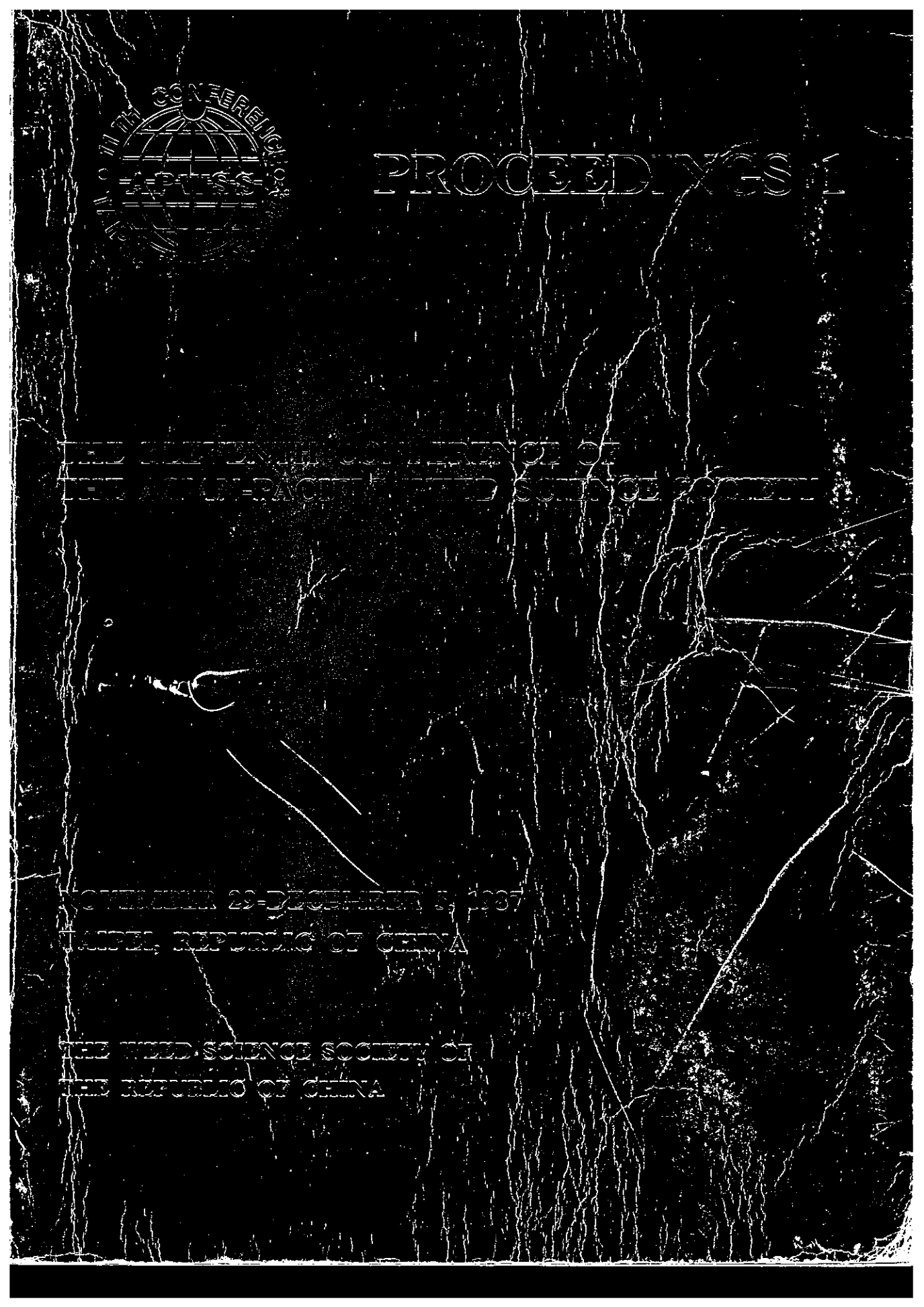
PROCEEDINGS

# THE ELEVENTH CONFERENCE OF
# THE ASIAN-PACIFIC WEED SCIENCE SOCIETY

# PROCEEDINGS OF THE ELEVENTH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE

1987

WEED SCIENCE SOCIETY OF
THE REPUBLIC OF CHINA

## 11th ASIAN-PACIFIC WEED SCIENCE SOCIETY

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# ALLELOPATHY IN SUSTAINABLE AGRICULTURE IN TAIWAN

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## ABSTRACT

The allelopathic phenomenon has been found in various habitats in Taiwan involving both natural and agricultural ecosystems, and research on it has included autointoxication of several monocultural crops, such as rice, sugar cane, asparagus, and some pasture grasses. The reduction of rice yield in the second crop season is due primarily to the phytotoxins produced during the decomposition of rice residues in soil. The cause f suppression of ratoon sugar cane growth and that of yield reduction of sugar cane were ascribable to phytotoxins produced by the debris of the plant and by fusaric acid produced by a soil borne pathogen, *Fusarium oxysporum*. And a significant reduction of yield and quality of asparagus plants found in old plantation soil was also due to phytotoxins released from the plant parts and those produced from the decomposition of residues remaining in soil. In agricultural practice, allelopathic concept was introduced into weed control in order to select an aggressive cultivar or variety, thus to minimize the use of herbicides. We found that under sufficient application of nitrogen fertilizer, *Digitaria decumbens* form a relatively pure stand among 12 subtropical grasses studied. This fact indeed is due to the phytotoxicity present in the aqueous extract of grass leaves; however, several years after planting the grass also exhibits autoinhibition leading to decline of productivity. Furthermore, in a pasture-forest intercropping, a kikuyu grass, *Pennisetum clandestinum* can suppress the growth of many weeds but not to the seedling growth of Chinese fir. In forest plantation, both *Phyllostachys edulis* and *Leucaena leucocephala*, exhibit distinguished allelopathic effect, which regulates the population of weeds grown beneath these two vegetations. Allelopathic compounds present in plant extracts can practically be used as natural herbicide to lessen the labor cost and to avoid soil pollution. The allelopathic patterns can be interacted by the environmental stresses, and the productivity of the autotoxic plants can be enhanced by eliminating the phytotoxins through field treatments, such as crop rotation, water drainage, water flooding, and through a detoxification process of phytotoxic phenolics by polymerizing phenolics into a humic complex. Allelopathy thus plays a significant role in sustainable agriculture.

## INTRODUCTION

The sustainable agriculture is a way to make efficient use of resources internal to the farm, to rely on a minimum of necessary purchased inputs, and to minimize the influence of agricultural practices beyond the farm boundaries (14). A well-designed sustainable system can

practically improve the production of resource over time. The sustainable agriculture is also known as organic, alternative, regenerative, biodynamic, intensive, low-input or resource-conserving. To achieve the goal of sustainable agriculture, the mainstream research currently conducted by the American scientists involved plant breeding, soil fertility and tillage, crop protection, and cropping systems. Moreover, allelopathy, which means a detrimental biochemical interaction between plants (1, 17, 23) ia an important phenomenon in agriculture and the research on it has recently been brought into the sustainable agriculture (1, 23, 24). For example, Putnam and Duke (21) introduced allelopathy into the breeding process of cucumbers to obtain weed control cultivars. Chou and coworkers (16) conducted experiments to select some aggressive cultivars of pasture grass with high potential of weed control. More recently allelochemicals were used as defensive compounds to control plant pathogens, soil borne diseases, and pests (23, 24). On the other hand, autointoxication, another phase of allelopathy also plays an appreciable role in reducing productivity of several important crops, such as rice, wheat, sugar cane, asparagus, pasture grasses and many others, where these crops are under a monoculture for years (1, 5, 8, 22, 23, 24, 29, 33, 34). However, alternative agriculture managements, such as crop rotation, intercropping, and multicropping systems are good ways to enhane crop productivity by reducing such an autotoxic effect. This paper thus describes most of our research findings done by the author and his coworkers in the last decade regarding the allelopathy in relation to the aforementioned mainstream research of sustainable agriculture in Taiwan.

## ALLELOPATHIC APPROACH OF WEED CONTROL

**Pasture grass with potential of weed control** An increasing amount of allelopathic research on grassland species has been conducted in many parts of the world during recent decades (6, 23). Most of the studies have been concerned with the interpretation of allelopathic phenomena in the field. Only a few studies have employed the allelopathic effect as a practical means of directly controlling weeds. In Taiwan, many grasses have been introduced into pasture but only a few varieties can be established as forage pasture. Twelve subtropical introduced species of forage grasses were selected to give aqueous leaf extracts evaluated for their phytotoxicity on tested species. Of them; *Acroceras macrum, Cynodon dactylon, Chloris gayana, Digitaria decumbens* (pangola grass), *Eragrostis curvula, Panicum repens*, and *P. maximum* exhibited significant inhibition of radicle growth of test plants. And *Digitaria decumbens* had the highest phytotoxicity upon the tested species even at a concentration as low as 10 milliosmols, in which no osmotic inhibition occurred (12, 13). *D. decumbens* was also shown to be an autotoxic species, and its productivity was significantly reduced after several years of planting (Chou, unpublished data). However, under sufficient application of nitrogen fertilizer, the pangola grass forms a pure stand where almost no other weeds can grow with. However, the growth performance and competitive ability of the grass varied with cultivars. Liang et al. (16) therefore selected eight varieties of pangola for field trials and laboratory assays, and concluded that the invasion ability of cultivars A65, A255, and A254 were highest in Hsinhwa, Hengchun, and Hwalien station, respectively; while A79 and A80 were inferior in all stations. Cultivars A84, A254, and A255 possessed the highest toxicity, which was due to phytotoxins, of which nine phytotoxic phenolics were identified. Although the grass interaction in the field is

Although the grass interaction in the field is very complicated and allelopathy cannot account for the only factor involved, the mechanism of dominance performed by the grass with high potential of weed control is surely related to the nature of allelopathy.

**Allelopathic compounds from *Leucaena leucocephala*** *Leucaena leucocephala* exhibits a unique pattern of almost lacking of understory species besides *Leucaena* itself in *Leucaena* plantations. This phenomenon is due primarily to an allelopathic effect that *Leucaena* releases a certain amount of phytotoxns including 8 phenolic acids, flavonoids, and mimosine from leaves and litter of the *Leucaena*. The compounds can suppress the growth of many weeds and forest species, namely *Acacia confusa, Ageratum conyzoides, Liquidambar formosana, Casuarina glauca*, and *Alnus formosana* (7). It is noticeable that the growth of *Mimosa pudica* was suppressed by *Leucaena* leaf leachate, although the leaf juice of *M. pudica* contains a relatively high amount of mimosine. Among 84 seedlings of *M. pudica* tested only 2 seedlings survived, showing that mimosine can be practically useful to control a notorious weed such as *M. pudica* in the field.

**Allelochemicals produced by *Vitex negundo*** *Vitex negundo*, a dominant component of coastal vegetation, is widely distributed in the southern parts of Taiwan. Chou and Tao (11) found that the biomass and density of its associated understories are relatively lower than in adjacent pasture. Field results showed that the natural leachate of *V. negundo* significantly retarded the growth of *Digitaria decumbens* but stimulated the growth of *Andropogon nodosus* as compared to the rainfall control. The growth of *D. decumbens* grown in pots under greenhouse conditions was significantly retarded by watering with a 1% aqueous extract of *V. negundo*, but the growth of *Andropogon nodosus* and *Mimosa pudica* was stimulated. The aqueous extract was phytotoxic to lettuce and rye grass seeds. The aqueous effluents obtained from a polyamide column chromatograph also bioassayed. Some fractions inhibited radicle growth of lettuce and rice seedlings, whereas other fractions had a stimulatory effect. The responsible substances were isolated and identified that included several phenolic acids and 10 flavonoids. One flavonoid, 3'- hydroxyvitexin, and nine other flavonoids were identified (11).

## ALLELOPATHY IN THE TILLAGE PROBLEMS OF MONOCULTURE CROPS

**Phytotoxic nature of decomposing rice residues in soil** Rice (*Oryza sativa*), the most important crop in oriental countries, is planted twice a year by a continuous monoculture system. For nearly a century, the yield of the second crop there has been generally lower by 25% than that of the first crop. This reduction of rice productivity has been particularly pronounced in areas of water drainage (31). The cropping system of rice in Taiwan is different from that of other countries. In the first crop season (from March to July) the temperature increases gradually from 15°C to 30°C but for the second crop (August to Decemder) it decreases from 30°C to 15°C. Between these two crops, there are usually 3 weeks for fallowing period as compare with a 10-week period elsewhere. The farmers always leave rice stubble in the field after hervesting, and submerge these residues in the soil for decomposition during the fallowing time. Chou and co-workers (9, 10) conducted a series of experiments, of which one pot experiment was taken as that a rice straw-soil mixture (100 g: 3kg) was saturated with distilled water and allowed to decompose for 1, 2, and 4 weeks under greenhouse conditions. Soil alone was treated in the same manner, as a control. The results showed that rice seedlings grow under control conditions were normal, while the seedlings grew significantly poorly in the straw-soil

mixture. The roots of retarded plants were dark brown and the root cells were abnormal and enlarged. Further experimental results showed that when the amount of rice straw mixed was increased to 100 g/3 kg soil, the phytotoxicity increased with the increase of straw added and the toxicity was persistent 16 weeks after decomposition (9). The compounds present in the extracts of decomposing rice residues in soil were identified that included *p*-coumaric, *p*-hydroxybenzoic, syringic, vanillic, *o*-hydroxyphenylacetic, and ferulic acids (9), and propionic, acetic, and butyric acids (31). Particularly, *o*-hydroxyphenylacetic acid, first reported to be a phytotoxin by us, was toxic to rice growth at a concentration of $1,64 \times 10^{-4}$M. And the concentration of o-hydroxyphenylacetic acid reached about $10^{-2}$M in the soil containing decomposing rice residues. Further evidences of phytotoxic effects in relation to other environmental factors will be discussed later.

**Phytotoxic effect of decomposing sugar cane residues in soil** Inadequate germination and growth of ratoon cane have been found to be the two major problems in the farms of Taiwan Sugar Corporation (TSC). The yield of monoculture sugar cane has declined in many sugar cane fields. The causes of this yield reduction have been investigated, but no single factor causing the reduction can be found. Wang et al. (29) demonstrated by field and laboratory experiments and showed that the phytotoxic effects of decomposing sugar cane residues in soil are one of the important factors involved. Five phenolic acids, *p*-hydroxybenzoic, ferulic, *p*-coumaric, syringic, and vanillic and 6 short chain fatty acids, acetic, oxalic, malonic, tartaric, and malic acids were identified in the decomposing sugar cane leaves in water-logged soil. At $3 \times 10^{-4}$M solution of these phenolic acids in water culture, the growth of young sugar cane root was inhibited. The aliphatic acids were also found to inhibit the growth of ratoon sugar cane at $10^{-3}$M. Furthermore, Wu et al. (30) found that the population of *Fusarium oxysporum* associated with the rhizosphere soil of poor ratoon cane roots was much greater than that of good growing ratoon or of newly planted sugar cane roots. They found that fusaric acid, a secondary metabolite of the organism, was toxic to the growth of young sugar cane plants in vitro (30).

**Phytotoxic effect of asparagus plant parts** *Asparagus officinalis* is a perennial ratoon crop widely planted in many plantations of Taiwan. A significant reduction of yield and quality of asparagus often occurs in old plantation soil. The wilting of asparagus plants has been found to be due to monoculture of the crop. Young (33) indicated that there was about 40% of asparagus seedling missing from the plantation. Young further showed that the root exudates of asparagus retarded the seedling growth of asparagus cultivars, namely Mary Washington, California 309 and California 711 (33). Exudate collected by use of the circular trapping with a XAD-4 resin significantly retarded radicle and shoot growth of asparagus seedlings (34). Six phytotoxic phenolics, namely 3,4-dihydroxybenzoic, 3,4-dimethoxybenzoic, 2,5-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, and b-(m-hydroxyphenyl) propionic acid, and 3,4-dimethoxy acetophenone were found in the extracts and exudates of asparagus plant parts. The amount of phytotoxins identified was significantly higher in the stem than in the root, and was well correlated to phytotoxicity (33). It is concluded that the reduction of asparagus productivity in old asparagus fields is due primarily to phytotoxins released from the plant parts and those produced from the decomposition of residues remaining in soil.

## DECOMPOSING PLANT RESIDUES UNDER ENVIRONMENTAL STRESSES

**Plant growth under water-logged and oxygen-deficient conditions** Many aquatic plants grow very well in a water-logged and oxygen deficient environment because of their adaptive mechanism. Although rice plants are not hydrophyte, they grow very well in the paddy soil. Patrick and Mikkelsen (20) indicated that the level of oxygen reached almost zero when it was measured at 25 cm below the soil surface in the paddy field. We obtained similar results in Taiwan paddy soils. In many areas of Taiwan, namely Tsingshui (the central part of Taiwan), Chiatung and Yuanlin (the southern part), and Tungshan (the east coast), where the paddy fields are either poor in water drainage or have a higher water table, leading to oxygen deficiency. This is even pronounced in the second crop season when the monsoon comes. As mentioned the farmers in Taiwan have always submerged rice straw into soil and allowed them to decompose. During the decomposition of rice residues in soil, a significant amount of phytotoxic substances, such as short-chain aliphatic acids and phenolic acids were produced. The amounts of these compounds produced reached its maximum the first month after rice residues are submerged into soil, resulting in the suppression of root growth and panicle initiation; thus, the rice yields decreases (6, 9, 31).

**Redox potential (Eh) of paddy soil affected by decomposing rice residues** As mentioned earlier, the oxygen level is nearly zero at a depth of 25 cm below the soil surface of paddy fields, resulting in the reduction of soil redox potential (Eh) (6). Chou and his co-workers found that the soil Eh ranged from -10 to 200 mV during the first crop season and from -200 to 100 mV during the second crop season in the Nankang paddy field. At the farm of the National Chungshing University of Taichung, the Eh was remarkably low, ranging from -500 to 100 mV during the second crop season. In pot experiments, we found that the soil Eh was below -300 mV in the treatment of rice straw mixed with soil and was above 100 mV in the treatment of soil alone. Thus, the reduction of soil Eh was apparently related to the decomposition of rice residues in soil. The reduced soil Eh was remarkable at the tillering stage (30-45 days after transplanting) and at the panicling stage (80-90 days after transplanting) (6). During this period, the growth of rice roots was retarded, the root cells swelled, and many adventitious roots developed. Wu et al. (31) postulated that the swelling of root cells could be a kind of adaptive mechanism in order to obtain more oxygen.

**Microbial activity in decomposing plant residues** During the decomposition of plant residues in soil, microbial activities are involved (19). Wu et al. (31) found that the denitrified bacterium *Pseudmonas putida* became dominant in the rhizosphere of the rice paddy, and the population of *P. putida* was positively correlated to phytotoxin production when rice residues were submerged in soil and to the poor water drainage. They pointed out that in the well-drained area of Tsaotune soil the number of *P. putida* was 701 x $10^5$ /g dry soil, while in the poor water drainage area of Lotung, Taan, and Taichung, the number of *P. putida* ranged from 347 to 3412 x $10^5$ /g dry soil. It was evident that the number of *P. putida* was exceedingly high in the poor water drainage soil, indicating that the organism might use the residues as its carbon source. Wu et al. (31) furthermore indicated that the phytotoxic phenolics did from the metabolites of this microorganism but were released from decomposing rice residues. Chou et al. (6) pointed out that ammonium sulfate mixed with rice residues enhanced phytotoxicity, reflecting that the addition of nitrogen fertilizer might favor the growth of decomposing microorganisms and thus expedite the decomposition rate of rice residues in soil. Wu et al. (31) also indicated that the application of ammonium sulfate fertilizer to paddy soil was

beneficial to the growth of P. *putida* but that may expedite the formation of $H_2S$, which is toxic to rice growth. Similarly, the cause of yield decline of sugar cane in Taiwan has been investigated by Wang and his associates (29) and Wu et al. (30). They found that the reduction is partly due to the phytotoxic effect of phenolic acids after decomposition of cane residues in soil and a fusaric acid produced by *Fusarium oxysporum*. They found that the F. *oxysporum* population was much greater in the rhizosphere of ratoon sugar cane soil than in the soil without planting (30). At 10 ppm of fusaric acid mixed in Murashige and Skoog's medium, leaves of sugar cane wilted and became chlorotic (31).

## ALLELOPATHY IN RELATION TO SOIL NUTRIENT AVAILABILITY

**Phytotoxins in relation to nitrogen availability** Chou et al. (10) concluded that the more rice stubble left in the paddy soil, the higher would be the phytotoxic phenolics and less amount of leachable nitrogen, reflecting that the phytotoxins produced may interact with nitrogen available in soil. They also found that the amount of leachable $NH_4$-N was about ten times as great as that of $NO_3$-N (6, 9). Chou and his co-workers (5) used $^{15}N$-isotope tracer techniques to study the distribution of nitrogen in soil or soil-rice residues mixture under different temperature regimes and sequences. In the absence of straw, most of the fertilizer N remained in the mineral form. Straw enhanced N immobilization only moderately. The gradual decrease in the proportion of fertilizer N in mineral form was accompanied by a steady increase of fertilizer N in amino acid fraction of organic N. Little accumulation of fertilizer N in the amino sugar or the insoluble humin fraction was found (5). Although the experimental results did not show a distinct trend in relation to temperature variations, the temperature range of 25-30°C tended to favor N transformation activities.

**Interaction of decomposing rice residues with soil leachable cations** During the decomposition of rice residues in soil, the amount of available minerals might be affected and consequently alter plant growth. Chou and Chiou (6) studied the effect of rice straw incorporated into on the dynamics of some cations in pot soil. The results revealed that the concentrations of cations, K, Cu, and Mn, were higher in the first crop season whereas those of Na, Ca, Mg, and Zn were higher in the second crop season in Nankang paddy soil regardless of nitrogen fertilizer application. Most of our findings agree with those of Patrick and Mikkelsen (20). In flooded soil, the concentrations of reducible iron and manganese were relatively low. When the pot soil was mixed with rice straw and allowed to decompose, the amount of K was significantly higher than that in soil alone, but the concentrations of Cu, Fe, Mn, and Zn were, on the average, significantly lower in the soil in terms of the ratio of soil to straw. It is interesting to note that in several poor water-drainage areas in Taiwan, such as Changhwa, Taitung, and Pingtung, Zn deficiency is particularly pronounced during the second crop season.

## ALLELOPATHY IN RELATION TO CROPPING SYSTEMS

**Phytotoxic effect of cover crops on orchard plants** Wu et al. (32) compared the phytotoxic effects of some cover crops, namely *Centrocema* sp., *Indigofera* sp., and *Paspalum notatum* (Bahia grass), on the growth of pea, mustard, cucumber, cauliflower, rape, chinese cabbage, mungbean, watermelon, tomato, and rice. They found that rape was most sensitive to the extracts of these

cover crops. Among them, *Centrocema* and *Indigofera* exhibited the greater phytotoxic effect; moreover, the leachate of *Centrocema* inhibited the growth of banana. More recently, several cover crops including *Bromus catherticus, Pennisetum clandestinum, Lolium multiflorum* (both chromosome 4X and 2X cultivars), *Paspalum notatum*, and white clover are now under investigation for allelopathic effects on the productivity of apple and peach plantations in the Lishan area of central Taiwan (3). A vast area of apple plantations has been situated on the hillsides of the Central mountain since the 1960s. The productivity of these plantations was exceedingly high in the first decade after planting but has gradually decreased in recent years. In fact, this problem has been encountered in many European countries and Northern America as well.

**Forest-pasture intercropping** Taiwan is an island, with two thirds of the land occupied by mountains, and its forests are extremely important for water conservation. The limited amount of agricultural land for crops and pasture forces farming activities to move upward to hillsides and higher elevations. A forest-pasture intercropping system has been thought to be a possible way to increase livestock production. Recently we have conducted several experiments in the forest area of Hoshe Experiment Station of National Taiwan University located at an elevation of about 1200 meters (7). An area of about one hectare was deforested, part was cleaned by removing the leaf litter of the conifer tree (*Cunninghamia lanceolata*), and part was left unchanged to serve as control. The cleaned and unchanged plots were planted with kikuyu grass (*Pennisetum cladestinum*) or left open. The experiment was designed to determine the reciprocal interaction of fir litter and kikuyu grass, and to evaluate the allelopathic potential of the two plants on weed growth under natural conditions. Results (7) indicated that the biomass of kikuyu grass in the cleaned plot was significantly higher than that in the control plot. In addition, the number of weeds that grew in the plot planted with kikuyu grass was lower than that in the control plot, indicating that the kikuyu grass may compete with and suppress weeds. The seedlings of fir regenerated in the deforested area grow well and seemed to not be affected by the neighboring newly planted kikuyu grass. However, the growth of kikuyu grass was inhibited by the fir litter left on the unchanged plot in the first three months after deforestation. Furthermore, bioassay of aqueous extracts showed that the fir litter extract exhibited higher phytotoxicity than the kikuyu grass. Nevertheless, four months after deforestation the kikuyu grass growth in the field was luxuriant, indicating that the phytotoxicity of fir litter disappeared (7).

## WAYS OF ENHANCING CROP PRODUCTIVITY BY ELIMINATING ALLELOPATHIC COMPOUNDS

**Improvement of water drainage** As mentioned earlier, the reduction of rice yield in the second crop season in Taiwan is partly due to the phytotoxic substances produced during the decomposition of rice residues in soil in areas of poor water-drainage. In addition, it was mentioned that the denitrified organism, *Pseudomonas putida*, was the dominant microbe during the decomposition of rice residues in soil. To eliminate the phytotoxins in paddy soil, a large scale experiment of improving drainage system has been conducted in Chiatung, where the water table is relatively high and water-drainage is poor. The results showed that the rice yield has been increased by at least 30% since the system was improved (1). We have analyzed the poor water-drainage and improved water-drainage soils for the phytotoxicity and phytotoxins present. Significantly greater phytotoxicity and higher amounts of phytotoxins were present in the poor

water drainage soil than in improved water drainage soil, indicating that the phytotoxins were leached from the poor water drainage paddy soil; thus the productivity of rice was greatly increased. Furthermore, Wu et al. (31) found that the total amount of phytotoxic phenolics in the well-drained Tsao-tun soil (0.52 x $10^{-2}$ mole/100 g soil) was significantly lower than that in the poorly drained Lotung soil (1.92 x $10^{-2}$ mole/100 g soil). They also reported that the amounts of nonvolatile and volatile fatty acids also were significantly lower in the well-drained soil (7.61 umole/100 g soil) than that in the poorly drained soil (37.16 umole/100 g soil). Rice yield in these poorly drained soils was lower. The amounts of phytotoxic phenolics were also correlated to that of denitrifying bacteria as described in the earlier session (31).

**Removal of soil phytotoxins by water flooding** It has mentioned that several years after growth of sugar cane, a significant reduction of yield usually occurred (29). This was due to the phytotoxins produced during the decomposition of sugar cane residues left in the soil and the imbalance of the microbial population. In order to eliminate the soil phytotoxins and to improve the soil condition of the microbial balance, the effects of water flooding on the sugar cane soil were studied (15, 29). The population of *Fusarium oxysporum* was exceedingly high in the low-yield sugar cane soil before water flooding, but the population decreased after flooding. In addition, the amount of phytotoxin, fusaric acid, produced by *F. oxysporum*, was significantly lower after-flooding, indicating that phytotoxins had been leached out (15).

**Crop rotation** Many monoculture fields often produce a soil-sickness problem, which is assumed to be due to the imbalance of soil microorganisms, accumulation of soil toxins, mineral deficiency, or abnormal soil pH, resulting in a decrease in crop productivity. Rotation of crop will avoid or eliminate the cause of the problem. Although many successful examples of crop rotation have been reported, only a few studies concerned allelopathic effects (23). In Taiwan, pangola grass (*Digitaria decumbens*) produces a highly productive pasture and constitutes a dominant species; however, the productivity declines several years after planting. Chou and his associates found that the pangola grass produced phytotoxins, which suppressed its own growth (1). The declined productivity of this grass has been particularly pronounced in the farm of Hengchun Experiment Station of Taiwan Livestock Research Institute. Thus, a crop rotation system of pangola grass-watermelon-pangola grass was established. The watermelon was planted in the drought winter season and pangola grass in the spring season following the harvest of watermelon. The yield of pangola grass after watermelon was significantly increased to about 40% as compared to that without the rotation. It is assumed that the increase of grass production could be a result of the disappearance of phytotoxins produced by pangola grass, and the phytotoxic effect is apparently lessened by the rotation.

**Detoxification of phytotoxins by polymerization of humic substance** Many phytotoxic substance bound to clay minerals or other organic compounds will result in decrease of phytotoxicity (25-29). Wang et al. (28) found that protocatechuic acid, one of the phytotoxins related to trans *p*-coumaric acid, can be polymerized with humic acid by using clay minerals as heterogeneous catalysts. In fact, humic acid can polymerize many kinds of substances, such as amino acids, flavonoids, terpenoids, aliphatic acids, and other nitrogen containing compounds, thus keeping the soil in a fertile state. However, it is also possible that the polymerization of phytotoxic phenolics fixed in humic substances can be reversed under certain environmental conditions, with subsequent release of free phenolic compounds that will exert a phytotoxic

effect on nearby susceptible plants (3). If this is the case, the natural device of organomineral complexing of humic acid would actually be a pool of detoxification of toxic substances produced by plants.

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# ECOTYPIC VARIATION IN *ECHINOCHLOA COLONA* I. COMPARATIVE MORPHOLOGICAL AND PHENOLOGICAL DIFFERENCES

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## ABSTRACT

Twelve ecotypes of *Echinochloa colona* (L.) Link were characterized based on the latitude and habitat at the collection sites. Within 20 days of growth there was significant variation in plant height, accumulative tiller length, leaf area, and dry weight of the ecotypes. However, variation decreased with time. During the experimental period, ecotypes from Batangas and Leyte grew consistently slower than the other ecotypes. The growth patterns between the ecotypes were different; the relative growth rate (RGR) of the IRRI green ecotype was related to leaf area ratio (LAR) whereas the RGR of the ecotype from Camarines Sur was associated with net assimilation rate (NAR). Days to panicle emergence were correlated to the latitude at which the ecotypes were originally growing. Ecotypes from higher latitudes produced panicles at least 10 days before ecotypes which originated from lower latitudes. There were significant differences in the total number of spikes per plant, accumulative length of the spikes, seed size, and total seed output among the ecotypes. Although there was a wide variation in seed weight and germination ability of the ecotypes, the seed weight and seed size were not correlated to the germination ability, but the subsequent seedling growth was dependent upon the seed weight. Ecotypes with heavier seed weight gave rise to more vigorous seedlings at 10 days after seeding (DAS) compared to those ecotypes with lighter seed weight.

## INTRODUCTION

A multitude of ecotypic species has arisen through natural selection by different combinations of environmental factors (8). The ecotypic diversity of weeds results from the sum of its organic attributes which are demonstrably related to environment (7). There are several examples of morphological variation between ecotypes of weeds of different altitudinal and latitudinal origins (1, 9, 13). These include variation of growth habit, plant height, length and size of leaf, and size and shape of inflorescence(3, 18, 21).

*E. colona* is an important weed in five of the world's major crops which grow between latitudes 23°N and 23°S (10). In dryland rice (*Oryza sativa* L.), *E. colona* causes profound reduction in yield by its severe infestation, rapid growth, and great competitive ability. It

occurs in a wide variety of habitats ranging from wetland to dryland and from sites at very low altitudes to sites located 2000 m above sea level (14).

Few studies of ecotypic variation have been previously conducted with *E. colona*. Ramakrishnan (16) observed two ecotypes of *E. colona* in India, a tall form growing in very moist to waterlogged soils and a short form thriving in drier soils. In the Philippines, Pagaspas (15) distiguished one ecotype commonly associated with wetland rice from another growing with upland crops on the basis of growth and developmental pattern, reproductive capacity, and factors affecting germination.

This study was conducted to determine the pattern of ecotypic variation of *E. colona* in the Philippines based on growth and developmental and phenological characteristics.

## MATERIALS AND METHODS

Twelve selections of mature seeds of *E. colona* were obtained from different areas throughout the Philippines (Fig. 1). For convenience, the names of the provinces were reduced to two letters to designate each ecotype. Two ecotypes were found at the International Rice Research Institute (IRRI); one which had a red seed coat was designated as ecotype IR and the other which had a green seed coat was designated as ecotype IG. Seeds of all ecotypes were germinated and grown to maturity on a greenhouse under one environment condition at IRRI to obtain sufficient and uniform seeds for this experiment. The greenhouse had natural sunlight with temperature ranging from approximately 18°C at night to 33°C during the day.

Five seeds of each ecotype were planted on the soil surface in a plastic pot (14 x 14 cm) which contained sieved sandy clay loam soil. Before the seedlings had reached the two-leaf stage, the number of seedlings per pot was reduced to one. After removal of the extra seedlings, the equivalent of 100 kg N/ha was applied as urea. The pots were subirrigated for the first 5 DAS and the soil was maintained at saturation during the rest of the experimental period by frequent watering. To obtain growth parameters such as RGR, NAR, and LAR, four replications were harvested at random at 10-day intervals and data on plant growth were collected 10, 20, and 30, DAS. Four replications were used to determine the morphological differences in panicles and the reproductive capacity 60 DAS.

After taking 100-seed weight and seed size, one hundred air-dried seeds of each ecotype were planted on the soil surface in a plastic pot (9 x 9 cm) to determine the germination ability of the ecotypes. There were four replications. At 10 DAS, the number of germinated seeds was counted and 10 seedlings per pot were selected at random to determine seedling growth.

Reproductive capacity (Salisbury, 1946 as cited in (7) of each of the ecotypes was calculated as follows: Reproductive capacity = (total number of seeds/plant x % germination)/100.

Growth parameter RGR, NAR, and LAR were calculated using the following equations (5).

RGR (mg $mg^{-1}$ $day^{-1}$) = $(\log_e W_2 - \log_e W_1)/(t_2 - t_1)$

NAR (mg $cm^{-2}$ $day^{-1}$) = $[(W_2 - W_1)(\log_e L_2 - \log_e L_1)]/[(t_2 - t_1)(L_2 - L_1)]$

LAR ($cm^2$ $mg^{-1}$)=$[(L_2 - L_1)(\log_e W_2 - \log_e W_1)]/(W_2 - W_1)(\log_e L_2 - \log_e L_1)]$

where $W_1$, $L_1$, $W_2$, and $L_2$ represent dry weights and leaf areas at the beginning and end of time interval, $t_1$ -$t_2$ days. The replicated values for statistical analysis were obtained by calculating the parameters for paired plants across each harvest interval: the largest at $t_1$

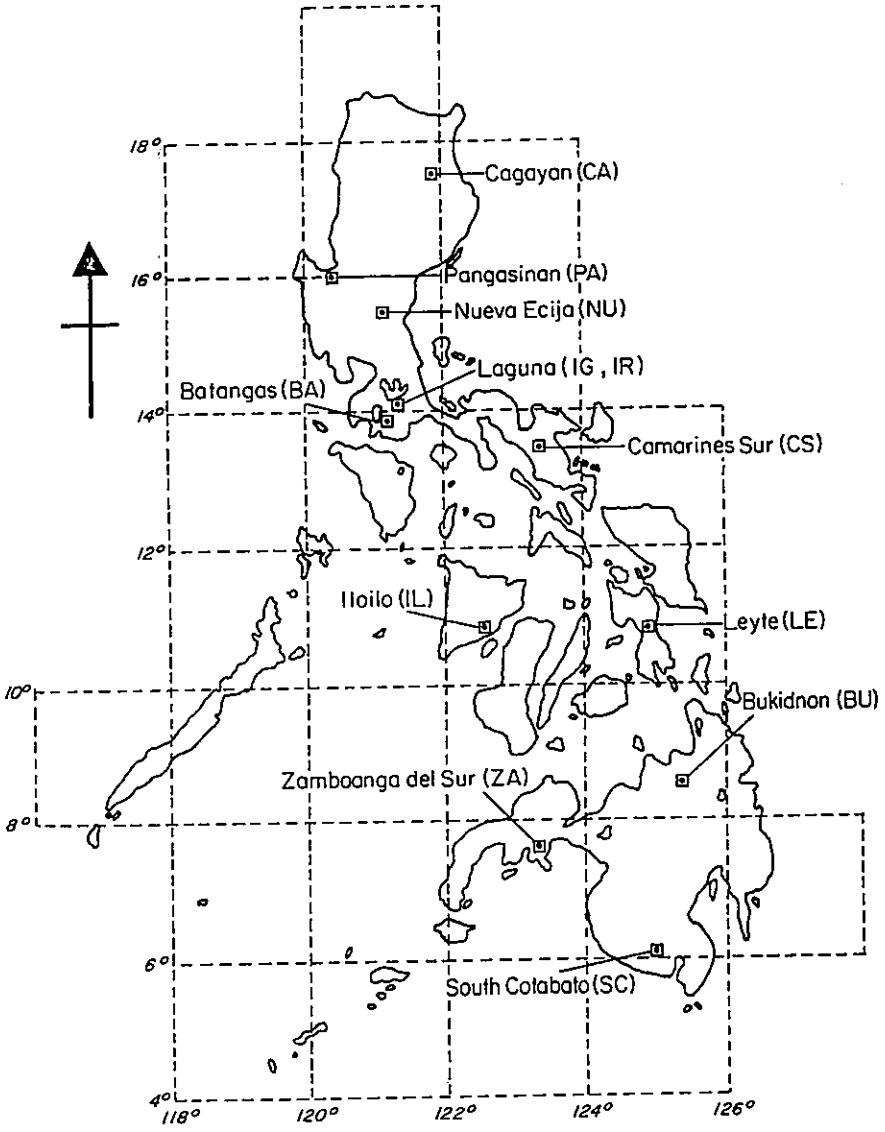



Fig. 1. Collection sites of Echinochloa colona in the Philippines (abbreviations denote each ecotype).

Table 1. Description of *Echinochloa colona* collection sites.

| Ecotype | Province | Location | Soil Moisture Condition | Associated Crop | Previous Crop |
|---|---|---|---|---|---|
| CA | Cagayan | 8 km from Solana on the way to Iraga | Moist | Mungbean | Lowland rice |
| PA | Pangasinan | Caaringayan, Laoac | Moist | None | Lowland rice |
| NU | Nueva Ecija | 5 km from San Jose on the way to Cabanatuan | Moist | None | None |
| IG | Laguna | IRRI, Los Banos | Dry | Maize | Maize |
| IR | Laguna | IRRI, Los Banos | Dry | Maize | Maize |
| BA | Batangas | San Felipe, Cuenca | Moist | Dryland rice | Maize |
| CS | Camarines Sur | 1 km from Pili on the way to Legaspi | Moist | Lowland rice | Lowland rice |
| IL | Iloilo | Sta. Monica, Oton | Saturated | Lowland rice | Lowland rice |
| LE | Leyte | Abuyog Experiment Station | Saturated | Lowland rice | Lowland rice |
| BU | Bukidnon | 8 km from Pangantucan on the way to Magsaysay | Flooded | None | Lowland rice |
| ZA | Zamboanga del Sur | 0.62 km from Pili on the way to Pagadian | Dry | Lowland rice | Lowland rice |
| SC | South Cotabato | 10 km from President Quirino on the way to Mariano Marcos | Flooded | Lowland rice | Lowland rice |

paired with the largest at $t_2$; and second largest at $t_1$ and the second largest at $t_2$; and so on (11). Means were then calculated for each harvest date.

## RESULTS AND DISCUSSION

**Description of collection sites** The latitude and longitude of the collection sites of the *E. colona* ecotypes ranged from 6° to 18°N and from 120° to 126°E, respectively (Fig. 1). Most ecotypes were collected from moist to flooded soils, except for ecotypes IG, IR, and ZA which were obtained from dryland soils (Table 1). When the ecotypes were collected, ecotype CA was associated with mungbean [*Vigna radiata* (L.) Wilczek] which had been planted after wetland rice. Ecotypes PA and BU were obtained during a fallow period in a wetland rice area. Ecotypes IG and IR were obtained from a maize (*Zea mays* L.) field. Ecotype BA was collected from a dryland rice field. The rest of the ecotypes except for ecotype NU were associated with wetland rice. Ecotype NU was not associated with any crop before or during the collection period.

**Variations at different growth stage** As the twelve ecotypes developed, considerable variation in plant height, accumulative tiller length (Table 2), leaf area, and dry weight (Table 3) was observed. There were significant differences in plant heights of the different ecotypes during the first 10 to 20 days of growth, but variation in plant height decreased after that period. None of the ecotypes produced tillers 10 DAS. At 20 DAS, there was a 2.8-fold variation in accumulative tiller length for the ecotypes whereas the variation decreased to 1.8-fold at 30 DAS. Variations in leaf area and dry weight of the ecotypes were similar to those for plant height (Table 3).

The fact that variation in the growth characters among the ecotypes decreased as the plants developed indicated differences in growth rate of the ecotypes. Ecotypes PA, NU, CS, and SC grew more rapidly for the first 10 DAS, resulting in greater plant height, leaf area, and dry weight when compared to ecotypes CA, IR, BA, LE, and ZA. For the second 10 DAS, ecotypes IG, CS, and ZA grew faster. Ecotypes BA and LE grew consistently slower than the other ecotypes and produced shorter accumulative tiller lengths and lower dry weights 30 DAS although the plant heights were not significantly different from those of the IG, CS, and ZA ecotypes.

The growth of the ecotypes during the periods after the first 10 DAS was expressed by growth parameters (Table 4). The RGR of ecotypes IG and CS during the second 10 DAS was not significantly different, but contribution of NAR and LAR to the RGR was different: NAR of ecotype CS was significantly greater than that of ecotyped IG while a significantly greater LAR was obtained from IG when compared with ecotype CS, indicating that the RGR of ecotype CS was correlated to NAR whereas the RGR of ecotype IG was associated with LAR. This indicates that although the dry weights of the plants were equal the growth patterns between ecotypes were different. On the other hand, during the third 10 DAS no significant difference was found between the growth parameters of ecotypes IG and CS. In addition, although a similar trend was the second and third 10 DAS.

