.06) |
| $T_9$ - 10 g plant product + Insects | 13.25 (5.26) | 36.32 (35.08) | 54.92 (66.97) | 70.59 (88.96) |
| $T_{10}$ - 15 g plant product + Insects | 24.40 (17.07) | 51.98 (62.07) | 90.00 (100.00) | 90.00 (100.00) |
| $T_{11}$ - 20 g plant product + Insects | 32.12 (28.27) | 62.64 (78.88) | 90.00 (100.00) | 90.00 (100.00) |
| $T_{12}$ - 25 g plant product + Insects | 38.29 (38.40) | 90.00 (100.00) | 90.00 (100.00) | 90.00 (100.00) |
| $SE_D$ | 1.81 | 1.01 | 1.32 | 1.35 |
| CD | 3.66 | 2.03 | 2.65 | 2.70 |

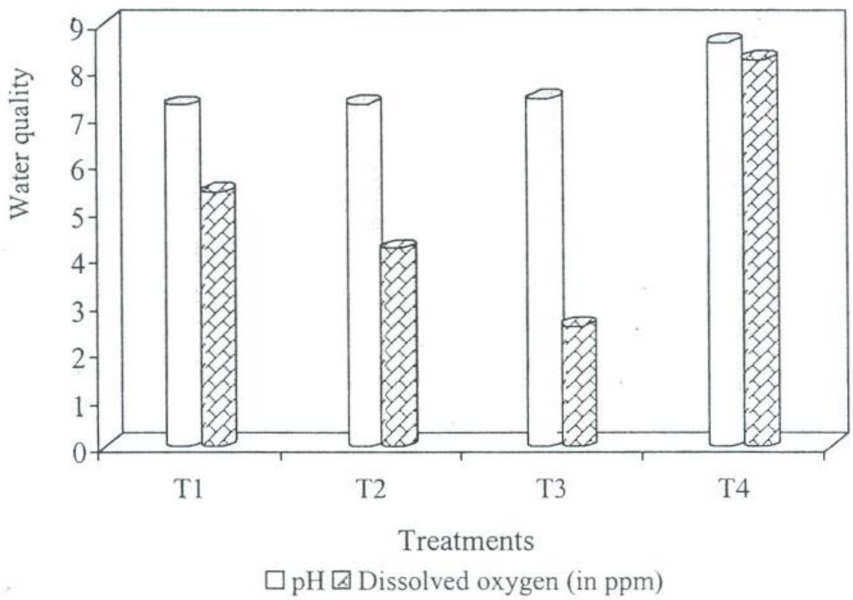
Figures in parentheses are original values
DAT – Days after treatment

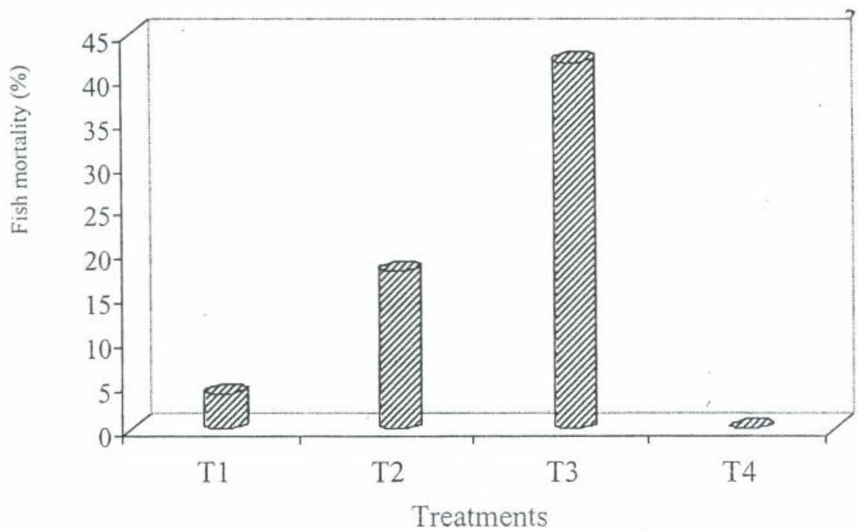
Table 2. Impact of the integrated bio-control involving the use of insect agents followed by foliar spray of plant product at different interludes on percentage reduction in fresh weight of *E. crassipes*

| Treatments | 10 DAIR | 30 DAIR | 60 DAIR | 90 DAIR |
|---|---|---|---|---|
| Main treatments | | | | |
| $M_1$ – 25% plant product spray | 28.54 (22.54) | 47.17 (53.80) | 90.00 (100.00) | 90.00 (100.00) |
| $M_2$ – 20% plant product spray | 28.51 (22.78) | 43.82 (47.94) | 74.84 (93.16) | 90.00 (100.00) |
| $M_3$ – 15% plant product spray | 29.17 (23.75) | 40.14 (41.55) | 52.96 (63.71) | 90.00 (100.00) |
| $M_4$ – 10% plant product spray | 29.11 (23.66) | 39.61 (38.87) | 50.11 (58.88) | 90.00 (100.00) |
| $M_5$ – 5% plant product spray | 28.48 (22.74) | 37.75 (37.48) | 43.71 (47.75) | .48.78 (56.59) |
| $M_6$ – Insect alone | 27.76 (21.70) | 36.88 (36.03) | 42.85 (46.25) | 45.79 (51.39) |
| $SE_D$ | 0.94 | 0.98 | 1.61 | 1.31 |
| CD | 1.87 | 1.98 | 3.24 | 2.63 |
| Sub treatments | | | | |
| $S_1$ – 10 DAIR | 28.58 (22.88) | 44.49 (49.11) | 57.27 (70.77) | 67.29 (85.10) |
| $S_2$ – 20 DAIR | 27.84 (21.80) | 41.49 (43.89) | 56.53 (69.59) | 66.89 (84.60) |
| $S_3$ – 30 DAIR | 29.17 (23.70) | 36.46 (35.32) | 53.39 (64.44) | 66.66 (84.30) |
| $SE_D$ | 0.97 | 1.12 | 1.63 | 1.42 |
| CD | 1.94 | 2.25 | 3.28 | 2.84 |

Figures in parentheses are original values
DAIR – Days after releasing of insects

The insects did not suffer any mortality due to direct exposure to the plant product spray at the highest concentration tried, viz., 25 % spray, and they also did not suffer any histological infection that led to altered histological architecture in respect of various tissues such as cuticular membrane, fat body, foregut, midgut, hindgut, salivary gland and testis. This as probably due to that fact that many of the active principles in the plant product are only allelopathic and not allelomediatory in terms of toxicity to adult insects (Lee et al. 2001). The integrated approach of releasing the insect agents at 2 plant$^{-1}$ followed by spraying plant product at 25 % over the weed 10 days later showed that the water quality in terms of pH and dissolved oxygen were not affected. The untreated *E. crassipes* infested system recorded pH 7.42 and dissolved oxygen of 2.56 ppm at 30 DAS whereas pH of 7.28 and dissolved oxygen of 5.41 ppm were recorded in the system subjected to the integrated approach. Fish mortality of 4 % was recorded in aquatic system wherein integrated approach was followed as against 42 % in aquatic system with untreated *E. crassipes* infestation (Fig. 1).




$T_1$ - Insects + 25% plant product spray ; $T_2$ - Insects alone
$T_3$ - Unsprayed check with *E.crassipes*; $T_4$ - Fishes alone without *E.crassipes*

Figure 1. Impact of integration of insect agents and plant product spray on water quality and fish mortality.

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# Goat grazing for perennial weed control in rainfed agriculture

T. Geetha Jebarathnam and RM.Kathiresan*
Department of Agronomy, Faculty of Agriculture
Annamalai University, Annamalainagar, Tamilnadu, INDIA – 608 002
rm.kathiresan@sify.com

**Abstract:** Rainfed and low input agriculture holds the key for sustainability in many developing countries. Due to uncertainty and poor distribution of rainfall in such situations, integration of farming elements other than crop raising plays a vital role in resource generation and sustenance under small holder farms practicing low input agriculture. Goat rearing is a preferred farming element in rainfed dry lands of India. This element, in addition to its contribution in terms of meat, milk and economic return to the farming enterprise, also compliments depletion of weed seed bank and weed control through grazing in the off-season. It could be particularly useful in terms of reducing the menace of perennial weeds that perpetuate through food reserves in underground propagules. However, penning or addition of goat waste might occasionally negate this complimentary weed control through enriched soil seed bank by virtue of endozoochory or it may also help deplete the weed seed reserves through the addition of allelomediatory principle. Hence, a three year study was conducted at Department of Agronomy, Annamalai University to compare the effects of integrating goat rearing with millets. In the off-season, goats were allowed to graze in a particular strip of land repeatedly, while in another strip, goat waste was added repeatedly and in another field both grazing and penning were combined. During the succeeding cropping season, maize was raised in the field and each strip was divided to subplot treatments, i.e., unweeded, twice hand weeding, intercropping with mungbean, alachlor at 1.5 kg ha$^{-1}$, alachlor + handweeding and alachlor + intercropping in a split plot design. Results showed that goat grazing during the off-season gradually brought down the *Cyperus rotundus* population to 69.04 % at the end of last year experiment. The reduction in *C. rotundus* population was 61.8 % in plots where grazing was combined with penning, but only 13.25 % in penning alone.

**Key words**: Goat grazing, penning, *Cyperus rotundus*, rainfed millet cultivation

## INTRODUCTION

Indian agriculture has been showing a higher degree of reliance on dry land farming for its progress in recent years. In India, 92 million ha are under dry land farming or rainfed agriculture, accounting for more than 70 % of the cultivated area in the country. However, it contributes 42 % of the food production. The yields in these dry tracts are generally low coupled with the everlasting risk of total crop failure due to vagaries of rainfall. Component technologies of soil, water, fertilizer and weed management have to be attended for conserving the meager resources available in such dry tracts and to effectively utilize them for farm production in a sustainable manner. As rainwater needs to be effectively harnessed on the soil surface, the land needs to be kept weed free. This enables maximum amount of rainwater to seep into the soil and also reduces evapo-transpiration loss of water from the weed vegetation. In many occasions, perennials like *Cynodon dactylon* and *Cyperus rotundus* dominate the floristic composition of weeds in dry lands. Repeated hand weeding using hand hoes or shallow depth implements often exposes the surface soil moisture for more evaporative loss. Use of herbicides is seldom preferred due to lack of optimum soil moisture for herbicide desorption. This makes weed management in dry lands a more complicated but much more significant technology for enhancing farm productivity. In view of the mounting demands of food, linking of enhanced food production with nutritional security, conservation of natural resources, enhancing farmer's income, employment generation, etc., diversification of agriculture becomes imperative (Mangala Rai 2004). Diversification of agricultural activities, which links farm-based enterprises with cultivation, would help the farmers to get more income and generate additional employment. The farming system approach was observed as a resource management strategy for achieving economic and sustainable agricultural production to meet the diverse requirement of farm household while preserving the resource base and maintaining high environmental quality (Rangasamy 1994). Small ruminants especially goats gain importance mainly on account of their short generation intervals, higher rates of prolificacy and the ease with which they can be marketed. They are very useful in semi arid and arid zones, where they can sustain themselves on sparse vegetation and extreme climatic conditions. Goats were shown to be effective in controlling leafy spurge by grazing on it, when stocked at a density of three to four goats per acre (Sedivec et al. 1995). Field studies at University of Idaho showed that goat grazing reduced the infestation of spotted knapweed and seed head production by the weed (Williams and Prather 2003). Accordingly, an integrated farming system with goat rearing and millets might serve all the above discussed prospects of weed suppression and moisture conservation. A study was conducted at Annamalai University, India with the objective of tracing the impact of integrating goat rearing in dry land farming on management of weeds.

## MATERIALS AND METHODS

Field experiments were conducted for two years in the Annamalai University Experimental Farm, Tamilnadu, India from 2003 in the same field to compare different modes of integrating goat component in maize crop under rainfed conditions. The experimental field was predominated by perennial weeds *Cyperus rotundus* L. and *Cynodon dacytlon* L. along with rare occurrence of annuals like *Trianthema portulacastrum* L. and *Eclipta alba* L. The fields were segmented to main plots of dimension 15 m x 24 m, fenced with naturally available semi-permanent fencing materials and goats were integrated in these segments for different purposes. These include grazing alone, goat manuring alone and grazing + manuring that were compared as main treatments along with a fallow as untreated control, during the off-season (January to June), under split plot design. Goats of local breed were allowed to graze in the field, 10 goats $ha^{-1}$ in treatment plots that involve goat grazing. Their wastes were collected and incorporated in the treatment plots that involve goat manuring. During the subsequent cropping season, July to October, maize crop was raised in these fields. Each segment accommodating a goat integration treatment, viz., grazing or manuring or grazing + manuring was further divided in to sub-plots wherein maize was raised with different weed control treatments. These sub-treatments comprised of an unweeded control, twice hand weeded, pre-emergence herbicide alachlor 1.5 kg $ha^{-1}$, intercrop of blackgram, alachlor + hand weeding and alachlor + intercrop. The maize cultivars Bhanu and Co-1 were raised during 2003 and 2004 respectively. An additive inter crop of mungbean cultivar ADT – 5 was raised in a row in between two rows of maize (with a spacing of 60 cm x 22 cm). Pre-emergence herbicide alachlor (Lasso 50 EC) was sprayed with a knapsack sprayer fitted with flood jet deflector nozzle using 500 L $ha^{-1}$ of spray fluid and 12pPsi of pressure, on 3rd day after sowing the crops in respective treatment plots. Observations recorded include weed counts and weed biomass on 60 DAT, weed control index and grain yield at harvest.