Contrasting results were found between ecotypes PA and LE. During the second 10 DAS, the RGR of ecotype PA was significantly greater than that of ecotype LE. However, the reverse was observed during the third 10 DAS, resulting from the higher capacity of ecotype LE to increase dry weight in terms of the area of its assimilatory surface. Differences in the growth patterns

Table 2. Plant height and accumulative tiller length of *Echinochloa colona* ecotypes at different growth stages.[1]

| Ecotype | Plant Height (cm) | | | Accumulative Tiller Length (cm) | |
|---|---|---|---|---|---|
| | 10 DAS | 20 DAS | 30 DAS | 20 DAS | 30 DAS |
| Cagayan | 12.3 de | 42.8 abc | 82.4 ab | 40.6 bcd | 247.2 cd |
| Pangasinan | 14.7 ab | 45.5 a | 81.6 ab | 64.8 a | 311.5 abc |
| Nueva Ecija | 13.8 abcd | 45.1 a | 87.7 a | 65.1 a | 307.8 abc |
| IRRI (green) | 13.2 bcde | 42.8 abc | 86.5 ab | 53.4 ab | 35.4 a |
| IRRI (red) | 12.0 ef | 38.2 bcde | 83.2 ab | 48.8 abc | 342.8 ab |
| Batangas | 10.4 f | 34.1 e | 75.3 b | 26.7 cd | 230.4 cd |
| Camarines Sur | 14.3 abc | 40.4 abcd | 80.1 ab | 47.7 abc | 309.9 abc |
| Iloilo | 15.5 a | 43.9 ab | 92.3 a | 55.4 ab | 314.6 abc |
| Leyte | 8.7 g | 35.0 de | 80.0 b | 23.4 d | 211.3 d |
| Bukidnon | 12.6 cde | 37.2 cde | 83.3 ab | 48.3 abc | 271.8 bcd |
| Zamboanga del Sur | 11.9 ef | 41.9 abc | 82.3 ab | 43.2 abcs | 280.3 bcd |
| South Cotabato | 13.8 abcd | 42.7 abc | 79.5 b | 55.2 ab | 250.0 bcd |

1 In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. DAS = days after seeding.

Table 3. Leaf area and dry weight of *Echinochloa colona* ecotypes at different growth stages.[1]

| Ecotype | Leaf Area ($cm^2$) | | | Dry Weight (g) | | |
|---|---|---|---|---|---|---|
| | 10 DAS | 20 DAS | 30 DAS | 10 DAS[2] | 20 DAS | 30 DAS |
| Cagayan | 3.5 bcd | 79.3 bc | 399.1 abc | 10.5 de | 0.26 bc | 2.60 ab |
| Pangasinan | 4.5 ab | 112.9 a | 478.4 ab | 15.7 a | 0.39 a | 3.25 a |
| Nueva Ecija | 4.1 abc | 86.3 ab | 499.6 a | 13.8 b | 0.31 abc | 2.91 ab |
| IRRI (green) | 3.7 bcd | 102.9 ab | 376.2 bc | 11.5 cd | 0.32 abc | 2.77 ab |
| IRRI (red) | 3.4 cd | 80.4 b | 428.4 abc | 10.3 de | 0.22 cd | 2.69 ab |
| Batangas | 2.1 e | 49.4 cd | 342.2 c | 6.9 f | 0.15 d | 1.58 c |
| Camarines Sur | 3.2 cd | 85.7 ab | 393.8 abc | 11.4 cd | 0.31 abc | 2.84 ab |
| Iloilo | 4.8 a | 83.6 ab | 467.0 ab | 13.5 b | 0.33 ab | 3.23 a |
| Leyte | 2.0 e | 31.2 d | 362.8 bc | 7.2 f | 0.15 d | 2.21 bc |
| Bukidon | 3.4 cd | 81.8 b | 416.4 abc | 12.8 bc | 0.29 abc | 2.89 ab |
| Zamboanga del Sur | 2.9 de | 78.3 bc | 427.5 abc | 9.4 e | 0.26 bc | 2.26 bc |
| South Cotabato | 3.9 abcd | 96.9 ab | 347.7 c | 12.0 bcd | 0.31 abc | 2.33 b |

1 In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. DAS = days after seeding.

2 Dry weightts are in mg.

Table 4. Growth parameters of *Echinochloa colona* ecotypes at different growth stages.[1]

| Ecotype | RGR (mg/mg/day) | | NAR (mg/cm$^2$/day) | | LAR (cm$^2$/mg) | |
|---|---|---|---|---|---|---|
| | 11-20 DAS | 21-30 DAS | 11-20 DAS | 21-30 DAS | 11-20 DAS | 21-30 DAS |
| Cagayan | 0.321 | 0.213 | 1.019 | 1.182 | 0.315 | 0.180 |
| Pangasinan | 0.321 | 0.212 | 1.113 | 1.129 | 0.288 | 0.188 |
| Nueva Ecija | 0.311 | 0.224 | 1.098 | 0.105 | 0.283 | 0.203 |
| IRRI (green) | 0.333 | 0.216 | 1.034 | 1.162 | 0.322 | 0.186 |
| IRRI (red) | 0.306 | 0.250 | 0.861 | 1.187 | 0.355 | 0.211 |
| Batangas | 0.308 | 0.235 | 0.955 | 0.945 | 0.323 | 0.249 |
| Camarines Sur | 0.330 | 0.222 | 1.190 | 1.252 | 0.277 | 0.177 |
| Lioilo | 0.320 | 0.228 | 1.148 | 1.301 | 0.279 | 0.175 |
| Leyte | 0.304 | 0.269 | 1.102 | 1.394 | 0.276 | 0.193 |
| Bukidnon | 0.312 | 0.230 | 1.125 | 1.265 | 0.277 | 0.182 |
| Zamboanga del Sur | 0.332 | 0.216 | 1.095 | 0.972 | 0.303 | 0.222 |
| South Cotabato | 0.325 | 0.202 | 1.029 | 1.029 | 0.316 | 0.196 |
| Mean | 0.319 | 0.226 | 1.064 | 1.160 | 0.301 | 0.197 |

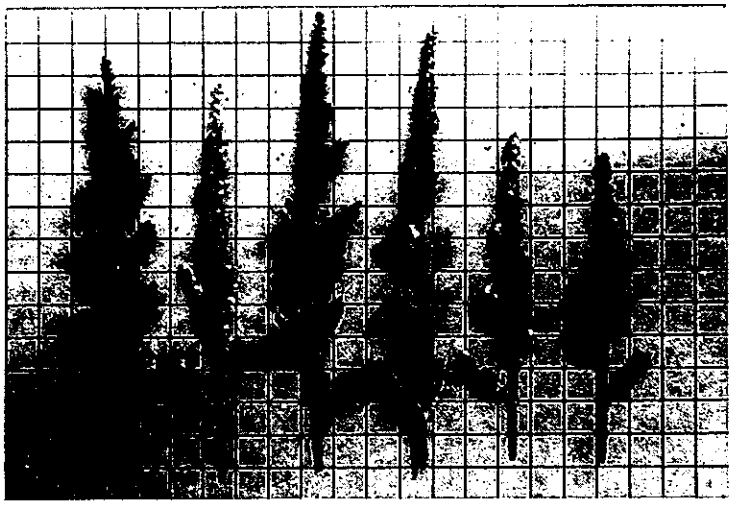
1 RGR = relative growth rate, NAR = net assimilation rate, LAR = leaf area ratio. DAS = days after seeding.

Table 5. Days required to panicle emergence for *Echinochloa colona* ecotypes and the leaf stage at which the panicle was produced.[1]

| Ecotype | Day | Leaf stage (Range) |
|---|---|---|
| Cagayan | 33 g | 10-12 |
| Pangasinan | 28 h | 9-10 |
| Nueva Ecija | 35 fg | 11-12 |
| IRRI (green) | 42 c | 12-14 |
| IRRI (red) | 40 d | 12-14 |
| Batangas | 36 ef | 10-13 |
| Camarines Sur | 37 e | 12 |
| Iloilo | 33 g | 9-10 |
| Leyte | 44 ab | 13-15 |
| Bukidnon | 46 a | 13-15 |
| Zamboanga del Sur | 43 bc | 11-13 |
| South Cotabato | 45 ab | 13-14 |

1 Means followed by a common letter are not significantly different at the 5% level by DMRT.

BA IG IR NU PA CA



SC ZA BU LE IL CS

Fig. 2. Typical panicles of the twelve ecotypes of Echinochloa colona showing variation in size and shape (each background grid is 1 x 1 cm).

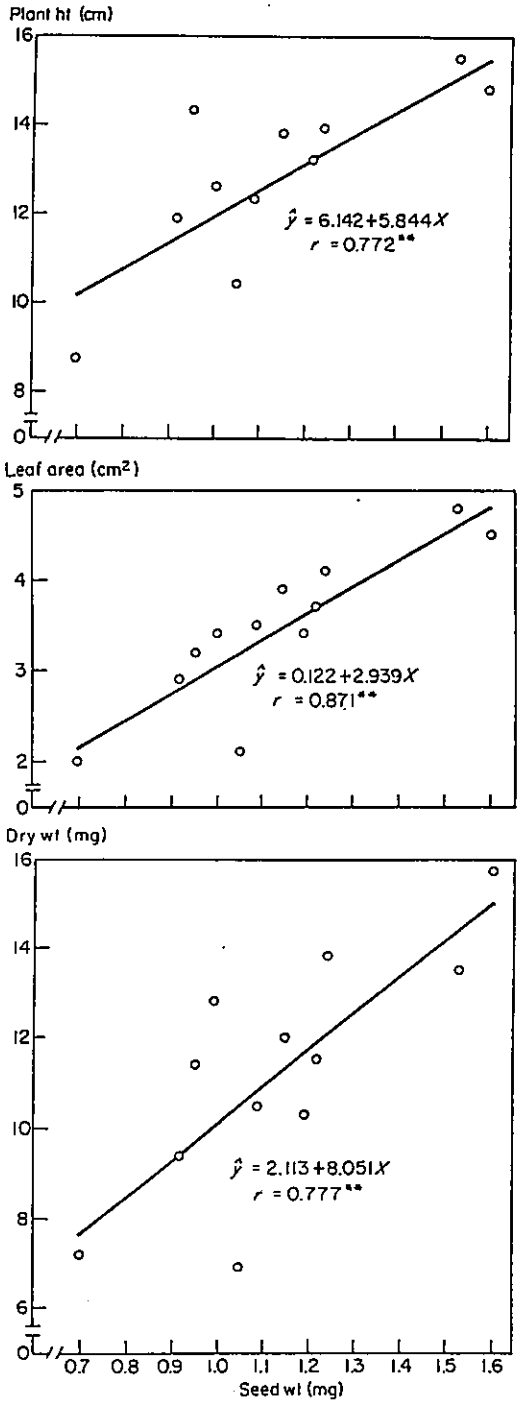



Fig. 3. Regression between seed weight and plant height, leaf area, and dry weight for Echinochloa colona ecotypes.

between the ecotypes would be an exhibition of persistent characters which had been obtained environmentally in the original collection sites according to ecological preferences.

**Panicle emergence** Significant differences were obtained in the time to panicle emergence among the ecotypes (Table 5). The ecotypes from higher latitudes (CA and PA) produced panicles at least 10 days before the ecotypes (BU, ZA, and SC) which originated from lower latitudes. However, the panicles of ecotype BA emerged earlier than ecotype IG and IR although they were collected from a similar latitude. Similarity on the time to panicle emergence was observed between ecotpyes IL and LE. This may have been due to the heritable patterns of growth which the ecotypes had obtained from their previous habitats.

The days required to panicle emergence were correlated to the latitude at which the ecotypes were originally growing. The correlation coefficient calculated between the time requirement for panicle emergence and the latitude of the original collection sites was -0.737 ($p < 0.01$).

Existence of latitudinal ecotypes was most strongly supported by the time required for floral initiation (2). This has been confirmed for several weed species (4, 22). The rapid growth of *E. colona* ecotypes collected from the higher latitudes appeared to be correlated with the environment in which the ecotypes had grown.

With increasing latitude, the daylength later in the year would be shorter. Under this condition, *E. colona* ecotypes would have had a shorter growing period which would result in the life cycle being completed more rapidly. By comparison, adaptation to long growing periods by maintaining from low latitudes. Ecotypes PA and CA coming from high latitudes emerged panicles at the 9- to 12-leaf stage, while the 13- to 15-leaf stage was required to emerge panicles for ecotypes BU and SC which were from low latitudes (Table 5).

In addition to the effect of the growing period, the original habitats would influence the phenological development of the ecotypes. Both ecotypes IG and IR were collected from a dryland habitat but the panicles emerged at least 4 days after ecotype BA which was collected from a wetland habitat. This was due probably to high soil moisture for the dryland ecotypes in the experiment. The dryland ecotypes would not tolerate the saturated growth condition to which the wetland ecotype was well adapted. A similar observation was made in an *Agrostis tenuis* Sibth population by Bradshaw (1), indicating flowering of a plant subjected to moisture stress (3) would result from the slow growth rate of the plant. However, early panicle emergence of ecotype IL compared to ecotype LE is difficult to explain in terms of the environmental factors. More complex factors might be involved in the adaptation of ecotype IL to the original collection site where climate and edaphic gradients might be greatly different from the other areas.

**Variation in panicle morphology** Great variability between the ecotypes was found in size and appearance of panicles (Table 6; Fig. 2). Relatively long panicles were found in ecotypes PA, IG, BA, LE, BU, ZA, and SC whereas ecotypes CA, NU, IR, CS, and IL had short panicles. The shape of the panicle in ecotype PA was an open panicle with relatively long spikes. A closed panicle was observed in ecotype IG. However, ecotypes IR, BA, LE, and ZA produced panicles in which the lower spikes were open and the upper were closed.

The total number of spikes per plant and accumulative length of the spikes were also significantly different among ecotypes. The average length of the spikes ranged from 1.31 cm for ecotype IL to 0.76 cm for ecotype BA. Bradshaw (1) determined panicle length and breadth

and angle of inflorescences in A. *tenuis* populations to represent intraspecific morphological variations.

**Differences in seed size and seed production** Seed size of ecotypes PA and IL were relatively larger than that of the other ecotypes (Table 7). The seeds of ecotypes BU, IG, and LE were the smallest with respect to length, breadth, and thickness, respectively. Differences in length, breadth, and thickness between the largest and the smallest seeds in the twelve ecotypes were 0.42, 0.27, and 0.18 mm, respectively.

The ecotypes varied greatly both in seed weight and number of seeds produced (Table 8). Both ecotypes PA and IL which had larger seeds produced significantly heavier seeds. However, significantly less seeds were produced by ecotype IL when compared with ecotype PA. Although ecotype LE produced the lightest seeds among the ecotypes, the amount of seed produced did not differ significantly from ecotypes PA and IL. Total seed output was closely related to accumulative spike length ($r = 0.711$, $p < 0.01$).

**Seed germination and reproductive capacity** Wide variation was observed in seed germination of the ecotypes(Table 8). A high germination rate was obtained with ecotypes CA, IL, and BU whereas the germination of seed of ecotype BA was only 25%. This was due probably to differences in seed dormancy characteristics of the ecotypes. Actual degree of dormancy may depend to a great extent upon climatic conditions during the seed maturation (20). Diverse environments for the *E. colona* selections at the original collection sites would have influenced differently the potential property of dormancy, which would result in the different germination behaviour under the experimental conditions.

The reproductive capacity of the ecotypes was obtained by multiplying the germination ability by the total number of seeds produced (Table 8). Although ecotype BA produced more seeds, its reproductive capacity was lower than that of ecotype IL which produced significantly less seeds that had a higher germination ability. This indicated that the production of a large number of surviving individuals was not necessarily achieved by simple increasing seed production. The different reproductive capacities of the different ecotypes would result in differences in the abilities for survival and dispersal.

**Regression of seedling vigour on seed weight** There was poor correlation between seed weight and percent germination of the ecotypes ($r = 0.541$, $p > 0.05$). There was no correlation between seed size and germination ability ($r = -0.054$ for seed length, -0.116 for seed breadth, and 0.501 for seed thickness; $P > 0.05$). However, weed weight affected subsequent seedling growth. Ecotypes with heavier seed weight gave rise to more vigorous seedlings compared to those ecotypes with lighter seed weights (Fig. 3). There was a linear relationship between seed weight and plant height, leaf area, and dry weight of 10-day-old seedlings. Differences in seedling vigour as affected by seed weight would be associated with the establishment ability of a plant species. During early growth more food reserves in the seed would supply more food (12), resulting in easier establishment of the seedlings. Gregor (7) reported that there was a tendency for large seeds to produce large seedlings and suggested that initial competitive advantage of the large seedlings would be maintained beyond the seedling stages.

The comparison of measurable characters for *E. colona* selections collected on the basis of the latitude and habitat at the collection sites provided evidence on the existence of *E. colona* ecotypes. Numerous differences in morphological and phenological characters between the ecotypes would have been due to the effects of the availability of suitable adaptive genes (6).

Table 6. Morphological differences of panicles of *Echinochloa colona* ecotypes

| Ecotype | Average Length of Panicles (cm) | No. of Panicles/ Plant | Total Number of Spikes/Plant (A) | Accumulative Length of Spikes (cm, B) | Average Length of Spikes (cm, B) A |
|---|---|---|---|---|---|
| Cagayan | 8.9 bcd | 7.3 abc | 69 bcd | 71.0 abc | 1.03 |
| Pangasinan | 10.7 ab | 5.0 efg | 62 cde | 78.9 abc | 1.27 |
| Nueva Ecija | 8.5 cd | 4.8 fg | 54 cde | 54.2 bcd | 1.00 |
| IRRI (green) | 10.8 ab | 6.5 bcd | 81 bc | 68.3 abc | 0.84 |
| IRRI (red) | 9.9 abcd | 8.3 a | 110 a | 98.5 a | 0.90 |
| Batangas | 11.4 a | 7.3 abc | 112 a | 85.4 ab | 0.76 |
| Camarines Sur | 8.1 d | 4.8 fg | 40 e | 33.5 d | 0.84 |
| Iloilo | 8.5 cd | 3.8 g | 39 e | 51.2 bcd | 1.31 |
| Leyte | 10.4 abc | 5.5 def | 69 bcd | 66.5 abcd | 0.96 |
| Bukidnon | 11.5 a | 6.3 cde | 79 bc | 77.7 abc | 0.98 |
| Zamboanga del Sur | 11.3 a | 4.0 g | 50 de | 48.7 cd | 0.97 |
| South Cotabato | 10.0 abcd | 7.8 ab | 93 ab | 97.6 a | 1.05 |

1 In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 7. Seed size of *Echinochloa colona* ecotypes.[1]

| Ecotype | Length (mm) | Breadth (mm) | Thickness (mm) |
|---|---|---|---|
| Cagayan | 2.46 ± 0.15 | 1.38 ± 0.13 | 1.11 ± 0.10 |
| Pangasinan | 2.75 ± 0.22 | 1.57 ± 0.13 | 1.18 ± 0.10 |
| Nueva Ecija | 2.56 ± 0.18 | 1.39 ± 0.10 | 1.09 ± 0.09 |
| IRRI (green) | 2.50 ± 0.14 | 1.30 ± 0.10 | 1.03 ± 0.06 |
| IRRI (red) | 2.45 ± 0.18 | 1.33 ± 0.08 | 1.03 ± 0.06 |
| Batengas | 2.71 ± 0.21 | 1.49 ± 0.15 | 1.05 ± 0.15 |
| Camarines Sur | 2.73 ± 0.19 | 1.44 ± 0.09 | 1.11 ± 0.07 |
| Iloilo | 2.80 ± 0.20 | 1.46 ± 0.12 | 1.19 ± 0.06 |
| Leyte | 2.40 ± 0.14 | 1.32 ± 0.11 | 1.01 ± 0.06 |
| Bukidnon | 2.38 ± 0.19 | 1.33 ± 0.11 | 1.04 ± 0.05 |
| Zamboanga del Sur | 2.48 ± 0.19 | 1.40 ± 0.12 | 1.04 ± 0.07 |
| South Cotabato | 2.56 ± 0.16 | 1.40 ± 0.13 | 1.06 ± 0.10 |

1 Numbers after means indicate standard error.

Table 8. Seed weight and seed production of *Echinochloa colona* ecotypes.[1]

| Ecotype | Weight of 100 Seeds (mg) | Germination (%, A) | No. of Seeds Produced/Plant (B) | Reproductive Capacity (A x B)/100 |
|---|---|---|---|---|
| Cagayan | 108.6 e | 94 a | 1441 ab | 1355 |
| Pangasinan | 159.9 a | 89 abc | 1919 a | 1708 |
| Nueva Ecija | 124.0 c | 85 abc | 1115 bc | 948 |
| IRRI (green) | 121.7 c | 86 abc | 1447 ab | 1244 |
| IRRI (red) | 119.1 cd | 76 bcd | 1803 a | 1370 |
| Batangas | 104.6 ef | 25 e | 1906 a | 477 |
| Camarines Sur | 95.2 gh | 71 cd | 711 c | 505 |
| Iloilo | 152.8 b | 93 a | 783 a | 728 |
| Leyte | 69.3 i | 57 d | 1393 ab | 794 |
| Bukidnon | 100.8 fg | 91 ab | 1459 ab | 1328 |
| Zamboanga del Sur | 91.5 h | 56 d | 998 bc | 559 |
| South Cotabato | 114.8 d | 73 bcd | 1820 a | 1329 |

1 In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Turrill (19) noted that spatially widely separated ecotypes may exhibit characters determined by genes restricted to the geographical regions in which they occur. The heritable characters which ecotypes had obtained through a process of adaptation to diverse environments in their habitat would appear as adaptational attributes that can be observed during various stages of evolutionary differentiation of a plant species.

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# *AZOLIA* : FRIEND OR FOE ?

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## ABSTRACT

In a long-term field trial, the use of azolla affected weed growth and species dominance. When no nitrogen and no azolla were applied, monochoria [*Monochoria vaginalis* (Burm. f.) Presl # MOOVA] was the dominant weed in all crops. In the azolla-inoculated plots, knotgrass (*Paspalum distichum* L. # PASDS) replaced monochoria in the later crops. In the nitrogen-treated plots without azolla, monochoria dominated in the first crop. In the later crops, it co-dominated with *Echinochloa glabrescens* Munro ex Hook. f. In the azolla-inoculated plots, *E. glabrescens* monochoria in the later crops. Weed growth was greater in the nitrogen-treated plots in the absence of azolla. Consistently higher grain yield was obtained with azolla inoculation regardless of nitrogen level. In greenhouse trials, azolla inoculation reduced light transmission ratio (LTR) and the $O_2$ content, temperature and pH of the flood water. When azolla was inoculated flooding to a depth of 10-15 cm or submergence for at least 24 hours immediately after transplanting, reduced plant height, tiller number, dry weight, and grain yield. Thus, azolla can be a friend or foe depending on the circumstances.

## INTRODUCTION

Azolla is a free-floating fern known for its nitrogen-fixing capacity through its association with a blue-green algae. For many years it has been used as a green manure in transplanted rice (*Oryza sativa* L.) in Vietnam and China (1).

Several workers (2, 3, 4, 7, 9) have reported that azolla suppresses weeds. Janiya and Moody (2) reported that azolla applied at 500g fresh weight/$m^2$ reduced total weed weight by 79%; small flower umbrella sedge (*Cyperus difformis* L. # CYPDI) which comprised 66% of the weed flora on a dry weight basis was suppressed 92%. The azolla-inoculated plots yielded as well as those that were hand weeded. In another trial (3), azolla suppressed weeds by 69-100% depending on the weed species.

In many regions of the world, azolla is considered to be a weed (5). It impedes water flow, clogs pipes and flood gates, disrupts fishing and livestock watering, interferes with crop cultivation, and hampers growth of rice seedlings. Suppression of young rice seedlings can occur if the water level rises after planting such that azolla is above the rice plants. When the water level falls, the azolla may pull down the rice and eventually kill it (6, 9, 11).

This paper reports the results of several trials conducted to quantify the benefits and detrimental effects of azolla on rice.

## MATERIALS AND METHODS

One field experiment and four greenhouse experiments were conducted at the International Rice Research Institute. For all experiments, IR58 was the test cultivar.

**Effect of azolla on weed growth and yield of transplanted rice** This experiment was conducted from May 1984 to June 1986 and seven crops were planted in succession. A split-split plot design with four replications was used. The treatments were nitrogen level (0 and 60 or 90 kg/ha), plant spacings (20 x 20 cm and 40 x 10 x 10 cm) and weed control methods (see Fig. 2).

Land preparation for each crop consisted of one plowing plus two or three harrowings. At the start of the experiment, the whole area was prepared. In the succeeding crops, each 5 x 4.5 m plot was prepared separately to prevent mixing of soil from different plots.

Sixty kg N/ha was applied during the wet season and 90 kg/ha during the dry season. Two-thirds of the N was applied and incorporated before planting together with 40 kg $P_2O_5$ and 40 kg $K_2O$/ha. The remainder of the N was applied at panicle initiation.

Two to three 18-day-old rice seedlings were transplanted per hill. Within two days after transplanting (DAT) the plots were flooded to a depth of 3-5 cm water, then 500 g/m$^2$ fresh weight of azolla (*Azolla microphylla* Kaulfuss, Collection #418) was inoculated into the plots except in the first crop when 150 g/m$^2$ was used.

Weeds were sampled from two 50 x 50 cm quadrats per plot at 45 DAT, bulked, sorted by species, dried at 80°C for 48 to 72 hours then weighed. The summed dominance ratios (SDR) of individual weed species were computed using the following equations:

SDR = (Relative density (RD) + Relative dry weight (RDW)) / 2
RD = [Density of a given species) / Total density] x 100
RDW = [(Dry weight of a given species) / Total dry weight] x 100

Rice yield was taken from a 5 m$^2$ harvest area in the center of each plot and converted to t/ha at 14% moisture.

**Effect of azolla inoculation on the soil-water environment** Two experiments were conducted to determine the effect of an azolla cover on the soil-water environment. The same treatments were used in each experiment.

Plastic buckets 60 cm in height and 38 cm in diameter were filled to within 10 cm of the top with soil. Forty kg N and 40 kg of $P_2O_5$ were incorporated before transplanting. Three 18-day-old rice seedlings were transplanted per hill and there were three hills/bucket. One DAT, the soil was flooded to a depth of 5 cm, then azolla was inoculated at the desired rate.

In the first experiment, LTR below the water level, $O_2$ content of water and pH of the water were taken at 2, 5, and 10 days after inoculation (DAI). In the second trial, LTR, $O_2$ content of the water, pH of the water and the soil, water temperature, and water loss were determined at 4, 8, 14, 21, and DAI.

**Effect of time and depth of flooding and azolla inoculation on the growth of rice and weeds** Factors studied were seedling age (10, 15, and 20 days), time of flooding (at transplanting, 5, 10, and 15 DAT), and flooding depth (2, 5, 10 and 15 cm). A split plot design with four replications was used.

Plastic buckets 50 cm high and 15 cm in diameter were used. The equivalent of 60-40-40 kg/ha of N, $P_2O_5$, and $K_2O$ was applied before transplanting. Three rice seedlings were transplanted per hill and there were two hills/bucket. Azolla was inoculated at a rate equivalent to 500 g fresh weight/$m^2$ immediately after transplanting.

The flooding depth was maintained for 24 hours. Thereafter, the water was drained to 2 cm which was maintained until harvest (35 DAT).

Plant height was taken before flooding and at harvest. Weed density, tiller number, leaf area, and dry matter of rice were taken at crop harvest.

**Effect of length of flooding and azolla inoculation on the growth and yield of rice** The experiment was laid out in a two-factor randomized complete block design with four replications. Factor A was with and without azolla, and factor B was the length of flooding (0, 1, 2, 4, 8, 24, and 48 hours).

Plastic buckets 50 cm high and 15 cm in diameter were filled to within 20 cm of the top with soil. Fertilizer was applied at 90-40-40 kg/ha N, $P_2O_5$, and $K_2O$. Two-thirds of N was applied before planting together with all of the $P_2O_5$ and $K_2O$. The remainder of the N was applied 40 DAT.

Three 15-day-old rice seedlings were transplanted per hill and there were 2 hills/bucket. Immediately after transplanting the soil was flooded to a depth of 2 cm and azolla was inoculated at a rate equivalent to 500 g/$m^2$. One DAT the water depth was increased to 15 cm, submerging the rice plants completely. After the desired length of flooding, the water was drained to a depth of 2 cm which was maintained until crop harvest.

Plant height, tiller number, number of productive tillers, dry matter yield, and grain yield were taken at crop harvest.

## RESULTS AND DISCUSSION

**Effect of azolla on weed growth and yield of transplanted rice** Data are averages of the two plant spacings used because they did not affect the results regardless of the weed control method and nitrogen level used. In some cases, data from the first, third, fifth, and seventh croppings are presented for ease of discussion. Similar trends were observed for the other croppings.

The dominant weed species in the unweeded plots were affected by azolla inoculation and nitrogen application. In the plots where no N was applied, monochoria dominated in the plots without azolla (Fig. 1). When the plots were inoculated with azolla, knotgrass replaced monochoria as the dominant weed. In the plots where nitrogen and no azolla was applied monochoria dominated in the first crop and co-dominated with *E. glabrescens* in the seventh crop. In the azolla-inoculated plots, *E. glabrescens* replaced monochoria as the dominant weed.

Weed weights were also affected by the application of N and the weed control method. Nitrogen application generally resulted in an increase in weed weight in the unweeded plots except for the azolla-no-weeding treatment in the first and fifth crops (Fig. 2). Nitrogen application had variable effects on weed weight in the hand weeded plots.

In the first crop, when no N was applied weed weight in the azolla-inoculated unweeded plots was higher than in the plots without azolla. This was due to the low amount of inoculum applied, poor azolla growth, and vigorous monochoria growth. In the third and fifth crops, weed

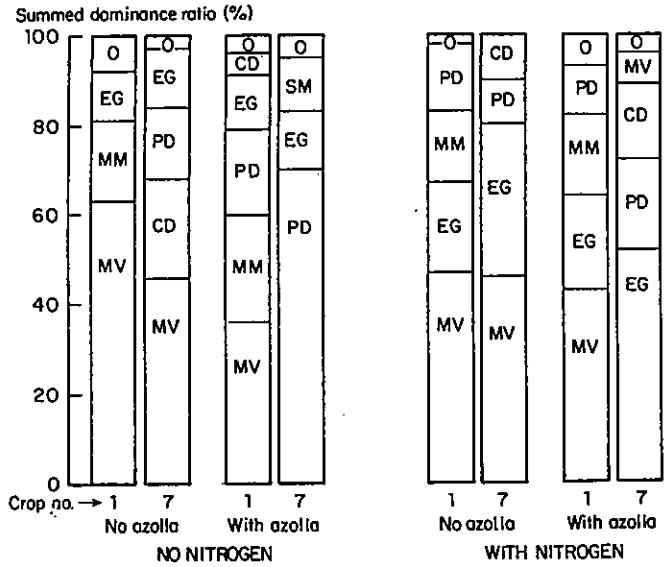


Figure 1. Summed dominance ratio of weed species in the unweeded plots as affected by azolla inoculation and nitrogen application (av. of two plant spacings). MV = Monochoria vaginalis, MM = Marsilea minuta EG = Echinochloa glabrescens, PD = Paspalum distichum, CD = Cyperus difformis, SM = Scirpus maritimus, O = others.

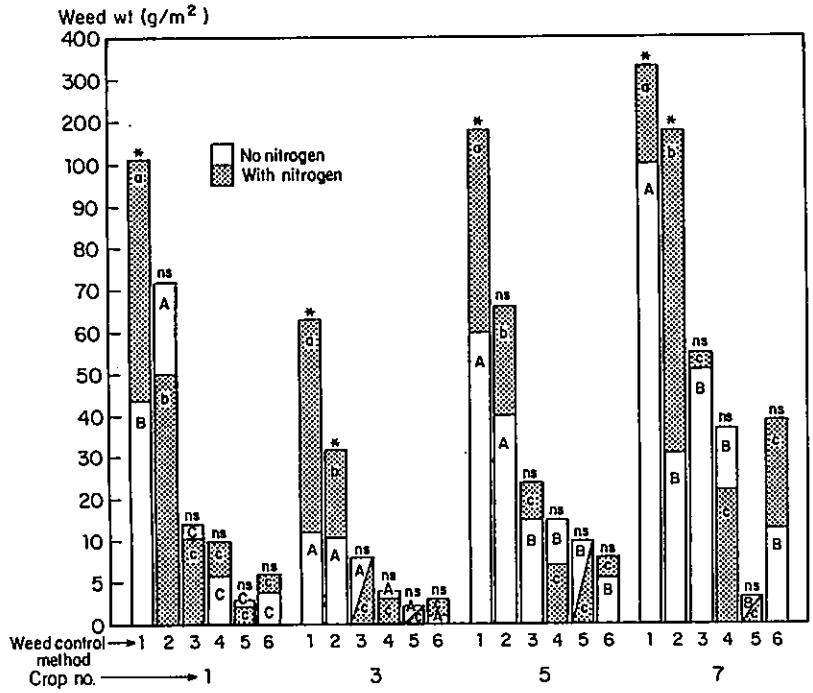


Figure 2. Weed weight at 45 days after transplanting as affected by azolla inoculation and nitrogen application (av. of four replications and two plant spacings). [*Significant at the 5% level, ns = Not significant] = for comparison between nitrogen levels. Capital letters = for comparison between weed control treatments when no nitrogen was applied. Small letters = for comparison between weed control treatments when nitrogen was applied. 1 = No azolla-no weeding, 2 = Azolla-no weeding, 3 = No azolla-hand weeded once, 4 = Azolla + hand weeding, 5 = No azolla-hand weeded twice, 6 = Azolla incorporated + hand weeding + inoculation.

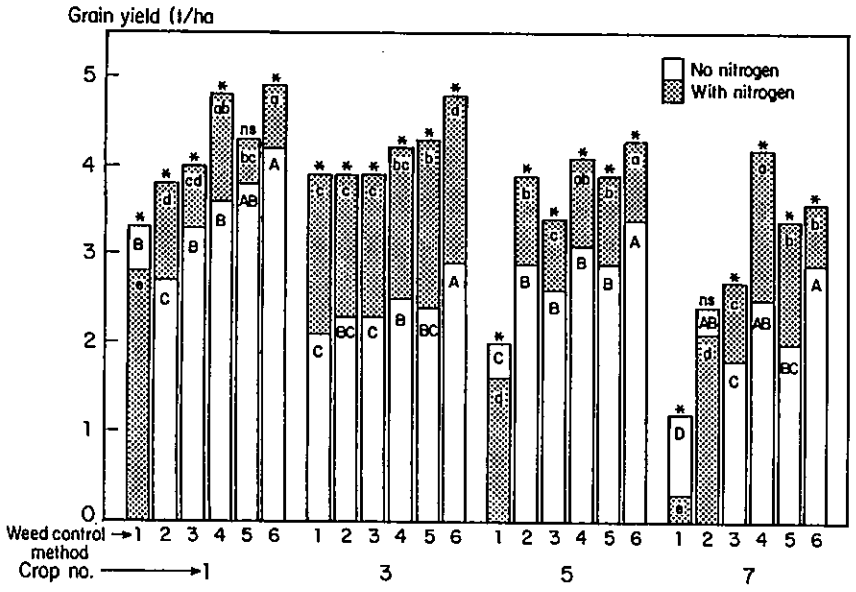


Figure 3. Grain yield as affected by azolla inoculation and nitrogen application (av. of four replications and two plant spacings). [*Significant at the 5% level) = for comparison between nitrogen levels. Capital letters = for comparison between weed control treatments when no nitrogen was applied. Small letters = for comparison between weed control treatments when nitrogen was applied. 1 = No azola-no weeding, 2 = Azolla-no weeding, 3 = No azolla-hand weeded once, 4 = Azolla + hand weeding, 5 = No azolla-hand weeded twice, 6 = Azolla incorporated + hand weeding + inoculation.

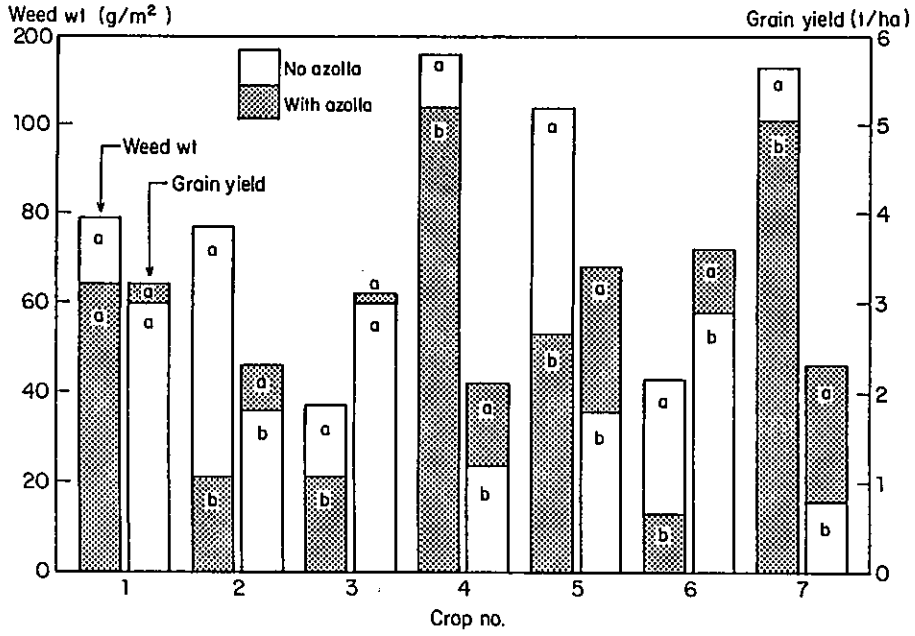


Figure 4. Weed weight and grain yield of IR58 rice cultivar in the unweeded plots across time as affected by azolla inoculation (av. of four replications, two nitrogen levels, and two plant spacings). Bars with the same letter are not significantly different at the 5% level.

weights in the unweeded plot with or without azolla were similar. In the seventh crop, weed weight in the azolla-inoculated plot was lower than in the one without azolla. Weeding resulted in a significant reduction in weed weights in all crops.

Grain yields were usually higher when fertilizer was applied regardless of the weed control method (Fig. 3). However, application of nitrogen in the weedy plots resulted in significantly lower yields in the first, fifth, and seventh crops due to greater weed competition.

In the unweeded plots, consistently higher yields and lower weed weights were observed in the plots inoculated with azolla (Fig. 4). There was an inverse relationship between grain yield and weed weight ($y=3.39 - 0.01X$, $r=-0.84^{**}$).

**Effect of azolla inoculation on the soil-water environment** In the first experiment, LTR and water pH were reduced by azolla inoculation at all sampling times (Table 1). The oxygen content of water was reduced significantly by 29% at 2 DAI and 45% at 5 DAI when 500 $g/m^2$ of azolla was inoculated but it was not significantly reduced at 10 DAI.

In the second experiment, the weight of azolla increased by three to eight times 8 DAI. Thereafter, it increased at a slower rate (1.0 to 1.6 times until the completion of the experiment at 34 DAI. The greatest increase occurring in both cases with the lowest rate of inoculum.

The LTR decreased with increase in the amount of azolla inoculated (Table 2). The oxygen content of the water also decreased in the presence of azolla with the greatest reduction occurring at the highest level of inoculum regardless of sampling time (Table 3).

The azolla cover did not affect soil pH regardless of sampling time (data not presented). The pH of the water from 8 DAI was significantly (an average of 0.6-0.8) lower when azolla was inoculated indicating less photosynthetic activity and a higher $CO_2$ concentration under the azolla mat. In contrast, Roger et al. (8) reported that the pH of the flood water was fairly stable under floating macrophytes such as azolla. The amount of inoculum did not affect the pH. From 8 to 21 DAI the azolla cover altered water temperature (Table 4). At 8 and 14 DAI the greatest reduction in water temperature occurred when 300 to 500 $g/m^2$ azolla was inoculated. At 21 DAI all the azolla inoculated pots had lower water temperatures than the pots without azolla. Water loss was not affected by azolla inoculation.

Ngo-gia-Dinh (7) attributed the suppresive effect of an azolla cover to limited air diffusion and reduced light intensity but this is the first time to our knowledge that these effects have been quantified.

**Effect of time and depth of flooding and azolla inoculation on the growth of rice and weeds** Seedling age and time of flooding had no effect on rice growth (data not presented). However, flooding depth and azolla inoculation did (Table 5). Plant height, dry weight, and leaf area decreased when the azolla-inoculated buckets were flooded to a depth of 15 cm. The tiller number was significantly reduced at the 15 cm flooding depth in the buckets without azolla. A further reduction in tiller number was observed when azolla was inoculated at the same flooding depth.

The average height of the rice plants immediately after transplanting was 15 cm. Thus, when the flooding depth was 15 cm, the rice plants were almost completely submerged. After the draining of water, the rice plants were pulled down by the azolla mat. This caused reduction in growth of the rice plants. These findings support the observations of Moody (6), Singh and

Table 1. Light transmission ratio and water pH as affected by azolla inoculation[1]. IRRI, 1985.

| Azolla rate ($g/m^2$) | Days after inoculation | | |
|---|---|---|---|
| | 2 | 5 | 10 |
| | Light transmission ratio (%) | | |
| 0 | 53.1 a | 54.1 a | 31.0 a |
| 100 | 29.4 bc | 28.3 b | 29.6 a |
| 200 | 30.0 b | 13.3 c | 11.6 b |
| 300 | 29.7 bc | 13.7 c | 8.2 b |
| 400 | 18.5 c | 11.8 c | 9.2 b |
| 500 | 18.9 bc | 12.4 c | 3.7 b |
| | Water pH | | |
| 0 | 9.1 a | 9.5 a | 8.9 a |
| 100 | 8.9 a | 9.5 a | 8.7 ab |
| 200 | 8.8 ab | 9.3 a | 8.4 bc |
| 300 | 8.6 bc | 9.1 ab | 8.4 bc |
| 400 | 8.5 c | 8.5 c | 8.4 bc |
| 500 | 8.5 c | 8.6 bc | 8.3 c |

1 In a column within each parameter, means followed by a common letter are not significantly different at the 5% level.

Table 2. Light transmission ratio (%) as affected by azolla inoculation[1]. IRRI, 1986.

| Azolla rate ($g/m^2$) | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 4 | 8 | 14 | 21 | 34 |
| 0 | 46.4 a | 57.4 a | 53.0 a | 40.5 a | 26.6 a |
| 100 | 15.0 b | 8.6 b | 3.6 b | 2.1 b | 0.8 b |
| 200 | 9.7 bc | 8.6 b | 1.6 bc | 1.4 b | 0.5 b |
| 300 | 7.3 cd | 3.7 c | 1.0 c | 0.4 b | 0.2 b |
| 400 | 4.5 cd | 3.1 c | 0.9 c | 0.4 b | 0.4 b |
| 500 | 3.3 d | 2.5 c | 1.0 c | 0.3 b | 0.2 b |

1 In a column, means followed by a common letter are not significantly different at the 5% level.

Table 3. Oxygen content of water (ppm) as affected by azolla inoculation[1]. IRRI, 1986.

| Azolla rate ($g/m^2$) | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 4 | 8 | 14 | 21 | 34 |
| 0 | 8.4 a | 10.6 a | 11.7 a | 13.9 a | 10.7 a |
| 100 | 6.8 b | 5.7 b | 6.7 b | 8.6 b | 6.5 b |
| 200 | 5.7 bc | 5.0 bc | 5.6 bc | 7.5 bc | 6.2 b |
| 300 | 4.9 cd | 4.7 c | 5.3 bcd | 7.0 cd | 6.2 b |
| 400 | 4.8 cd | 4.2 cd | 4.8 cd | 6.9 cd | 5.8 b |
| 500 | 3.5 d | 3.7 d | 3.9 d | 5.8 d | 5.4 b |

1 In a column, means followed by a common letter are not significantly different at the 5% level.

Table 4. Water temperature (°C) as affected by azolla inoculation[1]. IRRI, 1986.

| Azolla rate ($g/m^2$) | Days after inoculation | | | | |
|---|---|---|---|---|---|
| | 4 | 8 | 14 | 21 | 34 |
| 0 | 32.3 a | 29.1 a | 33.3 a | 31.4 a | 31.0 a |
| 100 | 32.3 a | 28.7 abc | 32.4 c | 30.1 b | 30.9 a |
| 200 | 32.3 a | 28.8 ab | 33.0 ab | 30.2 b | 30.8 a |
| 300 | 32.3 a | 28.4 bc | 32.6 bc | 30.2 b | 30.6 a |
| 400 | 32.3 a | 28.5 bc | 32.4 c | 29.9 b | 30.4 a |
| 500 | 32.3 a | 28.3 c | 32.2 c | 30.3 b | 30.7 a |

1 In a column, means followed by a common letter are not significantly different at the 5% level.

Table 5. Plant height, tiller number, leaf area, dry weight, and weed density as affected by depth of flooding and azolla inoculation[1]. IRRI, 1985.

| Depth of flooding[2] (cm) | Height (cm) | Tiller number[3] | Leaf area ($cm^2$)[3] | Dry weight (g)[3] | Weed density (no./pot) |
|---|---|---|---|---|---|
| Without azolla | | | | | |
| 2 | 60.0 a | 10.3 abc | 1203 a | 6.7 a | 13.9 a |
| 5 | 60.5 a | 10.3 abc | 1224 a | 6.7 a | 8.9 b |
| 10 | 60.3 a | 10.8 a | 1277 a | 6.8 a | 8.6 b |
| 15 | 60.1 a | 9.6 bc | 1072 ab | 6.1 a | 8.8 b |
| With azolla | | | | | |
| 2 | 60.0 a | 10.5 ab | 1057 ab | 6.2 a | 0.1 a |
| 5 | 60.0 a | 10.2 abc | 950 bc | 6.4 a | 0 c |
| 10 | 59.4 a | 9.3 c | 1063 ab | 5.7 a | 0 c |
| 15 | 54.2 b | 7.6 d | 743 c | 4.3 b | 0 c |

1 Average of four replications, three seedling ages, and four times of flooding. In a column, means followed by a common letter are not significantly different at the 5% level.