## RESULTS AND DISCUSSION

Integration of goats in dry land agriculture as a farming element along with millets (allowing the goats to graze on the weed vegetation in the off-season and adding the goat manure to the field) significantly influenced weed infestation and crop performance in millets raised subsequently. Grazing by goats in the off-season reduced weed infestation in millets during the cropping season and such a weed suppression was significantly superior to all the other main treatments compared. Grazing on annual weeds in their vegetative stage did not allow them to set seeds, thereby blocking the enrichment of soil weed seed bank contributed for suppression of annual weeds in plots grazed by goats. In respect of perennial weeds, repeated grazing led to the exhaustion of food reserves in the underground propagules that was reflected on reduced infestation during subsequent cropping seasons. Though the goat component effectively reduced the weed population in subsequent seasons through their feeding habit, addition of fresh goat manure slightly brought down weed control effect by favoring higher weed counts and bio-mass especially with annuals, compared to grazing alone. This is attributed to the re-infestation by annual weed seeds through the goat manure by virtue of the process of endozoochory. However, the total weed count and weed biomass in these treatments involving goat manure addition were significantly lesser than the untreated control. This weed control advantage in goat manuring compared to untreated control might be due to reduced soil pH and reduced recuperation of soil weed seed bank. When integrated as a farming component in dry lands where in millet was grown during cropping season, goats supplemented weed control and reduced weed infestation significantly by imparting weed control indices of 17.7 % and 31.34 % during the first and second year, respectively. Excluding goat manure addition, grazing alone supplemented weed control in millets recording weed control indices of 25.20 % and 45.17 %, respectively. These observations are in concomitance with earlier observations of Tim Johnson (1994). The impact of weed suppression on the yield performance of the millet was higher than the soil fertility enhancing advantage of goat waste. This could be appreciated from higher grain yields in goat grazing alone compared to treatments involving goat grazing + goat manuring. It could be suggested that instead of adding fresh goat manure to the field, allowing the goat manure to decompose throughout the off-season and incorporating it in the field just before raising the crop could yield better results in terms of reducing weed competition and favoring millet yields.

As regards weed control treatments, pre-emergence application of alachlor 1.5 kg $ha^{-1}$ followed by hand weeding was significantly superior once the other treatments, recording higher weed control indices of 67.2 % and 54.7 % and grain yields of 106.25 q $ha^{-1}$ and 45.3 q $ha^{-1}$ in 2003 and 2004, respectively. Efficient control of weed seed emergence by the application of alachlor with the prevention of late emerging weeds by the supplemental hand weeding was responsible for it's superior performance. These results are in conformity with the reports of Thakur and Sharma (1996) and Mundra et al. (2003).

Table 1. Effect of grazing component on weeds in maize.

| Treatment | Weed Density $m^{-2}$ | | Weed Biomass (g $m^{-2}$) | | Weed control index (%) | |
|---|---|---|---|---|---|---|
| | 2003 | 2004 | 2003 | 2004 | 2003 | 2004 |
| Main plots | | | | | | |
| 1. Control | 23.89 | 13.09 | 83.90 | 62.25 | - | - |
| 2. Grazing | 16.26 | 4.03 | 62.76 | 34.13 | 25.20 | 45.17 |
| 3. Penning | 20.36 | 11.36 | 74.45 | 42.74 | 11.26 | 32.95 |
| 4. Grazing & penning | 18.52 | 5.00 | 69.04 | 39.75 | 17.7 | 31.34 |
| LSD (p=0.05) | 2.13 | 1.69 | 6.09 | 4.92 | 1.46 | 3.06 |
| Sub plots | | | | | | |
| 1. Control | 21.4 | 14.13 | 163.81 | 145.95 | - | - |
| 2. Twice hand weeding | 7.13 | 6.49 | 7.46 | 6.73 | 95.45 | 95.39 |
| 3. Alachlor 1.5 kg $ha^{-1}$ | 12.63 | 11.29 | 49.38 | 47.58 | 69.89 | 67.40 |
| 4. Blackgram (Intercrop) | 15.78 | 12.26 | 55.87 | 51.51 | 65.89 | 64.71 |
| 5. Alachlor + 1 hand weeding 1.5 kg $ha^{-1}$ | 7.02 | 6.4 | 7.23 | 6.44 | 95.59 | 95.59 |
| 6. Alachlor + Intercrop 1.5 kg $ha^{-1}$ | 9.59 | 8.24 | 25.64 | 23.6 | 84.35 | 83.80 |
| LSD (p=0.05) | 2.4 | 2.15 | 5.02 | 3.51 | 6.46 | 6.40 |

the underground propagules that was reflected on reduced infestation during subsequent cropping seasons. Though the goat component effectively reduced the weed population in subsequent seasons through their feeding habit, addition of fresh goat manure slightly brought down weed control effect by favoring higher weed counts and bio-mass especially with annuals, compared to grazing alone. This is attributed to the re-infestation by annual weed seeds through the goat manure by virtue of the process of endozoochory. However, the total weed count and weed biomass in these treatments involving goat manure addition were significantly lesser than the untreated control. This weed control advantage in goat manuring compared to untreated control might be due to reduced soil pH and reduced recuperation of soil weed seed bank. When integrated as a farming component in dry lands where in millet was grown during cropping season, goats supplemented weed control and reduced weed infestation significantly by imparting weed control indices of 17.7 % and 31.34 % during the first and second year, respectively. Excluding goat manure addition, grazing alone supplemented weed control in millets recording weed control indices of 25.20 % and 45.17 %, respectively. These observations are in concomitance with earlier observations of Tim Johnson (1994). The impact of weed suppression on the yield performance of the millet was higher than the soil fertility enhancing advantage of goat waste. This could be appreciated from higher grain yields in goat grazing alone compared to treatments involving goat grazing + goat manuring. It could be suggested that instead of adding fresh goat manure to the field, allowing the goat manure to decompose throughout the off-season and incorporating it in the field just before raising the crop could yield better results in terms of reducing weed competition and favoring millet yields.

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| 3. Penning | 20.36 | 11.36 | 74.45 | 42.74 | 11.26 | 32.95 |
| 4. Grazing & penning | 18.52 | 5.00 | 69.04 | 39.75 | 17.7 | 31.34 |
| LSD (p=0.05) | 2.13 | 1.69 | 6.09 | 4.92 | 1.46 | 3.06 |
| Sub plots | | | | | | |
| 1. Control | 21.4 | 14.13 | 163.81 | 145.95 | - | - |
| 2. Twice hand weeding | 7.13 | 6.49 | 7.46 | 6.73 | 95.45 | 95.39 |
| 3. Alachlor 1.5 kg ha$^{-1}$ | 12.63 | 11.29 | 49.38 | 47.58 | 69.89 | 67.40 |
| 4. Blackgram (Intercrop) | 15.78 | 12.26 | 55.87 | 51.51 | 65.89 | 64.71 |
| 5. Alachlor + 1 hand weeding 1.5 kg ha$^{-1}$ | 7.02 | 6.4 | 7.23 | 6.44 | 95.59 | 95.59 |
| 6. Alachlor + Intercrop 1.5 kg ha$^{-1}$ | 9.59 | 8.24 | 25.64 | 23.6 | 84.35 | 83.80 |
| LSD (p=0.05) | 2.4 | 2.15 | 5.02 | 3.51 | 6.46 | 6.40 |

Table 2. Effects of grazing component on grain yield of maize (q $ha^{-1}$).

| Treatment | 2003 (Bhanu) | 2004 ($CO_1$) |
|---|---|---|
| Main plots | | |
| 1. Control | 67.75 | 36.00 |
| 2. Goat Grazing | 84.75 | 43.9 |
| 3. Penning | 71.35 | 37.3 |
| 4. Grazing & penning | 75.05 | 40.35 |
| LSD (p=0.05) | 2.62 | 1.94 |
| Sub plots | | |
| 1. Control | 61.7 | 35.16 |
| 2. Twice hand weeding | 79.9 | 38.5 |
| 3. Alachlor 1.5 kg $ha^{-1}$ | 75.8 | 40.6 |
| 4. Blackgram | 62.5 | 39.88 |
| 5. Alachlor + 1 hand weeding 1.5 kg $ha^{-1}$ | 106.25 | 45.3 |
| 6. Alachlor + Intercrop 1.5 kg $ha^{-1}$ | 64.35 | 41.46 |
| LSD (p=0.05) | 3.81 | 1.98 |

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# Herbicide Resistant Weeds and Crops

## Gene flow from rice cultivars to weedy relatives under field assessment

Nguyen thi Lang[1], Bui Chi Buu[1], and Allison Snow[2]
[1] CuuLong Delta Rice Research Institute, Can Tho, Vietnam
[2] Ohio State University, Columbus, USA

**Abstract:** The studies have sought to identify the gene flow between two populations of IR64 and weedy rices under natural hybridization. The experimental designs differing in the natural hybridization of IR64 and weedy rice plants were used in experiments conducted at CLRRI, CanTho, Vietnam. Natural hybridization events between the two populations were detected by codominant SSR markers' scoring. Distribution and frequency of alleles at the SSRs loci were analyzed within weedy rices. A total of nine hybrids were identified from 1,000 seeds collected from weedy rice mean 0.001%. The occurrence of the crop to weedy gene flow was significantly associated with wind direction and frequency of the gene flow, which significantly decreased with distance from the pollen sources. In both phenotypic analysis, molecular and segregation data showed that pollination of recipient plants with pollen of the $F_1$ source occurred at a significant frequency. Both designs could also prove to be very useful in studying the gene flow.

**Key words:** Gene flow, microsatellite marker (SSR), weedy rice

### INTRODUCTION

In plant breeding programs, the introgression of target genes from wild relatives to cultivars have often been very important to develop new improved varieties. Gene flow between cultivated rice and weedy relatives has been recently reviewed by Messehuer et al. (2001). The gene flow slightly lower than 0.1% was detected in the circular plot when the plants were placed at a 1 meter distance from the transgenic central nucleus. And a significantly lower value (0.01%) was found in the other circle 5 meters from the transgenic plants. Gene flow is a two-part process involving pollination of sexually compatible species followed by subsequent introgression of the trait into rice. This paper has been considered potential risk by gene flow from rice cultivars (supposed as transgenic rice) to weedy rice.

### MATERIALS AND METHODS

Five accessions of weedy rices were collected in Mekong delta (Can Tho, Ca Mau, Dong Thap and Kien Giang provinces) ( table 1)

**Experiment design**
Experiments were conducted for natural hybridization with IR64 in 2002 and 2003 wet seasons with the design namely "alternating population combination" with spacing of 20x20 cm (fig. 1). Pollination was observed in the field during October–November. Weedy rice panicles were covered with bags after flowering

Table 1: Weedy rice accessions used in the experiments

| Acc. No. | Collected in | Feature |
|---|---|---|
| 001 | Tra Vinh | tall, long awn, black hull |
| 002 | Can Tho | tall, awnless, black hull |
| 003 | Ca Mau | tall, yellow hull, awned |
| 004 | Kien Giang | short, short awn, yellow hull |
| 005 | Long An | tall, red hull, awnless |

| Rep I | Rep II | Rep III |
|---|---|---|
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |
| ====== | ====== | ====== |
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |
| ====== | ====== | ====== |
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |
| +++++++++ | +++++++++ | +++++++++ |

\+ IR64

= weedy rice genotypes

Figure 1: Alternating population combination for weedy rices and IR64

**DNA extraction for PCR analysis**

DNA suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A piece of young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Spot Test Plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 25mM EDTA, 300mM NaCl and 1% SDS). Grinding was done until the buffer turned green which is an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of the extraction buffer was added and mixed into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-dried and resuspended in 50 µl of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). An aliquot of 1 µl is sufficient for PCR analysis.

**Microsatellite analysis**

The primers used for amplifying are microsatellites listed in table 2. Each 25$\mu$l amplification reaction consisted of 10mM of Tris-HCL (pH=8.0), 10xPCR buffer (10mM Tris pH 8.4, 50mM KCl, 1.8mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 unit of *Taq* DNA polymerase in a total volume of 20 µ0mM of PCR buffer 0.1mM of dNTPs, 200nM of primers, 0.5 units of *Taq* polymerase, and 20ng of genomic DNA. All DNA amplifications were performed on a PTC 100 thermal cycler under the following conditions: 5 min at 94°C, followed by 1 minute at 94°C, 60 s at 55°C and 60 s at 72°C for 35 cycles and 7 min at 72°C for final extension. An aliquot of 10 µl of the PCR product was routinely taken for gel electrophoresis to determine if amplification was successful. When the primers detected an amplicon length polymorphism, the samples were readily scored. The PCR products or the DNA fragments produced by restriction digestion were resolved electrophoretically on 1.2% agarose gel in 1X TBE buffer.