2 Maintained for 24 hours. Thereafter the depth of flooding in all treatment was 2 cm.

3 Total of 2 hills.

Table 6. Plant height, dry weight, and grain yield as affected by length of flooding and azolla inoculation[1] IRRI, 1985.

| Length flooding (hours) | Plant height (cm) | | | Dry Weight (g/pot) | | | Grain yield (g/pot) | | |
|---|---|---|---|---|---|---|---|---|---|
| | No azolla | With azolla | Difference | No azolla | With azolla | Difference | No azolla | With azolla | Difference |
| 0 | 107 a | 105 a | 2 | 52 a | 52 a | 0 | 64 a | 70 a | -6 |
| 1 | 106 a | 102 a | 4 | 45 a | 46 a | -1 | 54 a | 59 ab | 5 |
| 2 | 108 a | 106 a | 2 | 50 a | 50 a | 0 | 60 a | 66 a | -6 |
| 4 | 104 a | 109 a | -5 | 53 a | 48 a | 5 | 61 a | 61 ab | 0 |
| 8 | 116 a | 100 a | 16 | 51 a | 42 ab | 9 | 64 a | 40 bc | 24 |
| 24 | 104 a | 56 d | 48 | 47 a | 25 bc | 22 | 60 a | 32 c | 28 |
| 48 | 104 a | 28 b | 76 | 53 a | 15 c | 38 | 62 a | 19 c | 43 |

1 In a column, means followed by a common letter are not significantly different at the 5% level.

* Significant at the 5% level, ** Significant at the 1% level, ns= Not significant.

Srivastava (10), and Tuzimura et al. (11). Weed density was significantly lower in the azolla-inoculated plots compared to the uninoculated ones (Table 5).

**Effect of length of flooding and azolla inoculation on the growth and yield of rice** Length of flooding had no effect on plant height, dry weight, and yield in the absence of azolla (Table 6). When azolla was inoculated significant reduction in plant height and dry weight was observed when there was flooding for 24 or 48 hours.

Grain yield decreased in the presence of azolla when there was at least 8 hours flooding. The greatest reduction in yield being observed when flooding lasted for 48 hours.

The use of azolla as a weed suppressant in transplant rice is undoubtedly helpful in reducing weed competition in transplanted rice. The reduction in weed growth due to azolla inoculation is explained by the reduction in light transmission, increase in $CO_2$ content, and decrease in oxygen content in water. However, in instances where water management is a problem, azolla could reduce crop growth and yield if flooding occurs early in crop growth and rice plants are submerged for 24 hours or more.

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# THE GENECOLOGICAL VARIATION OF THE PHOTOPERIODISM IN *ECLIPTA PROSTRATA* (L.) S.L. FROM JAPAN AND TAIWAN

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## ABSTRACT

The variations in the photoperiodic flowering responses of *E. prostrata* (L.) L. sensu lato were examined using 38 strains collected from Japan and Taiwan under three conditions; short day condition (8h), natural condition and long day condition (16h). Remarkable variations in flowering response were observed under the three photoperiodic conditions. The following three types of photoperiodic response were recognized. Type I : qualitative short day response; 35 strains from Japan. Type II : quantitative short day response; 2 from Okinawa Islands in the southernmost of Japan. Type III : day-neutral response; 1 strain from Taiwan. The variation in photoperiodic responses was discussed on the geography and history of *Eclipta prostrata*, then with special reference to the intraspecific two types (6).

## INTRODUCTION

*Eclipta prostrata* (L.) l. sens. Koyama and Boufford (3) (Compositae) has a vast variation and a wide geographic distribution extending from the tropics to the temperate regions of both hemispheres (1, 3).

*E. prostrata* has caused agricultural weed problems in many countries (1). Recently in Japan, frequent and increasing occurrence of this weed has been observed in arable land.

A wide variation in morphology, physiology and ecology of *E. prostrata* is observed in Japan. Intraspecific two types were found in Japan through genecological studies on *E. prostrata* (6, 7, 8): Round (R) type with winged large achenes (3 mm in length ) and Slender (S) type with wingless small achenes (2 mm in length).

Preliminary studies on *E. prostrata* showed some variation in the period to flowering in natural day condition using strains collected from Honshuu of Japan. The present paper concerns the variation in photoperiodic responses of *E. prostrata* collected from Japan and Taiwan.

## MATERIALS AND METHODS

Variation in the photoperiodic flowering responses of *E. prostrata* was examined using 38 strains collected form Japan and Taiwan (Fig. 1) under three conditions: short day (8 hours, SD), natural day (ND) and long day (16 hours, LD). Achenes were raised at alternate 30-15°C in 3000 lux fluorescent all day under wet condition. Dicotyledonous seedlings from properly germinated achenes were transplanted into the above three photoperiodic conditions on July 20,

1985. Repetition was five plants. Days to the first flowering after transplanting were counted until the end of November, 1985. The experiment was carried out at Kyoto university (35° 01'N 136°48'E). Voucher specimens are deposited in Laboratory of Weed Science, Faculty of Agriculture, Kyoto University.

## RESULTS

A wide variation was observed in all of the three photoperiodic conditions (Table 1).

**Variation in R type** Most of strains, 15 strains from Honshuu and 3 strains from Okinawa Islands of Japan, did not flower in LD. They continued vegetative growth for more than 100 days after transplanting and died of frost. In ND, a wide variation in the period to flowering after transplanting was observed among 18 strains. For expample, Strain No. 2 flowered in 33.0 days and No. 29 flowered in 75.6 days. Six strains, 3 strains, 3 from southern Honshuu (Nos. 24, 29 and 32) and 3 from Okinawa Islands (Nos. 33-35), flowered in as long as 62.2 to 75.6 days. In SD, the above 3 strains from Okinawa Islands flowered after more than 30 days while the remaining 15 strains from Honshuu flowered in less than 30 days. By SD treatment, the period to flowering of these strains, which had no flowering in LD, was significantly shortened (Table 1).

Three strains, 2 from Okinawa Islands (Nos. 36 and 37) and one from Taiwan (No. 38), flowered in LD ranging 32.6 to 44.2 days. In SD, they flowered in more than 30 days. In ND they flowered in 34.6 to 40.2 days. By LD treatment, the periods to flowering of these strains were not significantly affected. While the period to flowering of the strain from Taiwan was not significantly shortened by SD treatment, the periods to flowering of two strains from Okinawa Islands were significantly shortened (Table 1).

A statistically significant correlation between the period to flowering in ND and the degree of shortening by SD treatment (the days to flowering in ND minus the days to flowering in SD devided by the days to flowering in SD, %) was obtained ($y=-17.2+1.09x$; $r=0.92$ ($p$ 0.01), Fig. 2)

Considering the flowering responses in three photoperiodic conditions above mentioned, three types of photoperiodic responses were recognized in R type of *E. prostrata*.

Type I. The period to flowering was significantly shortened by SD treatment and flowering was not produced in LD. This response type was observed in 18 strains, 15 from Honshuu and 3 from Okinawa Islands.

Type II. The period to flowering was significantly shortened by SD treatment and flowered in virtually similar days in both ND and LD treatments, in 2 strains from Okinawa Islands.

Type III. The period to flowering was neither affected statistically significantly by SD nor LD, in the case of the only one strain from Taiwan.

**Variation in S type** All of 17 strains did not flower in LD and did flower in less than 30 days in SD. By SD treatment, the period to flowering was significantly shortened. Therefore, all strains of S type belonged to Type I. In ND, a wide variation in the period to flowering was also observed as R type. However, if excluding two strains which were estimated morphologically as putative hybrids, then little variation in the period to flowering in ND was observed, ranging from 33.6 to 39.2 days (Tables 1 and 2).

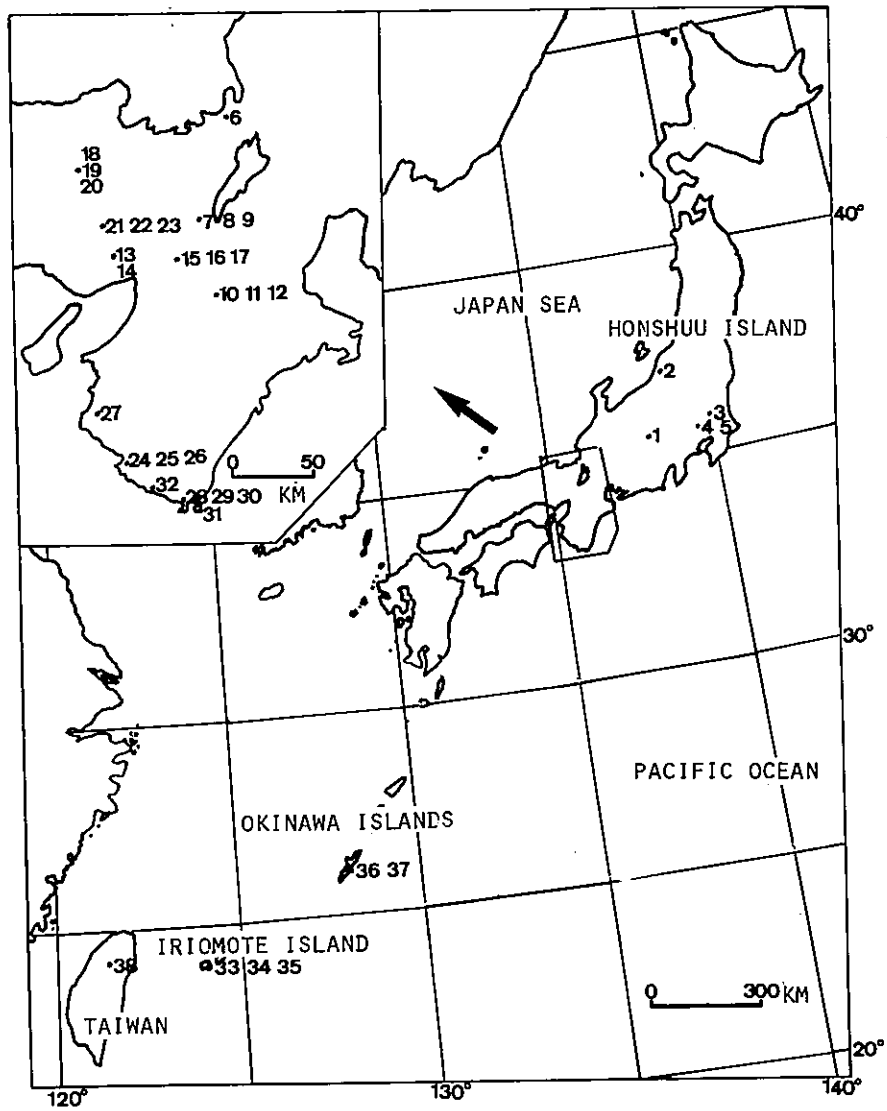


Fig. 1. Collection location of Eclipta prostrata strains.

Table 1. Photoperiodic response of Eclipta prostrata strains.

| Strain No. | Type | Days to flowering in three photoperiodic conditions | | | | Type |
|---|---|---|---|---|---|---|
| | | Short(A) | Natural(B) | (B-A)/B(%) | Long day | Response[1] |
| Honshuu of Japan | | | | | | |
| 1 | S | 27.5 | 36.8 | 25.3 | -[3] | I |
| 2 | R | 27.4 | 33.0 | 17.1 | - | I |
| 3 | S | 25.8 | 37.8 | 31.8 | - | I |
| 4 | R | 29.3 | 34.3 | 14.6 | - | I |
| 5 | S | 24.0 | 38.8 | 38.1 | - | I |
| 6 | R | 25.2 | 34.2 | 26.3 | - | I |
| 7 | R | 27.8 | 39.2 | 29.1 | - | I |
| 8 | R | 26.8 | 35.4 | 24.3 | - | I |
| 9 | S | 26.8 | 39.2 | 31.6 | - | I |
| 10 | R | 28.8 | 42.3 | 31.9 | - | I |
| 11 | S | 26.0 | 35.0 | 25.7 | - | I |
| 12 | S | 27.4 | 34.5 | 20.1 | - | I |
| 13 | S | 26.0 | 38.3 | 32.1 | - | I |
| 14 | R | 28.0 | 35.0 | 20.0 | - | I |
| 15 | R | 28.2 | 43.0 | 34.4 | - | I |
| 16 | R | 27.8 | 35.2 | 21.1 | - | I |
| 17 | R | 25.5 | 41.0 | 37.8 | - | I |
| 18 | S | 26.8 | 35.4 | 24.5 | - | I |
| 19 | S | 25.2 | 34.8 | 27.6 | - | I |
| 20 | S | 27.0 | 37.0 | 27.0 | - | I |
| 21 | S | 27.8 | 36.6 | 24.0 | - | I |
| 22 | R | 27.0 | 39.3 | 31.3 | - | I |
| 23 | S | 26.0 | 36.0 | 27.8 | - | I |
| 24 | R | 25.8 | 62.2 | 58.5 | - | I |
| 25 | S[2] | 25.6 | 49.5 | 48.3 | - | I |
| 26 | R | 26.8 | 39.8 | 32.7 | - | I |
| 27 | S | 24.6 | 34.8 | 29.3 | - | I |
| 28 | S | 26.4 | 33.8 | 21.9 | - | I |
| 29 | R | 28.0 | 75.6 | 63.0 | - | I |
| 30 | S[2] | 24.3 | 65.5 | 62.9 | - | I |
| 31 | S | 24.0 | 33.6 | 28.6 | - | I |
| 32 | R | 27.2 | 68.8 | 60.5 | - | I |
| Okinawa Islands of Japan | | | | | | |
| 33 | R | 31.5 | 65.4 | 51.8 | - | I |
| 34 | R | 31.7 | 70.0 | 54.7 | - | I |
| 35 | R | 31.6 | 69.8 | 54.7 | - | I |

| | | | | | | |
|---|---|---|---|---|---|---|
| 36 | R | 30.2 | 40.2 | 24.9 | 44.2 | II |
| 37 | R | 30.8 | 38.4 | 19.8 | 42.8 | II |
| Taiwan | | | | | | |
| 38 | R | 35.2 | 34.6 | -1.73 | 32.6 | III |

1 See the context; 2 Putative hybrid; 3 - : No flowering

Table 2. Variation of R and S types from Honshuu in the period to flowering under the natural day length.

| Type | No. of Strains | Days to flowering | | | |
|---|---|---|---|---|---|
| | | Max. | Min. | Mean | Range |
| R | 15 | 75.6 | 33.0 | 43.9 | 42.3 |
| S | 15[1] | 39.2 | 33.6 | 36.2 | 5.6 |

1 Excluding two putative hybrid strains.

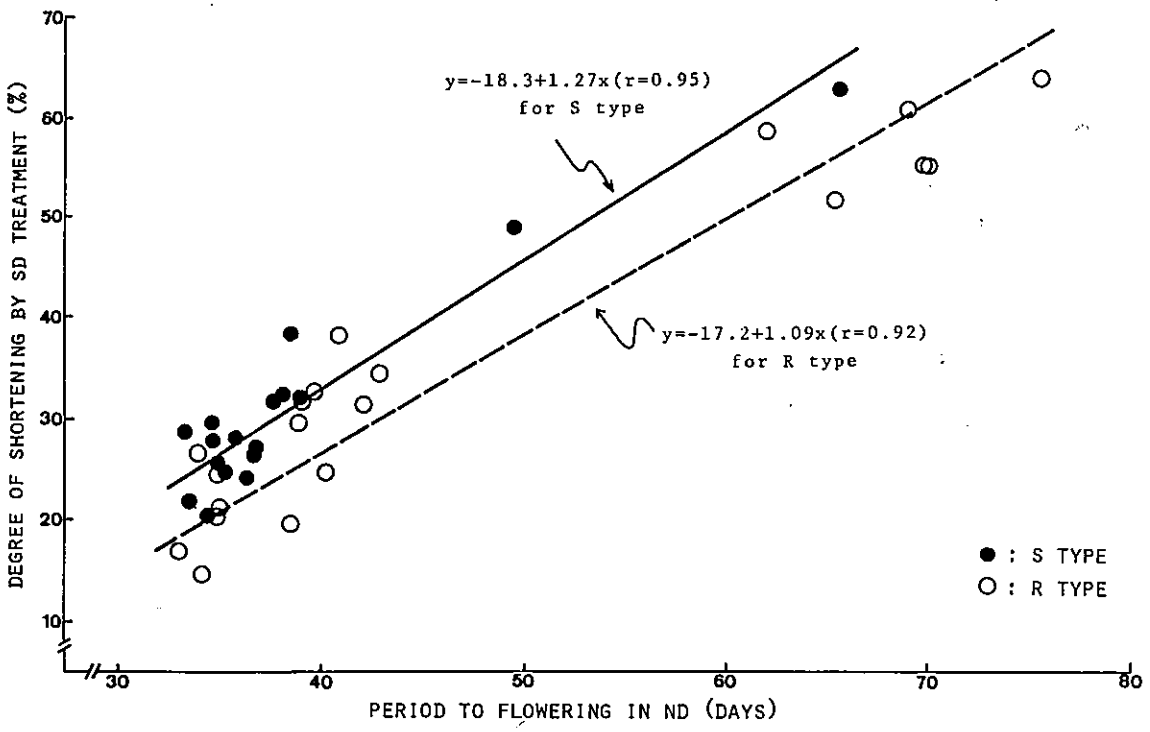


Fig. 2. Relationship between the period to flowering in ND and the degree of shortening by SD treatment. One strain from Taiwan is excluded.

A statistically significant correlation between the period to flowering in ND and the degree of shortening by SD treatment was also obtained (y=-18.3+1.27x; r=0.95 (p 0,01)) as R type (Fig. 2).

## DISCUSSION

Three types of photoperiodic responses were recognized (Table 1). Based on Lang (4) and Salisbury and Ross (5), Type I, II, and III were interpreted as qualitative short day, quantitative short day, and day-neutral respectively. Three response types were recognized in R type, while only Type I was recognized in S type. Furthermore, little variation of S type in the period to flowering in ND was also recognized (Table 2).

This lack of photoperiodic variation in S type may be attributed to its short history from the introduction to Japan. It was shown that S type had appeared since 1948, just after World War II by the examination of herbaria deposited in Kyoto University (KYO!), National Science Museum (TNS!) and Tokyo Metropolitan University (MAK!) (unpublished). The original immigrant population was estimated a small one and the founder effect may be concerned with the little variation of S type. Such examples can be seen in various introduced species. On the other hand, relict achenes in the Johmon era (ca. 2000 BP) reported by Kasahara and Takeda (2) showed characteristics of R type achenes this time. Such different histories between R and S types may be related to the different pattern of variation in photoperiodic responses between themselves.

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# BURIED WEED SEED POPULATION IN ARABLE SOILS

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## ABSTRACT

The buried seed population in the upper 15 cm soil horizon was determined by sieving/washing floatation and seedling emergence methods. Estimated total seed population was 48700 per m2 with 19230, 13326 and 16342 per m2 for the first (0-5 cm), second (5-10 cm) and the third (10-15 cm) soil layers respectively. There were 30 species found, with dominant species being *Digitaria* spp., *Panicum repens*, *Phylanthus niruri*, *Leucas lavandulifolia* and *Ageratum conyzoides*. The total number of germinated seeds was 7353 per $m^2$ with *Digitaria ciliaris*, *A. conyzoides*, *P. niruri* and *Cyperu* spp. as the most common species. Seeds of 16 species were overlooked in the sieving/washing floatation examination but were found in the seedling emergence examination while 13 species found in the sieving/washing floatation examination were not found in the seedling emergence test.

## INTRODUCTION

Weed seed population in soil varies with seed composition and is closely associated with the history of the land. If land was used for pasture, most of the seed found in that soil would be associated with pasture weeds, while cultivated land would have a seed population that is closely associated with cropland weeds (15). Seeds of many weed species mature with or can enter a state of dormancy that assures an extended life span in the soil. Weed seeds shed onto the soil surface may remain there or be incorporated into the profile by natural or artificial means (7).

Weed seed population in cultivated soils is generally composed of a few dominant species that are present in high numbers, a few species present in moderate levels, and a large variety of species present at low levels (12).

Very little information is available on the population of buried seed in arable soils of the tropics, and time of emergence of annual weeds.

The objective of the present research was to determine the species composition of the seedbank in different soil depth collected from arable, total population and their germination ability.

## MATERIALS AND METHODS

**Sampling** The sampled BIOTROP experimental field was located in Tajur, Bogor. The field had been under a corn/soybean/peanut rotation and subjected to various tillage regimes since 1980. Following soybean harvest in May 1986, 20 samples taken from an area of 1000 $m^2$ were selected and divided into 40 plots with plot sizes of 5 x 5 $m^2$ each. Twenty soil samples were taken randomly from these plots by using a square metal frame (25 x 25 $cm^2$). Each sample was carefully subdivided into three soil layers: 0-5, 5-10, and 10-15 cm (referred to as D1, D2 and D3, rspectively) using a 15 cm wide spade. Soil samples were carefully bagged and transported to laboratory, and after mixing were immediately divided into two parts for estimation of total weed seed population and viable weed seeds, respectively. Samples were then air dried under greenhouse conditions to an average soil moisture content of 5 % (w/w).

**Estimation of total weed seed population** Sub-samples of 1300 g airdried soil from different soil layers were used for the estimation of total weed seed population. The seeds in the soils were recovered by sieving with mesh of different sizes (No. 6, 12, 28, and 60). Large seeds found in the first three sieves could be easily removed by forceps but the last sieve retained almost all the seeds together with clay and organic materials. The separation of seeds in the last sieve was made by floating the seeds in 50 % $Na_2CO_3$ solution developed by Hayashi (4). The seeds and litter floated in the solution were airdried and the seed were selected from the litter under a binocular microscope using a forcep (referred as sieving/washing floatation method). Total population of buried seed found in different layers was expressed in square metres.

**Estimation of viable weed seeds** Sixty samples from different soil layers (D1, D2 and D3) were placed in plastic trays (15 x 20 x 5 cm3) with drainage holes which were kept in a greenhouse with average temperature of 29°C and watered daily. The soil samples were placed in thin layer mostly less than 2 cm deep.

Daily inspection was made and the seedlings noted and removed. Those that could not be identified were raised to maturity. The soil was turned over regularly, and the samples kept under greenhouse condition for 6 months, a period sufficient to obtain a reasonably accurate estimate of the number of viable seeds of arable weeds (referred to as seedling emergence method). The numbers of these apparently viable seeds in the sample were derived from the toatl subsamples and expressed in square metres basis.

## RESULTS

**Species composition of the buried seed** There was an appreciable number of species of the buried weed seeds in the soils ranging from 16 to 30 species per $m^2$. The most frequent species with 100% occurrence in all soil layers were *Digitaria* spp., *Cyperus* spp., *Phyllanthus niruri* L. and *Eleutheronthera ruderalis* Sw. Bip. Seeds of these species were found in all amples. *Panicum repens*, *Leucas lavandulifolia* Sm., *Ageratum conyzoides* L., *Polygala paniculata* L., and *Borreria* spp. also occurred in most samples (90%). Other species encountered frequently were *Porophyllum ruderale* L., *Cleome rutidosperma* L., *Centella asiatica*, *Oxalis corniculata* L. and *Eleusine indica* (L.) Gaertn (Table 1). Some of the dominant weed seeds are illustrated in Fig. 1.

**Total weed seed population** The total weed seed population (viable and non viable) found in the arable soils sampled from different layers in May 1986 is given in Table 2.

Table 1. Species composition of the seedbank and their frequency of occurrence (% of total samples) in different soil layers.

| Species | Soil layers | | |
|---|---|---|---|
| | $D_1$ (0-5 cm) | $D_2$ (5-10 cm) | $D_3$ (10-15 cm) |
| 1. *Digitaria* spp. | 100 | 100 | 100 |
| 2. *Cyperus* spp. | 100 | 100 | 100 |
| 3. *Panicum repens* L. | 100 | 95 | 100 |
| 4. *Phyllanthus niruri* L. | 100 | 100 | 100 |
| 5. *Leucas lavagdulifolia* Sm. | 95 | 100 | 95 |
| 6. *Ageratum conyzoides* L. | 85 | 95 | 100 |
| 7. *Eleutheranthera ruderalis* | 100 | 100 | 100 |
| 8. *Polygala paniculata* L. | 95 | 85 | 85 |
| 9. *Borreria* spp. | 100 | 90 | 85 |
| 10. *Porophyllum ruderale* L. | 90 | 80 | 75 |
| 11. *Mimosa pudica* L. | 0 | 5 | 0 |
| 12. *Cleome rutidosperma* | 80 | 80 | 80 |
| 13. *Centella asiatica* Lerb. | 80 | 75 | 80 |
| 14. *Oralis corniculata* L. | 85 | 85 | 80 |
| 15. *Eleusine indica* (L.) Gaertn | 85 | 50 | 75 |
| 16. *Echinochloa colonum* (L) Link | 55 | 40 | 50 |
| 17. *Amaranthus* sp. | 35 | 5 | 5 |
| 18. *Celosia argentea* L. | 15 | 25 | 20 |
| 19. *Bidens pilose* L. | 90 | 25 | 25 |
| 20. *Setaria glauca* P. Beauv. | 45 | 55 | 40 |
| 21. *Eclipta prostrata* L. | 45 | 45 | 40 |
| 22. *Richardia brassiliensis* Gomer | 60 | 45 | 45 |
| 23. *Euphorbia geniculata* Ortega | 65 | 35 | 20 |
| 24. *Paspalum* sp. | 55 | 30 | 35 |
| 25. *Melochia corchorifolia* L. | 20 | 20 | 15 |
| 26. *Chromolasena odorata* | 5 | 15 | 30 |
| 27. *Clibadium surinamense* | 10 | 0 | 5 |
| 28. *Minosa pigra* L. | 15 | 0 | 5 |
| 29. *Synedrella nodiflora* Gaertn | 10 | 5 | 10 |
| 30. *Amaranthus spinosus* L. | 5 | 0 | 5 |

Table 2. Weed seed population (viable and unviable) in different soil layers ($m^{-2}$.5cm) of arable field collected in May, 1985.

| Species | Soil layers | | |
|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ |
| 1. *Digitaria* spp. | 4861 | 3476 | 4020 |
| 2. *Cyperus* spp. | 5334 | 3734 | 3838 |
| 3. *Panicum repens* | 2291 | 1421 | 2580 |
| 4. *Phyllanthus niruri* | 1872 | 1170 | 1545 |
| 5. *Leucas lavandulifolia* | 1338 | 1086 | 1307 |
| 6. *Ageratum conyzoides* | 718 | 664 | 1037 |
| 7. *Eleutheranthera ruderalis* | 580 | 549 | 646 |
| 8. *Polygala paniculata* | 243 | 125 | 169 |
| 9. *Borreria* spp. | 189 | 189 | 217 |
| 10. *Porophyllum ruderale* | 267 | 145 | 158 |
| 11. *Mimosa pudica* | - | 2 | - |
| 12. *Cleome rutidosperma* | 211 | 176 | 94 |
| 13. *Centella asiatica* | 141 | 125 | 99 |
| 14. *Oralis corniculata* | 128 | 109 | 89 |
| 15. *Eleusine indica* | 155 | 56 | 65 |
| 16. *Echinochloa colonum* | 69 | 54 | 62 |
| 17. *Amaranthus* sp. | 165 | 2 | 14 |
| 18. *Celosia argentea* | 40 | 77 | 59 |
| 19. *Bidens pilosa* | 137 | 8 | 20 |
| 20. *Setaria glauca* | 45 | 33 | 56 |
| 21. *Eclipta prostrata* | 46 | 40 | 46 |
| 22. *Richardia brassiliensis* | 46 | 28 | 51 |
| 23. *Euphorbia geniculata* | 56 | 14 | 14 |
| 24. *Paspalum* spp. | 40 | 16 | 27 |
| 25. *Melochia corchorifolia* | 17 | 14 | 51 |
| 26. *Chromolaena odorata* | 5 | 11 | 24 |
| 27. *Ctibadium* sp. | 11 | - | 2 |
| 28. *Mimosa pigra* | 6 | - | 5 |
| 29. *Synedrella nodiflora* | 8 | - | 3 |
| 30. *Amaranthus spinosus* | 1 | 2 | 8 |
| Total | 19023 | 13326 | 16342 |

The mean weed seed population of the first (D1) was 19023 per $m^2$, the second (D2) and third (D3) layers was 13326 and 16342 per $m^2$, respectively. There was no significant difference ($p = 0.05$) among the layers in the total number of seed found per $m^2$, but there was a tendency for the number to decrease with increasing soil depth.

A striking feature of the arable field was the predominance of annual weeds. In all layers these made up more than 90% of the total species found. The most dominant species with a total seed population of more than 50% in all different layers were *Digitaria* spp., *Cyperus* spp., *P. repens*, *Phylanthus niruri*, *A. conyzoides*, *E. ruderalis* and *L. lavandulifolia*. These are species having no special modification for dissemination except for *A. conyzoides* which is wind dispersed. There were only three woody weeds i. e. *Clibadium surinamense* L., *Chromolaena odorata* R. B. King and *Mimosa pigra* L., which are common plants of abandoned fields.

Some species has a high number of buried seeds (per $m^2$) in the first layer (D1), e. g. *Digitaria* spp. (4861), *Cyperus* spp. (5334), *Leucas lavandulifolia* (1338), *Polygala paniculata* (234), *Peperophyllum ruderale* (267), *C. rutidosperma* (211), *Centella asiatica* (141), *Oxalis corniculata* (128), *E. indica* (155), and *Bidens pilosa* (137), however some species had a high number of buried seeds in the second (D2) or third (D3) layers, e. g. *Panicum repens* (3838), *A. conyzoides* (1073), *E. ruderalis* (646), *Borreria* spp. (217), *Celosia argentea* (77), and *Richardia braziliensis* (51).

**Total viable weed seeds** The total population of viable weed seeds from the different soil layers is given in Table 3. Thirty six species were found from germinated seeds in the different soil layers. The mean viable weed seed population in the first layer was 2473 per $m^2$, while the second and the third layers and 2510 and 2370 per $m^2$, respectively.

*Digitaria ciliaris* (Retz.) Koel. had the highest number of viable seeds in all the different layers. This species showed a definite decrease in viable seeds from 537 per $m^2$ in the first layer to 462 and 387 per $m^2$ in the second and the third layers, respectively. Other dominant species of viable seeds included *A. conyzoides* with 336, 334 and 374 per $m^2$; *Fimbristylis miliacea* (L.) Bahl, 210, 285 and 230 per $m^2$; *Borreria alata*, 125, 94 and 230 per $m^2$; *P. niruri*, 286, 274 and 195 per $m^2$; *Cyperus* spp., 118, 180 and 160 per $m^2$, *Digitaria nuda*, 137, 61 and 86 per $m^2$, *Euphorbia geniculata*, 102, 117 and 62 per $m^2$, and *Oldenlandia corymbosa* with 137, 177 and 150 per $m^2$ for D1, D2 and D3 respectively.

Pattern of emergence for ten dominant species was converted into a percentage of the total emerged during six months and is presented in Fig. 2. *E. geniculata* was one of the earliest emerging weeds and almost completed its emergence in a short period (after two weeks) and very few seedlings emerged after this period. Seedlings of *B. alata* began to emerge after two weeks and emerged at relatively high levels after one month. Thereafter, the species continued to emerge in numbers throughout the experimental period. *D. ciliaris* and *P. niruri* had the same pattern of emergence i.e. high levels of emergence after 20 days. Late seedling emergence (more than one month) was found for *Cyperus* spp., *E. ruderalis*, *F. miliacea* and *O. corymbosa*.

## DISCUSSION

The total population of buried weed seeds in the top 15 cm soils of arable field in Tajur, Bogor was about 48700 per $m^2$. This is extremely high when compared with the total population of buried seeds recorded form arable soils of Scotland (16000 per $m^2$), or corn field of Colorado

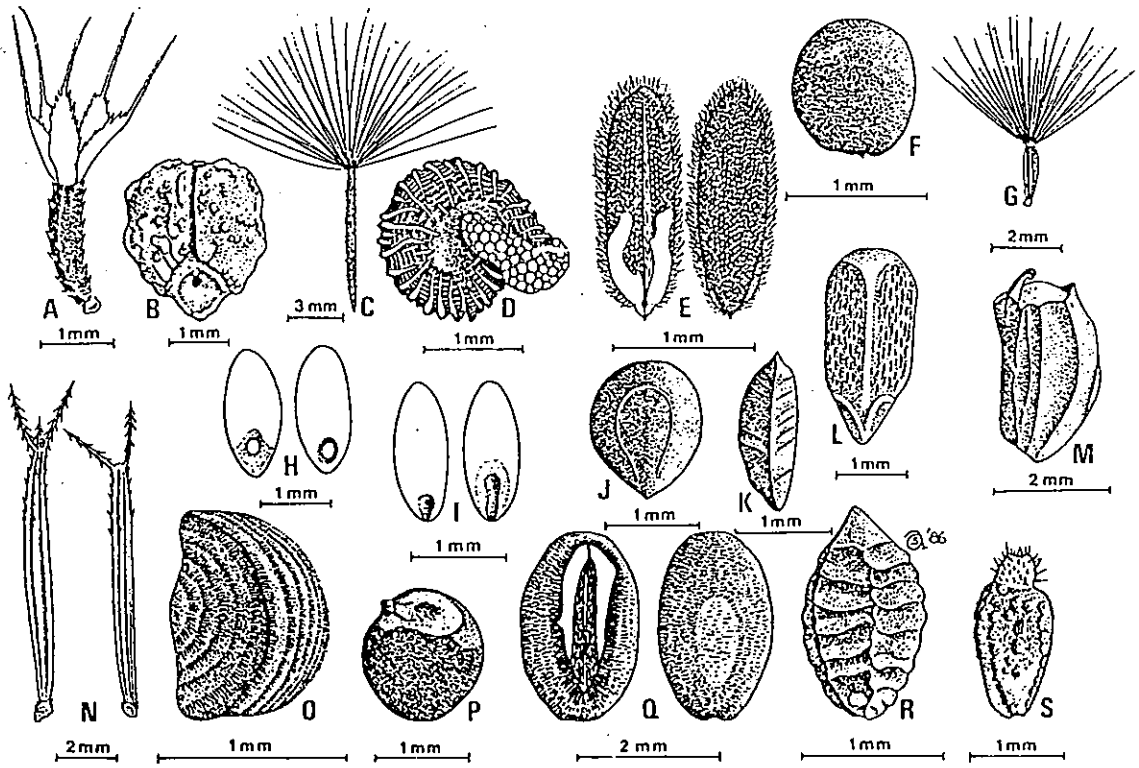


**Fig. 1. Some dominant weed seeds isolated from the top 15 cm soil of arable land.**

A : *Ageratum conyzoides*
B : *Euphorbia geniculata*
C : *Porophllum ruderale*
D : *Cleome rutidosperma*
E : *Polygala paniculata*
F : *Amaranthus spinosus*
G : *Chromolaena odorata*
H : *Digitaria adcendens*
I : *Digitaria sanguinalis*
J : *Mimosa pudica*
K : *Cyperus rotundus*
L : *Leucas lavandulifolia*
M : *Centella asiatica*
N : *Bidens pilosa*
O : *Phyllanthus niruri*
P : *Celosia argentea*
Q : *Borreria alata*
R : *Oxalis corniculata*
S : *Eleutheranthera ruderalis*

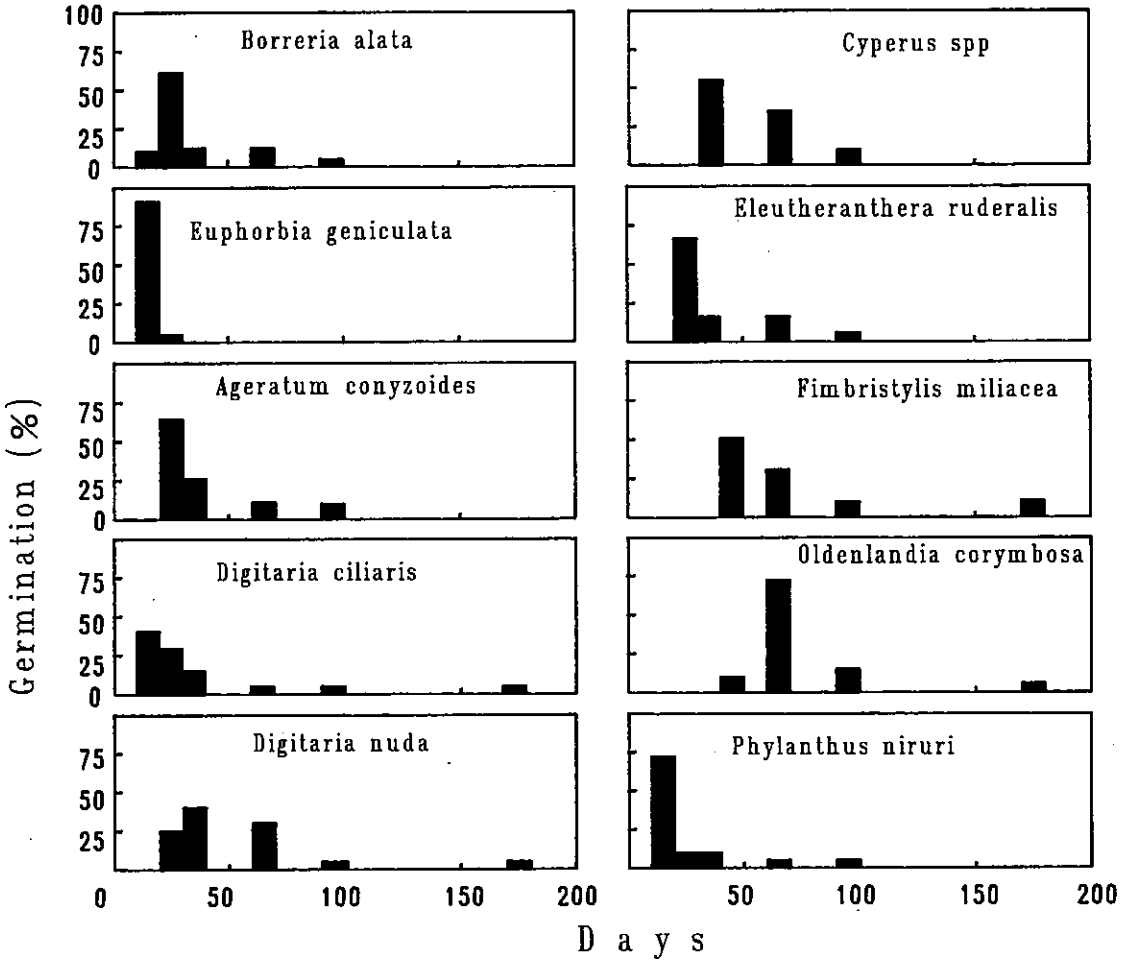


**Fig. 2. Pattern of emergence for ten dominant species in the top 15 cm soil during six months period.**

(8-26000 per $m^2$), but low when compared with the total population of buried seeds recorded from oak forest soils in Japan (66000 per $m^2$), vegetable soils in England (16-80000 per $m^2$), or ricefield soils in the Philippines (80400 per $m^2$) (6, 8, 10, 11, 13).

There was no significant difference among the number of buried weed seeds in the first, second or third layers. This is probably due to the extensive cultivation of the soils up to 15 cm depth so that the buried seed in this layers were mixed up.

Based on the total population and viable weed seeds the percentage of the germinated seed to the total population of some dominant species was calculated (Table 4). It was found that the number of germinated seeds for dominant species was very low except for *Borreria* spp. (95%). The lowest was found in *Leucas lavandulifolia* with less than 1 % germination. No germinated seed was found for *Panicum repens*. It was due to the fact that in Indonesia no fertile seeds were found for this species.

The total population of viable weed seeds in this study, i.e. 7353 per $m^2$ was also extremely high when compared with the total population of viable weed seeds recorded from soil cultivated with pineapple for 10 years in Malaysia (6200 per $m^2$) or uncultivated soil in Alaska (5313 per $m^2$), but low when compared with the total population of viable weed seeds recorded from soil cultivated with pineapple for 0.5-6 years in Malaysia i.e. 9560-12192 per $m^2$) (2, 14).

The importance of using both seedling emergence and sieving/washing floatation methods to determine buried seed population is manifest in our results. Seeds of 16 species were overlooked in the sieving and floating examination but were found in the seedling emergence examination. These species included *Commelina benghalensis* L., *Euphorbia hirta* L., *Erechtites valerianifolia* (Wolf), F. *miliacea*, *Hyptis capitata*, *Emilia sonchifolia*, *Ludwigia* sp., *Portulaca oleracea* L., *Sida rhombifolia* L., *Centipeda minima* L., *Althernanthera sessilis*, *Tridax procumbens*, *Oldenlandia corymbosa*, *Molugo stricat*, *Piperomia pellucida* and *Rotala rocea*. Most of these species had minute sized seeds (less than 1 mm) and it is possible that the seeds were located inside soil aggregates so that separation from this using Hayashi's method was impossible. Pareja et al. (7) have indicated that some factors such as sampling instrument used, sieving procedure, and the number of aggregate size fractions separated may all have affected the results.