The amplification products were mixed with an equal volume of formamide dye (98% formamide, 10mM of EDTA pH 8.0, 0.1% bromophenol blue and xylene cyanol). After being denatured at 94°C for 3 min and immediately chilled on ice, 5$\mu$l of the sample was run for through a 5% polyacrylamide gel for 2 hours using due by silver staining

**For the performance test**

Agronomic characteristics such as plant height, panicle length, panicle per hill, spikelets per panicle, filled grain percentage, grain yield were investigated and compared to check parents in the RBD with three replications. Line assessment was done at maturity. Analysis of variance and mean comparisons of the data from field were statistically carried out by IRRISTAT

## RESULTS AND DISCUSSION

### *RESULTS*

**Survey of flowering time and seed set**

*Oryza sativa* (IR64 and weedy rice) is predominately self-pollinated. When plants with overlapping flowering periods are grown in close proximity, they may outcross naturally at low rates. The floral structure of *O. sativa* and the short viability of its pollen present biological barriers to cross-pollination. Natural cross-pollination is highly dependent on proximity in time and space. Typically, a rice floret opens only once for a brief period, during which fertilization takes place. Under CLRRI's field conditions, the time of flowering of four varieties on a given day varied as much as 8:00-10:00 am whereas the time overlapses between opening and closing of florets was only 30 minutes. Overlap of flowering in rice and weedy rice is complicate. Flowering dates among weedy rice types is highly variable. Several awnless weedy rice types have reached 50% heading nearly one week earlier, and several awned weedy rice types have flowered more than 2 week later than a midseason maturity rice variety. Furthermore, the total flowering period for red rice types typically ranges from one to several weeks, whereas that for rice varieties typically lasts for only a few days. The time of day when the flower opens and the period of flowering in rice depend on accessions used and environmental conditions such as humidity and temperature. A number of studies of pollen completion focused on the occurrence of competition when pollen grains were deposited simultaneously on stigmas. Sequential pollination is usually defined as foreign and conspecific pollen grain being deposited on stigmas at different time (Snow et al. 2000). The experiment offered only two accessions of weedy rice overlapping flowering periods with IR64 and OM1490.

The flowering period of weedy rice populations, IR64 and OM1490 was notices as from late October to early December. Based on the observation, experiment was correctly designed how to synchronize flowering time.

**Field pollination**

Hybridization rate of 1000 IR64 offsprings was detected as 0.00% and 0.01% in case of the treatments including IR64 *versus* weedy rice accession 001 (acc. 001), and acc. 005, respectively. The morphology of the hybrids exhibited intermediate features between that of the parental species.

The stable introgression of transgene within natural populations depends on fertility, genomic structure and generations. This experiment indicated that the introgression exhibited slowly with very low probability under natural optimal conditions. So under normal agricultural conditions, introgression can be much rarer in terms of female weedy rice.

Differences among varieties were thought to be varied due to the differential flowering times of weedy and cultivated genotypes. Phenotypically, 999 plants were noticed as short plant type as IR64. Of these, one tall plant was addressed as mid traits of weedy rice parents. For the rice hull, of 1000 seeds only one exhibited black color in primary branch with awnless seeds.

Table 2: Seed set and number of $F_1$ plants obtained in IR64 x weedy rice (acc. 005) based on pollen receiver distance

| Pollen receiver distance(cm) | Number of seeds | Percentage of gene flow (%) | Remark |
|---|---|---|---|
| A (20 cm) | 1000 | 0.000 | Non-overlap flowering time |
| B (40 cm) | 1000 | 0.000 | Overlap flowering time |
| C (60 cm) | 1000 | 0.001 | Overlap flowering time |
| D (80 cm) | 1000 | 0.000 | Non-overlap flowering time |
| E (100 cm) | 1000 | 0.000 | Non-overlap flowering time |
| F (120 cm) | 1000 | 0.000 | Non-overlap flowering time |

Seed set and number of plants obtained in the IR64 and weedy rice population (acc. 005) indicated that in treatment of distance C (60 cm), awned grain mutants and colorful hull grain mutants accounted for 0.03% and 0.01%, respectively.

Table 3: Grain analysis of F1 from crosses based on pollen receiver distance in 2002 wet season

| Pollen receiver distance(cm) | Awn | Grain length (mm) | Grain width (mm) | Color hull | Color grain | Number of seeds |
|---|---|---|---|---|---|---|
| A (20 cm) | 0 | 7.30 | 2.3 | yellow | white | 1000 |
| B (40 cm) | 0 | 7.20 | 2.3 | yellow | white | 1000 |
| C (60 cm) | 0:1 (997:3) | 7.26 | 2.3 | yellow | white | 1000 |
| D (80 cm) | 0 | 7.23 | 2.3 | yellow: black (999:1) | white | 1000 |
| E (100 cm) | 0 | 7.14 | 2.3 | yellow | white | 1000 |
| F (120 cm) | 0 | 7.40 | 2.3 | yellow | white | 1000 |
| IR64 (check) | 0 | 7.15 | 2.3 | yellow | white | 1000 |
| Weedy rice acc.005 | 1 | 4.50 | 1.1 | black | red | 1000 |

**Rice pollen dispersal study:** Natural outcrossing among rice plants is generally low as reflected in the summary of pollen dispersal among rice plants (Table 3).

**Agronomic characterization of IR64 and weedy rice lines**

Among tested lines, according to the mean agronomic data, the $F_1$s obtained the highest value of 1000-grain weight. All of these lines showed higher yield performance than commercial IR64 and weedy rice acc.005. ANOVA showed that the differences among treatments were significant at the level of 0.01 in term of grain yield potential of $F_1$s. Similarly, heading date, plant height, panicle length, total grains per panicle, filled grains per panicle, panicles / hill, 1000-grain weight exhibited significant differences at the level of 0.01 (table 4)

Table 4: Agronomic traits of cross between weedy rice x IR64 based on pollen receiver distance in 2002 wet season

| Pollen receiver distance(cm) | Plant height (cm) | Panicles / hill | Grain length (mm) | Filled grains / panicle | Unfilling grain (%) | 1000-grain weight |
|---|---|---|---|---|---|---|
| A (20 cm) | 99c | 12.33b | 24.33bc | 88.77a | 19 | 30.17a |
| B (40 cm) | 101b | 11.17b | 24.10bc | 77.69c | 26 | 25.37bc |
| C (60 cm) | 98cd | 13.33b | 21.83d | 81.78b | 17 | 27.08b |
| D (80 cm) | 99cd | 11.17b | 24.67b | 78.13c | 19 | 26.83bc |
| E (100 cm) | 97d | 12.50b | 23.67c | 82.34b | 21 | 32.08a |
| F (120 cm) | 103cd | 13.17b | 23.60c | 75.60c | 25 | 30.67a |
| IR64 (check) | 98cd | 23.17a | 24.83ab | 83.97b | 19 | 32.67a |
| Weedy rice acc.005 | 102ab | 11.33b | 25.50a | 81.40b | 32 | 23.92c |
| CV (%) | 3.07 | 7.79 | 5.94 | 7.06 | | 19.35 |
| F (0.01) | ** | ** | ** | ** | | ** |

**Genotyping**

Weedy rice populations are often genetically diverse. In an attempt to better understand the complex genetics and origins of weedy rice evaluated variations in SSR markers

SSR marker technology was used to evaluate the natural hybridization between weedy and cultivated rices at 43 loci.