On the other hand, 13 species were not found in the seedling emergence examination, but were found in the sieving/washing floatation examination. These species included *Panicum repens*, *Mimosa pudica*, *Echinochloa colonum*, *Amaranthus* spp., *Setaria glauce*, *Eclipta prostrata*, *Richardia brassiliensis*, *Melochia corrchorifolia*, *Chromolaena odorata*, *Clibadium surinamense*, *Synedrella nodiflora* and *Amaranthus spinosus*. It was assumed that the seeds of these species were non-viable or in a state of deep seed dormancy. Another reason could be that the seeds were lost due to wind, or died of poor germination condition before setting up germination test. Factors that influence seed longevity in soil includes depth of burial, tillage practices, and the dormancy of each weed species. In general longevity increases with depth of burial and is greater in undisturbed soil than in tilled soil (10). Currie (3) also stated that germination, dormancy, and viability of weed seed in the soil could be regulated by microsite characteristics.

Kropac (5), reviewing various seed recovery methods, noted that although floatation method had superseded the seedling emergence method in seed soil surveys, the examination of the fractions was time consuming, tedious and complete recovery of all seeds could not be

Table 3. Total number of viable seeds ($m^{-2}$.5cm) from different soil layers of arable field collected in May 1985.

| Species | Soil layers | | | |
|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_3$ | Total |
| 1. *Ageratum conyzoides* | 336 | 344 | 374 | 1054 |
| 2. *Bidens pilosa* | 11 | 17 | 62 | 90 |
| 3. *Borreria alata* | 125 | 94 | 131 | 350 |
| 4. *B. latifolia* | 69 | 70 | 75 | 214 |
| 5. *Celosia argentes* | 52 | 41 | 13 | 106 |
| 6. *Cleome rutidosperma* | 17 | 21 | 38 | 76 |
| 7. *Cyperus* spp. | 118 | 180 | 160 | 458 |
| 8. *Commelina benghalensis* | 16 | 2 | 10 | 28 |
| 9. *Digitaria ciliaris* | 537 | 462 | 387 | 1386 |
| 10. *D. ruda* | 137 | 61 | 86 | 284 |
| 11. *Elentheranthera rederalis* | 113 | 73 | 121 | 307 |
| 12. *Euphorbia geniculata* | 102 | 117 | 62 | 281 |
| 13. *E. hirta* | 3 | 6 | 6 | 15 |
| 14. *Erechtites valerianifolia* | 8 | 6 | 9 | 23 |
| 15. *Fimbristylis miliacea* | 210 | 285 | 230 | 275 |
| 16. *Hyptis capitata* | - | 3 | 3 | 6 |
| 17. *Emila sonchifolia* | 8 | 3 | 6 | 17 |
| 18. *Leucas lavandulifolia* | 17 | - | 16 | 33 |
| 19. *Ludwigia* sp. | 2 | 16 | 3 | 21 |
| 20. *Mimosa* sp. | - | 3 | 2 | 5 |
| 21. *Oxalis corniculata* | 14 | 35 | 30 | 79 |
| 22. *Paspalum* sp. | 16 | 21 | 8 | 45 |
| 23. *Porophyllum ruderale* | 9 | 13 | 32 | 54 |
| 24. *Portulaca oleracea* | 5 | 3 | 9 | 17 |
| 25. *Polygala paniculata* | 24 | 26 | 33 | 83 |
| 26. *Phylanthus niruri* | 286 | 274 | 195 | 755 |
| 27. *Centela asiatica* | 22 | 45 | 16 | 83 |
| 28. *Sida rhombifolia* | 38 | 46 | 35 | 119 |
| 29. *Centipeda minima* | 37 | 47 | 45 | 129 |
| 30. *Althernanthera sessilis* | 6 | 6 | 5 | 16 |
| 31. *Tridax procumbens* | 5 | 3 | - | 8 |
| 32. *Oldenlandia corymbosa* | 137 | 177 | 150 | 364 |
| 33. *Eleusine indica* | 3 | 3 | 6 | 12 |
| 34. *Piperomia pellucida* | - | 2 | 2 | 4 |
| 35. *Mollugo stricta* | - | 3 | 8 | 11 |
| 36. *Rotala rosea* | - | 2 | 2 | 4 |
| Total | 2473 | 2510 | 2370 | 7353 |

Table 4. The percentage ratio between total buried weed seeds and viable weed seeds ($m^{-2}$.15cm) found in arable soils collected in May 1985.

| Dominant species | Total buried seeds | Viable seeds | Ratio (%) |
|---|---|---|---|
| 1. *Digitaria* spp. | 12357 | 1670 | 13.5 |
| 2. *Phyllanthus niruri* | 4587 | 755 | 16.5 |
| 3. *Leucas lavandulifolia* | 3731 | 33 | 1 |
| 4. *Cyperus* spp. | 12906 | 458 | 3.5 |
| 5. *Ageratum conyzoides* | 2455 | 1054 | 42.9 |
| 6. *Eleutheranthera ruderalis* | 1775 | 307 | 17.3 |
| 7. *Borreria* spp. | 595 | 564 | 94.8 |

guaranteed. He recommended the seedling emergence method similar to that used by Brenchley and Warrington (1). Apparently most workers in soil seed population studies in England and Wales used the seedling emergence method, although Robert and Ricketts (9) used floatation method with satisfactory results.

## ACKNOWLEDGEMENTS

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# MECHANISM OF PARAQUAT TOLERANCE IN SOYBEAN CULTIVARS

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## ABSTRACT

Activities of superoxide dismutase, fatty acid composition and electroconductivity have been determined in Kwangkyo, a paraquat-tolerant cultivar and Hood, a paraquat-susceptible one of soybean (*Glycine max* L.) grown under the controlled environment. A paraquat-tolerant cultivar showed markedly higher activities of superoxide dismutase than susceptible one. Paraquat treatment did not alter membrane lipid composition. However, a ratio of total saturated over unsaturated fatty acids decreased in Kwangkyo from 0.32 to 0.23 while increased in Hood from 0.24 to 0.37, meaning that the unsaturated fatty acids like linolenic and arachidonic acids increased in tolerant cultivar such as Kwangkyo. Electroconductivity was much higher in paraquat-susceptible cultivar, Hood, than a paraquat-tolerant one, Kwangkyo, indicating that the membrane of a susceptible cultivar was greatly affected by paraquat. It is strongly proposed that paraquat tolerance in soybean cultivars may be due to destruction of superoxide anion by elevated concentration of superoxide dismutase in the tolerant cultivar together with less damage of membrane and probably increased unsaturated fatty acid composition.

## INTRODUCTION

Elevated activities of superoxide dismutase provide a basis for paraquat tolerance in green plants and tobacco callus (3, 4). A copper chelate of D-penicillamine which protects photosynthetic tissues against paraquat damage verified the important role of superoxide ion in the inhibition of the phytotoxic effects of bipyridylium herbicides (10). Further, this compound was found to reduce the loss of both chlorophyll and carotenoid pigments and to prevent the oxidation of unsaturated fatty acids in paraquat treated cotyledon leaves of flax (5).

Oxidants and free radicals arising from the reduction of bipyridylium compound interacted each other and other cell component, and can rapidly initiate a chain reaction resulting in peroxidation of unsaturated fatty acids (7), which are essential constituents of cell membranes, and finally causing bleaching and desication of leaf.

Paraquat is contact herbicide but rapidly penetrate into leaf as soon as it contacts. The rapid damage caused by paraquat may be related to destruction of cell membrane. Wright (9) indicated that an increased electrolytic concdutivity increased in membrane permeability allowing electrolytes to leak from cell. This study was attempted to determine different

susceptibility of soybean cultivar against paraquat, using the parameter such as superoxide dismutase activity, electroconductivity and fatty acid composition.

## MATERIALS AND METHODS

**Plant growth** Soybean cultivars (Kwangkyo and Hood) were grown in a vermiculite under a phytotron. The phytotron was maintained at 30°C/20°C (day and night, respectively) under light intensity, 2000lux and photoperiod, 16 hrs (light) and 8 hrs (dark). A hyponex solution was applied as a nutrient source before seeding and 1/4 strength of MS basal solution was applied twice at 2 and 4 weeks after seeding.

**Activity of superoxide dismutase** The foliar materials of soybean which developed the 3rd main leaves were harvested for enzyme assay at 6-48 hrs after paraquat application and harvested materials were homogenized with 0.2M phosphate buffer (pH 7.8). Sonication of the homogenate was done for 3 minutes under the cooled condition. The insoluble residue was removed by centrifugation at 20000g for 30 minutes and the clear supernatant was used for the determination of superoxide dismutase. Activity of superoxide dismutase was determined spectrophotometrically at 540nm according to Elstner et al. (1) based on the inhibition by superoxide dismutase of the rate of oxidation of hydroxylamine driven by superoxide anion produced by the anthraquinone (AO)/diaphorase system.

**Fatty acid composition** GC 304 Gas Chromatography (Pye Unicam Ltd., England) equipped with heated FID was used to analyze the methylesters of fatty acids from phospholipid by Folch et al. (2). The column used for GLC was packed with 10% Alltech CS-10cm Chromosorb W (Alltech Associates, Deerifield, IL), which efficiently separated C:16 and C:18 fatty acids. The column temperature was 170°C (after staying for 2 min.) initially followed by an increase at 1.5°C/min. to 200°C (maintaining for 8 min.) with a carrier gas ($N_2$) flow rate of 30 ml/min. Both injector and detector temperatures were 240°C.

**Electroconductivity** After cutting 25 segments of square millimeter of leaf disc prepared from the 3rd main leaves were put into 20ml of 10 and 100 mg/l for 1 to 6 hrs under the controlled environment at 25°C and 40000lux of light. Electrolyte leakage was measured with conductivity Bridge Barnsted PM-70CB. Three replicates were taken at one hour interval from 1 to 6 hrs after floating leaf disc in paraquat solution.

## RESULTS AND DISCUSSION

Different activities of superoxide dismutase were observed in two different soybean cultivars. A greater activities of superoxide dismutase was obtained in Kwangkyo than Hood, indicating existance of varietal response in between soybean cultivars (Fig. 1). This is supported by Harper et al. (4) and Furusawa et al. (3), who showed that an elevated concentration of superoxide dismutase upon paraquat application is the main mechanism to result in paraquat tolerance in various plant species. An increased activities of superoxide dismutase in Kwangkyo seems to be related to exhibition of different responses of soybean cultivars.

Electrolytic conductivity as a measure of cell membrane disruption on two soybean cultivars which had been floated with paraquat solution at 10 and 100 ppm. Paraquat at 100 ppm resulted in significant tissue damage compared to 10 ppm. Regardless of varieties tested, the

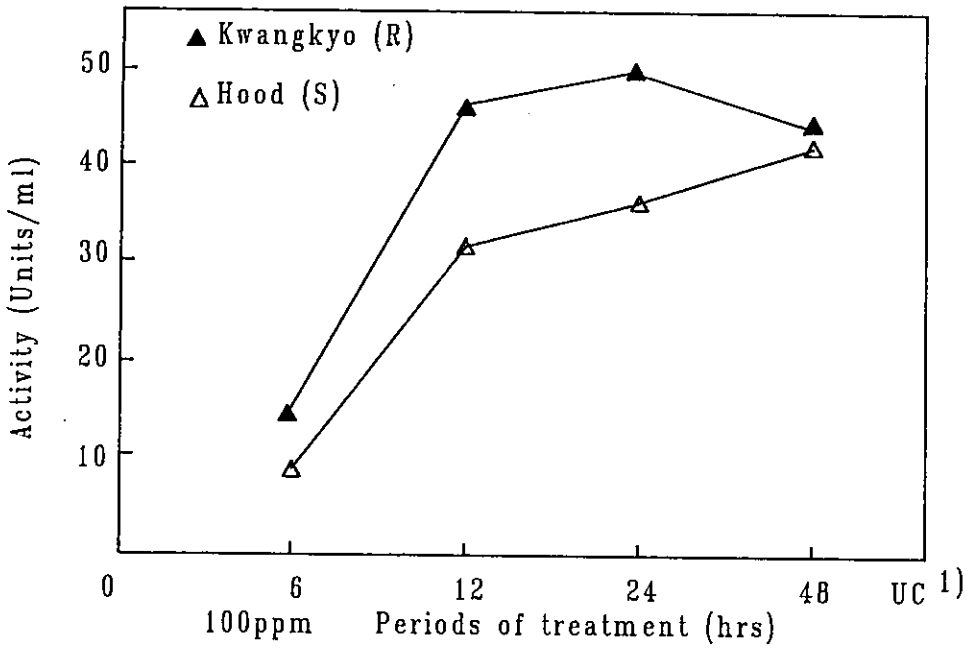


Fig. 1. Changes in superoxide dismutase activity of soybeans as affected by paraquat treatment.

1) UC : untreated control, determined at 48 hours after incubation.

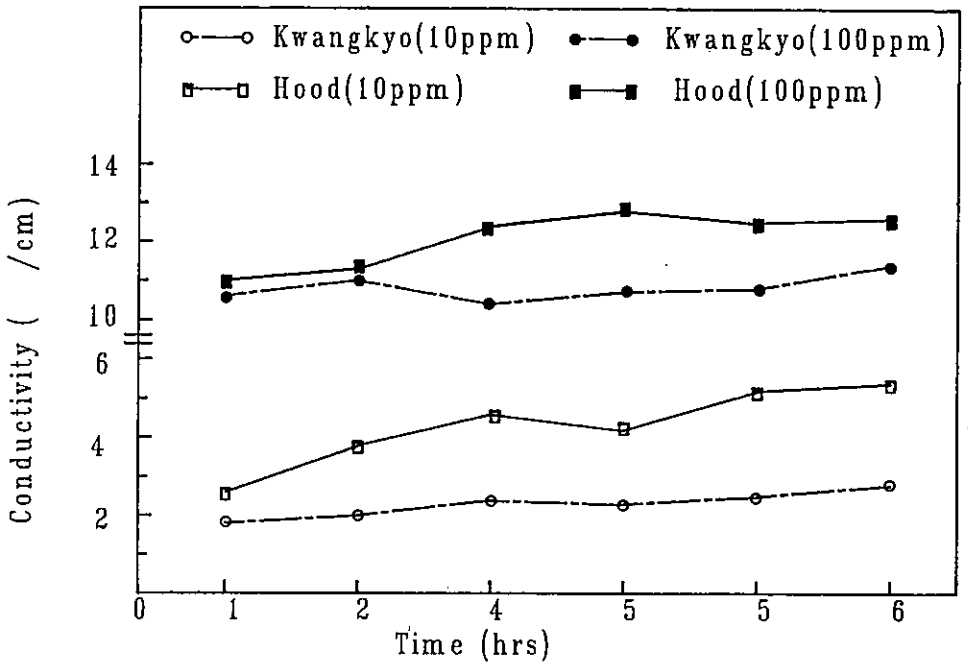


Fig. 2. Electroconductivity of Glycine max leaf treated with paraquat at various concentrations and times at the growth chamber (light intensity : 40,000 lux, temperature : 25° C)

Table 1. Effect of paraquat on relative fatty acid composition of soybean leaf.

| Cultivar | Herb.[1] | Duration[2] (hrs) | Composition of fatty acid[3] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 | S/US |
| Hood | Untreated control | 0 | 0.11 | 12.52 | 2.14 | 4.80 | 5.65 | 10.43 | 45.89 | 9.98 | 0.24 |
| | Paraquat | 3 | 3.12 | 17.98 | 0.98 | 5.73 | 3.47 | 7.26 | 34.93 | 18.63 | 0.34 |
| Kwangkyo | Untreated control | 0 | 4.23 | 11.66 | 0.24 | 8.44 | 3.91 | 3.90 | 28.36 | 39.30 | 0.32 |
| | Paraquat | 3 | 3.44 | 9.44 | 0.31 | 5.99 | 2.75 | 2.09 | 34.58 | 41.39 | 0.23 |

1 Concentration of paraquat applied was 100 ppm.

2 Duration indicates duration of soybean leaves exposured to paraquat.

3 14:0; myristic acid, 16:0; palmitic acid, 16:1; palmitoleic acid, 18:0; stearic acid, 18:1; oleic acid, 18:2; linoleic acid, 18:3; linolenic acid, 20:4; arachidonic acid.

electrolytic conductivity increased with increased visual injury. Five to six times greater conductivity was measured in 100 ppm concentration of paraquat than that of 10 ppm (Fig. 2). The greater electrolytic conductivity was observed in Hood when comparison were made with Kwangkyo. Hood which showed a great injury symptom than Kwangkyo, increased electroconductivity as the exposure of soybean leaf to paraquat prolonged 1 to 6 hours in both 10 and 100 ppm concentrations. No significant change of electroconductivity was observed in Kwangkyo unlike Hood. The highest conductivity, 1293.33 uv/cm, was obtained in the 4 hours after 100 ppm paraquat treatment in Hood. The lower electroconductivity together with higher superoxide dismutase activity in Kwangkyo indicates that it is definitely tolerant to paraquat as compared with Hood. This is supported by the work of Vanstone et al. (1977) who reported that visual symptoms caused by paraquat toxicity appear to be closely related with increase in electrolytic in buckwheat (*Fagopyrum esculentum* Moench cv. Tokyo) plant. They further pointed out that the highest concentration of paraquat and oxyfluorfen gave higher conductivity than lower concentrations, and a sublethal concentration of paraquat resulted in significant decrease in conductivity at 6 and 8 hours after application.

The data in Table 1 summerize the effects paraquat on the relative fatty acid composition of soybean leaves. The similar number of fatty acids was observed in two cultivars whether applied with paraquat or not. One of significant differences between two cultivars was that the ratio of total saturated over unsaturated fatty acids was decreased in Kwangkyo from 0.32 to 0.23, meaning that the unsaturated fatty acids increased in tolerant soybean cultivar, but the reverse was observed in Hood, a susceptible one, showing an increase from 0.24 to 0.37 when applied with 100 ppm of paraquat and determined at 3 hours after application. The unsaturated

fatty acids such as linolenic and arachidonic acid present in great amount in Kwangkyo differs greatly from Hood. It is not clear, however, that this may be directly attributed to different response of two soybean cultivars against paraquat. The differential responses of plant species and tissues to substituted pyridazinones and triazine herbicides may depend upon linolenic acid biosynthesis and lipid composition of chloroplast membranes (6). On this basis, it is assumed that the varied percentage of composition of fatty acids between Hood and Kwangkyo may play some role in different sensitivity of two soybean cultivars against paraquat. It needs further evaluation for explaining different response exerted by paraquat on fatty acid composition.

Based on results and observation, it can be summerized that soybean cultivar, Kwangkyo seem to be genetically tolerance to paraquat than Hood.

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# USE OF BASTA FOR WEED CONTROL IN VEGETABLES

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## ABSTRACT

Basta® is a new non-selective herbicide for annual and perennial weed control in various plantation crops and other situations. The normal use rate is in the range of 0.28 and 3 kg/ha a.i., corresponding to 1.4 - 15 l/ha of the standard formulation. This product has been tested in the last few years including use in vegetables. Three possible application times were examined: 1. Prior to planting or seeding the crop; 2. After planting or seeding: directed spray between the rows, mostly with a spray shield; 3. Immediately before emergence of slowly emerging crops. Rates necessary for weed control prior to seeding or planting and for interrow application varied between 0.6 and 1.0 kg/ha a.i. depending on weed growth stage. For the late preemergence use in slowly emerging crops, such as onions or carrots, for the control of weeds just emerged not more than 0.6 kg/ha a.i. are necessary. Crop species tested so far include asparagus, bitter gourd, burdock, cabbage, carrot, chinese cabbage, chinese yam, cucumber, eggplant, green pepper, kidney bean, leeks, lettuce, melon, mung bean, onion, pea, pumpkin, radish, soya bean, spinach, sweet potato, taro, tomato and watermelon. Weed species controlled by 0.6 kg/ha a.i. include a wide spectrum of annual and biannual species.

## INTRODUCTION

Basta, proposed common name of the active ingredient: Glufosinate- Ammonium, a new non-selective foliar herbicide, was developed especially for weed control in different type of plantation crops, such as vineyards, orchards, tropical plantations, or in non-crop land (6). It was found that a broad spectrum of annual and perennial weeds is controlled by rates of 1.4 - 5 l/ha (formulation: aquaeous solution with 200 g/l a.i.), under difficult conditions higher rates are required, and especially for *Imperata cylindrica* control 10 - 15 l/ha are necessary (3, 4, 5). In addition to the use in perennial crops, Kassebeer et al. (2) in a summary report on first experiences in Japan, mentioned the possibility of using the product for annual weed control in vegetables as a directed spray between the rows or before land preparation prior to planting. The objective of further trials was to define the details of both weed control and crop tolerance in vegetables. These trials were mainly conducted in Japan and in the

Philippines. Furthermore, the application of Basta preemergence to a slowly emerging crop was tested in Germany as a completely different type of use.

## MATERIALS AND METHODS

**Crop tolerance trials** Normal field trial methods were used with plots of 6 - 10 m plot size, 3 or 4 replicates, randomized block design; for herbicide applications, experimental plot sprayers mostly with flat fan nozzles were used. For standard inter-row applications, a spraying shield was normally used. Standard water volumes ranged from 300 l/ha in Germany, 500 l/ha in the Philippines to 1000 l/ha in Japan. Evaluations were made in the form of a scoring system with figures from 0 (no damage to 100 (complete kill).

In pot trials, all made in Japan, plastic pots with 15 cm diameter or Wagner-pots were used. The pots were kept in the greenhouse.

**Efficacy trials** Principally the methods were very similar. In some cases, very small plots (1 - 5 m), replicated only twice, were used, depending on the occurrence of particular weeds.

If necessary, standard herbicides containing paraquat (200 g/l or 24 w/w %) or paraquat + diquat (5 + 7 % w/w) were included.

## RESULTS

### Crop tolerance

**Application before seeding of transplanting** In a trial on a medium loam soil in Germany, Basta was applied to the soil 3 days before transplanting lettuce. No phytotoxicity was recorded and yields were not significantly different from untreated (Table 1).

In some trials in the Philippines soil applications of Basta were made prior to seeding or transplanting the following crops (Table 2). No damage was recorded up to 4 weeks after application.

The organic matter content was 2.1 and 2.6 %, respectively. Soil applications were made with up to 80 l/ha Basta before planting or sowing some test crops. On soil type A, only slight damages could be recorded at 80 l/ha Basta, 4 weeks after application (Table 3). On the sandy soil, however, all 4 crop species reacted with some phytotoxicity at 40 and 80 l/ha; the damages to carrots being lower than those to other crops.

In official trials in Japan, conducted by the Japan Association for the Advancement of Phytoregulators (JAAPR) under different conditions from 1982 - 1986, no damages could be observed when 3 - 5 l/ha Basta was applied prior to seeding or planting spinach (3), asparagus (2), burdock (2), carrot (2), radish (2), onion (1), strawberry (1), cabbage (15), watermelon (1), or chinese cabbage (3 trials).

**Inter row application**

In a model trial (glasshouse), accidental contact of spraying liquid to the crop plants was simulated by painting the first 2 leaves with a brush. The product concentrations were based on 3 or 5 l/ha Basta and 1000 l/ha water, and 6 l/ha of a paraquat + diquat standard product. All painted leaves (see table 4) were completely necrotic within 5 days; after a Basta application, some slight yellowing was observed on upper leaves of cucumbers or tomatoes; the paraquat + diquat mixture, however, caused very severe damages to all plants.

In another model study, drift was simulated by spraying low amounts of a spray solution with concentrations of herbicides above the crop (glasshouse experiment). The figures given in Table 5, demonstrate very serious damages caused by the recommended rate of the paraquat + diquat standard, whereas the damage caused by Basta was significantly lower.

**Standard product: paraquat + diquat**

Official trials in Japan, conducted by the JAAPR, with 3 and 5 l/ha Basta and 3 l/ha of a paraquat product, demonstrated that Basta did not cause any phytotoxic symptoms, provided the product was applied correctly. Only in a very few cases, some slight damage was observed after drift. Damage scores did not exceed those caused by paraquat, in some cases they were lower. In very few cases, some slight damage was observed after drift. Damage scores did not exceed those caused by paraquat, in some cases they were lower. Crops tested were: cabbage (19), lettuce (3), leeks (5), asparagus (3), pumpkin (2), melon (3), watermelon (13), cucumber (5), tomato (2), green pepper (2), eggplant (1), taro (2) and chinese yam (3 trials).

**Preemergence application**

In Germany, a field trial was conducted in onions with an application 3-4 days before emergence of the crop. No crop damage could be observed (Table 6). Similar results could be obtained with carrots.

**Weed control** Japan: *Digitaria adscendens*, one of the most important weeds in vegetables, occurred in a greater number of trials over several years. As the efficacy of Basta differed to a certain extent according to growth stages, all results were sub-divided : Group 1 included applications where the target weed was less than 20 cm high at application (until end of tillering), and the 2nd one where it was taller and more developed (shooting, flowering, or fruiting). As far as available, paraquat results were included (Table 7). When Basta was treated to younger plants, 3 or 3.5 l/ha were sufficient with 96 - 98% efficacy, whilst applications to older plants required 5 l/ha.

In Table 8, the annual and biannual weed spectrum controlled with 3 and 5 l/ha Basta, is summarized. This list is based on Hoechst data as well as on official trials (JAAPR). The dose rate classification refers to normally growing weeds in vegetables between the rows or even in fields prior to seeding or transplanting. Very advanced plants (flowering to fruiting) need higher rates in many cases. On the other hand, extremely small weeds at the cotyledon or 1-leaf-stage may be effectively controlled by lower dose rates.

**Philippines**

A number of trials were conducted against different weeds. The spectrum controlled by 3 - 5 l/ha includes the species *Amaranthus spinosus, Cyperus iria, Digitaria sanguinalis, Echinochloa colona, Eleusine indica, Euphorbia hirta, Fimbristylis littoralis, Galinsoga parviflora, Poa annua, Polygonum* sp and *Portulaca oleracea*. Even here the higher rate was required if the plants had reached a more advanced growth stage, e.g., flowering, at time of application. In earlier growth stages even 2 or 2.5 l/ha of Basta were sufficient.

**Germany**

When Basta was applied immediately before emergence of a slowly emerging crop such as onions or carrots, a first flush of annual weeds usually was already emerged, and these weeds which had reached the cotyledon or first true leaf-stage, were easily controlled by 3 l/ha Basta. As an example average weed control figures in an onion trial are given in Table 6. Weed species present in this trial were *Capsella bursa-pastoris, Chenopodium album, Matricaria chamomilla,*

Table 1. Phytotoxicity and yield of transplanted lettuce after herbicide application to the soil 3 days before planting; all plots handweeded; absolute yield in control: 49.2 t/ha. (Germany)

| Treatments | % phytotoxicity 18 DAA | Yield (relative) |
|---|---|---|
| Control | 0 | 100 a |
| Basta 6 l/ha | 0 | 101 a |

Table 2. Crops which were included in crop tolerance trials in the Philippines with seeding (s) or transplanting (t) 0 to 7 days after a herbicide application; rates Basta 5 to 10 l/ha, Paraquat 5 l/ha.

| | |
|---|---|
| Soya bean (s) | Cucumber (s) |
| Mung bean (s) | Bitter gourd (s) |
| Kideny bean (s) | Lettuce (s) |
| Pea (s) | Cabbage (t) |
| | Tomato (t) |

Soil type : sandy loam or loamy clay
In Japan, 2 field trials were made on 2 different soils
- soil type A, a sandy loam, containing 21 % clay, 17 % silt, and 62 % sand;
- soil type B, a sandy soil, with 4 % clay, 17 % silt and 89 % sand.

Table 3. Phytotoxicity (in %) of different crops after soil applications of Basta immediately prior to planting or sowing.

| | | Sweet potato soil type | | | Soybean soil type | | | Cucumber soil type | | | Carrot soil type | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A[1] | | B[1] | A | | B | A | | B | A | | B |
| Treatment | l/ha | DAA[2] | | DAA | DAA | | DAA | DAA | | DAA | DAA | | DAA |
| | | 28 | 9 | 35 | 28 | 9 | 35 | 28 | 9 | 35 | 28 | 9 | 35 |
| Control | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Basta | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | 40 | 0 | 45 | 80 | 0 | 55 | 55 | 0 | 0 | 60 | 0 | 0 | 10 |
| | 80 | 10 | 42 | 80 | 0 | 75 | 70 | 0 | 0 | 60 | 5 | 0 | 30 |

1 Soil type A: sandy loam; Soil type B: sand; 2 DAA=days after application

Table 4. Phytotoxicity (in %) of different crops after application of herbicides to the first true leaves by brush), glasshouse, 14 days after application; standard product: paraquat + diquat. A score of 5 was given when the painted leaf was dead, scores 5: damage to the rest of the plant.

| Crop and height (cm) at application | | Basta 0.3 % | Basta 0.5 % | Standard product 0.6 % |
|---|---|---|---|---|
| Cucumber | 40 | 7 | 10 | 70 |
| Tomato | 35 | 6 | 13 | 46 |
| Eggplant | 40 | 5 | 5 | 28 |
| Green pepper | 50 | 5 | 5 | 9.5 |
| Onion | 40 | 5 | 5 | 10 |

Table 5. Phytotoxicity (in %) of different crops after a simulated drift application (greenhouse trial).

| Crop and height (cm) at application | | Basta 0.3 % | Basta 0.5 % | Standard product 0.6 % |
|---|---|---|---|---|
| Trial 1, assessment 10 days after application | | | | 70 |
| Rice | 25 | 10 | 24 | 62 |
| Tomato | 20 | 7 | 18 | 24 |
| Cucumber | 25 | 28 | 48 | 100 |
| Eggplant | 20 | 9 | 22 | 100 |
| Cabbage | 15 | 4 | 11 | 68 |
| Onion | 20 | 0 | 1 | 5 |
| Carrot | 10 | 1 | 5 | 48 |
| Green pepper | 10 | 16 | 28 | 98 |
| Trial 2, assessment 16 days after application | | | | |
| Chinese cabbage | 25 | 30 | 32 | 38 |
| Broccoli | 30 | 7 | 26 | 46 |
| Japanese radish | 15 | 4 | 13 | 24 |
| Potato | 20 | 1 | 5 | 2 |
| Taro | 20 | 3 | 4 | 20 |
| Lettuce | 20 | 5 | 6 | 28 |
| Strawberry[1] | 20 | 2 | 8 | 28 |

1. transplanted

Water volume in trial 1: 140 l/ha; in trial 2: 100 l/ha

*Poa annua*, *Polygonum convolvulus*, and *Stellaria media*. Average weed control figures which were obtained 2 weeks after application by 3 or 5 l/ha Basta, were 89.3 and 95.3 % respectively, and the same rates of a paraquat product gave 90.2 and 92.0 % control. Later in this and similar trials, new flushes of weeds emerged since the active ingredient of Basta is not persistant in the soil.

## DISCUSSION

Non-selective foliar herbicides of different type have been used widely under different conditions for many years. Basta is a newcomer in this scenario, and thanks to the quick activity and the generally low toxicity (1), it was well received by many institutes and weed experts following the first publications. One relatively new field of use is the use in vegetables, and 3 different possibilities of application could be shown in the trials presented in this paper.

a) Application to an existing weed flora prior to seeding or transplanting the crop. The question to be answered was whether soil activity of Basta could cause damage to the subsequent crop. The results presented in Tables 1, 2 and 3 very clearly show that there is no danger by soil activity. Only under extreme conditions (very light sandy soil) and with extreme rates (40 - 80) l/ha product =8 - 16 kg/ha a.i. corresponding to a 8 to more than 25 fold overdosage) a significant phytotox was found. The good crop tolerance at normal dose rates found in our trials, is in excellent agreement with the results of a great number of no-till trials to maize (corn), soya beans or sugarbeets in North America and Western Europe (internal reports of Hoechst AG).

b) Application to the weeds between the rows of crop plants. The trials were set out to investigate the effect of possible drift on the crop during application. The model studies presented in Tables 4 and 5 showed, that even if the higher Basta rate is used, the probability of severe phytotox seems to be lower than by the lowest recommended rate of the standard (paraquat + diquat) product (6 l/ha). The 2nd of these trials (Table 5) was certainly a very hard one, as the amount of spraying liquid applied to the crop in order to imitate drift (100 - 140 l/ha under a situation were 1000 l/ha is the normal amount to be used) seems relatively high. In practice the amounts which may contaminate the crop plants are certainly lower, and consequently the crop damage caused by such contaminations will be lower as well; however, the objective of this trial was to show whether a phytotox caused by Basta was equal to, worst or weaker than that caused by the standard product, and it could be shown to be weaker. However, for interrow applications it is advisable to use a spraying shield anyway in order to avoid drift.

c) Application to newly emerged weeds before the emergence of slowly emerging crops such as onions or carrots. In this paper only one example could be given, as this type of herbicide application was tested only in Germany, but not in Asia. Details will be published later.

As far as weed control is concerned, a broad spectrum of annual or biannual weeds is covered by 3 l/ha Basta, if the plants are not too far developed at the time of application. Since under the conditions described in this paper, the occuring weed plants are mostly small, the recommended rate will be 3 l/ha for both, application between the rows as well as before sowing or transplanting the crop plants.

Table 6. Phytotoxicity and weed control (average of 6 annual species) in % after herbicide application to onions prior to emergence; application 12 days after seeding, emergence 3 - 4 days later (Germany).

| Product | l/ha | Phytotoxicity DAA | | Average weed control DAA |
|---|---|---|---|---|
| | | 15 | 28 | 16 |
| Basta | 3 | 0 | 0 | 89.3 |
| | 5 | 0 | 0 | 95.3 |
| Paraquat | 3 | 0 | 0 | 90.2 |
| (200 mg/l) | 5 | 0 | 0 | 92.0 |

Table 7. *Digitaria adscendens* control in % according to growth stage at application (field trials in Japan).

| Product | rate l/ha | Growth stage: less than 20 cm end of tillering | | more than 25 cm shooting, flowering etc. | |
|---|---|---|---|---|---|
| | | WAA[1] | | WAA | |
| | | 2 | 4 | 2 | 4 |
| Basta | 2-2.5 | 91.2 (9)[2] | 85.3 (7) | 70.6 (5) | 54.4 (5) |
| | 3-3.5 | 96.2 (8) | 97.7 (6) | 86.6 (5) | 78.4 (5) |
| | 5 | - | - | 94.5 (4) | 92.5 (4) |
| Paraquat (200 g/l) | 2-2.5 | 74.2 (4) | 65.5 (4) | - | - |
| | 3-3.5 | 87.7 (4) | 83.7 (4) | - | - |

1 WAA= weeks after application; 2 in brackets ( ): number of trials

Table 8. Spectrum of annual and biannual weeds in Japan, based on own and official trials.

| a) 3 l/ha Basta required for the control of: | b) 5 l/ha Basta were required for the control of: |
|---|---|
| *Acalypha australis* | *Alopecurs aequalis* |
| *Amaranthus lividus* | *Commelina communis* |
| *A. retroflexus* | *Cyperus microiria* |
| *Amphicarpaea edgeworthii* | *Poa annua* |
| *Capsella bursa-pastoris* | *Portulaca oleracea* |
| *Cardamine flexuosa* | *Rorippa atrovirens* |
| *Chenopodium album* | *R. islandica* |
| *C. album* spp. *centrorubrum* | *Solanum nigrum* |
| *C. ficifolium* | |
| *C. serotinum* | |
| *Centipeda minima* | |
| *Cerastium glomeratum* | |
| *Digitaria adscendens* | |
| *Echinochloa crus-galli* | |
| *Eclipta prostrata* | |
| *Eleusine indica* | |
| *Eragrostis multicaulis* | |
| *Erigeron anuus* | |
| *E. canadensis* | |
| *Euphorbia supina* | |
| *Fatoua villosa* | |
| *Galinsoga ciliata* | |
| *Galium spurium* | |
| *Lamium amplexicaule* | |
| *Lindernia procumbens* | |
| *Polygonum aviculare* | |
| *P. blumei* | |
| *P. lapathifolium* | |
| *P. nodosum* | |
| *P. thunbergii* | |
| *Senecio vulgaris* | |
| *Setaria viridis* | |
| *Sonchus asper* | |
| *S. oleraceus* | |
| *Stellaria alsine* | |
| *Stellaria aquatica* | |
| *Stellaria media* | |
| *Veronica arvensis* | |
| *Veronica persica* | |
| *Vicia angustifolia* | |
| *Youngia japonica* | |

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# SETOFF® - A NEW RICE HERBICIDE FOR S. E. ASIA

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## ABSTRACT

SETOFF, previously identified as CGA 142464, is a sulfonylurea compound, chemical name 3-(4,6-dimethoxy-1, 3, 5-triazin-2-yl)-1-[2-(2-methoxyethoxy)- phenylsulfonyl]-urea. It has a wide activity spectrum against most major lowland rice weeds in S. E. Asia, such as *Monochoria vaginalis, Sphenoclea zeylanica, Marsilea crenata, Limnocharis flava, Sagittaria guyanensis, Rotala indica, Cyperus* spp. and *Scirpus* spp. SETOFF is selective to both transplanted (TPL) as well as direct seeded wet sown (DSWS) rice when applied 10-40 g active ingredient per hectare (g ai/ha.). Slight crop thinning with 40 g ai/ha applied 3-6 days after sowing (DAS) disappears within 1-2 weeks. The selectivity and weed control properties are relatively independent of application timing and water management when applied 3-25 DAS or days after transplanting (DAT). Applications of SETOFF result in rice grain yield increases of 10-42% over those from untreated rice. Its broad weed control spectrum, its flexible application timing and its non-reliance on strict water management make SETOFF a potential herbicide for use in both transplanted as well as direct wet sown rice in S. E. Asia.

## INTRODUCTION

One of the major constraints to higher rice yields is the proliferation of weeds. Uncontrolled, they interfere with normal growth of rice by competing for available nutrients, sunlight and water. In addition they may harbour insect vectors of several rice virus diseases. According to De Datta (1) in IRRI estimate suggests that weeds may reduce rice grain yield by as much as 34 % in transplanted rice and 45% in direct seeded rainfed lowland rice.

Chemical weed control is now more widespread as the labour needed for handweeding has become both scarce and expensive. Development of new chemical weed management agents has to consider not only efficacy against major weeds, but timing flexibility, and selectivity in both transplanted as well as DSWS rice systems.

One such development has resulted in the product SETOFF, the chemical and toxicological properties of which are described by Quadranti, Rufener and Zoschke (2).

## MATERIALS AND METHODS

Since 1984 SETOFF has been tested intensively in S. E. Asia with numerous field trials in the Philippines, Thailand, Indonesia and Malaysia.

In such trials, SETOFF 5 W. P., and occasionally 10 W. P., has been applied by knapsack sprayers using 300-450 litres of water per hectare (l/ha). Rates from 10 to 80 g ai/ha have been tested although the main concentration has been with 20-40 g ai/ha. Application timings have ranged from 3 to 25 DAT or DAS, and all trials have consisted of 3-4 replications of 15-25 square metre plots arranged in random complete block designs.

Evaluations of crop tolerance (% phytotoxicity) and of weed control efficacy (% weed control) have been made around 10, 20, 30 and 50 days after application (DAA). Visual assessments of crop damage, and of any reduction in the biomass of a weed, compared to growth in an adjacent untreated crop strip have been made.

Rice grain yields recorded from whole plots have been calculated and are given at 14% moisture content.

## RESULTS AND DISCUSSION

**Crop tolerance of SETOFF** A slight crop thinning, not exceeding 5% in transplanted rice and 15% in DSWS rice is observed when 20 g ai/ha SETOFF is applied between 3-9 DAT/DAS (Table 1.). This crop effect is outgrown within 1-2 weeks. Applications made 10-25 DAT/DAS do not result in phytotoxicity.

**Weed control efficacy of SETOFF** When applied at 15 DAS or 10 DAT, SETOFF at 20 g ai/ha controls the most important broadleaved weeds and sedges found in the Philippines. At 20 DAS or DAT, 40 g ai/ha of SETOFF gives equally good weed control (Table 2).

Similarly in Thailand, SETOFF selectively controls the major broadleaved weeds and sedges in both transplanted and DSWS rice when applied between 10-18 DAT or DAS at 20 g ai/ha. It is especially effective in controlling the important weeds *Marsilea crenata* and *Sphenoclea zeylanica* (Table 3).

It is clearly demonstrated in trials from Indonesia that applying SETOFF early at 3 DAS or DAT suppresses more obstinate weeds like *Ludwigia* spp., and *Fimbristylis miliacea*, as well as giving excellent control of the other important broadleaved weeds and sedges (Table 4).

In Malaysian trials almost perfect weed control is achieved in transplanted rice following a 14 DAT application of 40 g ai/ha SETOFF. Most other commonly occurring weeds are well controlled at this timing (Table 5).

Table 1. Crop tolerance of SETOFF in direct seeded wet sown (DSWS) and transplanted (TPL) rice in Indonesia, Malaysia, Philippines and Thailand (1985 - 87).

| Treatment | Rate g ai/ha | Timing DAS/DAT | Range of Phytotoxicity % DSWS | TPL |
|---|---|---|---|---|
| SETOFF | 10 | 3-9 | 3-10 | 0 |
| | 20 | | 10-15 | 0-5 |
| | 40 | | 10-20 | 0-10 |
| 2,4-D IBE | 700 | | 10-15 | 4-10 |
| SETOFF | 10 | 10-25 | 0 | 0 |
| | 20 | | 0 | 0 |
| | 40 | | 0 | 0 |
| 2,4-D IBE | 1000 | | 10-20 | 10-20 |

Table 2. Weed control and yield efficacy of SETOFF in direct seeded wet sown (DSWS) and transplanted (TPL) rice in the Philippines, WS 1985 and DS 1986.

| Treatment | Rate g ai/ha | Timing DAS[1]/DAT[2] | Weed[4] Control % 30 DAA[3] DSWS | | | | TPL | | | | Yield MT/HA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mv. | Sz. | Cs. | Ss. | Mv. | Sz. | Cs. | Ss. | DSWS | TPL |
| SETOFF | 10 | 15/10 | 96 | - | 78 | 79 | 100 | - | 94 | 90 | 5.4 | 4.7 |
| | 20 | | 98 | 90 | 92 | 82 | 100 | - | 100 | 90 | 4.8 | 4.8 |
| | 40 | | 100 | 96 | 98 | 96 | 100 | - | 100 | 96 | 5.2 | 4.7 |
| 2,4-D IBE | 800 | | 98 | 84 | 93 | 85 | - | - | - | - | 3.5 | 4.8 |
| SETOFF | 20 | 20 | 95 | 83 | 88 | 90 | 90 | 91 | 98 | 90 | 4.5 | 4.8 |
| | 40 | | 100 | 91 | 100 | 90 | 98 | 100 | 100 | 100 | 4.3 | 4.8 |
| 2,4-D IBE | 800 | | 100 | 83 | 90 | 80 | 100 | 88 | 80 | 75 | 2.7 | 4.9 |
| Untreated[5] | | | | | | | | | | | 3.8 | 4.4 |

1 DAS - days after sowing
2 DAT - " " transplanting
3 DAA - " " application
5 Weed cover; 80% TPL; 95% DSWS

4 Mv ... *Monochoria vaginalis*
Sz ... *Sphenoclea zeylanica*
Cs ... *Cyperus* spp.
Ss ... *Scirpus* spp.

Table 3. Weed control of SETOFF in direct seeded wet sown (DSWS) and transplanted (TPL) rice in Thailand (1985-1986).

| Weeds | SETOFF 5 W. P. 20 g ai/ha DSWS[1] | TPL[2] | 40 g ai/ha DSWS | TPL | 2,4 D IBE 1000g ai/ha DSWS | TPL |
|---|---|---|---|---|---|---|
| *Marsilia crenata* | 90 | 87 | 91 | 95 | 37 | 5 |
| *Sphenoclea zeylanica* | 99 | 99 | 98 | 100 | 43 | 57 |
| *Monochoria vaginalis* | 100 | - | 100 | - | 74 | - |
| *Limnocharis flava* | - | 88 | - | 100 | - | 85 |
| *Cyperus difformis* | 100 | 100 | 100 | 100 | 100 | 96 |
| *Cyperus iria* | 67 | 86 | 89 | 99 | 96 | 100 |
| *Fimbristylis miliacea* | 50 | 49 | 66 | 68 | 83 | 90 |

1 data from 11 trials, applic. 15 DAS, assess. 30-40 DAS
2 data from 3 trials, applic. 10-18 DAT, assess. 33-45 DAT

Table 4. Weed control and yield efficacy of SETOFF in direct seeded wet sown (DSWS) and transplanted (TPL) rice in Indonesia, WS 1986/87.

| Treatment | Rate g ai/ha | Timing DAS[1]/DAT[2] | Weed[4] Control % 50 DAA[3] DSWS Mv. | Ls. | Fm. | Cs. | TPL Mv. | Mc. | Fm. | Cs. | Cs. | Yield MT/HA DSWS | TPL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SETOFF | 20 | 3 | 80 | 68 | 74 | 83 | 69 | 68 | 75 | 64 | 62 | 4.7 | 5.3 |
| | 40 | | 90 | 73 | 89 | 91 | 82 | 73 | 78 | 73 | 82 | 5.5 | 5.5 |
| | 60 | | 93 | 77 | 88 | 93 | 90 | 87 | 88 | 91 | 91 | 6.1 | 6.3 |
| 2,4-D IBE | 700 | | 38 | 22 | 37 | 37 | 57 | 53 | 58 | 50 | 54 | 2.8 | 4.5 |
| SETOFF | 20 | 9 | 86 | 30 | 65 | 83 | 95 | 82 | 70 | 78 | 93 | 5.7 | 5.3 |
| | 40 | | 90 | 57 | 84 | 87 | 91 | 87 | 64 | 69 | 86 | 6.2 | 5.7 |
| | 60 | | 92 | 58 | 87 | 90 | 98 | 86 | 87 | 92 | 98 | 6.6 | 6.5 |
| 2,4-D IBE | 700 | | 67 | 37 | 72 | 62 | 53 | 47 | 68 | 61 | 62 | 4.8 | 4.9 |
| Untreated[5] | | | | | | | | | | | | | |

1 DAS - days after sowing
2 DAT - days after transplanting
3 DAA - days after application
4 Mv: *Monochoria vaginalis*
Ls: *Ludwigia* spp.
Fm: *Fimbristylis miliacea*
Cs: *Cyperus* spp.
Mc: *Marsilea crenata*
Ss: *Scirpus* spp.
5 Weed cover; 93% TPL; 87% DSWS

Table 5. Weed control and yield efficacy of SETOFF in transplanted rice in Malaysia, DS 1986.

| Treatment | Rate | Timing | Weed[4] Control % 49 DAA[3] | | | | | | | Yield |
|---|---|---|---|---|---|---|---|---|---|---|
| | g ai/ha | DAS[1]/DAT[2] | Mv. | Ri. | Lf. | Sg. | Mc. | Sj. | Scg. | MT/HA |
| SETOFF | 40 | 14 | 100 | - | 100 | 100 | 95 | 100 | 97 | 3.9 |
| | 60 | | 99 | 100 | 98 | 100 | 96 | 99 | 100 | 3.4 |
| 2,4-D IBE | 1000 | | 94 | 68 | - | - | 18 | 96 | - | 3.0 |
| SETOFF | 40 | 21 | 96 | 100 | 98 | 98 | 68 | 98 | 100 | 3.4 |
| | 60 | | 98 | 100 | 98 | 100 | 70 | 99 | 100 | 3.4 |
| 2,4-D IBE | 1000 | | 85 | 66 | 40 | 95 | 24 | 88 | 95 | 3.3 |
| Untreated[5] | | | | | | | | | | |

1 DAS - days after sowing
2 DAT - days after transplanting
3 DAA - days after application
5 Weed cover 66%

4 Mv: *Monochoria vaginalis*
Ri: *Rotala indica*
Lf: *Limnocharis flava*
Sg: *Sagittaria guyanensis*
Mc: *Marsilea crenata*
Sj: *Scirpus juncoides*
Scg: *Scirpus grossus*

Table 6. Effect of water management on the weed control efficacy of SETOFF in direct seeded wet sown (DSWS) and transplanted (TPL) rice in Indonesia, WS 1986-87.

| | | | Weed[5] Control % 25 DAA[2] | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | Timing | DSWS | | | | | | | | TPL | | | | | | | |
| Treatment | g ai/ha | DAS/DAT[1] | Mv. | | Fl. | | Cs. | | Ss. | | Mv. | | Fl. | | Cs. | | Ss. | |
| | | | F[3] | S[4] | F | S | F | S | F | S | F | S | F | S | F | S | F | S |
| SETOFF | 10 | 9 | 97 | 84 | 87 | 77 | 95 | 88 | 91 | 82 | 93 | 90 | 91 | 87 | 90 | 92 | 86 | 87 |
| | 20 | | 97 | 97 | 81 | 87 | 92 | 94 | 89 | 95 | 95 | 95 | 98 | 93 | 98 | 95 | 97 | 92 |
| | 40 | | 98 | 97 | 95 | 90 | 98 | 95 | 97 | 94 | 97 | 95 | 98 | 96 | 98 | 96 | 98 | 95 |
| 2,4-D IBE | 700 | | - | - | - | - | - | - | - | - | 81 | 90 | 94 | 93 | 96 | 95 | 91 | 93 |
| SETOFF | 10 | 21 | 92 | 90 | 72 | 28 | 77 | 28 | 86 | 87 | 93 | 78 | 63 | 37 | 78 | 45 | 87 | 55 |
| | 20 | | 96 | 94 | 63 | 30 | 63 | 49 | 97 | 92 | 87 | 75 | 50 | 42 | 82 | 52 | 82 | 63 |
| | 40 | | 96 | 92 | 68 | 53 | 70 | 62 | 98 | 95 | 94 | 75 | 67 | 58 | 87 | 65 | 87 | 70 |
| 2,4-D IBE | 800/1000 | | 83 | 95 | 82 | 87 | 75 | 57 | 91 | 93 | 77 | 85 | 92 | 87 | 90 | 88 | 82 | 83 |
| Untreated[6] | | | | | | | | | | | | | | | | | | |

1 DAT - days after transplanting
2 DAA - days after application
3 F - soil flooded (1-3 cm) at application
4 S - soil saturated (1-3 cm) at applicaton
6 Weed cover untreated check: 76% DSWS (F); 82% TPL (F): 87% DSWS (S); 87% TPL (S)

5 Mv: *Monochoria vaginalis*
Fl: *Fimbristylis littoralis*
Cs: *Cyperus* spp.
Sc: *Scirpus* spp.

Table 7. Effect of SETOFF 40 g ai/ha on yields in direct seeded wet sown (DSWS) and transplanted (TPL) rice in S. E. Asia, 1985-86.

| Application Timings DAS/DAT | Production System TPL No. of observation | TPL Yield difference[1] % | DSWS No. of observation | DSWS Yield difference[1] % |
|---|---|---|---|---|
| 3 - 6 | 7 | 117 | 2 | 142 |
| 7 - 10 | 9 | 110 | 4 | 133 |
| 14 - 15 | 17 | 110 | 4 | 130 |
| 20 - 25 | 17 | 115 | 5 | 119 |
| Handweeded check[2] | | 117 | | |
| Total | 50 | | 15 | |
| Mean | | 113 | | 130 |
| Untreated check (MT/HA) | 4.11 | | 3.70 | |

1 % of untreated check

2 handweeding at 20/40 DAT

3 Mean weed cover in untreated check at 50 DAA: TPL - 55%; DSWS - 92%

**Effect of water management on efficacy of SETOFF** Data from Indonesian trials hardly show any difference in the effectiveness of SETOFF when the application into normal flooded plots is compared with that onto saturated mud. A slight decrease in activity is seen mainly where the application has been made at 21 DAS or DAT (Table 6).

**Effect of SETOFF on rice grain yield** The results of 40 g ai/ha applications of SETOFF made at various timings to transplanted and to DSWS rice are summarized (Table 7). On average, rice grain yield is increased 10-42% over the untreated check, depending on the application timing of SETOFF. From 50 transplanted rice yields there is an average increase over the untreated rice of 13%, and in DSWS rice the mean of 15 yields shows a 30 % increase over the untreated check. In particular, the effect of early control of weeds especially in DSWS rice is shown.

## CONCLUSION

SETOFF is shown to be a good, versatile herbicide for rice especially where broadleaved weeds and sedges are dominant. In particular its outstanding activity against *Marsilea crenata* and *Sphenoclea zeylanica* is demonstrated.

It is seen to be selective in both DSWS as well as transplanted rice, and is effective when applied within 3 to 25 DAS or DAT. Slightly superior efficacy results from applications into flooded fields, but when the soil is in a saturated non-flooded condition at application time weed control is still satisfactory.

For effective weed management of nearly all major non-grass weeds found in S. E. Asian lowland rice cultivations about 20 g ai/ha of SETOFF applied between 3-15 DAS or DAT is the optimum treatment.

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# THE MODE OF ACTION OF THE NEW EXPERIMENTAL HERBICIDE QUINCLORAC (BAS 514 H)

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## ABSTRACT

BAS 514 H (3,7-dichloro-8-quinolinecarboxylic acid) a new experimental herbicide of the BASF Aktiengesellschaft, is particularly effective in controlling *Echinochloa* spp. in rice. Other target weed species for quinclorac are *Monochoria vaginalis*, *Aeschynomene* spp., *Sesbania* spp. and *Ipomoea* spp. In other crops, BAS 514 H is effective against *Aethusa*, *Veronica* and *Galium*. After applications of the compound, newly formed leaves of *Echinochloa* appeared chlorotic, a symptom succeeded by wilting and dying back of the entire plant. The symptoms expressed by dicotyledonous weeds and some other grasses differed from those described above. Here the plants were stunted, their leaves were reduced in size and twisted, and they had swollen nodes. BAS 514 H was absorbed by the leaves and translocated both basipetally and acropetally. On the other hand, the active ingredient could also be rapidly absorbed the roots and translocated to the green parts of the plants. Several test systems demonstrated the auxin-type character of BAS 514 H. An example was the root growth inhibition of cucumber seedlings which was similar to that shown by 2,4-D, picloram or IAA. The extension of wheat coleoptiles and ethylene biosynthesis by leaf discs were both induced. The results clearly demonstrated that quinclorac is a hormone-type herbicide. However, the symptoms shown by treated barnyard grass were not typical for hormone-type herbicides. Thus it is proposed that BAS 514 H has an additional mode of action which has yet to be clarified.

## INTRODUCTION

In 1985, the BASF Aktiengesellschaft presented two new herbicides out of the group of quinolinecarboxylic acids (12, 13). One of them, BAS 518 H (quinmerac), is being developed to control cleavers (*Galium aparine*), one of the major weeds in cereals, rapeseed, and sugarbeets in central and northern Europe. In rice, barnyard grass (*Echinochloa crus-galli*) is regarded as a major grass weed. A further quinolinecarboxylic acid, BAS 514 H (quinclorac) (Fig. 1) has provided consistent control of barnyard grass when applied from germination up to tillering (6). Besides *Echinochloa crus-galli*, other weeds such as *Aeschynomene* spp., *Monochoria vaginalis*, *Oenanthe javanica*, *Sesbania* spp., *Ipomoea* spp. and others may be suppressed by BAS 514 H. In this paper, results from experiments dealing with the uptake, translocation and the mode of action of quinclorac will be presented.

## MATERIALS AND METHODS

**Auxin tests** The cucumber test was performed with cucumber seedlings as described by Sloan and Camper (10). For this purpose, seeds of *Cucumus sativus* L. cv. "Robusta" were surface sterilized with a 2% sodium hypochlorite solution for 30 min and then transfered to Petri dishes lined with three discs of filter paper treated with different herbicide solutions. The samples were incubated at 25°C for 4 days. The root length was then measured and compared with that of seedlings incubated with distilled water.

Ethylene biosynthesis was determined using leaf discs from soybeans (*Glycine max*, cv. "Maple Arrow") which were grown under controlled environmental conditions. Leaf discs were transferred to filter paper discs treated with herbicide solutions. After a preincubation period of about 18 h, the leaf discs were placed in test tubes and sealed. After 5 h, ethylene production was measured by gas chromatography (Packard, model 419).

The wheat coleoptile test was performed according to Mitchell and Livingstone (7). Wheat seeds (*Triticum aestivum* cv. "Kolibri") were surface sterilized and allowed to germinate in vermiculite in darkness for 80 h. Thereafter, seedlings were selected for use under green safety light. Seven cm long coleoptile sections were cut with a razor blade and transferred to a buffer (50 mM sucrose, 5mM $KH_2PO_4/K_2HPO_4$, 30 um $CoCl_2$, pH 5) containing different herbicide concentrations. After incubation at 25°C for 20 h, the coleoptile length was measured and compared with that following incubation in a buffer without supplements.

**Uptake and translocation** Barnyard grass (*Echinochloa crus-galli*) and rice *Oryza sativa* cv. "Bahia") were grown in vermiculite in controlled environmental chambers (16 h day, 8 h night; 75% r.h.). After the emergence of the third leaf, plants were transferred to a hydroponic solution (8).

For leaf uptake studies, $^{14}$C-BAS 514 H (spec. radioactivity 360 MBq/mM) was applied together with an adjuvant to the second leaf of the plants with the aid of a micro syringe. After different time intervals, the plants were divided into four parts (treated leaf, 3rd leaf, primary leaf, root). The treated leaf was washed twice with water/nekanil (0.05%) to remove residues that had not penetrated. All samples were dried and then combusted in an oxidizer (Zinser, Oxymat OX 300). The evolved $^{14}CO_2$ was absorbed in a liquid scintillation cocktail and radioassayed in a scintillation counter (Packard, Tricarb, 460 CD). The radioactivity in the nutrient solution was determined separately.

Root uptake studies were also performed in a hydroponic solution. $^{14}$C-BAS 514 H was added to the nutrient solution at a final concentration of $10^{-5}$M. After different time intervals, plants were removed from the culture medium and the roots were washed. Radioactivity was determined separately in the roots and shoots as described above.

**Chemicals** All experiments were performed with analytical grade chemicals. 2,4-D, picloram, dicamba and IAA were purchased from Riedel de Haen, Seelze, FR-Germany.

## RESULTS

*Echinochloa* plants treated with BAS 514 H at first showed chlorotic discolouration of the youngest leaves. These symptoms were followed by wilting and dying back of the entire plant. In contrast, dicotyledonous weeds were stunted, their leaves reduced in size and twisted. Here phytotoxic symptoms resembled morphological changes following the application of hormone-type herbicides such as 2,4-D, benzoic acids or some pyridine compounds.

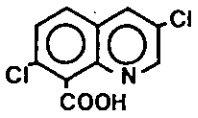


Figure 1 : Chemical structure of BAS 514 H (quinclorac)

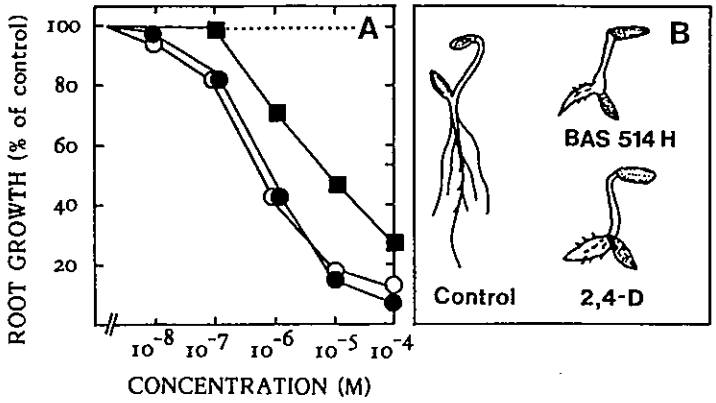


Figure 2 A: Influence of BAS 514 H (-○-), 2,4-D (-●-) and IAA (-■-) on root growth of cucumber seedlings.
B: Morphological changes of cucumber seedlings after the application of BAS 514 H and 2,4-D ($10^{-5}$M).

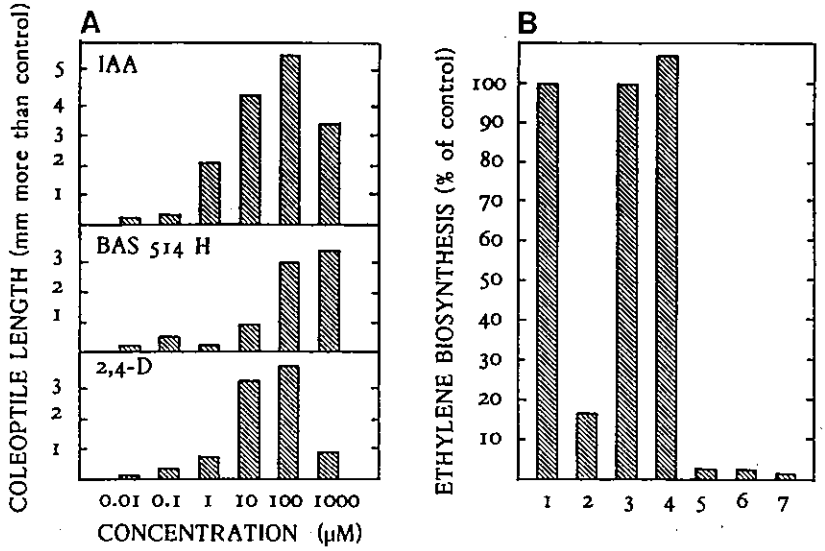


Figure 3 A: Influence of BAS 514 H, 2,4-D and IAA on the elongation of wheat coleoptiles.
B: Ethylene biosynthesis of soybean leaf discs incubated with 2,4-D (1 = control), BAS 514 H (2), dicamba (3), picloram (4), bentazone (5), sethoxydim (6) and water (7).

The above observation was verified by different auxin test systems. Root growth of cucumber seedlings was inhibited by BAS 514 H to the same extent as by 2,4-D and significantly greater than by IAA (Fig. 2 A). Moreover the morphological symptoms appeared to be identical. The primary root was stunted and thickened, the swelling resembling callus. Secondary root growth remained rudimentary (Fig. 2 B).

Two further in vitro test systems also demonstrated the auxin activity of BAS 514 H (Fig. 3). It caused an elongation of wheat coleoptiles as is known for IAA. The optimum effect was attained at a concentration of $10^{-4}$M, whereas 2,4-D and IAA were effective to the same degree at $10^{-5}$M (Fig. 3 A).

Ethylene biosynthesis of soybean leaf discs was induced by BAS 514 H. Although quinclorac activity was lower than that of 2,4-D, dicamba or picloram, the experiments clearly showed that BAS 514 H had a significant auxin activity when compared with water, or herbicides such as sethoxydim or bentazone that have a completely different mode of action (Fig. 3 B).

**Uptake and translocation** When applied to the second leaf, BAS 514 H was well absorbed by *Echinochloa crus-galli*. Within 24 h, nearly 100% of the applied substance could be found in the plant (Fig. 4 A), with most of the compound remaining in the treated leaf. Only small amounts of quinclorac were translocated basi- and acropetally. After 4 days, 10% of the applied radioactivity was found in the roots and 10% in the 3rd leaf. Transport to the primary leaf was limited.

Leaf-applied quinclorac penetrated into the leaves of rice as fast as in barnyard grass, but the radioactivity did not remain in the treated leaf as it was translocated acropetally to the 3rd leaf and basipetally to the roots (Fig. 4 B), from which a considerable amount of BAS 514 H was exudated into the nutrient solution.

When applied to a hydroponic solution, quinclorac was rapidly absorbed by the roots of barnyard grass and rice. Within a few hours, a 10 fold concentration of the active compound could be detected in the roots (Fig. 5), from which BAS 514 H was well translocated to the green parts of the plants. Radioautographs revealed that most of the compound was transported to the growing areas of the shoot, whilst small amounts could be detected in the older parts of the plant, such as the primary leaf. The experiments indicated a faster quinclorac translocation in rice than in barnyard grass.

## DISCUSSION

Morphological changes in dicotyledonous and some monocotyledonous plants, following a BAS 514 H application, were similar to those induced by hormone-type herbicides and revealed that quinclorac has auxin activity. This assumption was confirmed by different auxin test systems. Although hormone activity of BAS 514 H was lower than that of 2,4-D, picloram or dicamba, a significant auxin activity could be detected. This was also true for BAS 518 H (quinmerac), another quinolinecarboxylic acid (1), which is being developed by the BASF Aktiengesellschaft for the control of cleavers in cereals, rapeseed and sugarbeets.

Consequently, quinolinecarboxylic acids must be regarded as a new class of hormone-type herbicide in addition to the compounds known out of the group of pyridines, benzoic acids or phenoxy carbonic acids. Quinolinecarboxylic acids with a totally different substitution pattern, namely the -acid and the zeanic acid, are known to be natural auxins (5, 9).

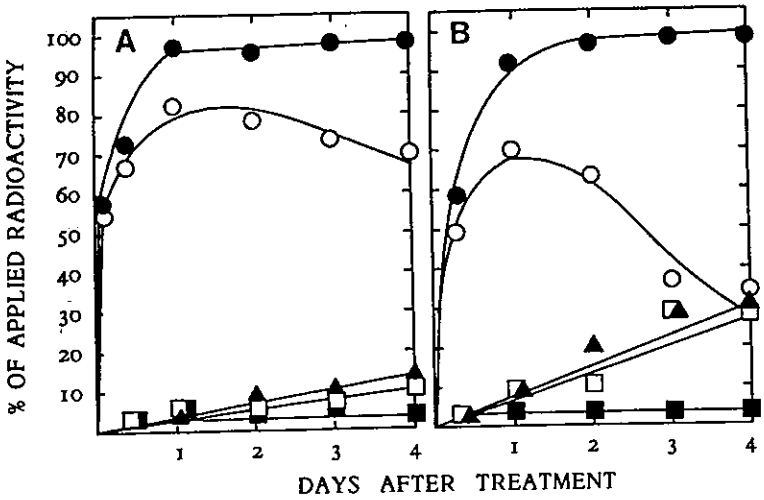


Figure 4 : The uptake and translocation of BAS 514 H after application to the second leaf of Echinochloa crus-galli (A) and Oryza sativa (B).
Total uptake (-●-), treated leaf (-○-), primary leaf (-■-), 3rd leaf (-▲-), root and nutrient solution (-□-).

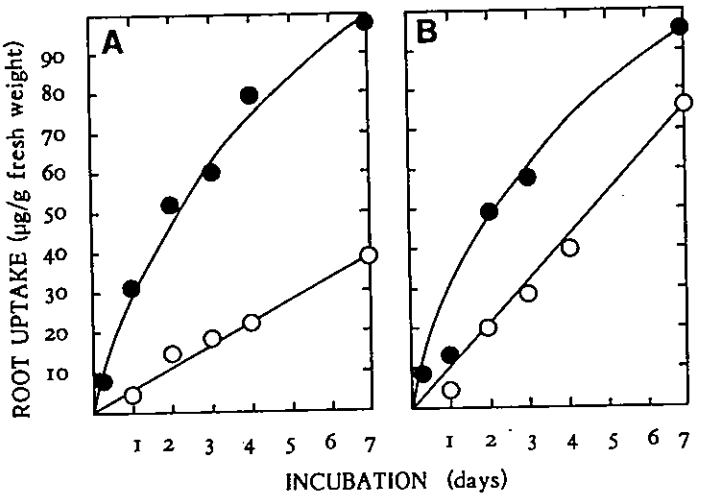


Figure 5 : The uptake and translocation of BAS 514 H after root application to Echinochloa crus-galli (A) and Oryza sativa (B).
Radioactivity in the root (-●-), and in the shoot (-○-).

Although it is common knowledge that hormone-type herbicides lead to an imbalance in the auxin level of plants, the exact mode of action for this type of compound has not been fully elucidated. Hallam (2) has shown that 2,4-D inhibited the chlorophyll biosynthesis in chloroplasts. However, it is not clear whether the symptoms observed in *Echinochloa*, after a BAS 514 H treatment, are solely due to the hormone effect of this substance, or whether quinclorac has an additional, as yet unknown, mode of action.

Quinclorac was rapidly absorbed by the leaves of both rice and barnyard grass. Translocation was observed to take place basi- and acropetally, as is known for herbicides that are regarded as weak acids (4). Quinclorac appeared to be more mobile in rice than in barnyard grass and furthermore, rice exhibited a greater extrusion of radiolabelled compound through the roots into the nutrient solution, which could be regarded as a protective mechanism (3). Root uptake was very fast and an accumulation of the herbicide in both the roots and shoots revealed a higher concentration than in the nutrient solution. Similar results are described for amiben by Stoller (11). Greater mobility of quinclorac in rice than in barnyard grass was also apparent in the root uptake study. More BAS 514 H was transported into the rice shoot than is the case in barnyard grass.

Whereas BAS 514 H gives an excellent control of *Echinochloa crus-galli*, the damage to rice plants is negligible. Whether this selectivity is based on sensitivity differences between rice and barnyard grass towards quinclorac or whether there is a variation in metabolic processes, is still under investigation.

## ACKNOWLEDGEMENT

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# QUINCLORAC - A NEW *ECHINOCHLOA* -HERBICIDE FOR RICE AND AN EXCELLENT PARTNER FOR BROAD SPECTRUM RICE HERBICIDES

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## ABSTRACT

Quinclorac (Facet®) belongs to the chemical group of quinoline carboxylic acids. It is being developed by the BASF Aktiengesellschaft as a specific herbicidal component to control *Echinochloa* species in the rice growing environments of the world. It was tested in the major rice growing areas of Asia, America, Africa and Europe. Outstanding control of *Echinochloa* and excellent selectivity are the essential features of quinclorac. Due to its selectivity it can be applied to transplanted rice as well as to all systems of direct seeded rice. Its timing flexibility permits quinclorac to be used from presowing to mid tillering of *Echinochloa*. Rates of 0.25-0.5 kg ai/ha are sufficient to provide excellent control of all species of *Echinochloa*. Having foliar and soil activity quinclorac is applicable to all rice water management systems. Beside *Echinochloa* spp. quinclorac effectively controls *Aeschynomene* spp. and *Sesbania*. When sprayed it has a considerable side effect on *Monochoria vaginalis*, *Oenanthe javanica*, *Cassia* spp. and *Ipomoea* spp. Due to its specific and longlasting effect on *Echinochloa* spp., quinclorac is a suitable combination partner for all rice herbicides which are ineffective on *Echinochloa* spp. or which only control *Echinochloa* spp. at an early stage. A combination with quinclorac results in more timing flexibility and rate reduction, for the partner component to control the above mentioned weeds.

## INTRODUCTION

Weeds are a serious constraint to rice production because they reduce yield and quality up to about 65% (10). A switch in production method from transplanting to direct seeding, the transition from tall local varieties to semi-dwarf varieties and a higher nitrogen input have reduced the effectiveness of rice competition with weeds. In recent years weed management often became the critical factor in rice production (4, 5). Hand weeding is still the most widely used method of control in rice and requires more than 200 hours/ha when accomplished at the proper time (3). An increase in price of labour relative to herbicide price resulted in many countries in a switch to herbicides. US rice farmers willingly pay about 7% of the crop production value for weed control and regard this expenditure as a definite bargain in view of the loss they might sustain without weed control (11).

On a world wide basis *Echinochloa crus-galli* is considered to be one of the most competitive weeds with rice. It is a cosmopolitan weed that is troublesome in both temperate and tropical rice cultivation areas (7), and certain ecotypes of *Echinochloa* spp. cannot be distinguished

from rice in the early stages of growth (14). It is suggested that rice mimicry arose through natural selection by hand weeding practices conducted under the intensive rice cultivation systems of Asia (1). The ecological requirements of *Echinochloa crus-galli* and rice are similar. *Echinochloa* prefers wet soils and will grow when partially submerged, and also grows best in heavy soils with a high nitrogen content. The fibrous root system of the weed overlays the rice roots, and a seasonlong competition for nutrients is inevitable (7). *Echinochloa crus-galli* has a $C_4$ type photosynthetic pathway which is superior in photosynthetic activity to the $C_3$ pathway of the rice crop (8).

Chemical weed control of *Echinochloa crus-galli* in direct seeded rice is usually accomplished through the use of sequential applications of propanil. Butachlor and Butachlor-combinations are mainly used to control *Echinochloa crus-galli* at early growth stages in transplanted rice. BASF Aktiengesellschaft is developing a new herbicide, quinclorac (BAS 514 H), which has demonstrated outstanding activity on *Echinochloa crus-galli* (6, 9). The field research reported herein deals with the performance of quinclorac in various rice cultivation systems.

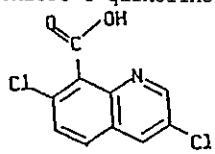
## MATERIALS AND METHODS

**Data on quinclorac**

Trade name: Facet®

Chemical name : 3,7-dichloro-8-quinoline carboxylic acid

Structural fromula :




Physico-chemical properties and toxicological data are presented (9, 13) and the mode of action of quinclorac is described (2).

Formulations for use in transplanted and direct seeded rice are:

BAS 514 00 H containing 50 % (w/w) quinclorac: WP

BAS 514 06 H containing 1 % (w/w) quinclorac: granule.

**Weed control spectrum**

susceptible:

*Echinochloa crus-galli, E. oryzicola, E. crus-pavonis, E. colona, E. glabrescens,*

susceptible weeds depending on rate, timing and formulation used:

*Aeschynomene* spp., *Alternanthera philoxeroides, Brachiaria platyphylla, Cassia* spp., *Digitaria sanguinalis, Eclipta* spp., *Ipomoea* spp., *Marsilea crenata, Monochoria vaginlis, Myriophyllum spicatum, Oenanthe javanica, Paspalum* spp., *Sesbania exaltata, Setaria* spp., *Sida* spp.

Not listed are susceptible weeds of other crops in which quinclorac is being developed as well.

**Field trials** Quinclorac was tested in transplanted and various direct seeded rice cultivation systems under tropical, subtropical and temperate climate conditions since 1983. The small plot trials with plot sizes up to 25 $m^2$, were completely randomized with 3-4 replications. The plots were separated by frames and were kept closed after application. The crop was rainfed and irrigated by flooding on an as-need basis. Granular formulations were spread by hand and a

Gloria pneumatic knapsack boom sprayer with four S 110 02 nozzles at a pressure of 2-4 bar and a spray volume of 200-400 1/ha was used for the wettable powder. Visual scoring of crop injury and weed control were made on a percentage scale in comparison to untreated check plots.

## RESULTS AND DISCUSSION

**Selectivity** Quinclorac is selective in transplanted rice. It can be applied immediately after transplanting. The age and variety of the rice seedling do not influence the selectivity as long as the recommended rates are used. Mechanical transplanted seedlings are occasionally not placed in the proper depth and roots are exposed on the soil surface. In that case temporary injury may occur, particularly at an early application timing and under high temperatures (11).

In direct seeded rice, the selectivity depends on the sowing technique. In drilled rice, quinclorac can be applied presowing, preemergence and postemergence at rates required for a satisfactory control of *Echinochloa crus-galli* (Table 1). Field research indicate that in wet-seeded rice the selectivity of quinclorac is marginal within the first days after seeding, especially with water-seeded and pregerminated rice. In that situation, quinclorac should not be applied as long as the radicals are exposed. From the 2nd leaf stage of the crop onward the herbicide is well tolerated. The selectivity depends neither on the variety nor on the ecotype of rice.

**Efficacy** The results gained thus far indicate that quinclorac can be integrated into the major rice cultivation systems. In transplanted rice the granule formulation of quinclorac controlled *Echinochloa* up to the 4 leaf stage of the weed (Fig. 1). Besides this outstanding timing flexibility, quinclorac provided a season-long *Echinochloa* control with a single application. Locally practiced water manegement was used in the field trials. The optimum water level in flooded fields for the granule application was in the range of 0-5 cm. In drill-seeded rice the spray formulation of quinclorac successfully controlled *Echinochloa* from preemergence up to tillering stage (Fig. 2). Due to its combined soil and leaf activity quinclorac was rather independent of water management used in drill-seeded systems. However, in water-seeded cultivation systems, water levels above 5 cm high reduced its effectiveness and resulted in less reliable grass control. The soil residual activity of quinclorac in preemergence application is partially influenced by the soil texture (Table 2). A Louisiana clay soil needed a higher rate of quinclorac for adequate control of *Echinochloa*. The soil moisture content at the time of the preemergence application significantly influenced the efficacy of quinclorac. A higher soil moisture content enhanced the control. The postemergence activity of quinclorac can be considerably improved by additives which act as penetration agents (Fig. 3). The additives not only improve the effectiveness of quinclorac on *Echinochloa* but also on the leguminous weeds *Aeschynomene* spp. and *Sesbania* spp. The great timing flexibility of quinclorac raises the question about the optimum time of postemergence application with regard to weed competition and yield of rice. The field research of Brazil in drill-seeded rice brought about that the best benefit on yield was accomplished when applications were made at the 2-3 leaf stage of *Echinochloa crus-galli* at 0.375 and 0.5 kg ai/ha respectively (Fig. 4). Obviously, weed competition of *Echinochloa crus-galli* is rather strong and starts very early in the growing season. Hence, the late applications of quinclorac at tillering stage accomplished

Table 1. Selectivity of quinclorac in drill-seeded rice at different timing, spray application, Brazil, 1983-1986.

| Rate kg ai/ha | n[1] | Time applied | 5-10 DAT[4] | 16-26 DAT | 27-50 DAT |
|---|---|---|---|---|---|
| 0.250 | 9 | 2-3 l.s[2]. | 2 | 0 | 0 |
| 0.375 | 6 | | 3 | 0 | 0 |
| 0.500 | 7 | | 4 | 0 | 0 |
| 0.250 | 6 | 4 l.s[2].- | 4 | 0 | 0 |
| 0.375 | 5 | tillering | 4 | 0 | 0 |
| 0.500 | 10 | | 2 | 0 | 0 |

1 number of field trials
2 leaf stage of rice at application time
3 preemergence application
4 days after treatment

Table 2. Influence of soil texture and soil moisture on the preemergence activity of quinclorac, spray application, U.S.A., 1986[1].

| State | Soil texture | Timing | n[3] | Quinclorac(kg ai/ha) 0.250 | 0.375 |
|---|---|---|---|---|---|
| Texas | sandy loam | pre - dry[2] | 1 | 100 | 100 |
| | | pre - moist | 1 | 100 | 100 |
| Texas | loam | pre - dry | 1 | 95 | 95 |
| | | pre - moist | 1 | 89 | 95 |
| Arkansas | clay loam | pre - dry | 1 | 100 | - |
| | | pre - moist | 1 | 100 | - |
| Louisiana | clay | pre - dry | 1 | 71 | 81 |
| | | pre - moist | 1 | 88 | 97 |

1. evaluation: 77 days after treatment
2. pre - dry and pre - moist refer to preemergence applications on dry and moist soils, respectively.
3. number of field trials

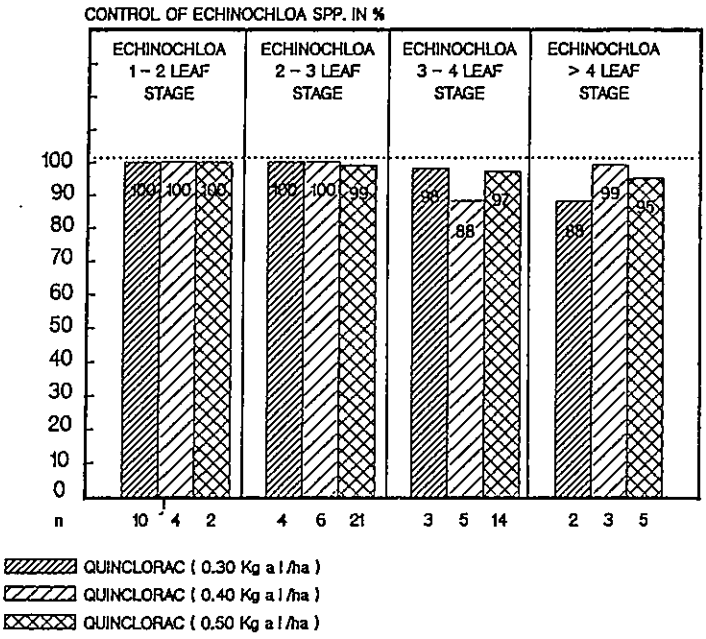



Fig. 1. Activity of quinlorac on *Echinochloa* spp. at different growth stages in transplanted rice, granular apllication, Japan, 1984-1986

evaluation: 30 days after treatment

n = number of field trials

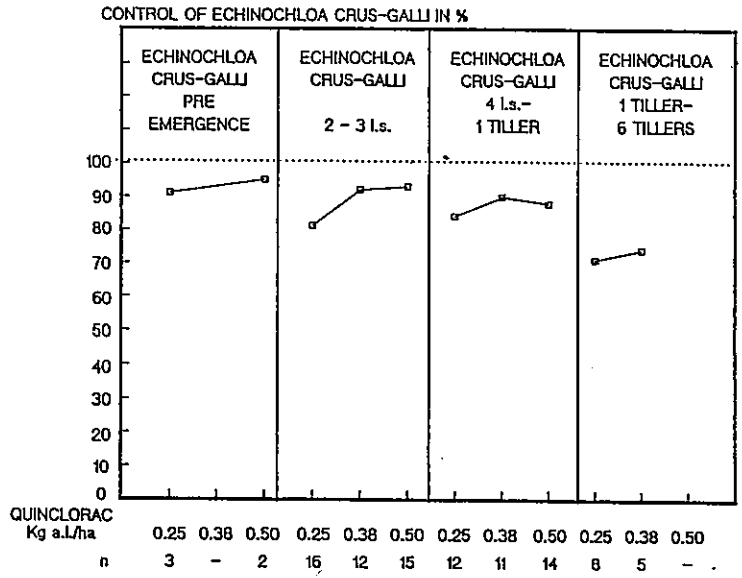



Figure 2. Activity of Quinclorac on *Echinochloa crus-galli* at different growth stages in drill-seeded rice, spray application, Brazil, 1983-1986.

evalution: 30-40 days after treatment

n = number of field trials

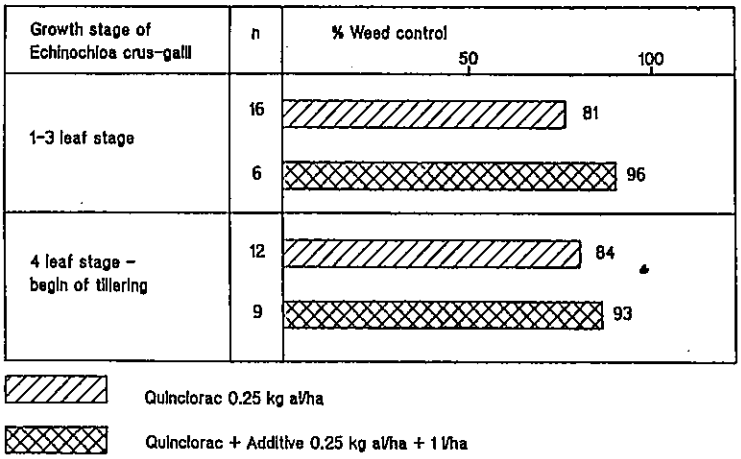
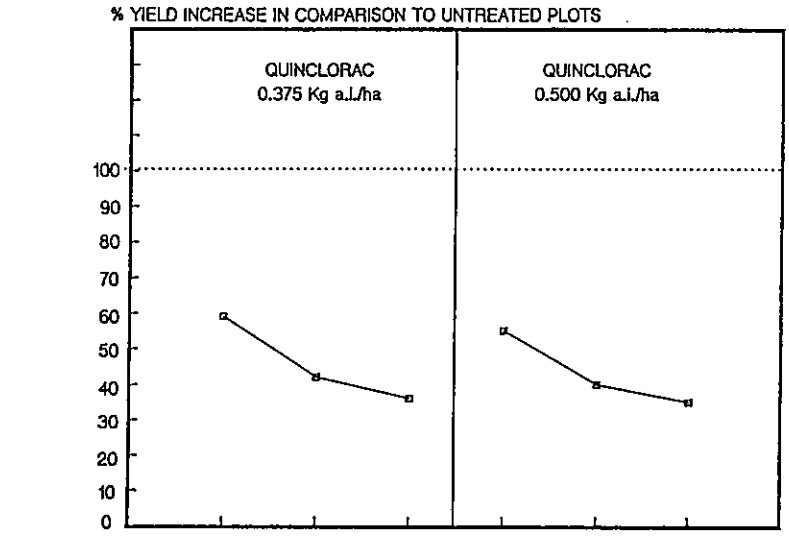



Figure 3. The influence of adjuvants on the activity of Quinclorac on *Echinochloa crus-galli*, spray application, Brazil, 1985-1986.

evaluation: 30-40 days after treatment

n = number of field trials




| leaf stage: ECHCG | 2–3 l.s. | 4 l.s. –1 tiller | 1 tiller –6 tillers | 2–3 l.s. | 4 l.s. –1 tiller | 1 tiller –6 tillers |
|---|---|---|---|---|---|---|
| n | 7 | 7 | 1 | 9 | 8 | 1 |
| % yield increase | 59 | 42 | 36 | 55 | 40 | 36 |

Figure 4. Influence of rates and timing of Quinclorac on rice yield, spray application, Brazil, 1983-1986.

n = number of field trials

l.s. = leaf stage

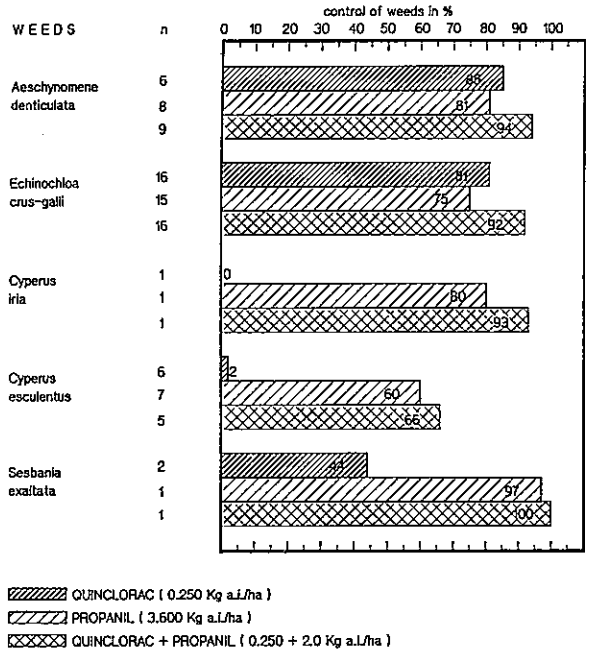



Figure 5. Activity of Quinclorac + Propanil on weeds of drill-seeded rice at 2-3 leaf stage (ECHCG) timing, spray application, Brazil, 1983-1986.

evalution: 30-40 days after treatment

n = number of field trials

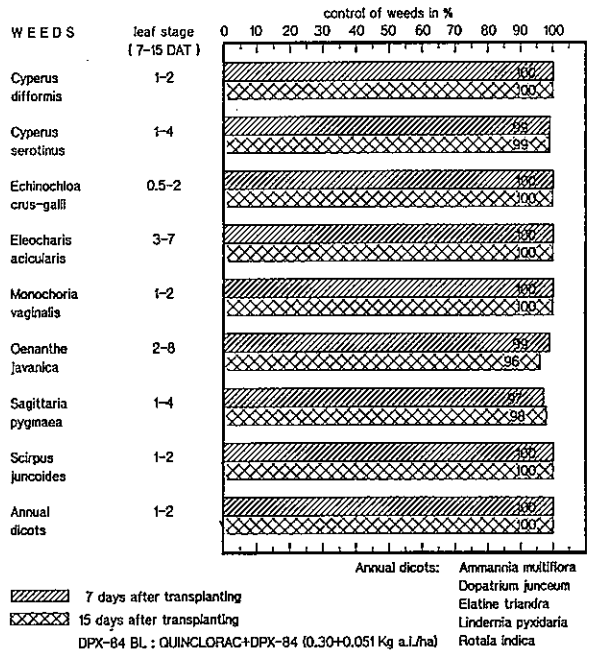



Figure 6. Activity of DPX-84 BL at different timing in transplanted rice, granular application, Japan, 1986.

evaluation: 30 dayss after treatment

2 trials

an effective control of *Echinochloa crus-galli* but missed a critical period of the crop-weed competition.