Of 43 SSRs, polymorphism was only recorded at 13 loci of weedy rices and IR64 through DNA survey (Figure 2)


Figure 2: Polymorphism at 13 loci of weedy rice (lane 1) and IR64 (lane 2)

After DNA survey, of 13 polymorphic markers, RM315 and RM223 were noticed to be effective to detect natural crossing between weedy and cultivated rices (Fig. 3). For 100 plants from natural crossing in 2001 wet season, no hybrid plants were found. However, among 600 plants from natural crossing in 2003 wet season, four hybrid plants (0.005%) were detected by DNA markers (figure 3). It means that natural outcross in such case was low rate.

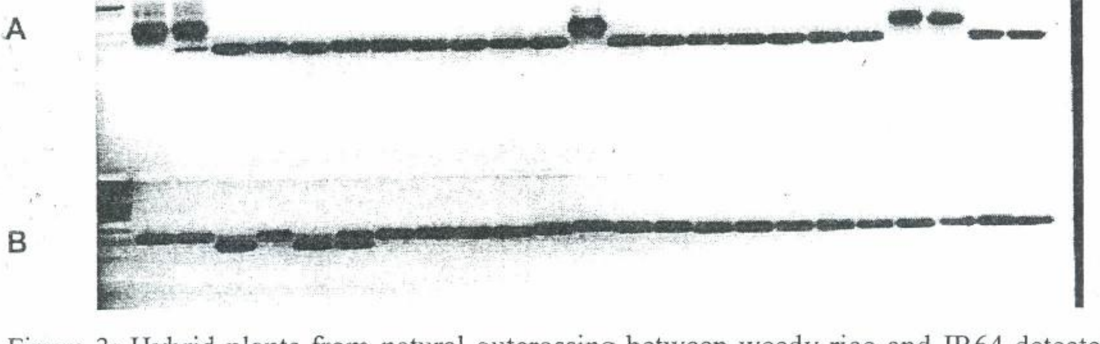


Figure 3: Hybrid plants from natural outcrossing between weedy rice and IR64 detected by DNA markers, [A]: RM223 and [B]: RM315

This study obviously showed that target genes from IR64 can introgress to weedy rice. Under natural condition with a distance of less than 100 cm, hybridization can occur (Song et al. 2003). Weedy rice population (acc.005) was more encouragingly to consider as point to the mechanism by which adaptive divergence can take place in the face gene flow. Determining the number of loci adaptive divergence is therefore of key importance for gene flow (Latta 2003).

## *DISCUSSION*

The maximum frequency of gene flow from cultivated rice to weedy rice in this study 0.02%, this is much lower than that Song et al. (2003), Messeguer et al. (2001) reported as 2.94% and 0.5% under similar conditions, respectively. The frequency of gene flow from cultivated rice to wild relative *Oryza rufipogon* is significantly lower than weedy rice (Langevin et al. 2000). Cultivars and weedy rices are primarily self-pollinated, but they can outcross with one another providing an avenue for the transfer of genetic traits such as herbicide resistance from transgenic rice to weedy rice (Davis et al. 2003). In natural crossing, *Oryza sativa* is predominately self-pollinated. So when plants are grown in close proximity with overlapping flowering periods, they may naturally outcross at (very) low rates. The consistent association of particular genotypes or phenotypes with particular environmental conditions makes a strong case that hybrid populations were noticed in weedy rice (acc. 005) x IR64 at the pollen receiver distance of 60 cm.

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# Field evaluation of transgenic maize carrying *$cp_4$ epsps* gene conferring herbicide (glyphosate) resistance

S. Patil, R. M. Sirohi* and S. Mehetre
Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, RAHURI, India 413722
sudampatil59@yahoo.co.in

**Abstract:** This field study was conducted under rainfed conditions during 2004. Genetically modified corn carrying the herbicide (glyphosate) resistance gene $cp_4$ *epsps* was planted in three replications. Eight treatments of herbicide sprays including farmers practice and weedy check were included to evaluate the bioefficacy of glyphosate on transgenic corn. The experimental area had high intensity of both dicot and monocot weeds. However, *Parthenium* (28.7 to 39 %) and *Digera arvensis* (11.7 to 16.7%) were the major weeds. Observations were recorded at 15, 30 and 45 days after spray treatments. It was observed that there was no crop injury, stand loss and phytotoxicity symptoms on corn due to herbicide (glyphosate) spraying treatments though it is a non-selective broad-spectrum herbicide. Results confirmed that the transgenic corn carrying herbicide resistance gene is stable under field condition. Glyphosate had 98 % weed control efficiency (WCE) up to 45 days when single spray was given with a dosage of 900 g ae ha$^{-1}$ as single spray after 18 days after planting. WCE was about 100 percent at all stages of crop growth in glyphosate-treated plots over the weedy check. This influenced shelling percent and grain weight, which resulted in higher yield. The highest grain yield (13.13 t ha$^{-1}$) with maximum cost benefit ratio (1: 1.86) was also observed in the least glyphosate sprayed treatment. The grain yield reduction was up to 41 % in weedy check (7.7 t ha$^{-1}$) plot when compared with treated highest yielding plot.

**Key words**: Bioefficacy, herbicide resistant, glyphosate, Roundup, transgenic corn.

## INTRODUCTION

Weed menace in agriculture is increasing in spite of constant efforts to get rid of them. Weeds cause major losses in crop yields. A large number of weed species cause economic losses in may field crops in India. Although manual and mechanical practices are important components of weed management, some weeds and weed seeds can reduce the crop quality to great extent (Padgette et al. 1996). Due to the scarcity and expensiveness of labor more emphasis needs to be bestowed on use of herbicide (Nagraju and Mohan Kumar 2005). Corn is one of the major cereal crops in India and fifth largest area in the world but has lowest productivity (1.81 t ha$^{-1}$). Poor weed management is one of the factors responsible for the low productivity. All cultivation practices are mechanized except weeding of crop (Senthilkumar et al. 2005). Crops made resistant to herbicide by biotechnology are being adopted in North America and are entering other parts of world (Duke 2004). Herbicide tolerant crops account for 72 % of total biotech cropped area. The herbicide tolerant maize occupies 4.3 million ha (Clive 2004). Glyphosate has been used for over 20 years in various ways in pre- planting, directed, spot or post harvest weed management system with no known report of weed resistance (Holt 1993). Herbicide resistant corn will speed the adoption of reduced or low tillage agriculture that may reduce environmental damage by conservation of soil moisture and soil erosion. Glyphosate-tolerant corn developed by Monsanto Company that confers the tolerance to herbicide by production of $cp_4$–enolpyruvylshikimate -3 phosphate synthase (*$cp_4$ epsps*) proteins. Glyphosate is the active ingredient of agricultural herbicide Roundup® that kills the plant by inhibiting the enzyme *epsps*. The experiment was aimed to test the phenotypic performance of transgenic corn carrying *$cp_4$ epsps* gene and assess the effect of herbicide under bioefficacy protocol and the results are described in this paper.