**Combinations** Effective control of *Echinochloa crus-galli*, even at advanced growth stages of the weed, is the main feature of quinclorac. This property makes quinclorac a suitable combination partner for all rice herbicides which are ineffective or control *Echinochloa crus-galli* only at an early growth stage. A combination with quinclorac provides more timing flexibility and rate reduction and accomplishes a consistent season-long control of *Echinochloa crus-galli* with a single application. As propanil has no residual activity, multiple applications and flooding after the last application are necessary to prevent continuous emergence of *Echinochloa crus-galli*. A single treatment of a combination with quinclorac (Fig. 5) could permit lower pumping cost by delaying flooding and eliminate multiple propanil applications. Quinclorac was also successfully tested in combination with bentazon, benthiocarb, moinate and pyrazolate. In transplanted rice, quinclorac combinations with sulfonyl ureas, pyrazoles and anilides accomplish a taylored one-shot treatment with an excellent residual activity (Fig. 6).

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# SELECTIVE MODE OF ACTION OF ROOT-APPLIED BENSULFURON METHYL AMONG RICE CULTIVARS

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## ABSTRACT

Root absorption, translocation, and metabolism of $^{14}$C-bensulfuron methyl (methyl 2-(((((4,6-dimethoxypyrimidine-2yl)amino)carbonyl)amino)sulfonyl)benzoate) were investigated in tolerant (Milyang 30, Shingwang) and susceptible (Sangpung, Shinseonchal, Nihonbare) rice cultivars. Root-applied bensulfuron methyl phytotoxicity to 5 rice cultivars was also examined under growth chamber conditions. Milyang 30 was the most tolerant to bensulfuron methyl among rice cultivars tested. The translocation rate was higher in Nihonbare, Shinseonchal, and Sangpung. In rice roots, parent bensulfuron methyl was 35.4%, 34.8% in Sangpung and Nihonbare, susceptible cultivars, whereas Milyang 30 and Shingwang, tolerant cultivars, showed 29.5% and 26.3%, respectively. Therefore, cultivar differences in phytotoxicity of bensulfron methyl could strongly be accounted for by the different metabolizing ability and partially by translocation rate in rice plants.

## INTRODUCTION

Bensulfuron methyl (DPX-F5384), a member of sulfonylurea herbicides, is a broad-spectrum herbicide for the control of broadleaved weeds and sedges in paddy rice field. As a selective herbicide or transplanted and direct-seeded rice, bensulfuron methyl was selected from among many sulfonylurea compounds (7). This compound is active at a rate as low as 20-50 g ai/ha and has good herbicidal activity on most annual and perennial weeds which infest rice paddy fields (6, 10).

The mode of action of sulfonylurea herbicides has been studied by Ray (4). The primary site of these compounds is the inhibition of acetolactate synthase, an important enzyme in the pathway for branched-chain amino acid biosynthesis. Secondary effects include the cessation of DNA synthesis, cell division and plant growth (3). DPX-F5384 also inhibits acetolactate synthase from weeds associated with rice (9). The inhibition of acetolactate synthase can account for the herbicidal action of DPX-F5384 in weeds. However, through metabolism study of DPX-F5384 in rice leaves, Takeda et al. (8) suggest that inactivating metabolism plays an important role in the tolerance of rice to DPX-F5384 since rice acetolactate synthase is readily inhibited by this sulfonylurea. Takeda et al. (8) concluded that the safety of DPX-F5384 to rice was due to metabolism to a hydroxylated compound which did not readily inhibit rice acetolactate synthase and was not herbicidal. High tolerance of rice to DPX-F5384 is related to

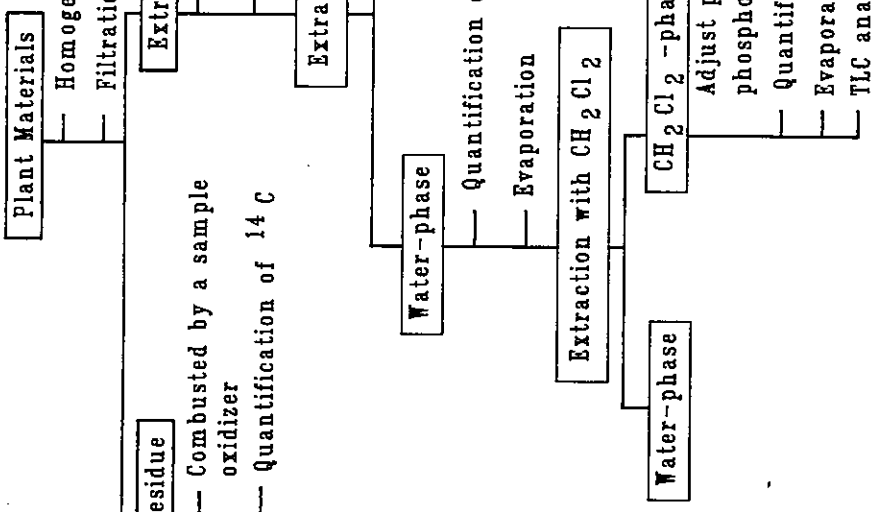


Fig. 1. Flow digram of extraction, separation and quantification of $^{14}$C-bensulfuron methyl and its metabolites in shoots and roots of rice plants.

the ability of rice to metabolize DPX-F5384 to an inactive compound at a rate much faster than sensitive weeds such as *Alisma trivale*, *Sagittaria latifolia*, and *Cyperus difformis*. The metabolic half life of DPX-F5384 in the rice leaves ranges from 2-9 hours depending upon the age of the leaf and the growth conditions.

Nakayama et al. (1) reported the difference in susceptibility to DPX-F5384 among rice cultivars. Ohno et al. (2) also reported that Japonica type rice cultivars were often more susceptible to DPX-F5384 than Indica type or Japonica x Indica type cultivars.

The objectives of the study were to : a) determine differential tolerance to bensulfuron methyl, and b) investigate the absorption, translocation, and metabolism of bensulfuron methyl among 4 rice cultivars to determine basis for differential response of rice cultivars to bensulfuron methyl.

## MATERIALS AND METHODS

**Plant culture** For comparison of tolerance of rice cultivars to bensulfuron methyl, 5 rice cultivars, Milyang 30, Shingwang (Japonica x Indica type), Sangpung, Shinseonchal and Nihonbare (Japonica type), were selected through the preliminary experiment.

Rice seeds were germinated in darkness for 2 days at 25°C in an incubator. Uniform seedlings were selected and transferred to plastic trays. The plants were grown in Kasugai nutrient solution until the 3 leaf stage in a growth chamber at 25°C daytime and 20°C during the night, at 55-60% relative humidity with 12 hours daylength and light intensity of 15 klx.

**Root application of bensulfuron methyl** Roots of intact 3 leaf stage seedlings were soaked in Kasugai nutrient solution adjusted to $10^{-5}$, 5 x $10^{-6}$ and $10^{-6}$M concentration. of bensulfuron methyl for 4 days. The roots were washed with distilled water and then seedlings were transferred to bensulfuron methyl-free Kasugai nutrient solution and grown another 6 days in the growth chamber. Plant height, leaf length, and dry weight of roots and shoots were measured after plant harvest. Each treatment was replicated 3 times using 3 plants for each replication.

**Absorption and translocation of bensulfuron methyl** The $^{14}$C-bensulfuron methyl supplied by Du Pont Company was uniformly phenyl ring-labelled with a specific activity of 15.0 uCi/mg. Plants were cultured and treated as previously described. Roots intact seedlings were soaked in 0.5 ℓ of $10^{-6}$ and 0.99 x $10^{-5}$M $^{14}$C-bensulfuron methyl for 3, 6, 24, and 48 hours. After soaking in the solution, plants were removed and roots were throughly washed with distilled water and blotted dry. The plants were sectioned into shoots and roots; oven dried at 90°C for 24 hours; and weighed. The plants were combusted in a sample combustion system (Aloka ASC-113) and the radioactivity was quantified using a liquid scintillation spectrometer (Beckman LS-1801), With correction for quenching. Translocation rate was calculated by the ratio of radioactivity in shoots to that in whole plants. Each treatment was replicated 3 times using 2 plants.

**Metabolism of bensulfuron methyl** Roots of intact seedlings were soaked in 0.8 ℓ of 0.99 x $10^{-6}$M $^{14}$C-bensulfuron methyl solution for 6 and 24 hours. Following absorption periods the plants were removed from the solution, rinsed with distilled water and sectioned into shoots and roots and fresh weight was weighed. Each treatment was duplicated using 30 plants. Each plant part was separately homogenized and extracted 2 times with 80% acetone (Fig. 1). The radioactivity of the combined extracts and non-extractable residues was determined and then their acetone was

evaporated in vacuo at 35°C. Water extract was adjusted to pH 7 by addition of ammonium hydroxide and extracted with dichloromethane. The remained water fraction was adjusted to pH 3 by addition of phosphoric acid and extracted again with dichloromethane. The radioactivity of dichloromethane extract at pH 7 and pH 3 and water extract was determined by liquid scintillation spectrometry. Extracts of dichloromethane at pH 7 and pH 3 were assayed by thin-layer chromatography. Chromatograms were obtained by developing pH 7-extract with a mixture of methylene chloride/methanol/concentrated ammonia (144/50/6, v/v/v), and pH 3-extract with a mixture of methylene chloride/acetonitrile/glacial acetic acid/water (150/27/2.5/0.3, v/v/v/v).

## RESULTS AND DISCUSSION

**Growth response of bensulfuron methyl among rice cultivars** Varietal differences in the phytotoxicity of root applied bensulfuron methyl was studied among 5 rice cultivars under nutrient culture conditions. Plant height of Milyang 30 exposed to $10^{-6}$M bensulfuron methyl was not much reduced compared to the other rice cultivars which were shown more susceptible to bensulfuron methyl (Table 1). Bensulfuron methyl significantly inhibited growth of rice leaves in Nihonbare, Sangpung, and Shinseonchal, susceptible cultivars. At harvest, Milyang 30 was developed to the 6 leaf stage but Shingwang, Sangpung, and Shinseonchal to the 5 leaf stage, and Nihonbare only to the 4 leaf stage when the 3 leaf stage rice seedlings were exposed to $10^{-5}$M bensulfuron methyl (Table 1).

Dry weight of rice seedling was decreased by exposure to bensulfuron methyl in the nutrient solution as a result of growth inhibition. Root growth of rice seedlings was remarkably retarded by bensulfuron methyl. Rice roots were more remarkedly inhibited in Sangpung, Shinseonchal, and Nihonbare, susceptible cultivars. Above results indicated that Milyang 30 was found to be the most tolerant to bensulfuron methyl among cultivars tested. Ohno et al. (2) reported that Japonica type rice cultivars were more sensitive to bensulfuron methyl than Indica type or Japonica x Indica type rice cultivars, and also varietal difference in the phytotoxicity of bensulfuron methyl was confirmed among 24 rice cultivars in pot experiments (1).

**The absorption and translocation of $^{14}$C-bensulfuron methyl** The amount of $^{14}$C-bensulfuron methyl absorbed by rice roots when exposed to $10^{-6}$M was found higher in Nihonbare, susceptible cultivar, than in the other cultivars at 48hr-absorption. Shingwang and Nihonbare absorbed greater amount of bensulfuron methyl than the other cultivars at $10^{-5}$M at 48hr-absorption (Fig. 2). Ohno et al. (2) reported that tolerant cultivar, RD1, absorbed higher amount of bensulfuron methyl compared to susceptible cultivar, Koshihikari. Therefore, inconsistant differences in absorption among rice cultivars does not account for the differences in response.

The translocation rate from roots to shoots of absorbed $^{14}$C-bensulfuron methyl at $10^{-6}$M was found higher in Nihonbare, Shinseonchal, and Sangpung which showed susceptible response to bensulfuron methyl (Fig. 3). Sangpung and Shingwang showed higher translocation rate when exposed to $10^{-5}$M bensulfuron methyl. Accordingly it may be considered that the translocation rate from roots to shoots may be partially one of the factors affecting the differential sensitivity of bensulfuron methyl among rice cultivars.

Table 1. Growth of 5 rice cultivars as affected by bensulfuron methyl treatment under solution culture condition.

| Rice cultivar | Bensulfuron methyl(M) | Plant height (cm) | Leaf length(cm) | | | Dry weight(g/3 plants) | |
|---|---|---|---|---|---|---|---|
| | | | 4th | 5th | 6th | Shoot | Root |
| Milyang 30 | 0 | 20.8$^{a}$ | 9.7$^{a}$ | 12.7$^{a}$ | 5.3$^{a}$ | 0.185$^{a}$ | 0.070$^{a}$ |
| | $10^{-6}$ | 17.4$^{b}$ | 9.1$^{ab}$ | 10.3$^{b}$ | 2.4$^{b}$ | 0.148$^{b}$ | 0.059$^{b}$ |
| | $5x10^{-6}$ | 14.9$^{c}$ | 8.5$^{bc}$ | 8.0$^{c}$ | 2.1$^{b}$ | 0.124$^{c}$ | 0.045$^{c}$ |
| | $10^{-5}$ | 13.7$^{c}$ | 8.1$^{c}$ | 8.0$^{c}$ | 0.9$^{c}$ | 0.122$^{c}$ | 0.048$^{c}$ |
| Shingwang | 0 | 26.2$^{a}$ | 13.1$^{a}$ | 16.0$^{a}$ | 2.5$^{a}$ | 0.180$^{a}$ | 0.055$^{a}$ |
| | $10^{-6}$ | 18.3$^{b}$ | 11.6$^{b}$ | 9.1$^{b}$ | 0.0$^{b}$ | 0.120$^{b}$ | 0.041$^{b}$ |
| | $5x10^{-6}$ | 17.8$^{b}$ | 11.4$^{b}$ | 8.9$^{b}$ | 0.0$^{b}$ | 0.121$^{b}$ | 0.036$^{c}$ |
| | $10^{-5}$ | 18.1$^{b}$ | 10.6$^{b}$ | 5.5$^{b}$ | 0.0$^{b}$ | 0.110$^{b}$ | 0.031$^{c}$ |
| Sangpung | 0 | 31.9$^{a}$ | 17.6$^{a}$ | 20.3$^{a}$ | 1.9$^{a}$ | 0.214$^{a}$ | 0.077$^{a}$ |
| | $10^{-6}$ | 22.9$^{b}$ | 14.9$^{b}$ | 10.7$^{b}$ | 0.0$^{b}$ | 0.147$^{b}$ | 0.049$^{b}$ |
| | $5x10^{-6}$ | 22.5$^{b}$ | 13.3$^{b}$ | 7.3$^{c}$ | 0.0$^{b}$ | 0.143$^{b}$ | 0.036$^{c}$ |
| | $10^{-5}$ | 20.4$^{b}$ | 13.4$^{c}$ | 2.4$^{d}$ | 0.0$^{b}$ | 0.145$^{b}$ | 0.037$^{c}$ |
| Shinseonchal | 0 | 30.8$^{a}$ | 18.7$^{a}$ | 18.5$^{a}$ | 5.3$^{a}$ | 0.230$^{a}$ | 0.073$^{a}$ |
| | $10^{-6}$ | 24.9$^{b}$ | 16.8$^{b}$ | 11.2$^{b}$ | 0.0$^{b}$ | 0.166$^{bc}$ | 0.044$^{b}$ |
| | $5x10^{-6}$ | 23.3$^{bc}$ | 15.4$^{c}$ | 7.2$^{c}$ | 0.0$^{b}$ | 0.158$^{cd}$ | 0.034$^{c}$ |
| | $10^{-5}$ | 22.2$^{c}$ | 14.9$^{c}$ | 5.2$^{d}$ | 0.0$^{b}$ | 0.143$^{d}$ | 0.030$^{c}$ |
| Nihonbare | 0 | 32.5$^{a}$ | 18.5$^{a}$ | 20.5$^{a}$ | 3.6$^{a}$ | 0.246$^{a}$ | 0.079$^{a}$ |
| | $10^{-6}$ | 24.2$^{b}$ | 15.6$^{ab}$ | 7.4$^{b}$ | 0.0$^{b}$ | 0.159$^{b}$ | 0.047 $^{b}$ |
| | $5x10^{-6}$ | 24.0$^{b}$ | 13.8$^{bc}$ | 1.4$^{c}$ | 0.0$^{b}$ | 0.159$^{b}$ | 0.040$^{c}$ |
| | $10^{-5}$ | 23.8$^{b}$ | 11.6$^{c}$ | 0.0$^{d}$ | 0.0$^{b}$ | 0.144$^{b}$ | 0.032$^{d}$ |

Means followed by the same letters in columns are not significantly different at 5% level by DMRT.

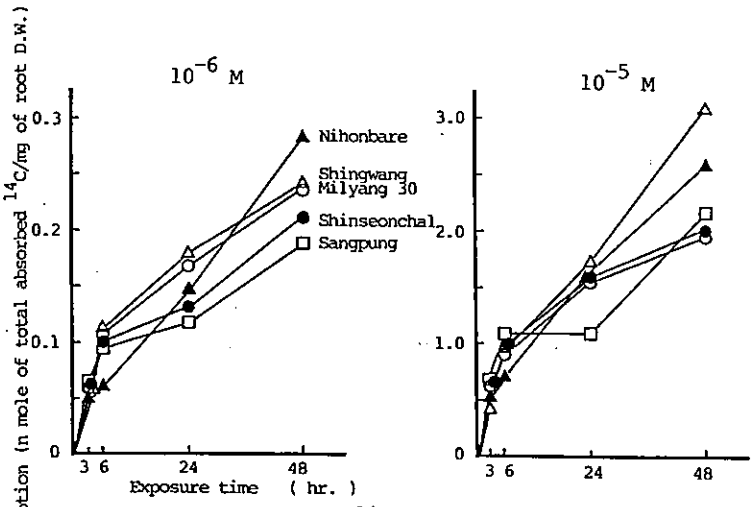


Fig. 2. Absorption of $^{14}$C-bensulfuron methyl by roots of rice cultivars at the 3 leaf stage.

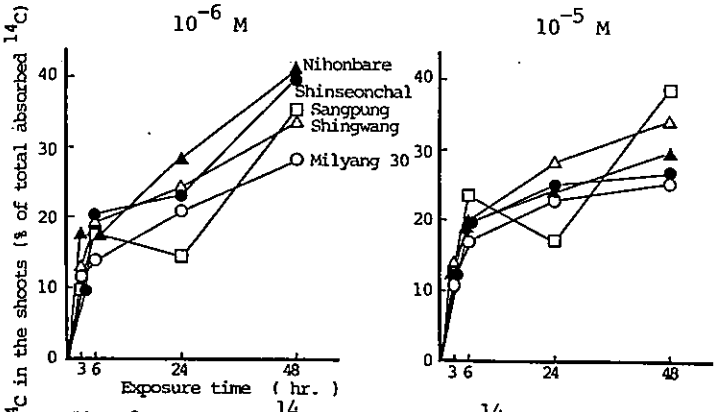


Fig. 3. Rate of $^{14}$C derived from $^{14}$C-bensulfuron methyl translocation from the roots to the shoots in rice cultivars at the 3 leaf stage.

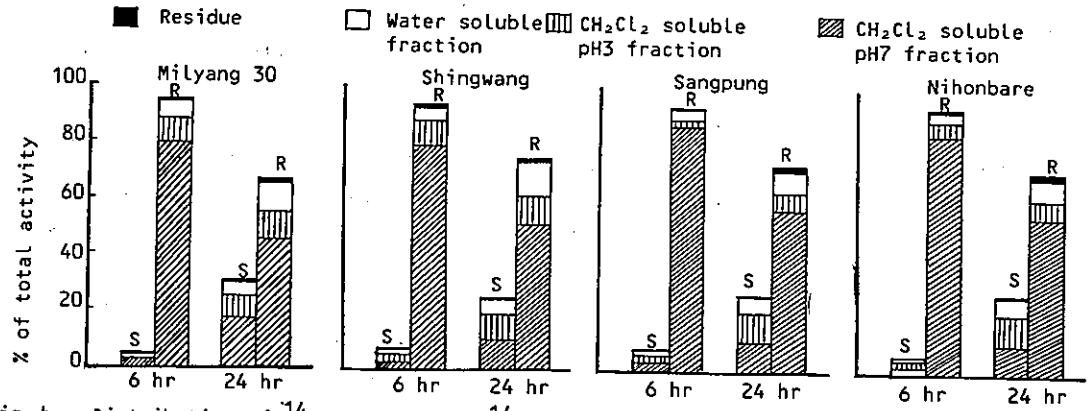


Fig. 4. Distribution of $^{14}C$ derived from $^{14}C$-bensulfuron methyl at each fraction in shoot and root of 5 rice cultivars at the 3 leaf stage.

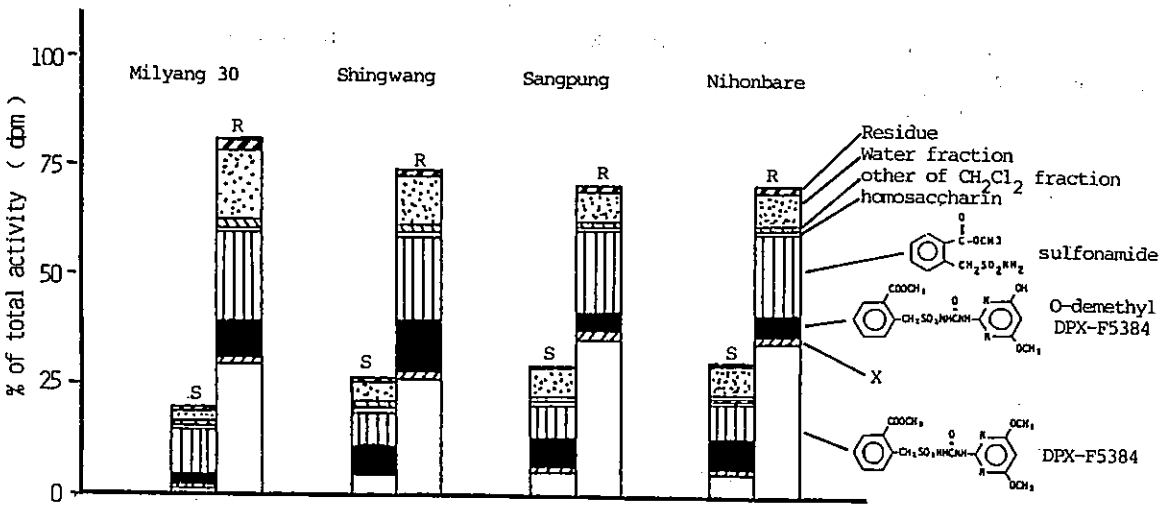


Fig. 5. Distribution of $^{14}C$ derived from $^{14}C$-bensulfuron methyl in each fraction of roots and shoots at the 3 leaf stage of rice plants after 24 hour exposure and the details of $CH_2Cl_2$ fraction analyzed by TLC.

**Metabolism of $^{14}$C-bensulfuron methyl** Results on $^{14}$C-bensulfuron methyl metabolism in roots and shoots of rice cultivars are given in Figs. 4 and 5. Rice roots contained a larger percentage of total absorbed $^{14}$C-radioactivity than shoots. Total radioactivity was increased in shoots as exposure time extended to 24 hours.

Dichloromethane-soluble fraction at pH 7 in which the parent compound was dissolved was higher in Sangpung and Nihonbare, susceptible cultivars, than Milyang 30 and Shingwang. This trend was also observed in case of 6 hour exposure to bensulfuron methyl compared to 24 hour exposure (Fig. 4).

The dichloromethane-soluble fraction of shoots and roots was further investigated in detail by thin-layer chromatography. In rice roots, parent compound level of bensulfuron methyl was 35.4% and 34.8% in Sangpung and Nihonbare, susceptible cultivars, whereas Milyang 30 and Shingwang showed 29.5% and 26.3%, respectively, those values were calculated from percentages of the chemicals existing in dichloromethane fractions (Fig. 5). Yuyama et al. (11) reported that the ratio of parent bensulfuron methyl to metabolites was higher in roots than shoots. But based on results obtained by metabolism study following root absorption, the high distribution of parent compound in roots of Sangpung and Nihonbare, susceptible cultivars, which may be caused by slower metabolism in roots might be related to phytotoxicity of bensulfuron methyl. In contrast Milyang 30 and Shingwang, tolerant cultivars, showed much lower distribution of parent compound in roots because of faster metabolizing ability in roots. Takeda et al. (9) reported that high tolerance of rice to bensulfuron methyl was related to faster inactivating metabolism in rice compared to sensitive weeds. The metabolic half life of bensulfuron methyl in rice leaves ranged from 2 - 9 hours, whereas that of *Alisma trivale*, *Sagittaria latifolia*, and *Cyperus difformis* showed longer than 50 hours since these weeds have little or no metabolism in plants. Sweetser et al. (5) found that chlorsulfuron, one of sulfonylurea herbicides, was tolerant to barley and wheat because these species rapidly metabolized chlorsulfuron to a polar, nonphytotoxic product.

Considering the absorption, translocation, and metabolism of bensulfuron methyl in rice seedlings, we may suggest that cultivar differences in the phytotoxicity of bensulfuron methyl applied to roots is strongly due to the different metabolizing ability in roots, and partially the differential translocation rate of bensulfuron methyl into shoots.

## ACKNOWLEDGMENTS

The authors express their apprecitation to Du Pont de Nemours & Company for providing $^{14}$C-labelled bensulfuron methyl and to Japan Society for Promotion of Science in cooperation with Korea Science & Engineering Foundation for awarding a research fellowship to the senior author.

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# HERBICIDAL ACTIVITY AND MOLECULAR FATE OF CHLOMETHOXYNIL IN THE LIGHT AND DARK

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## ABSTRACT

Effect of light on the mode action of diphenyl ether herbicide chlomethoxynil (2,4-dichlorophenyl-3'-methoxy-4'-nitrophenyl ether) was investigated in rice (*Oryza sativa* L.) and barnyardgrass (*Echinochloa oryzicola* Vasing.) plants. The herbicide showed phytotoxic activity on barnyardgrass in the light, but not in the dark. Rice plant showed no phytotoxic symptom both in the light and dark. Barnyardgrass absorbed the herbicide from shoots faster than rice, however, no difference in the rates was observed between the light and dark in the plants. Metabolic activity of the herbicide was much greater in rice, especially in the light, compared with slight changes in barnyardgrass. Metabolic activity and absorption from shoots were considered as factors of selective action of the herbicide between the plants. It was found that light increased the herbicide detoxifying activity in rice plant.

## INTRODUCTION

Chlomethoxynil (2,4-dichlorophenyl-3'-methoxy-4'-nitrophenyl ether), a diphenyl ether herbicide, is used to control selectively broadleaf weeds and barnyardgrass in paddy rice. It was reported that ortho and/or para-substituted diphenyl ether herbicides absolutely required light for their herbicidal activity (2, 7), however the role of the light involved is still unclear.

Niki et al. (9) reported absorption, translocation and metabolism of root-treated chlomethoxynil in rice (tolerant) and barnyard millet (susceptible) plants. Since translocation of the chemical from roots to shoots was highly limited and no phytotoxic action was observed in roots treatment, no information on a mechanism of selective action between the two plant species was provided.

The objectives of the study were to investigate a) an effect of light on herbicidal activity of root- or shoot-applied chlomethoxynil, b) rates of absorption, translocation and metabolism of the chemical and c) an effect of light on its absorption, translocation and metabolism.

## MATERIALS AND METHODS

**Plant culture** Rice (*Oryza sativa* cv. Nihonbare) and barnyardgrass (*Echinochloa oryzicola* Vasing.) were grown in a greenhouse to the 3- or 4-leaf stage in a nutrient solution as previously described (8). At 2 days before herbicide treatment, plants were transferred to a growth chamber which controlled for temperatures at 30 and 25°C for day (12 hours) and night respectively, with relative humidity at 67% and light intensity of 15 klx.

**Effect of light on chlomethoxynil activity** In shoots treatment, shoot parts of intact plants were soaked for 3 hours in designated concentrations (0, 1, 5, 20 ppm) of the herbicide solution. These solution contained 0.1% of Sorpol and 1% of acetone as solvents. The treatment was carried out both in the light and dark in the growth chamber. After the herbicide treatment, the plants were taken out from the solution and the shoots were washed with distilled water, then plants were transferred to a herbicide-free nutrient solution. At 2 days after treatment, the plants were harvested and their fresh weight was determined. Roots treatment was also carried out with same concentrations for the herbicides. The treatments were performed with 3 replicates of 5 plants each.

**Absorption and translocation** Absorption of chlomethoxynil through shoots was determined by using 2,4-dichloro substituted phenyl ring-$^{14}$C(U)-labeled chlomethoxynil (specific activity of 11.5mCi/mmole). Shoots of the plants were soaked in the herbicide solution (0.28 ppm) in the light and dark. After the prescribed periods of soaking, the plants were sampled and radioactivities in roots and shoots were separately determined by a liquid scintillation spectrometer (Beckman LS-8100) with 3 replicated of 5 plants each. Absorption and translocation of chlomethoxynil from roots of the plants were investigated by autoradiography.

**Metabolism** Shoots of the plants were soaked for 3 hours in 0.25 ppm solution of 3'-methoxy-4'nitro substituted phenyl ring-$^{14}$C(U)-labeled chlomethoxynil (specific activity of 9.85 mCi/mmole). After the treatment, followed by washing the shoots with distilled water, plants were transferred to the herbicide-free nutrient solution and then sampled at 3, 9 and 21 hours. The shoots of the plants were homogenized in 100 ml of acetone/water (1/1 v/v). The homogenates were filtered and the residues were extracted twice with 50 ml of the same solution. The combined extract was concentrated *in vacuo* at 40°C and partitioned twice with *n*-hexane and water. The water fraction was acidified with HCl and extracted twice with ethyl ether. Then remaining water fraction was refluxed for 2 hours at 90°C for analysis of hydrolysates. After neutralization the fraction was extracted twice with *n*-hexane. Radioactivities in a *n*-hexane, an acid-ether, a hydrolysis-hexane and a water fractions were determined by liquid scintillation spectrometry. Radioactivities in a insoluble residues and in roots were determined similarly after combustion. The *n*-hexane and the hydrolysis-hexane fractions were further investigated by thin-layer chromatography with developing solvents of benzene/dichloromethane (1/1, v/v) and *n*-hexane/toluene(2/3, v/v). Followed by autoradiography, radioactive spots were scraped off from the plates. Radioactivity of each spot was extracted in a counting vial with 2 ml of 95% ethanol and determined as mentioned above.

## RESULTS AND DISCUSSION

**Effect of light on the growth** Effect of light on herbicidal activity of chlomethoxynil was studied in rice and barnyardgrass plants. Shoots or roots of the plants were soaked for 3 hours in the herbicide solution and their fresh weight were determined at 2 days after treatment (Figs. 1 and 2).

In shoots treatment (Fig. 1) phytotoxicity of the herbicide was observed only in the light. Barnyardgrass was susceptible to chlomethoxynil and showed considerable severe damage. Rice was tolerant so that selective action of the herbicide was observed between the two plant species. In the dark, both plant species showed no phytotoxicity. In roots treatment (Fig. 2), no reduction of the growth was detected in the light and dark. The data demonstrated that light and direct absorption into shoots were essentially required for the activity of chlomethoxynil.

**Absorption and translocation** Absorption of $^{14}$C-chlomethoxynil from shoots of rice and barnyardgrass plants was determined in the light and dark (Fig. 3). The rate of absorption from shoots was found higher in barnyardgrass than in rice. Little difference in the rate between in the light and dark was detected in both plants. The data demonstrated that chlometoxynil showed no herbicidal activity in the dark notwithstanding concentration of the herbicide was same with in the light. In the light, differential absorption rates from shoots may contribute to the differential effect on the growth of the plants.

Autoradiographs of root-applied $^{14}$C-chlomethoxynil showed that no translocation was occured from roots to shoots(data not shown). It may contribute to the insensitiviness of the plants to chlomethoxynil in roots treatment.

**Metabolism** Metabolism of shoot-applied chlomethoxynil in shoots of rice and barnyardgrass plants was studied both in the light and dark. The percentages of radioactivity in the *n*-hexane, the water and the insoluble residue fractions and in the roots were determined (Fig. 4). In the light, radioactivity in the *n*-hexane fraction in rice was decreased rapidly with time and radioactivities in the water and the insoluble residue fractions were increased, while barnyardgrass showed much slower activity to change the *n*-hexane fraction into the water and the insoluble residue fractions. At 21 hours after treatment, radioactivities of the n-hexane, the water and the insoluble residue fractions were 35.8, 49.9 and 13.6% of total radioactivity absorbed in rice, and 86.4, 6.0 and 7.0% in barnyardgrass respectively. In the dark, although rice plant showed higher metabolic activity than barnyardgrass, percentages of the water and the insoluble residue fractions were considerably less than those in the light.

A ratio of the parent compound and its metabolites in the *n*-hexane fraction was assayed by thin-layer chromatography (Table 1). In barnyardgrass, a little amount of metabolite was detected and almost all radioactivity was originated from chlomethoxynil itself. Decrease of the parent compound was slightly faster in the light. In rice, 2,4-dichlorophenyl-3'-hydroxy-4'-nitrophenyl ether (abbreviated as 3'-desmethyl compound) was identified as a main metabolite. Decrease of the parent compound was considerably faster in the light. These data indicate that metabolism of chlomethoxynil in the plants is promoted by the light, being remarkable in rice plant which is tolerant to the chemical. The mechanism of this promotion should be investigated.

After extracted with acid-ether, the remaining water fraction of rice in the light treatment was hydrolyzed and extracted again with *n*-hexane (Table 2). By thin-layer chromatography of the hydrolysis-hexane fraction, 3'-desmethyl compound was identified as a major component of the fraction (data not shown). It was assumed that chlomethoxynil was conjugated with cell

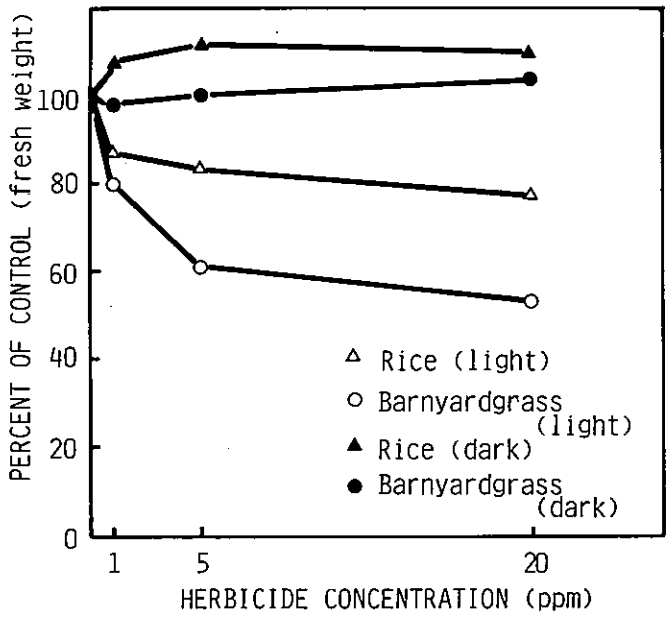


**Fig. 1. Herbicidal activity of shoot-applied chlomethoxynil in the light and dark.**

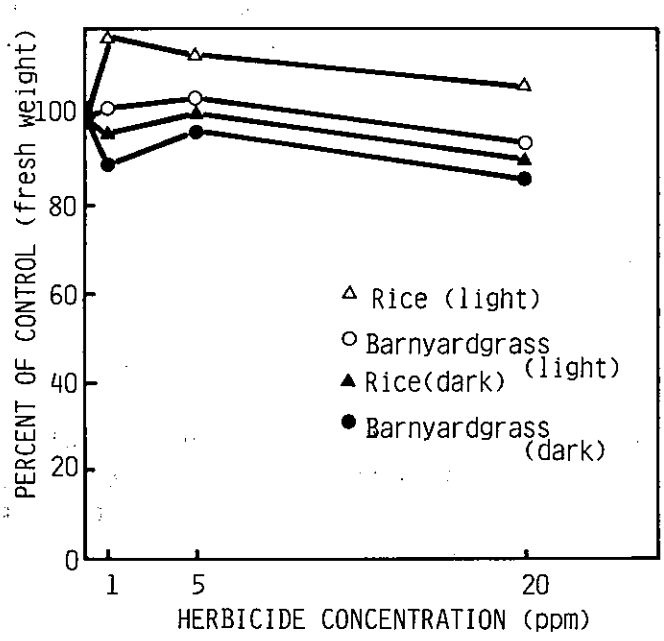


**Fig. 2. Herbicidal activity of root-applied chlomethoxynil in the light and dark.**

Table 1. Radioactivities of chlomethoxynil and its metabolites in the *n*-hexane fraction.

| Plants | Hours | Light | | | | Dark | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Chlomethoxynil | 3'-OH[1] | Others | Total | Chlomethoxynil | 3'-OH[1] | Others | Total |
| | | % | | | | % | | | |
| Rice | 3 | 66.0 | 3.7 | 5.2 | 74.9 | 85.4 | 5.7 | 1.5 | 92.6 |
| | 9 | 53.3 | 11.1 | 1.4 | 65.8 | 67.1 | 7.7 | 1.7 | 76.5 |
| | 21 | 28.0 | 5.8 | 2.0 | 35.8 | 52.9 | 14.8 | 2.3 | 70.0 |
| Barnyardgrass | 3 | 90.6 | 0.3 | 0.4 | 91.3 | 91.3 | 0.4 | 0.9 | 92.6 |
| | 9 | 88.9 | 0.4 | 0.3 | 89.6 | 91.3 | 0.3 | 0.7 | 92.3 |
| | 12 | 85.7 | 0.4 | 0.3 | 86.4 | 90.0 | 0.2 | 0.7 | 90.9 |

1 3'-OH : 2,4-dichlorophenyl-3'-hydroxy-4'-nitrophenyl ether.

Table 2. Distribution of radioactivity of the water fraction.

| Plants | Hours | Light | | | | Dark | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Acid-ether | Hydrolysis[1] -hexane | Water[2] | Total | Acid-ether | Hydrolysis[1] -hexane | Water[2] | Total |
| | | % | | | | % | | | |
| Rice | 3 | 5.6 | 3.6 | 11.9 | 21.1 | 1.9 | - | 2.3 | 4.2 |
| | 9 | 11.8 | 5.6 | 11.1 | 28.5 | 4.6 | - | 10.9 | 15.5 |
| | 21 | 15.2 | 12.4 | 22.3 | 49.9 | 2.7 | - | 14.8 | 17.5 |
| Baryna-rdgrass | 3 | 3.3 | - | 2.2 | 5.5 | 0.8 | - | 1.7 | 2.5 |
| | 9 | 3.0 | - | 3.0 | 6.0 | 1.1 | - | 2.5 | 3.6 |
| | 21 | 2.8 | - | 3.2 | 6.0 | 1.5 | - | 2.3 | 3.8 |

1 Hydrolysis was attempted only in rice plant in the light.

2 Radioactivity in the fraction contained metabolite(s) not extracted with acid-ether and *n*-hexane (after hydrolysis).

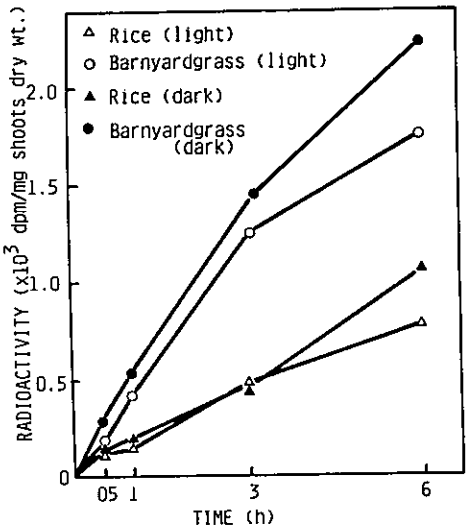


Fig. 3. Absorption of $^{14}$C-chlomethoxynil from shoots in the light and dark.