## MATERIALS AND METHODS

This study was conducted during the monsoon season 2004 at Mahatma Phule Krishi Vidyapeeth, Rahuri, India. The genetically modified herbicide tolerant hybrid corn (Hishell NK603), expressing *$cp_4$ epsps* gene was planted on protected area as bio-safety measures. The plant spacing was 60 cm x 25 cm, with plot size 18 $m^2$. The experiment consisted of eight treatments (Table 1) and was replicated thrice in RBD design. The post emergence application schedule was followed as per treatment detail. Standard agronomical operations were followed to raise the normal crop.

Table 1. Treatment details of the experiment.

| Treatment | Dosage (Formulated product) (L $ha^{-1}$) | Time of Application |
|---|---|---|
| Glyphosate Single application | 2.5 (900 g a.e. $ha^{-1}$) | Single Roundup® Application at V2-V4 stage of crop or 10 cm weeds. |
| Glyphosate Single application | 5 (1800 g a.e. $ha^{-1}$) | Single Roundup® Application at V2-V4 stage of crop or 10 cm weeds. |
| Glyphosate Single application | 10 (3600 g a.e. $ha^{-1}$) | Single Roundup® Application at V2-V4 stage of crop or 10 cm weeds. |
| Glyphosate Double application | 2.5 (900 g a.e. $ha^{-1}$) + 2.5 (900 g a.e. $ha^{-1}$) | V2 – V4 State of crop or 10 cm weeds followed by at V6-V8 stage of crop |
| Glyphosate Double application | 5 + 5 (1800 g a.e. $ha^{-1}$) + (1800 g a.e. $ha^{-1}$) | V2 – V4 State of crop or 10 cm weeds followed by at V6-V8 stage of crop |
| Farmers practice | 1 inter cultivation followed by 1 hand weeding between rows and earthing up | Inter culture at 25-30 days followed by 1 hand weeding between rows and earthing up |
| Weedy check | No weeding from sowing to harvest. | -- |
| Weed free plot. | Hand weeding 2 to 3 times to maintain the plot weed free throughout the growing season. | -- |

Data gathered included percent germination, plant population and crop safety observations (chlorosis, necrosis, scorching, malformed plants). The relevant observations on phenotype, yield and yield contributing traits influenced due to treatments were taken from randomly selected 80 plants in each plot to assess the effect of glyphosate spray on growth and yield of corn. The data were subjected to computational statistics Unistat 5.5 UK software.

## RESULTS AND DISCUSSION

Observations were recorded on weed species, weed intensity, crop injury after spray, weed control efficiency, flowering period, plant height, earhead length and grain yield. Ten major weeds comprised the weed flora in the experimental field. However, *Parthenium hysterophorus* had the highest (28.7 to 39 %) intensity followed by *Digera arvensis* (11.7 to 16.7 %), *Digitaria sanguinalis* (5.7 to 11.7 %), *Phylanthus niruri* (7.7 to 18.3 %), *Panicum isachmi* (9.7 to 14.3 %), *Amaranthus polygamus, Chorchorus acutangulus* and *Cyperus rotundus* ranged from 1 to 2.7 %. The intensity of other weeds like *Comellina benghalensis, Ipomoea* sp., *Comellina nudiflora* etc. was 1.7 to 4.3 %.

The weeds had 2.5 leaves with 3.7 to 14.7 cm height prior to the spray treatment. No symptoms of crop injury, stand loss and phytotoxicity were observed at 14 and 21 days after treatment (DAT). Weed dry matter per square meter at 15, 30 and 45 DAT (Table2) revealed that weed dry weight reduced significantly due to herbicide treatments; weed control efficiency (WCE) of herbicide treatment was more than 98 % in treated plots.

Table 2. Weed dry matter (g) $m^2$ meter and weed control efficiency at 15, 30 and 45 DAT.

| Treatment | 15 DAT | | 30 DAT | | 45 DAT | |
|---|---|---|---|---|---|---|
| | Dry matter | WCE | Dry matter | WCE | Dry matter | WCE |
| Roundup® 2.5 L $ha^{-1}$ | 4.00 | 98.74 | 5.33 | 98.08 | 6.67 | 97.54 |
| Roundup® 5.0 L $ha^{-1}$ | 3.67 | 98.84 | 2.67 | 99.02 | 4.33 | 98.40 |
| Roundup® 10.0 L $ha^{-1}$ | 4.00 | 98.74 | 3.67 | 98.66 | 3.33 | 98.77 |
| Roundup® 2.5 Seq 2.5 L $ha^{-1}$ | 3.33 | 98.95 | 5.00 | 98.18 | 3.33 | 98.77 |
| Roundup® 5.0 Seq 5.0 L $ha^{-1}$ | 4.67 | 98.53 | 4.67 | 98.30 | 2.00 | 99.26 |
| Farmers Practice | 36.67 | 88.49 | 1.40 | 85.81 | 30.00 | 88.95 |
| Weedy Check | 318.67 | -- | 275.00 | -- | 271.67 | -- |
| Weed free plot | 1.06 | 99.66 | 0.75 | 99.72 | 0.83 | 99.69 |
| C.D. at 5 % | 41.43 | -- | 26.27 | -- | 12.22 | -- |

Observations on various biometric and agronomic traits are shown in Table 3. The parameters ear length, shelling percentage, grain weight and yield were significantly influenced by various treatments. All glyphosate treated plots were significantly superior over the control (weedy check) on all the parameters. The phenotypic parameters like stay green, ear length, number of ears, were not significantly affected by glyphosate treatments when compared with farmer's practice and weed free plot. The highest test weight (29.98 %) and grain yield (13.13 t $ha^{-1}$) was obtained in treatment 900 g a.e. $ha^{-1}$ glyphosate sprayed 20 days after sowing (Table 3). This was the reflection of higher performance of other yield contributing characters like shelling percentage. The cost benefit ratio (C:B) was also higher (1.86) at lower dosage application of glyphosate. Dixit (1995) reported that weeds in maize were controlled at lower dose. From this study it is confirmed that the transgenic corn carried stable herbicide-resistance gene and spray of glyphosate at 900 g a.e. $ha^{-1}$ was efficient to control all the dicot and monocot weeds in the corn field.