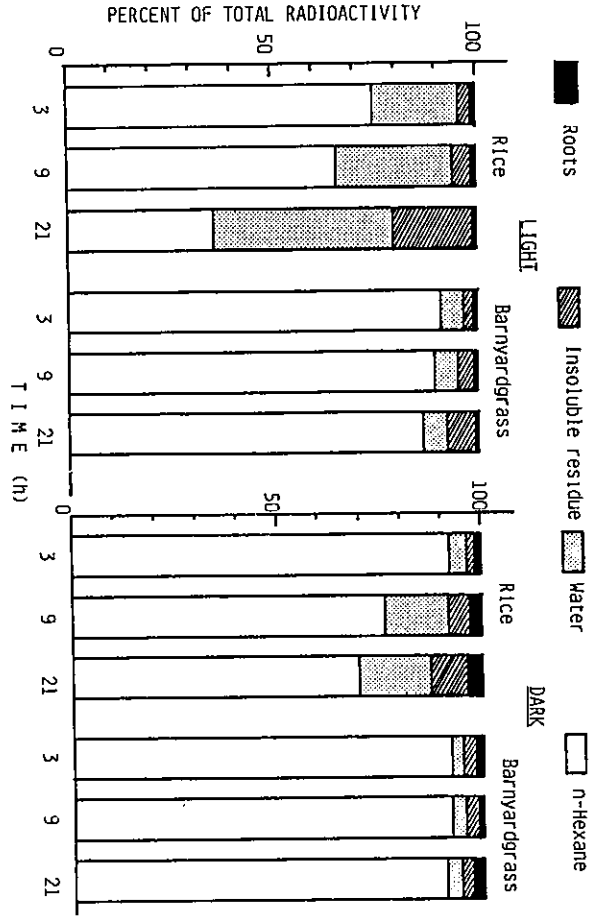


Fig. 4. Distribution of radioactivity from shoot-applied chlomethoxynil in the light and dark.

component(s) after demethylation. Although phenols, products by cleavage of ether bond, were supposed to be contained in the acid-ether fraction and analysis of the fraction was not attempted the metabolite(s) in the fraction is to be analyzed in detail. The metabolism of fluorodifen and nitrogen was shown to involve reduction of 4'-position from $NO_2$ to $NH_2$ and cleavage of ether bond (1, 4). In chlomethoxynil, demethylation followed by conjugation was considered to be major metabolic pathway.

From the above data obtained, it is concluded that rate of metabolism is, in combination with rate of absorption, a major factor of selectivity of chlomethoxynil in the light. In the dark, in spite of absorption rate was similar with in the light and little degradation occured, no phytotoxicity was observed even in the susceptible plant. It is indicated that chlomethoxynil absolutely requires light for its herbicidal activity. Recently, the role of light to activate diphenyl ether herbicides has been studied and lipid peroxidation of cell membranes by the herbicides was reported (3, 5, 6, 10, 11). Present study clarified that light acted to increase metabolic transformation of chlomethoxynil in rice plant. It is still uncertain that the light enhanced transformation of the chemical is related to the light activated peroxidation. The mechanism of this promotion is to be investigated.

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# CGA 142'464 : A NEW HERBICIDE FOR WEED CONTROL IN DIFFERENT RICE PRODUCTION SYSTEMS

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## ABSTRACT

CGA 142'464 is herbicide discovered by CIBA-GEIGY Ltd., Basel (Switzerland); its chemical name is 3-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1-[2-(2- methoxyethoxy)-phenylsulfonyl]-urea. A common name has not veen ascertained yet; the tradename is SETOFF® CGA 142'464 was first tested in the field during 1984 and since then it has been developed for use in different rice (*Oryza sativa* L.) production systems. CGA 142'464 acts as an inhibitor of cell division and growth of susceptible plants. It has an excellent broad-spectrum activity and most of the important weeds in rice such as Scirpoideae and broadleaved weeds in annual as well as perennial forms are well controlled. Depending on CGA 142'464's application timing and rate, a reasonable degree of control (suppression) of grassy weeds such as *Echinochloa* spp. can also be achieved. CGA 142'464 is entirely safe to rice when the crop is transplanted. A pre-emergence application may cause slight phytotoxicity in wet-sown rice; however, CGA 142'464 can be safely used after emergence of the rice crop. The high degree of weed control with CGA 142'464 is reached at the low dose of 20-60 g ai/ha. In addition, the timing of application is flexible and not limited to a specific developmental stage of the weeds or the rice crop.

## INTRODUCTION

Weed control has a major impact on rice production since a large portion of the total labour is devoted to weeding (2).

Weed species that cause problems in rice vary with soil, climate, rice culture, seedling method, water management and weed control technology (7). *Echinochloa crus-galli* is certainly worldwide the most troublesome weed in rice (5). Besides *Echinochloa* spp., however, other rice field weeds such as *Cyperus difformis, Cyperus iria, Fimbristylis littoralis, Monochoria vaginalis* etc. are of world importance (8). Yield losses in rice caused by the weeds are presented in Table 1.

CGA 142'464 is a new herbicide discovered and being developed by CIBA-CEIGY Ltd. for use in various rice production systems. CGA 142'464 has been tested worldwide in the major rice growing areas. It has an excellent broad-spectrum activity and important weeds in rice such as Scirpoideae (= sedges) and broadleaved weeds in annual as well as perennial froms are well controlled. The tradename of CGA 142'464 is Setoff$^{R}$.

Some physico-chemical and toxicological properties of the technical material of CGA 142'464 are presented in Talbe 2. This paper describes the characteristics of this new herbicide and summarizes some results obtained from greenhouse studies and field experiments. More detailed results from field trials will be published separately (1).

## MATERIALS AND METHODS

Field tests have been conducted worldwide in the major rice growing areas (Indonesia, Philippines, Thailand, Japan, etc.) Representative test have been selected to illustrate the activity of CGA 142'464 (= Setoff®. Experimental details are presented for each of these trials. The plot design was always a randomized complete block with 10 - 100 $m^2$ plots and 3 to 4 replicates. Adjacent to each plot was an untreated checkstrip in order to assess weed control better. All crop phytotoxicity or weed control evaluations are based on a (%) scale. All rates are expressed in active ingredient (ai).

**Experiments under greenhouse conditions** Trials were carried out at the CIBA-GEIGY Research Station, St. Aubin, Switzerland, in pots containing 140 litres of a standard sandy loam. Construction of such pots allows to vary leaching-conditions. Day/night-temperature was 30/25 °C, photoperiod 13 hours.

Granules (extruder) of Setoff® were applied by hand into the water.

**Field experiments under tropical climate conditions**

**Indonesia** The trials were conducted at the CIBA-GEIGY Research Station, Cikampek, in West Java. Soil preparation was done manually, puddling twice before levelling.

**Transplanted rice** Wet bed seedling of the rice cv. IR-36 were transplanted by the Indonesian standard spacing of 25 cm x 25 cm.

**Direct seeded rice** Pregerminated rice (pregermination according to IRRI recommendations) cv. IR-36 was sown into the mud.

**Water management** Several trials were carried out in which the water regime was manipulated. The two basic water regimes were a) normal flooded conditions, where level was maintained at a height of 3-6 cm, and b) saturated conditions, where the plots were only occasionally flooded to keep the soil wet and to prevent it from cracking.

**Thailand** Trials were conducted in the Chainat-area. Pregerminated rice (1 day soaking + 2-3 days incubation) cv. RD 7 was sown into the mud. Soil preparation, water management etc. were made according to local practices.

Spraying was done with a knapsack sprayer, either equipped with a single Cooper-Pegler flood jet nozzle or with a special trial spray boom consisting of 3 fanjet nozzles. Spray volume was 500 l/ha. The formulations used of Setoff® included WP- and WDG- type.

**Field experiments under temperate/subtropical climate conditions** Trials were conducted at the CIBA-GEIGY Experimental Station in Ono (Osaka). Seedlings of the rice cvs. Yamabiko and Nihonbare were transplanted mechanically or by hand. Soil preparation, water management etc. were made according to Japanese practices. Granules (extruder) of Setoff® were applied by hand into the water.

## RESULTS AND DISCUSSION

Table 1. Interference of selected species of weeds in rice. Data adapted from Smith (1981).

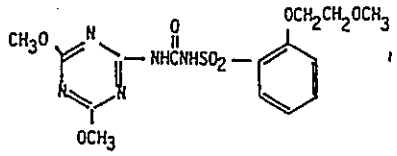
| Weed species | Country | Rice production system[1] | Yield loss in (%) |
|---|---|---|---|
| *Cyperus difformis* | Taiwan | TP | 49-90 |
| | Australia | DP | 33-44 |
| *Marsilea minuta* | Taiwan | TP | 45-87 |
| *Monochoria vaginalis* | Taiwan | TP | 31-86 |
| *Heteranthera limosa* | USA | DP | 6-27 |
| *Echinochloa crus-galli* | Japan | TP | 5-75 |
| | USA | DP | 25-95 |

1 TP = transplanted rice DP = direct-seeded paddy rice

Table 2. Some pypsico-chemical and toxicological properties of CGA 142'464.

Chemical name: 3-(4,6-dimethoxy-1,3,5-triazin-2-yl)-1-[2-(2-methoxyethoxy)-
(IUPAC) phenylsulfonyl]-urea.

Structural formula :




Molecular formula : $C_{15}H_{19}N_5O_7S$

Molecular weight : 413.41

Physico-chemical properties of the active ingredient

- Physical state at 20°C : crystalline, colourless.
- Melting Point : 144.6°C
- Vapour pressure at 20°C : 7.5 x $10^{-3}$mm Hg
- Solubility 3700 mg/1 in water (pH 7) at 20°C.

Toxicity of technical material

- Mammals $LD_{50}$ oral (rat) 5000 mg/kg
  $LD_{50}$ dermal (rat) 2000 mg/kg
  $LD_{50}$ inhalation (rat) 5000 mg/$m^3$
- Skin irritation (rabbit) : none
- Eye irritation (rabbit) : none
- Wild life : practically non-toxic to fish and daphnia.

Additional toxicology studies are in progress.

Table 3. Greenhouse studies on the performance of CGA 142'464 = Setoff$^R$ in relation to leaching conditions in transplanted and wet-sown rice. (application timing: 3 DAT/DAS; rate of CGA 142'464: 30 g ai/ha)

| Leaching | % Crop phytotoxicity[2] | | | | % Weed control | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Monochoria vaginalis* | | | *Alisma plantanginea* | | | *Eleocharis acicularis* | | | *Scirpus hotarui* | | |
| | 17 | 24 | 44 | DAA[3] | 17 | 24 | 44 | 17 | 24 | 44 | 17 | 24 | 44 | 17 | 24 | 44 |
| With[1] | 14 | 9 | 4 | | 90 | 100 | 100 | 90 | 100 | 98 | 90 | 85 | 95 | 100 | 100 | 92 |
| Without | 13 | 8 | 7 | | 97 | 100 | 99 | 95 | 95 | 90 | 95 | 90 | 98 | 100 | 100 | 96 |

1 Leaching: 6 mm per day

2 average on 3 varieties: Yamabiko, IR-36 (both transplanted), S-201 (wet-sown)

3 DAA : Days after application

Table 6. Performance of CGA 142'464 = Setoff$^R$ in relation to soil type in transplanted rice under tropical climate conditions, Indonesia (rate of CGA 142'464: 20 g ai/ha)

| Application | soil type[2] | % Crop phytotoxicity 12 - 17 DAA[3] | % Weed control at 50 DAA | | | |
|---|---|---|---|---|---|---|
| | | | *Monochoria vaginalis* | *Scirpus* spp. | *Fimbristylis* spp. | *Cyperus* spp. |
| 3 DAT[1] | light | 7 | 93 | 94 | 92 | 94 |
| | medium | 2 | 97 | 97 | 98 | 97 |
| | heavy | 3 | 91 | 87 | 82 | 85 |
| | x soil type | 4 | 94 | 93 | 91 | 92 |
| 15 DAT | light | 9 | 94 | 94 | 95 | 96 |
| | medium | 5 | 97 | 95 | 98 | 97 |
| | heavy | 2 | 97 | 97 | 87 | 90 |
| | x soil type | 5 | 96 | 95 | 93 | 94 |

1 DAT = Days after transplanting

2 soil type - light: 30% sand, 41% silt, 29% clay
- medium: 35% sand. 26% silt, 38% clay
- heavy: 25% sand, 45% silt, 41% clay

3 DAA = Days after application

Table 4. Weed control of CGA 142'464 = Setoff in transplanted rice under tropical climate conditions, Southeast Asia (Indonesia, Malaysia, Philippines, Thailand). (Average of 33 trials, rate of CGA 142'464: 40g a.i./ha).

| Application timing | % Weed Control at about 50 DAA[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Monochoria vaginalis* | *Scirpus juncoides* | *Fimbristylis* spp. | *Cyperus* spp. | *Marsilea crenata* | *Rotala indica* | *Sphenoclea zeylanica* | *Ludwigia* spp. |
| 3 DAT[1] | 97 | 88 | 95 | 99 | - | - | - | - |
| 9 - 12 DAT | 99 | 98 | 86 | 97 | 98 | 100 | 100 | 73 |
| 14 - 15 DAT | 97 | 93 | 78 | 93 | 93 | 100 | 99 | 78 |
| 18 - 21 DAT | 97 | 92 | 45 | 91 | 90 | 100 | 96 | 74 |
| 25 - 35 DAT | 96 | 90 | 60 | 80 | - | - | 95 | 68 |

1 DAT = Days after transplanting

2 DAA = Days after application

Table 5. Performance of CGA 142'464 = Setoff® in transplanted rice in relation to water management under tropical climate conditions, Indonesia (Average of 8 trials).

| Water management | Rate of CGA 142'464 g ai/ha | Application timing | % Crop phytotoxicity 10 DAA[2] | 45 DAA | % Weed control at 55 DAA *Monochoria vaginalis* | Scirpoideae[3] | *Echinochloa crus-galli* |
|---|---|---|---|---|---|---|---|
| permanently | 10 | 3-9 | 6 | 0 | 91 | 86 | 40 |
| flooded | 20 | DAT[1] | 7 | 0 | 93 | 88 | 52 |
| condition | 40 | | 10 | 0 | 94 | 99 | 57 |
| rainfed | 10 | 3-9 | 6 | 0 | 89 | 77 | 35 |
| condition | 20 | DAT | 9 | 0 | 91 | 83 | 48 |
| (saturated) | 40 | | 11 | 0 | 94 | 86 | 66 |

1 DAT = Days after transplanting

2 DAA = Days after application

3 Scirpoideae = *Cyperus difformis, Cyperus iria, Fimbristylis* spp. *Scirpus* spp.

**Greenhouse experiments** A large number of paddy weeds were included in such greenhouse experiments. Results from these trials as summarized in Talbe 3 show that CGA 142'464 at a low rate of 30 g ai/ha only will control important weed species of rice such as *Monochoria, Alisma, Eleocharis, Scirpus* (and others).

The mode of action of CGA 142'464 is not fully elucidated yet. It seems, however, that this compound acts in a similar way to that of other sulfonylurea herbicides, i.e. by inhibiting growth of meristematic tissues.

Of CGA 142'464 it is known to be taken up by both roots and shoot and afterwards translocated to the growing tips. Growth is then completely suppressed, chlorosis and anthocyanin accumulation occur. Later necrotic symptoms appear and normally susceptible plants will die 2 to 4 weeks after application.

From this it can be concluded, that the speed of activity of CGA 142'464 obviously is not very fast. Such slow action is demonstrated by the fact that in our greenhouse studies the level of control (depending on weed species) continued to increase with time (compare Table 3). Seedlings and young plants generally are more susceptible to this agrochemical than plants at later development stages.

In contrast to the increasing weed control level, crop phytotoxicity in transplanted as weed as wet-sown rice is comparatively high at early evaluation timings and decreases with time to a negligible level.

Water leakage has little influence on the performance of CGA 142'464. At a leaching of 6 mm per day neither in the weed control figures nor in the level of crop phytotoxicity any significant differences were observed (compare Table 3).

**Field experiments** The results presented in Tables 5 - 9 show that Setoff® can be safely applied in both transplanted and direct seeded rice. A slight early phytotoxicity was observed following a preemergence application of Setoff® in direct seeded rice in Indonesia; however, the phytotoxicity disappeared with time. (compare Table 8).

Generally, climatic conditions, soil type, rice cultivar and water management do not influence the good crop tolerance of Setoff®.

Based on these results Setoff® can be safely applied at different growth stages of rice.

Under field trial conditions, Setoff® has shown to provide effective control of a broad range of weeds in transplanted as well as in wet-sown rice. The spectrum of weeds controlled include broadleaved weeds and Scirpoideae in both annual and perennial forms (Table 10). Grasses such as *Echinochloa crus-galli* are only partially controlled and usually better so (which may be sufficient at a low *Echinochloa*-infestation) at early application timings (Tables 5 and 8).

For obtaining full spectrum weed control in areas with high *Echinochloa* spp. infestation, the addition of a grasskiller (such as pretilachlor) to Setoff® has shown to be very promising; such results will be published separately.

There is a good flexibility with regard to application timing of Setoff® without a need to restrict the application to a specific development stage of weeds. Early application timings (3 - 9 DAT/DAS) provide a high degree of weed control at rates as low as 10 - 20 g ai/ha. Somewhat higher rates of 20 up to 45 g ai/ha are required at later timings (Tables 7 and 8). As a matter of fact, CGA 142'464's activity will be reduced on some weed species at application timings as late as about 3 weeks after planting. However, the negative impact on rice grain yield of too

Table 7. Performance of CGA 142'464 = Settof® in transplanted rice under temperate/subtropical climate conditions, Japan.

| Rate CGA 142'464 g ai/ha | Season | Application timing | % Crop Phytotoxicity 21-31 DAT | % Weed control at 63 DAT | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | *Scirpus hotarui* | *Monochoria vaginalis* | *Sagittaria pygmaea* | *Linderia pyxidaria* | *Cyperus difformis* |
| 45 | cool[1] | 10 DAT[2] | 5 | 100 | 100 | 90 | 97 | - |
| | | 14 DAT | 3 | 100 | 100 | 100 | 90 | - |
| 60 | | 10 DAT | 4 | 100 | 100 | 100 | 96 | - |
| | | 14 DAT | 0 | 100 | 100 | 100 | 90 | - |
| 45 | hot[1] | 7 DAT | 2 | 100 | 99 | 100 | 85 | 100 |
| | | 11 DAT | 5 | 100 | 100 | 100 | 100 | 100 |
| 60 | | 7 DAT | 7 | 100 | 99 | 100 | 99 | 100 |
| | | 11 DAT | 11 | 100 | 100 | 100 | 100 | 100 |

1 transplanting date - cool: May 8, hot: June 3

2 DAT = Days after transplanting

Table 8. Perfomance of CGA 142'464 = Setoff® in wet seeded rice under tropical climate conditions, Indonesia. (Average of 8 trials).

| Rate of CGA 142'464 g ai/ha | Application timing | % Crop Phytotoxicity | | % Weed control at 50 DAA | | |
|---|---|---|---|---|---|---|
| | | 30 DAA | 50 DAA[2] | *Monochoria vaginalis* | Scirpoideae[3] | *Echinochloa crus-galli* |
| 10 | 4 DAS[1] | 9 | 0 | 94 | 94 | 49 |
| 20 | | 10 | 2 | 96 | 94 | 62 |
| 10 | 9 DAS | 9 | 0 | 92 | 89 | 45 |
| 20 | | 10 | 0 | 97 | 94 | 58 |
| 10 | 15 DAS | 0 | 0 | 83 | 79 | 24 |
| 20 | | 0 | 0 | 91 | 88 | 41 |

1 DAS = Days after sowing

2 DAA = Days after application

3 Scirpoideae = *Cyperus difformis, Cyperus iria, Fimbristylis* spp., *Scirpus* spp.

Table 9. Performance of CGA 142'464 = Setoff® in direct seeded rice under tropical climate conditions, Thailand. (Average of 9 trials; rate of CGA 142'464: 20 g ai/ha).

| Application timing | % Crop Phytotoxicity 15 DAA[2] | % Weed control at 45 - 55 DAA | | | | |
|---|---|---|---|---|---|---|
| | | *Monochoria vaginalis* | *Cyperus* spp. | *Limnocharis flava* | *Marsilea crenata* | *Scirpus juncoides* |
| 12-18 DAS[1] | 0 | 100 | 86 | 100 | 99 | 100 |

1 DAS = Days after sowing

2 DAA = Days agter application

Table 10. Weed control spectrum of CGA 142'464 = Setoff$^{R}$.

| Susceptible | Moderately susceptible |
|---|---|
| *Alisma canaliculatum* | *Commelina benghaliensis* |
| *Alisma plantago-aquatica* | *Cyperus serotinus* |
| *Ammania coccinea* | *Eleocharis kuroguwai* |
| *Cyperus difformis* | *Fimbristylis* spp. |
| *Cyperus iria* | *Ludwigia* spp. |
| *Elatine triandra* | *Sagittaria* trifolia |
| *Eleocharis acicularis* | |
| *Limnocharis flava* | |
| *Lindernia pyxidaria* | |
| *Marsilea crenata* | **Moderately resistent** |
| *Marsilea quadrifolia* | (Partial control) |
| *Monochoria vaginalis* | *Echinochloa colonum* |
| *Potamogeton natans* | *Echinochloa crus-galli* |
| *Rotala indica* | *Echinochloa oryzicola* |
| *Sagittaria guyanensis* | |
| *Sagittaria pygmaea* | |
| *Scirpus grossus* | |
| *Scirpus mucronatus* | |
| *Scirpus supinus* | |
| *Sphenoclea zeylanica* | |

long lasting weed/rice crop-competition (6) is well known; therefore, when having the maximization of yield in mind, it does not seem to be recommendable to the farmer in the tropics to apply any weed control measure later than 3 weeks after planting.

The duration of weed control is an important parameter. Vega et al. (1967, cited in (1)) indicate that, for example, 20 days of weed-free growth appears best in short-statured plant types such as IR-8 but for C4-63, an intermediate-statured variety, the weed-free period should be extended to the first 30 days after transplanting.

All our results of trials carried out under a broad range of different conditions indicate a duration of full weed control of at least 50 to 55 days after treatment (Tables 4 - 9).

There is only litte effect of soil composition and water leakage on the activity of this new herbicide. This was demonstrated in a set of trials with CGA 142'464 carried out on three different soils in Indonesia varying in their texture (Table 6).

The ambient temperature during early crop and weed growth also does not significantly effect the performance of Setoff® In Japan crop tolerance and weed control were excellent under both early season (cool) and late season (hot) conditions (Table 7).

Further the moisture regime is a key factor in weed growth and herbicide effectiveness may vary significantly with different moisture regimes (4).

The results presented in Table 5 show that weed control with Setoff® is similar under different water management systems. Since water management practices commonly vary especially in the tropics, Setoff® will offer, besides its excellent broad spectrum of weeds being controlled and flexible usage (Table 8), an additional advantage to the farmer.

## CONCLUSION

Greenhouse experiments followed by an important number of field trials have demonstrated excellent efficacy of Setoff® in transplanted as well as direct seeded wet-sown rice. Good crop tolerance, broad spectrum weed control, very low use rates, application timing flexibility and little dependency on environmental parameters are the strengths of this new agro-chemical. Setoff® should, therefore, contribute to improve further weed control technologies in the major rice growing systems.

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# EFFECT OF BUTACHLOR ON PRODUCTION OF HYDROLYTIC ENZYMES AND CELL GROWTH

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## ABSTRACT

This study was conducted to investigate the effects of butachlor (2-chloro- 2',6'-diethyl-N-Butoxymethyl acetanilide) on production of α-amylase and protease in seed germination and cell division and cell elongation in early seedling development. Production of α-amylase and protease in germinating rice (*Oryza sativa* L.) seeds was inhibited by butachlor. However, butachlor did not affect hydrolytic reaction per se of the two enzymes. A significant decrease in mitotic index of oat (*Avena sativa* L.) roots occurred when incubated with $10^{-6}$M butachlor for 18 h. Butachlor did not disrupt the mitotic sequence, but induced an inhibition of mitotic entry, resulting in the delayed dividing process. Elongation of oat coleoptile was significantly reduced with $10^{-5}$M butachlor. Butachlor primarily inhibited cell division and secondarily cell elongation.

## INTRODUCTION

Butachlor is one of the α-chloroacetamide group of herbicides and selectively controls most annual seedling grasses and certain broadleaf weeds and sedges in direct-seeded and transplanted rice. Since the α-chloroacetamide herbicides inhibit initial seed germination and development of germinating seedlings (11), butachlor is generally used as a pre-emergence treatment.

Many studies suggest that the α-chloroacetamide herbicides have a basic inhibitory effect on one or several metabolic processes upon which seed germination and early seedling growth are dependent. Devlin and Cunningham (6) reported that alachlor (2-chloro- 2',6'-diethyl-N-methoxymethyl acetanilide) and propachlor (2-chloro-N- isopropyl acetanilide) inhibited gibberellic acid ($GA_3$)-induced -amylase production in deembryonated barley (*Hordeum vulgare* L.) seed and suggested that this effect could be related to an effect on protein synthesis. Duke et al. (8) confirmed that propachlor caused a prior inhibition of protein synthesis.

Inhibition in cell division and cell elongation by α-chloroacetamide herbicides occurs in combination to produce the overall inhibition of growth. Dhillon and Anderson (7) reported that propachlor inhibited cell division in onion (*Allium cepa* L.) tip. Deal and Hess (4) also observed that alachlor and metolachlor [2-chlor-N- (2-ethyl-6- methylphenyl)- N-(2-methoxy-1-methylethyl) acetamide] inhibited cell division in oat roots. Oat coleoptile cell enlargement has been reported to be significantly inhibited by alachlor (3) metolachlor (4) and propachlor

(7). However, little information is available concerning mode of action of butachlor or its developmental effects on treated plants.

This study reported herein was conducted to determine the effect of butachlor on production and activity of hydrolytic enzymes and to investigate the influence on two components of cell growth; cell division and cell elongation. Further investigation was done to observe the effect of butachlor on mitotic phases.

## MATERIALS AND METHODS

**Effect on production and activity of hydrolytic enzymes**

**α-Amylase** Dehulled rice seeds were transversely cut to remove the embryo and the deembryonated seeds were surface sterilized for 20 min in 1 % sodium hypochlorite and rinsed several times in sterile water. They were transferred aseptically to sterile petri dishes containing silica sand moistened with 10 ml of sterile distilled water. The petri dishes were wrapped in aluminum foil and allowed to incubate at 30°C for 3 days. After the incubation, the twenty deembryonated seeds were transferred aseptically to 250 ml Erlenmeyer flasks containing 20 ml incubation medium consisting of 2 u moles of acetate buffer (pH 4.8), 200 u moles of $CaCl_2$, 0.01 u moles of chloramphenicol, 2.3 u moles of $GA_3$ and varying amounts of butachlor. The flasks were then incubated in a 30°C shaking water bath for 24 h. After the incubation period, the incubation media were ground and centrifuged at 2500 g for 10 min. The supernatant was used for determining production of α-amylase. The α-amylase was assayed using the starch-iodine method (13).

In vitro study on hydrolysis of starch by endogenous and exogenous α-amylase was employed to determine the effect of butachlor on hydrolytic reaction per se of α-amylase. Endogenous α-amylase was prepared from seeds grown in the absence of butachlor. For exogenous α-amylase 15 mg of crystalline α-amylase (Sigma, St.Louis) was dissolved in 100 ml of 0.01 N acetate buffer previously adjusted to pH 4.8. The α-amylase solution was added to the starch solution containing varying amounts of butachlor.

**Protease** Dehulled rice seeds were sown in petri dishes containing silica sand moistened with 10 ml butachlor solutions and placed in a 30°C incubator in the dark. After 5 days the coleoptile and radicles were removed, and a crude enzymatic extract was prepared by grinding the seeds in 1/15 M phosphate buffer (pH 7.0). One g of the seeds was used for each five ml of buffer. The temperature was maintained at 0 to 5°C with ice. The homogenates were centrifuged at 10000g for 5 min. The supernatant was subjected to dialysis for 24 h and then filtered through a Whatman No. 2 filter paper. The filtrate was used for the assay of proteolytic activity.

After a 10 min equilibration period in a 35°C water bath, 4 ml of the filtrate were added to 2 ml of a 2 % casein solution dissolved in the phosphate buffer (pH 7.0). The reaction mixture was incubated for 2 h in a 35°C shaking water bath. The reaction was terminated by adding 5 ml of a 5 % trichloroacetic acid solution. After filtration, optical density of the filtrate was measured at 280 nm. Proteolytic activity was determined as the amount of tyrosine liberated from 1 ml enzyme-substrate mixture per hour. The Lowry method (14) was used for determining protein content of the enzyme solution.

For an in vitro study, extract of endogenous protease was obtained from seeds grown in the absence of butachlor. Exogenous protease was prepared by dissolving 20 mg of crystalline trypsin (Sigma, St. Louis) in 100 ml of 1/15 M phosphate buffer (pH 7.0). Hydrolytic activity of the proteases on casein was determined as described above.

**Effect on cell growth**

**Cell division** Oat seeds were germinated in petri dishes on a Whatman No. 2 filter paper moistened with 3 ml of distilled water for 2 days and thence transferred to other petri dishes containing 5 ml of butachlor solution at 22°C. After the desired treatment period, ten seedlings were sampled and the terminal 1 cm of the roots were harvested into an ethanol and acetic acid solution (3:1 v/v).

After washing the roots in distilled water three times, the roots were hydrolyzed in 1 N HCl at 60°C for 13 min and then washed in distilled water. The roots were stained 25 min in Shiff's reagent in the dark. After washing in distilled water, 5 ml of 5% pectinase (pH 4.0) were applied to the roots for 8 to 12 h. The root tips (2 mm) were then placed on microscope slides in small drops of 40% HCl. The meristem tips were then squashed under cover slips and examined for cell division. The number of dividing cells in 1000 total cells was determined. Mitotic index was defined as the number of dividing cells as a percentage of the total number of cells observed. For the kinetic studies, 1000 cells were differentiated into prophase, metaphase, anaphase and telophase. There were four replications.

**Cell elongation** Oats were germinated in moist vermiculite in the dark at 25°C for 5 days. To prevent phytochrome growth responses, all manipulations of seedlings were conducted in a dark room illuminated with a green light. The 3 to 4 cm oat coleoptiles were uniformly sectioned with a premeasured double razor blade cutter. Five mm sections were cut 3 mm below the tip and placed into sterile petri dishes containing 10 ml of 10 mM potassium phosphate buffer (pH 5.3) with various amounts of butachlor. The petri dishes were wrapped in aluminum foil to exclude light, and placed on a 25°C shaking water bath for 24 h. The sections were measured with a dissecting microscope. The experiment was repeated three times. The data represent the average of all 9 replications.

## RESULTS AND DISCUSSION

**Effect on production and activity of hydrolytic enzymes** Production of $GA_3$-induced α-amylase in deembryonated rice seed was inhibited by butachlor (Fig. 1). A significant inhibition occurred at $10^{-5}$M butachlor. Increase in the butachlor concentration resulted in increase in inhibition of α-amylase production. However, hydrolytic activity of α-amylase was not affected by butachlor (Table 1). When either endogenous or exogenous α-amylase was added to starch, butachlor did not inhibit the amylase-starch reaction even at $10^{-3}$M.

Butachlor also inhibited production of protease in germinating rice seeds. Total activity of protease as measured by content of tyrosine liberated was significantly reduced by butachlor (Table 2). The reduction increased as the butachlor concentration increased. The inhibitory effect of butachlor on protease production was confirmed by determining water soluble protein. There was a significant decrease in the water soluble protein content with increasing butachlor concentration. Since in most cases enzymes having proteolytic activity in germinating seeds are water soluble (15), decrease in water soluble protein due to butachlor indicates inhibition of

Table 1. Effect of butachlor on in vitro hydrolysis of starch by exogenous and endogenous α-amylases.

| Concentration (M) | Hydrolytic activity of α-amylase (%)[1] | |
|---|---|---|
| | Exogenous | Endogenous |
| Control | 100 | 100 |
| $10^{-6}$ | 99 | 100 |
| $10^{-5}$ | 99 | 99 |
| $10^{-4}$ | 100 | 97 |
| $10^{-3}$ | 98 | 98 |

1 All values are averages across two separate experiments and each treatment was replicated three times in each experiment.

Table 2. Effect of butachlor on total activity of protease and water soluble protein content in rice seed[1].

| Concentration (M) | Total activity (Tyrosine ug/ml) | Water soluble protein content (mg/g) |
|---|---|---|
| Control | 4043 a | 0.88 a |
| $10^{-5}$ | 1859 b | 0.63 b |
| $10^{-4}$ | 1215 c | 0.39 c |
| $10^{-3}$ | 828 d | 0.29 d |

1 In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Effect of butachlor on in vitro hydrolysis of casein by endogenous and exogenous proteases.

| Concentration (M) | Hydrolytic activity of protease (%)[1] | |
|---|---|---|
| | Exogenous | Endogenous |
| Control | 100 | 100 |
| $10^{-5}$ | 100 | 98 |
| $10^{-4}$ | 99 | 100 |
| $10^{-3}$ | 101 | 100 |

1 All values are averages across two separate experiments and each treatment was replicated three times in each experiment.

Table 4. Mitotic index of oat root tips as affected by butachlor at different incubation times[1]

| Concentration (M) | Mitotic index (%) Incubation time(h) 6 | 12 | 18 | 24 |
|---|---|---|---|---|
| Control | 10.9 a | 10.0 a | 10.2 a | 10.5 a |
| $10^{-6}$ | 10.5 a | 9.5 a | 8.0 b | 7.4 b |
| $10^{-5}$ | 10.8 a | 6.6 b | 5.4 c | 4.0 c |
| $10^{-4}$ | 10.8 a | 4.8 c | 3.5 d | 2.6 d |
| $10^{-3}$ | 7.1 b | 4.6 c | 1.4 e | 1.4 e |

1 In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 5. Percent distribution of mitotic phases as affected by butachlor at different incubation times[1].

| Incubation time (h) | Concentration (M) | Percent distribution of mitotic phases Prophase | Metaphase | Anaphase | Telophase |
|---|---|---|---|---|---|
| 6 | Control | 41.3 b | 27.5 b | 16.5 a | 14.7 a |
| | $10^{-6}$ | 38.1 b | 29.5 b | 18.0 a | 14.3 a |
| | $10^{-5}$ | 40.7 b | 30.6 b | 15.7 a | 13.0 a |
| | $10^{-4}$ | 39.6 b | 31.1 b | 16.0 a | 13.2 a |
| | $10^{-3}$ | 46.5 a | 35.2 a | 12.7 b | 5.6 b |
| 12 | Control | 47.0 b | 26.0 a | 15.0 a | 12.0 a |
| | $10^{-6}$ | 47.3 b | 26.3 a | 14.7 a | 11.6 a |
| | $10^{-5}$ | 47.6 b | 24.7 a | 15.1 a | 12.6 a |
| | $10^{-4}$ | 43.8 b | 24.3 a | 18.5 a | 13.4 a |
| | $10^{-3}$ | 60.9 a | 23.9 a | 8.7 b | 6.5 b |
| 18 | Control | 44.1 b | 28.4 a | 13.7 a | 13.7 a |
| | $10^{-6}$ | 47.5 b | 26.3 a | 13.8 a | 12.5 a |
| | $10^{-5}$ | 46.2 b | 26.9 a | 13.5 a | 13.5 a |
| | $10^{-4}$ | 48.6 b | 25.7 a | 15.1 a | 10.6 a |
| | $10^{-3}$ | 57.1 a | 14.3 b | 14.3 a | 14.3 a |
| 24 | Control | 45.7 b | 29.5 a | 14.3 a | 10.5 a |
| | $10^{-6}$ | 47.3 b | 29.7 a | 12.2 a | 10.8 a |
| | $10^{-5}$ | 50.0 b | 27.5 a | 13.5 a | 9.0 a |
| | $10^{-4}$ | 46.2 b | 28.5 a | 15.6 a | 9.7 a |
| | $10^{-3}$ | 58.3 a | 17.3 b | 13.1 a | 11.3 a |

1 In a column within each incubation time, means followed by a common letter are not significantly different at the 5% level by DUNCAN's multiple range test.

enzymatic protein synthesis. Although butachlor inhibited production of protease, the proteolytic reaction per se was not affected by the presence of butachlor in reaction mixture (Table 3).

Inhibitory effects of α-chloroacetamide herbicides on α-amylase and protease production have previously been observed by several workers (6,16). No inhibition of the herbicides on activity of the enzymes has also been determined. Jaworski (12) reported that allidochlor (N-N-diallyl-2-chloroacetamide) and propachlor did not inhibit α-amylase activity per se at $10^{-3}$ M. Activity of proteolytic enzymes was also affected by allidochlor (1). The effects of butachlor on the hydrolytic enzymes were similar to those reported for the other α-chloroacetamides.

One of the modes of action involved in α-chloroacetamides is inhibition of protein synthesis (2,5,12,16). Since hydrolytic enzyme synthesis in response to $GA_3$ is dependent on protein and nucleic acid synthesis (10,17), inhibitory effect of the α-chloroacetamides on α-amylase and protease could be interpreted as a result of a direct effect on protein and nucleic acid synthesis. Based on these findings, it would seem that butachlor is acting on biosynthetic reactions required for α-amylase and protease production.

**Effect on cell growth** When oat roots were exposed to treatment solutions containing $10^{-6}$ to $10^{-4}$M butachlor for 6 h, the mitotic index was not significantly affected (Table 4). Exposure for 12 h $10^{-6}$M did not cause a significant reduction, but after 18 h the treatment significantly reduced the mitotic index. At $10^{-5}$M a significant reduction was obtained when exposed for 12 h. A complete reduction in mitotic index did not occur with $10^{-3}$M butachlor even at the end of 24 h. Exposure of oat roots to concentrations of $10^{-6}$ to $10^{-3}$M butachlor for 12 to 24 h reduced the mitotic index to a great extent than did treatment for 6 h.

The observed reductions in mitotic index at different concentrations of butachlor is due probably to arrest of the cells at a stage of the cell cycle preceding mitosis. When the percent distribution among different division phases was checked, no deviation from controls could be detected, except for high butachlor concentration (Table 5). In mitosis, there was a uniform decrease in proportion of cells of respective mitotic phases. Proportion of cells observed in prophase was always greater than those observed in the later phases of mitosis. The proportion of cells occurred in respective mitotic phases was not altered by butachlor concentrations and incubation times employed, except for $10^{-3}$M butachlor. A similar result was observed by Deal and Hess in 1980 (4) who reported that alachlor and metolachlor did not disrupt mitosis, but rather inhibited the onset of mitosis. No effect of butachlor on the mitotic sequence but decrease in the number of dividing cells (as measured by the mitotic index) apparently indicates that butachlor blocks some preparatory step or process during interphase which is required for cell division, resulting in delaying the dividing process.

The effect of butachlor treatments on cell elongation in oat coleoptile is shown in Fig. 2. A significant inhibition of cell elongation occurred at $10^{-5}$M butachlor. After the first significant inhibition, severity of inhibition increased as the butachlor concentration increased. Elongation of oat coleoptile after 24 h of treatment reduced by 16.7% at $10^{-6}$, 33.4% at $10^{-4}$ and 75.0% at $10^{-3}$M butachlor when compared to the control.

Butachlor inhibits both cell division and cell elongation. However, the inhibition was greater on cell division than on cell elongation. When oat roots were exposed to butachlor at $10^{-6}$, $10^{-5}$, $10^{-4}$ and $10^{-3}$M for 24 h, inhibitions in the mitotic index were 29.6%, 62.0%, 75.3%

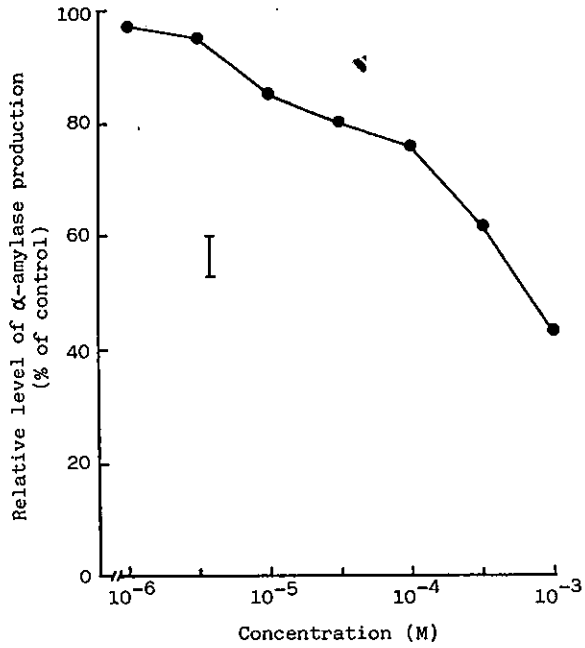


Fig. 1. Effect of butachlor on $GA_3$-induced α-amylase production in deembryonated rice seed. Vertical bar represents the LSD at the 5% level.

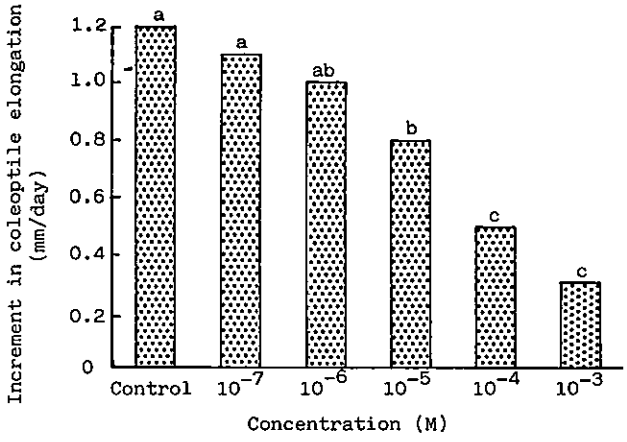


Fig. 2. Effect of butachlor on cell elongation of oat coleoptile. Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

and 86.7%, respectively, as compared to the control (Table 4). The percent inhibitions of cell division was greater than was observed in the cell elongation tested at the respective butachlor concentrations. The available evidence indicates that butachlor affects primarily cell division and secondarily cell elongation. Fedtke (9) noted a similar effect for alachlor.

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# METSULFURON METHYL - A NEW HERBICIDE FOR USE IN RICE AND PLANTATION CROPS

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## ABSTRACT

"Ally" (metsulfuron methyl), a sulfonylurea herbicide widely used in cereal crops throughout Europe and North America, is being developed for use in rice and plantation crops (oil palm and rubber) in Southeast Asia. The compound is active at low use rates, is extremely safe to fish and wildlife and has an excellent toxicological profile. In direct-seeded and transplanted rice, rates of 3 to 6 g ai/ha effectively control many broadleaf weeds. Crop safety has proven to be excellent. Broadleaf weed control in oil palm and rubber is achieved at 10 to 20 g ai/ha with good crop safety. Additionally, effective control of major brush species can be obtained at 10 to 30 g ai/ha. Information regarding product chemistry, toxicology, environmental fate and biological efficacy is summarized.

## INTRODUCTION

"Ally" (metsulfuron methyl) was developed for use in cereal crops in Europe and North America (2, 3, 6). More recently, the compound has proven effective for use in rice and plantation crops (1, 5).

In rice, metsulfuron methyl selectively controls most broadleaf weeds at 3 to 6 g ai/ha with good crop safety to transplanted and direct-seeded rice (5). The product is marginally effective on sedges but can be used in combination with half-rates of 2,4-D, thereby providing broad-spectrum weed control with the exception of grasses (5).

In oil palm and rubber, 10 to 20 g ai/ha provides excellent control of key broadleaf weeds and noxious brush species (1) - with good residual activity. Metsulfuron methyl is compatible with many existing products commonly used in plantation crops, thus providing an opportunity for broader-spectrum weed control (1).