Table 3. Biometric and agronomic parameters.

| Treatment | Ear length (cm) | Shelling (%) | 100 grain weight (g) | Yield mt $ha^{-1}$ at 15 % moisture | C : B ratio |
|---|---|---|---|---|---|
| Roundup® 2.5 L $ha^{-1}$ | 21.57 | 80.33 | 29.98 | 13.13 | 1.86 |
| Roundup® 5.0 L $ha^{-1}$ | 21.20 | 79.10 | 29.37 | 13.00 | 1.74 |
| Roundup® 10.0 L $ha^{-1}$ | 20.50 | 78.95 | 26.96 | 11.69 | 1.30 |
| Roundup® 2.5 Seq 2.5 L $ha^{-1}$ | 20.00 | 79.80 | 27.96 | 12.8 | 1.70 |
| Roundup® 5.0 Seq 5.0 L $ha^{-1}$ | 21.10 | 76.81 | 26.19 | 10.83 | 1.13 |
| Farmers Practice | 20.53 | 81.15 | 26.79 | 11.35 | 1.40 |
| Weedy Check | 18.79 | 71.55 | 25.28 | 7.70 | 0.74 |
| Weed free plot | 20.60 | 77.90 | 26.26 | 10.48 | 1.07 |
| C.D. at 5 % | 1.39 | 4.56 | 4.12 | 2.85 | -- |

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**Establishment of a test method of glyphosate-resistant *Conyza canadensis* in China**

X L Song, J J Wu, S Qiang
Weed Research Laboratory, Nanjing Agricultural University, Nanjing,China, 210095
wrl@njau.edu.cn

**Abstract:** Glyphosate, non-selective herbicide, has been used for the control of annual and perennial weeds in orchards for more than 25 years in China. *Conyza canadensis*, which has been reported to have evolved glyphosate resistance in some countries, is one of the main weeds in orchards in China. It is not known whether glyphosate resistance has evolved in *C. canadensis* in China. In order to investigate and monitor the glyphosate resistance evolvement in China at the long-term, a methodology for glyphosate resistance test was established first to test a large number of glyphosate- resistant weed samples in China and to be comparable to the test methods used in the documented cases of glyphosate-resistant weed. The method of glyphosate resistance test was established here as follows. Sample seeds were sown over the mixed soil surface of a shallow tray to be cultured into seedlings. Single seedling was transplanted into separate 5 cm x 5cm pot containing mixed soil at 5-7 leaf stage of the seedling. The isopropylamine salt of glyphosate at rate 0, 35, 70, 140 280, 560, 1120, 2240, 4480, 8960 g ai $ha^{-1}$ could be applied when the seedling grew to 11-13 leaf stage. The first observation was made 3 days after application and herbicide injury rate was recorded based on visual assessment. The same work was consistently conducted every four days until the injury symptom did not develop further. All data were fitted to a log –logistic regression model. By comparison of the test experiment results in two times, it was confirmed that the method could be repeated very well. So the method has been applied to test more 27 horseweed samples during past two years.

**Key words**: *Conyza canadensis*, glyphosate, resistance, test method.

## INTRODUCTION

Glyphosate, a broad-spectrum and nonselective-herbicide, has been used widely in the world since the 1970s and became one of the most important pesticides (Ren 1998). With the successful development and commercialization of glyphosate-resistant transgenic crops, glyphosate will be applied to a wide range of crops (Xiang 1998; Fu 2002). At present, China has been become the biggest producer and consumer of glyphosate.

With its widespread and long-term use during the past 30 years, glyphosate resistance has been reported in six kinds of weeds in the world. It was first reported in 1996 from rigid ryegrasss (*Lolium rigidum*) in an orchard in Australia (Powles et al. 1998). Subsequently, several additional glyphosate-resistant weed populations have been identified: rigid ryegrass in a wheat production system in Australia and in California (Pratley et al. 1999; Simarmata et al. 2003), Italian ryegrass (*Lolium multiflorum*) in Chile (Perez and Kogan 2003), goosegrass (*Eleusine indica*) in Malaysia (Lee et al. 2000), and horseweed (marestail) (*Conyza canadensis*) in the eastern, midwestern, and southeastern United States (Van 2001; Rogers 2003). However, there are few reports about the research of glyphosate-resistant weed in China so far.

Bioassay has been proven an effective method to identify glyphosate resistance of weeds. Two main bioassay methods for glyphosate-resistance test were conducted in petri dish experiment or pot experiment. Seed bioassay in petri dish has been reported to be used successfully for documenting *L. multiflorum* (Perez & Kogan 2003). Seedling bioassay in pot has been documented for *Conyza canadensis* (Mark and VanGessel 2001), *L. multiflorum* (Perez and Kogan 2003), *L. rigidum* (Pratley 1999). In petri dish test, root length and shoot length at different glyphosate concentrations were usually measured as the response index to establish a standard non-linear regression analysis while the fresh weight or injury severity based on visual assessment was taken in pot experiment.

In order to evaluate the current status of glyphosate-resistant weeds and carry out a long-term monitoring of glyphosate-resistant weeds in China, it is imperative to establish the glyphosate-resistance testing methods at the beginning. *Conyza canadensis*, in which the evolvement of glyphosate resistance has been reported in some countries else, is one of main weeds in orchards in China. *C. canadensis* was used as material in this paper to determine whether the glyphosate resistance has evolved in *C. canadensis* in China.

## MATERIALS AND METHODS

Seeds of *C. canadensis* population

Seeds of *C. canadensi* collected from roadside of CangBoMen in Nanjing were used as the materials in order to establish the method of glyphosate resistance test.

Herbicide

Isoproplayamine salt of glyphosate, a commercial product named Nong Wang produced by XinAnJiang Chemical Company of ZheJiang in China, was used as experimental herbicide.

Experimental container

Shallow trays (22 cm x 15cm surface by 6 cm depth) and small plastic pots (5 x 5 surface and 8 cm depth) were used as experimental container.

Soil

Soil collected from the garden of Nanjing Agriculture University was mixed with humic fertilizer at the ratio of 2:1.

Other materials

The other materials included the rose nozzle watering pot, manual sprayer and muslin.

Methods

The experiment was conducted in a green house of Nanjing Agriculture University from September to October in 2003.

Method for culturing *C. canadensis*

The shallow trays were filled with prepared soil then watered using a rose nozzle equipped watering pot. Test seeds were sown over the soil surface evenly. Each tray was watered lightly using a fog nozzle that provided gentle watering. Immediately each tray was covered with muslin to keep other weed seeds out. These trays were put in a green house randomly. The temperature was kept at a range of 25℃-30℃. Each day, the muslin cover was opened and the trays were watered lightly to keep the soil surface damp.

Transplant seedlings