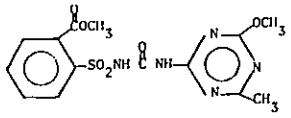
## MATERIALS AND METHODS

**Chemical and physical properties** Metsulfuron methyl is a sulfonylurea herbicide, and pertinent physical (Table 1) and chemical characteristics are listed in Table 1.

**Toxicology** Acute and chronic toxicological data are summarized in Tables 2 to 4. As evident from the data, metsulfuron methyl exhibits low acute, subchronic and chronic toxicity.

Table 1. Physical and chemical properties of metsulfuron methyl.

Common Name: Metsulfuron Methyl

Structure: 

Chemical Name: Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]benzoate

Physical Form: Off-White Solid

Specific Gravity: 1.47 g $cc^{-1}$

Melting Point: 158°C

Vapor Pressure: 2.5 x $10^{-12}$ mm Hg @ 25°C

Solubility in Water:

pH 5.0 270mg $L^{-1}$; pH 6.0 1750mg $L^{-1}$; pH 7.0 9500mg $L^{-1}$

Table 2. Acute toxicity and mutagenicity of metsulfuron methyl.

| | |
|---|---|
| Oral (rat, male and female) | $LD_{50}$ 5000 mg $kg^{-1}$ |
| Dermal (rabbit) | $LD_{50}$ 2000 mg $kg^{-1}$ |
| Inhalation (rat, male and female) | $LC_{50}$ 5.0 mg $L^{-1}$ |
| Not mutagenic in: | Ames bacterial assay |
| | Chinese hamter ovary cell assay |
| | DNA rat liver repair assay |
| | in vivo bone marrow cytogenetic assay |

Table 3. Summary of subchronic, chronic, reproduction and developmental studies for metsulfuron methyl.

| Study | No-Observed-Effect Level (NOEL) |
|---|---|
| | (ppm) |
| 90 Day Rat | 1000 |
| 90 Day Mouse | 5000 |
| 90 Day Dog | 5000 |
| 2 Year Rat | 500 |
| 18 Month Mouse | 5000 |
| 12 Month Dog | 500(males) |
| | 5000(females) |
| Multigeneration Reproduction, Rat | 500 |
| Developmental Toxicity, Rat | 400(maternal) |
| | 1000(conceptus) |

**Mode of action** Metsulfuron methyl inhibits the activity of acetolactate synthase, an enzyme involved with the synthesis of valine and isoleucine (3). The compound is rapidly absorbed by plant roots and foliage and is readily translocated (3).

## RESULTS AND DISCUSSION

**Herbicidal efficacy and selectivity - cereals** As noted in the introduction, metsulfuron methyl effectively controls a wide range of broadleaf weeds prevalent in wheat and/or barley in Europe and North America (Table 5). Use rates are between 4 and 8 g ai/ha and crop selectivity is excellent (2, 3, 6).

**Herbicidal efficacy and selectivity - rice** In rice, 3 to 6 g ai/ha effectively controls many broadleaf weeds indigenous to rice paddies in Southeast Asia (Table 6). Crop safety has proven to be excellent. In a comprehensive field test conducted in Thailand, safety of metsulfuron methyl was investigated in 10 Thai rice varieties (Fig. 1). Metsulfuron methyl at rates as high as 8 g ai/ha had virtually no effect on tillering, plant height or root length. Tests conducted in Indonesia also revealed that the compound was safe when used in transplanted rice (5 varieties - data not shown) at rates up to 16 g ai/ha. These data indicate that several commonly grown rice varieties have a high degree of tolerance to metsulfuron methyl.

Application in direct-seeded rice should not be made prior to 5-7 days after seeding, since the potential for crop injury is greater in newly-germinated rice.

The herbicidal efficacy and crop selectivity of metsulfuron methyl alone and in combination with 2,4-D is detailed in another paper of this proceeding (5).

**Herbicidal efficacy and selectivity - plantation crops** A summary of the herbicidal efficacy of metsulfuron methyl in oil palm and rubber is presented in Table 7. The product effectively controls major broadleaf and brush species at rates between 10 and 20 g ai/ha. Tests currently in progress indicate that safety to oil palm and rubber is excellent. More detailed information regarding the use of metsulfuron methyl in plantation crops can be obtained by referring to the paper by Chang and Tsay (1).

**Breakdown in soil** Metsulfuron methyl is broken down in the soil by microbial metabolism and chemical hydrolysis (4). Degradation is fairly rapid, with a half-life of 2 to 3 weeks (Fig. 2). Carbon dioxide is the major metabolite and a few minor non-volatile metabolites are also formed (4). The rate of dissipation can be influenced by soil pH. Breakdown is more rapid in acidic soils as compared with more alkaline soils (pH 7.0).

## CONCLUSION

Metsulfuron methyl is a highly active herbicide with activity in cereal crops, rice and plantation crops. Some of the key features of this herbicide are:

1. A high degree of safety in rice and cereal crops.
2. Activity on a range of broadleaf weeds found in rice, oil palm, rubber, wheat and barley.
3. Excellent activity on noxious brush species found in plantation crops.
4. Herbicidal activity at extremely low use rates.
5. Low mammalian and wildlife toxicity.

Table 4. Fish and wildlife toxicity of metsulfuron methyl.

| | | | |
|---|---|---|---|
| Rainbow Trout | $LC_{50}$ | (96-hr) | 150 ppm |
| Bluegill Sunfish | $LC_{50}$ | (96-hr) | 150 ppm |
| Mallard Duck | $LC_{50}$ | (oral) | 2510 mg $kg^{-1}$ |
| Mallard Duck | $LC_{50}$ | (dietary) | 5620 mg $kg^{-1}$ |
| Bobwhite Quail | $LC_{50}$ | (dietary) | 5620 mg $kg^{-1}$ |
| *Daphnia magna* | $LC_{50}$ | (48-hr) | 150 ppm |

Table 5. Herbicidal efficacy of metsulfuron methyl in Europe and North America. Weeds controlled ( 80%) with a post emergence application of metsulfuron methyl at 4 to 8 g ai/ha. More detailed information can be found in Doig et al. (3).

| | |
|---|---|
| *Ambrosia artemisiifolia* | *Papaver rhoeas* |
| *Anagallis arvensis* | *Polygonum convolvulus* |
| *Arabidopsis thaliana* | *Polygonum persicaria* |
| *Brassica napus* | *Ranunculus sardous* |
| *Chenopodium album* | *Rumex obtusifolius* |
| *Cirsium arvense* (seedlings) | *Senecio vulgaris* |
| *Eupatorium capillifolium* | *Sinapsis arvense* |
| *Kochia scoparia* | *Stellaria media* |
| *Lamium purpureum* | *Veronica persica* |
| *Matricaria* spp. | *Viola arvensis* |

Table 6. Herbicidal efficacy of metsulfuron methyl in transplanted and direct-seeded rice. Data from field tests conducted in Thailand, Malaysia and Indonesia.

| Weed Species | Country | Rate Range (g ai/ha) | Efficacy (% Control) |
|---|---|---|---|
| *Sphenochlea zeylanica* | Thailand | 2-6 | 60-99 [1] |
| *Monochoria vaginalis* | Indonesia/Malaysia | 2-8 | 80-100 |
| *Cyperus iria* | Indonesia | 2-8 | 0-40 |
| *Selvinia molesta* | Indonesia | 3-8 | 70-90 |
| *Fimbristylis miliacea* | Thailand | 3-6 | 0-20 |
| *Marsilea crenata* | Malaysia/Indonesia | 3-8 | 40-100 |
| *Echinochloa crus-galli* | Thailand/Indonesia | 3-6 | 0-20 |

1 Data represent treatment means for evaluations made 21 days after application. Data from 4 to 10 test were pooled (depending on species) for analysis.

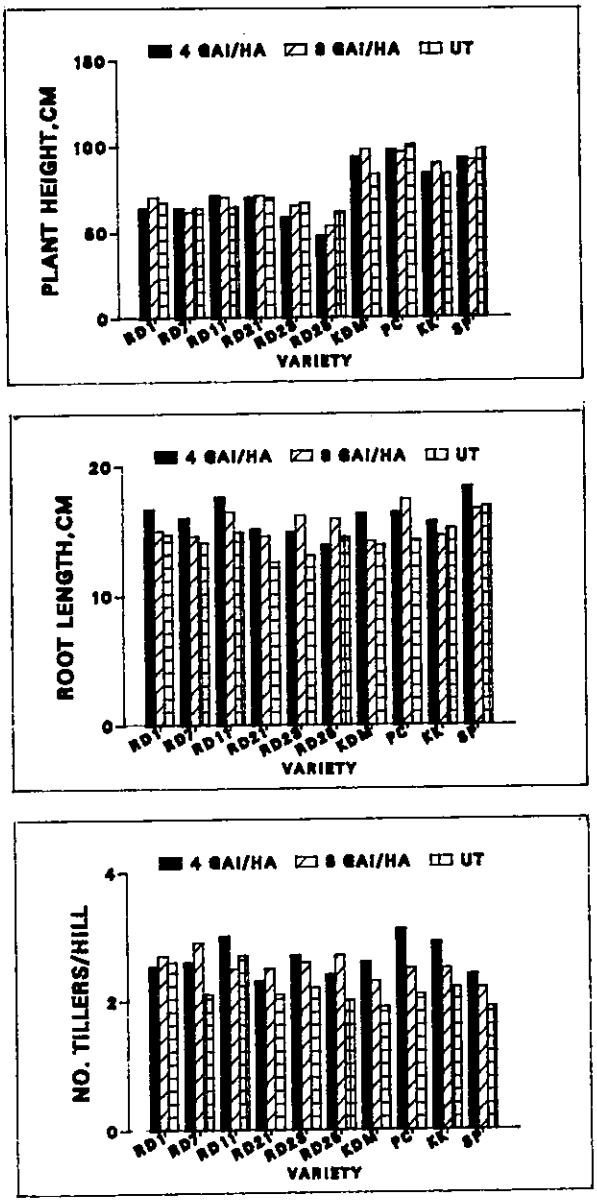



Fig. 1. Effects of metsulfuron methyl on rice plant height (upper), root lenght (middle) and tillering (lower). Varietal designation is by number (RDI - RD26) or abbreviation (KDM = KHAO DOK MALI; PC - PHAYA CHOM; KK - KHAO KAEW; SP = SAN PATONG). Treatments were 4 and 8 gai/ha, applied 21 days after seeding. Evaluation was 6 weeks after treatment. UT = untreated.

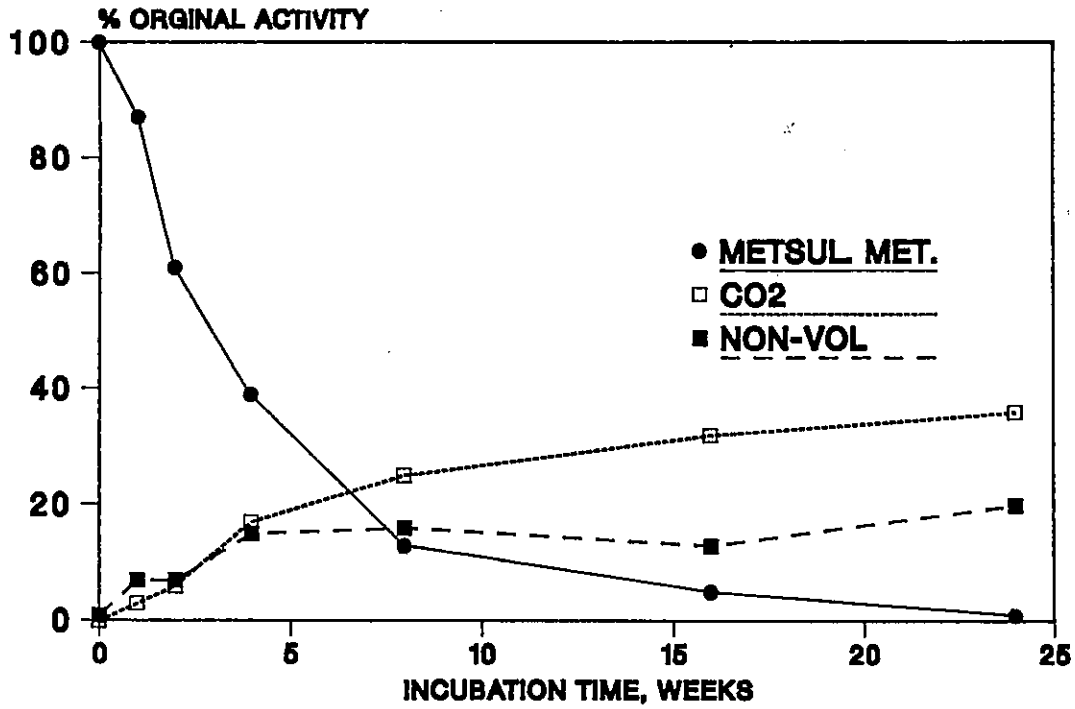



Fig. 2. Aerobic soil metabolism of metsulfuron methyl. Data represent time course changes in metsulfuron methyl, $CO_2$ and non-volatile components. Initial concentration of metsulfuron methyl was 1.0 ppm. Soil type was a silt loam, pH 6.4.

Table 7. Herbicidal efficacy of metsulfuron methyl on selected weeds in oil palm and rubber. Data compiled from 15 tests conducted in Malaysia and Thailand.

| Weed Species | Rate Range (g ai/ha) | Efficacy (% Control) |
|---|---|---|
| *Borreria latifolia* | 10-20 | 80-97[1] |
| *Calapogonium caerulium* | 10-20 | 75-100 |
| *Mikania micrantha* | 10-20 | 10-20 |
| *Clidemia hirta* | 10-20 | 71-100 |
| *Lantana camara* | 10-20 | 93-98 |
| *Melastoma malabathricum* | 10-20 | 94-97 |
| *Imperata cylindrica* | 10-40 | 0 |
| *Paspalum conjugatum* | 10-20 | 0-35 |

1 Values represent treatment means for evaluations made 40 days after application.

Based on these characteristics, metsulfuron methyl may have applicability for crop and non-crop uses as yet undiscovered.

## ACKNOWLEDGEMENTS

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# UPTAKE AND DISTRIBUTION OF BENSULFURON METHYL (DPX-F5384) IN PADDY RICE

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## ABSTRACT

The short-term and long-term uptake and distribution of bensulfuron methyl (formerly DPX-F5384, BSM for short) have been studied using $^{14}C$-labeled material and HPLC. In the short-term study, with roots being directly exposed to a solution containing $^{14}C$-BSM, considerable uptake and distribution of $^{14}C$ material was observed in roots compared with shoots. In contrast, in the long-term study with rice transplanted in a sandy soil shoots tended to accumulate more $^{14}C$ material than roots with surface water treatment of BSM. The extraction of $^{14}C$ material from roots and shoots of plants exposed to BSM for 24 hours revealed rapid metabolism of the compound by shoots. Shoots converted approximately three quarters of the accumulated $^{14}C$ material to a polar metabolite, whereas in roots, three quarters of the extracted $^{14}C$ was associated with parent compound. However, in the long-term study in a simulated paddy environment, a treatment of BSM at the rate of 100 g ai/ha resulted in 30% reduction in shoots fresh weight, but 0% in roots. These data indicated that rice roots can potentially accumulate BSM to a significant extent when directly exposed to the compound. However, due to soil binding kinetics in the paddy ecosystem, relatively little BSM would be available for root uptake.

## INTRODUCTION

Bensulfuron methyl (BSM) is a highly active herbicide for use in paddy rice developed out of sulfonylurea group of chemistry (3, 7, 10). At the 10th Conference of APWSS, the mode of selectivity of this compound was presented (6) indicating the selectivity of BSM is due to differential metabolism in rice and weeds. For effective and safe use in actual field conditions, it is also important to understand the extent and kinetics of uptake and accumulation of herbicides, since these factors often impact on herbicidal performances.

In this paper we describe the uptake and accumulation of BSM by rice in short-term hydroponic studies and in long-term studies with rice growing in a simulated paddy environment.

## MATERIALS AND METHODS

**Short-term uptake** Rice (*Oryza sativa* L. 'Nihonbare') seedlings at the 2.5 leafstage were placed in test tubes containing a half-strength complete nutrient solution, pH 5.8. In one set of plants, the roots were exposed to the solution and in a second set, the roots plus 3.0 cm of shoot tissue were exposed to the solution. Plants were placed in a growth chamber controlled at 25/20 + 2°C (day/night: 14-hour photoperiod) and 70 + 5% relative humidity. One hour after the plants had been placed in the chamber (at the beginning of the photoperiod), $^{14}C$-bensulfuron methyl (phenyl labeled; specific activity of 2.98 uci.mg$^{-1}$) was added to the solution to achieve a final concentration of 0.515 ppm in 20 ml (1.54 x $10^{-3}$ uci.ml). Test tubes were sealed to prevent evaporation of the solution. Two plants were placed in each test tube and were selected based on uniformity of height and fresh weight.

Plant material was harvested at the specific times, washed with distilled water and frozen for subsequent analysis. Total $^{14}C$ activity was determined by combusting samples (100mg fresh weight) in a Packard 306 sample Oxidizer using unlabeled tissue as background. Radioactivity was determined by liquid scintillation counting. Duplicate sets of plants were harvested and analyzed at each time point (0 to 24 hours).

Samples collected at 24 hours were also extracted and analyzed for $^{14}C$- labeled metabolites. Solvent extraction procedures and HPLC analysis of intact BSM and metabolites were previously described (1, 4, 9).

**Long-term studies in soil** Rice seedlings at the 2.5 leafstage were transplanted 3.0 cm deep in pots (16 cm diameter) containing Sassafras sandy loam soil. The pots were flooded to achieve a water depth of 3.0 cm above soil level. There was no water leakage and water level was maintained constant throughout the course of experiment. The experiment was carried out in a growth chamber with environmental conditions the same as described for the short-term uptake studies.

Two days after transplanting, BSM was added to the paddy water at rates of 100 and 400 g ai/ha. A portion of the BSM added was $^{14}C$-labeled (as described above), and each paddy received a total of 1.623 uci (both application rates). Plant samples were harvested on specific days following treatment and total $^{14}C$ activity was determined by combustion. Each pot contained three sets of plants, and four independent subsamples were pooled from the total plant biomass for analysis on each sampling date.

After plant material was harvested, 2.0 ml aliquots of paddy water (3 per pot) and 20 grams of soil (obtained from the top 2.0 cm of soil) were obtained from each pot. Paddy water samples were counted directly for radioactivity. Soil samples were frozen and then analyzed for BSM subsequently.

## RESULTS

**Short-term uptake** When rice was exposed to BSM, root exposure alone resulted in rapid accumulation of $^{14}C$-labeled material in both root and shoot tissue (Fig. 1). Significantly more $^{14}C$ was recovered in root tissue as compared with shoot tissue. Translocation from the roots to the shoots was evident based on the accumulation of $^{14}C$ in shoots when only roots were exposed to BSM. Root plus shoot exposure resulted in more accumulation of $^{14}C$ than when roots only were incubated in the solution, but after 24 hours, root plus shoot exposure resulted in only 33% more accumulation of labeled material by shoot tissue as compared with root exposure alone.

Table 1. Injury to rice plants grown in soil and treated with 100 and 400 g ai/ha bensulfuron methyl. injury was determined by measuring fresh weights of roots and shoots 7 days after treatment.

| | % Injury [1] | |
|---|---|---|
| | 100 g ai/ha | 400 g ai/ha |
| Roots | 0 | 5 |
| Shoots | 30 | 57 |

1 Compared with untreated controls. Each value represents the mean of 4 replications, and each replicate was comprised of 3 sets of 3 plants.

Table 2. Distribution of $^{14}C$ activity in authentic bensulfuron methyl and an unidentified polar metabolite in rice roots and shoots.

| | % $^{14}C$ Activity[1] | |
|---|---|---|
| | Bensulfuron Methyl | Polar Metabolite |
| Shoots | 27.5 | 72.4 |
| Roots | 75.8 | 23.8 |

1 Samples were taken after 24-hour exposure to $^{14}C$-bensulfuron methyl (0.515 ppm). Rice seedlings were obtained from the short-term uptake studies with roots and 3.0 cm shoots exposed to BSM.

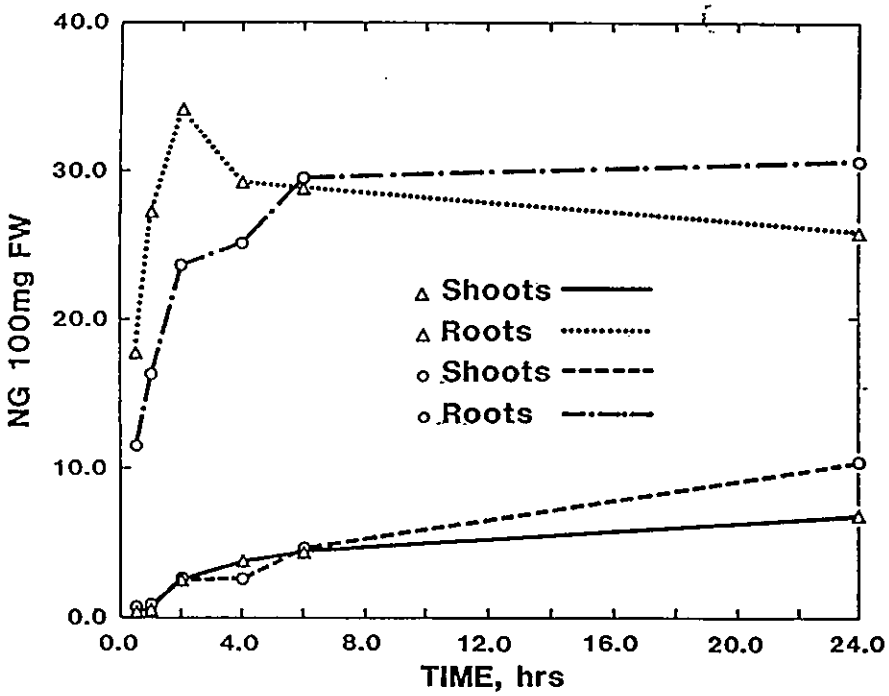


Figure 1. Accumulation of bensulfuron methyl by rice as a function of time assuming all $^{14}C$ activity was in the form of parent material. Circles represent data when roots plus 3.0 cm of shoot tissue were exposed to the uptake medium, and triangles represent data when roots only were exposed.

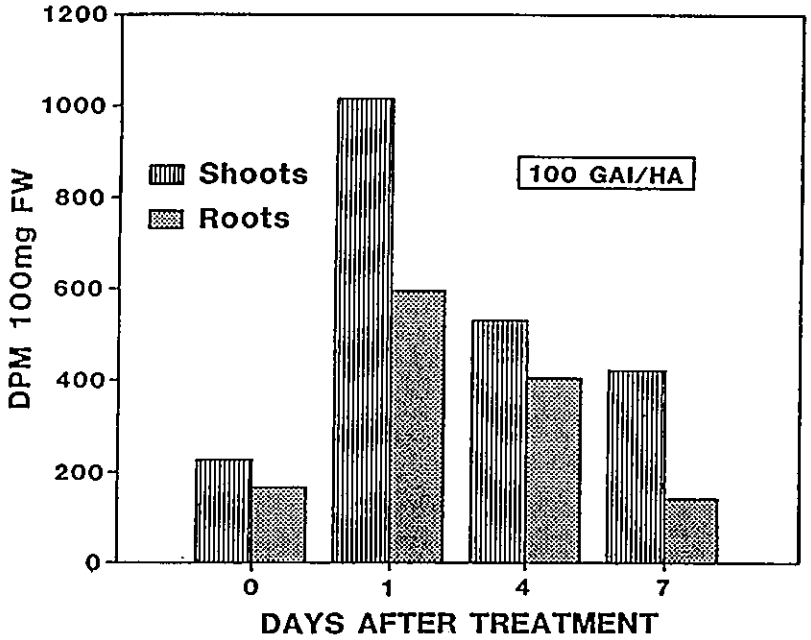


Figure 2. Accumulation of $^{14}C$ activity by rice plants grown in a simulated paddy with use rate of 100 g ai/ha.

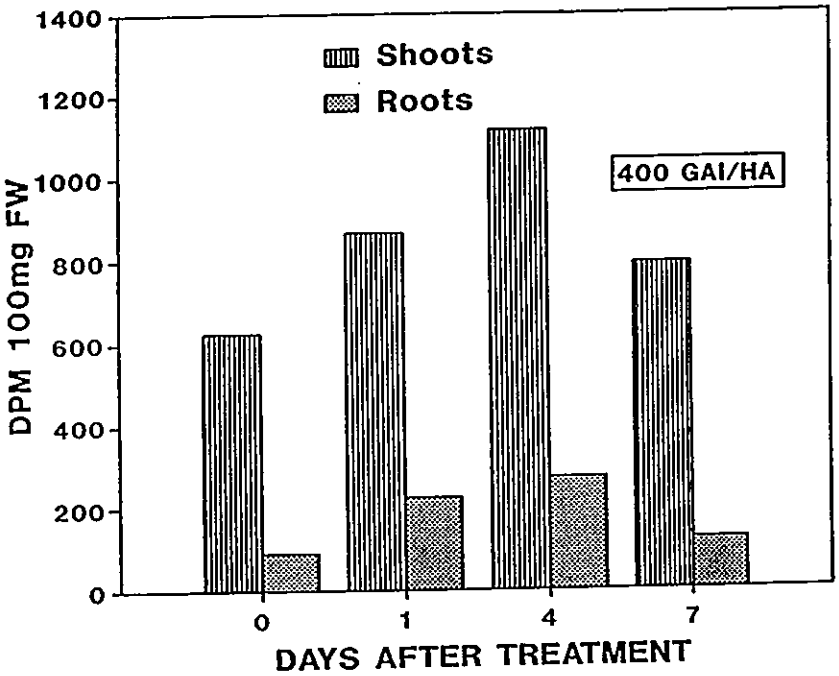


Figure 3. Accumulation of $^{14}C$ activity by rice plants grown in a simulated paddy with use rate of 400 g ai/ha.

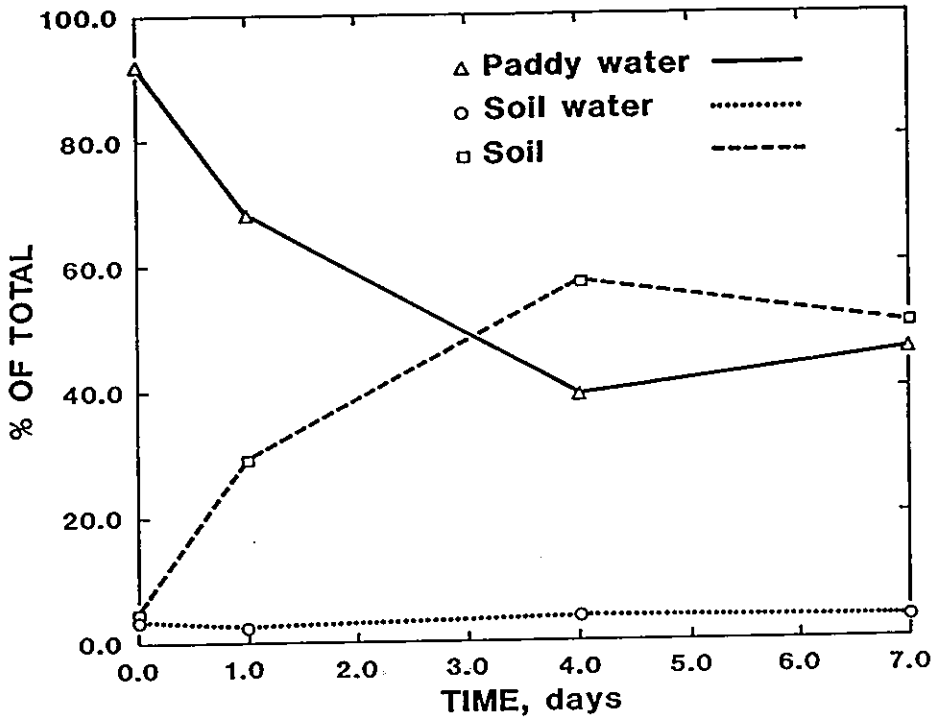


Figure 4. Distribution of $^{14}C$ activity in paddy water, soil and soil water as a function of days after application with use rate of 100 g ai/ha.

This suggests that shoot (foliar) uptake accounted for about one-fourth of the BSM accumulated by rice plants in these experiments. Nonetheless, these experiments indicate that root uptake is potentially much greater than foliar uptake. Recently, Ohno et al. (2) have presented data showing similar uptake kinetics of BSM in rice seedlings.

**Long-term studies in soil** The previous experiments indicated that both root and shoot uptake of BSM occurred. However, when rice was grown in soil and BSM was added to the water, shoots consistently accumulated more $^{14}C$ material than roots (Figs. 2 and 3). At a 100 g ai/ha use rate, peak levels of $^{14}C$ occurred on the day following application, whereas peak level in the 400 g ai/ha treatment occurred four days after treatment. Rice injury observed in this experiment is shown in Table 1. At both use rates, shoot injury was significantly greater than root injury (reduction in fresh weight).

**Distribution of BSM in the paddy** The relative amounts of $^{14}C$ activity recovered in the paddy water, soil (top 2.0 cm), and soil water (aqueous extract) are presented in Fig. 4. Initially, all $^{14}C$ activity was recovered in the water. Thereafter, decreases in water concentration paralleled increases in the amount of $^{14}C$ associated with soil fraction. There was a relatively constant amount of $^{14}C$ recovered in the soil water fraction associated with the top 2.0 cm of soil (Fig. 4).

**Metabolism of BSM in roots and shoots during short-term uptake** After 24-hour exposure to $^{14}C$-BSM, the majority (72.4%) of $^{14}C$ activity recovered in shoot tissue was in the form of a polar metabolite (Table 2). This is in accordance with the rapid rate of metabolism observed in rice leaves (5, 6). In contrast, root tissue did not appear to metabolize BSM as rapidly as shoots, since the majority (75.8%) of $^{14}C$ activity in the roots was in the form of parent material. In these short-term uptake experiments, all ( 99%) $^{14}C$ activity was associated with parent material or a polar metabolite (not identified), presumably the 4-hydroxy analog of BSM (5, 6). The polar nature of the metabolite was assumed due to significantly earlier elution of the compound compared with BSM on a reversed-phase liquid chromatography colum operating isocratically using 45% acetonitrile.

## DISCUSSION

Both foliar and root uptake of BSM can occur in rice. Even though the potential for root uptake is significant (Fig. 1), roots did not accumulate as much $^{14}C$ material as shoots when rice was grown in soil (Figs. 2 and 3). Accordingly, even at the 400 g ai/ha use rate, root injury was relatively insignificant compared to shoot injury (Table 1). Due to the rapid metabolism of BSM by rice leaves (Table 2), it is conceivable that the $^{14}C$ material accumulated by roots of plants grown in soil was not parent material, but rather, a translocatable metabolite such as 4-hydroxy analog BSM (5, 6). Since the metabolite is non-herbicidal (5, 6), this would explain the lack of injury observed in the long-term uptake studies in soil (Table 1). The decline in paddy water concentration of BSM over time (Fig. 4) should also minimize foliar uptake, thus limiting potential crop injury. It should also be noted that in actual paddy rice situation, most herbicides are formulated in granules which normally allows slower release of active ingredients than technical materials as used in this study, and consequently, results in later as well as lower peak of chemical concentration in the paddy water which would be reflected in the compound accumulation in rice plants. Moreover, most rice paddy soils in

Japan normally have much more organic matter and clay contents than in the soil used in these experiments, and that also result in lower initial concentration due to more readily absorption to the soil which has been confirmed in separate studies.

Although roots can accumulate BSM (Fig. 1), it is probable that under normal paddy conditions, root uptake will be minimal. However, shallow transplanting and transplanting rice on soils that exhibit significant downward water percolation could potentiate herbicide injury in some cases.

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# EFFECT OF BENZSULFURON ON GROWTH INHIBITION AND REGROWTH OF *SAGITTARIA PYGMAEA*

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## ABSTRACT

A series of experiments were conducted in a growth chamber to investigate growth inhibition of *Sagittaria pygmaea* Miq. caused by benzsulfuron methyl-2- [[[[[(4,6- dimethoxy pyrimidine-2-yl) amino]carbonyl] amino]sulfonyl] methyl]benzoate and regrowth of the depressed plants. Benzsulfuron did not affect sprouting of tubers of *S. pygmaea*. Growth of *S. pygmaea* treated with benzsulfuron ceased at 2 to 3 leaf stages and thereafter remained in a depressed growth stage. When benzsulfuron was applied at 17 and 51 g ai/ha, growth cessation of *S. pygmaea* lasted for 30 and 80 days, respectively. No weed natures in the depressed plant were observed. During the period of the depressed growth stage photosynthetic activity as determined by $^{14}C$-assimilation decreased, whereas slight increase in respiration was observed. Tetrazolium test indicated that tubers of the depressed plants were biologically viable. After the regrowth *S. pygmaea* was capable of producing normal offshoots and new tubers. There was no sprouting in the new tubers when they were connected to the parent plant. The new tuber separated, however, began sprouting soon after planting.

## INTRODUCTION

Benzsulfuron is one of the sulfonylurea herbicides developed by E. I. Du Pont de Nemours and Co., Inc. It is a new broad spectrum herbicide characterized by very low use rates and excellent crop selectivity in transplanted and direct-seeded rice (*Oryza sativa* L.). The herbicide is highly effective for control of most annual and perennial broadleaf weeds and sedges (4).

One of phytoptoxic symptoms caused by benzsulfuron is an inital growth cessation of some susceptible weeds. Following this growth cessation, the weed remains in a depressed growth stage for certain period of time. This effect is often observed in *S. pygmaea*, a perennial weed occurring in lowland rice fields, after application of benzsulfuron.

Study on the mode of action of the sulfonylureas has revealed that they act in plants to block the production of the essential amino acids valine and isoleucine by inhibiting the enzyme acetolactate synthase (3, 5). Inhibition of the action of this enzyme results in a rapid growth cessation of the plants. Ray (2) has reported that inhibition of growth and cell division are early physiological responses induced by chlorsulfuron §2-chloro-N- [[(4-methoxy-6- methyl-

1,3,5- triazin-2-yl) amino] carbonyl] benzenesulfonamide . Initial growth inhibiting effect of benzsulfuron is thought to be based on the mode of action observed in other sulfonylureas. However, it has not been investigated whether plant remained in depressed growth stage after benzsulfuron treatment is physiologically viable and is capable of proliferating and producing new plants. Therefore, the objectives of this research were to evaluate the effect of benzsulfuron on growth inhibition and regrowth of *S. pygmaea* and to determine the physiological activity of *S. pygmaea* remained in the depressed growth stage.

## MATERIALS AND METHODS

**Effect on growth and regrowth of *Sagittaria pygmaea*** Tubers of *S. pygmaea* were collected in the fall and stored at 4°C before planting in a growth chamber. Tubers that weighed 70 to 120 mg each were selected for use in the experiments. Tubers, five per pot, were planted 1 cm deep in a lowland soil in 15 cm diameter plastic pots. The soil was clay loam with 2.5% organic matter and pH 5.8. All pots were surface-irrigated to maintain the standing water level at 1 cm deep. Benzsulfuron granule containing 0.17% active ingredient was then applied at the rates of 17, 34 and 51 g/ha as a preemergence treatment. There were four replications. Throughout the course of the experiments, the pots were kept in a growth chamber maintained at 29°C ± 2 and illuminated for 24 h with fluorescent lamps at 80 $_{\mu}E.m^{2}.s^{-1}$ photosynthetic photon flux density at plant level.

Effect of benzsulfuron on sprouting of tubers was determined 10 days after treatment (DAT). Tubers were considered sprouted when the second leaf was emerged above the soil surface. Growth inhibition caused by benzsulfuron was measured 30 DAT. After harvesting plant height, number of roots, total root length and number of offshoots were recorded. Dry weight was obtained after the plant had dried overnight at 85°C.

Effect of benzsulfuron on growth of *S. pygmaea* was determined using growth paramter relative growth rate (RGR). *S. pygmaea* was established in the pots with 16 replications. After applying benzsulfuron, four replications were selected at random at 10-day intervals and dry weights were obtained 10, 20, 30 and 40 DAT. RGR was calculated from the formula: $(\log_e W_2 - \log_e w_1)/(t_2 - t_1)$ then $w_1$ and $w_2$ represent dry weights at the beginning and end of time interval, $t_1$ - $t_2$ days.

Regrowth of *S. pygmaea* remained in a depressed growth stage after the application of benzsulfuron was observed. After planting the tubers in pots, benzsulfuron was applied at 17, 51 and 85 g/ha. At 5 days after treatment the number of *S. pygmaea* sprouted was counted and thereafter the number of *S. pygmaea* regrown was recorded at 25-day intervals. The plants were considered regrown when the new leaf from the depressed plants was developed.

To relate regrowth of the depressed plants with the residual activity of benzsulfuron, two lots of the pots were prepared. For one lot tubers were planted and benzsulfuron at 0 and 51 g/ha was applied. At 10-day intervals the plants were harvested and number of roots was counted. For the other lot, however, planting of tubers at 10-day intervals was started from the time of benzsulfuron application. At 10 days after planting the plants were harvested and number of roots was measured. This was compared with the 10-day old seedlings obtained from the untreated control.

**Physiological activity of the depressed *Sagittaria pygmaea***

Table 1. Effect of benzsulfuron on sprouting and early growth of *Sagittaria pygmaea*.[1]

| Application rate (g/ha) | Percent sprouting[2] | Plant height (cm) | Number of roots | Total root length (cm/plant) | Number of offshoots (No./plant) | Dry weight (g/plant) |
|---|---|---|---|---|---|---|
| 0 | 100 | 5.7 a | 51 a | 565 a | 3 a | 86 a |
| 17 | 100 | 2.3 b | 35 b | 97 b | 1 b | 40 b |
| 34 | 100 | 0.9 c | 21 c | 64 c | 1 b | 28 c |
| 51 | 100 | 0.8 c | 18 c | 20 d | 0 b | 26 c |

1 The data was obtained 30 days after benzsulfuron treatment. Means in a column followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

2 percent sprouting was obtained 10 DAT.

Table 2. Relative growth rate (RGR) of *Sagittaria pygmaea* as affected by benzsulfuron.

| Application rate (g/ha) | R G R (mg/mg/day) | | |
|---|---|---|---|
| | Days after treatment | | |
| | 10 - 20 | 20 - 30 | 30 - 40 |
| 0 | 0.077 | 0.050 | 0.051 |
| 17 | 0.047 | 0.022 | 0.107 |
| 34 | 0.053 | 0.028 | 0.023 |
| 51 | 0.050 | 0.025 | 0.027 |

Table 3. Percent regrowth of *Sagittaria pygmaea* remained in the depressed growth stage after application of benzsulfuron.

| Application rate (g/ha) | Regrowth (%) | | | | |
|---|---|---|---|---|---|
| | Days after treatment | | | | |
| | 30 | 55 | 80 | 105 | 130 |
| 17 | 7.7 | 22.8 | 60.0 | 62.8 | 86.7 |
| 51 | 0.0 | 0.0 | 8.3 | 20.0 | 31.7 |
| 85 | 0.0 | 0.0 | 2.5 | 7.5 | 17.5 |

Table 4. Light absorbance of extracts of *Sagittaria pygmaea* tubers from untreated plants and plants treated with benzsulfuron.

| Application rate | Absorbance | | | |
|---|---|---|---|---|
| | Days after treatment | | | |
| (g/ha) | 10 | 35 | 100 | 130 |
| 0 | 0.52 | N[1] | N | N |
| 17 | 0.47 | 0.46 | N | N |
| 51 | 0.49 | 0.49 | 0.46 | N |
| 85 | 0.48 | 0.43 | 0.32 | 0.36 |

1 N = No tuber remained.

Table 5. Oxygen uptake in leaves, roots and tuber after application of benzsulfuron to *Sagittaria pygmaea*.

| Application rate | Oxygen uptake (nmole/ml/mg/h) | | | | | |
|---|---|---|---|---|---|---|
| | Leaf | | Root | | Tuber | |
| (g/ha) | 20 DAT[1] | 50 DAT | 20 DAT | 50 DAT | 20 DAT | 50 DAT |
| 0 | 10.8 | 13.2 | 8.7 | 11.5 | 21.7 | - |
| 17 | 32.5 | 28.0 | 14.5 | 20.7 | 17.3 | 27.1 |
| 34 | 21.7 | 24.9 | 14.5 | 25.5 | 14.5 | 28.2 |
| 51 | 18.6 | 25.6 | 8.7 | 21.2 | 10.8 | 29.7 |

1 DAT = days after treatment

**Tuber viability** Tubers were collected from plants remained in a suspended growth stage after application of benzsulfuron. The viability of these tubers was determined using 2,3,5-triphenyl-2H-tetrazolium chloride (TTC). Approximately 300 mg of tuber fragments was placed in a test tube containing 5 ml of the 0.4% TTC solution and 5 ml of 0.1M phosphate buffer (pH 7.0). After 3 h storage in the dark at 30°C, the tuber was rinsed with distilled water and ground in a mortar with 12 ml of ethyl acetate. The extract was then filtered through a Whatman No. 1 filter paper, and light absorbance of the filtrate was determined in a colorimeter at a wavelength of 585 nm.

**Respiration** Following the preemergence application of benzsulfuron, dark respiration of the depressed plants was measured 20 and 50 DAT. After harvesting the plants were separated into leaves, roots and tuber. Fifteen mg of the plant parts was placed in a biological oxygen monitoring system (Cole-Parmer Instrument Co.) to measure the $O_2$ uptake.

**Photosynthetic activity** Assimilation of $^{14}CO_2$ by plants remaining in a depressed growth stage after the application of benzsulfuron was determined. The plants were placed in a plant chamber having 10 $\mu$Ci of $NaH^{14}CO_2$ (specific activity 56 mCi/mM). Ten ml of 1N lactic acid was allowed to run into the $NaH^{14}CO_2$ and the plants was allowed to assimilate the $^{14}CO_2$ for 4 h. At the end of this period, 15 ml of 2.5N KOH was added to absorb the remaining $^{14}CO_2$. The plants were then harvested and dried for 24 h at 85°C. Fifteen mg of the leaves was oxidized in a combustion apparatus, and the resulting $^{14}C$ was captured in a liquid scintillation cocktail. Samples were assayed in a liquid scintillation counter, and counts per minute were corrected for efficiency and converted to disintegrations per minute (dpm).

## RESULTS AND DISCUSSION

**Effect on growth and regrowth of *Sagittaria pygmaea*** Benzsulfuron treatment did not affect sprouting of tubers of *S. pygmaea*. However, it caused initial growth inhibition after the sprouting (Table 1). There were significant reductions in plant height, number of roots, total root length, number of offshoots and dry weight at the rate of 17 g/ha benzsulfuron. The inhibition increased with increasing the application rate. When benzsulfuron was applied 0 days after planting of tubers, the tubers were usually sprouted in 1 to 2 days and produced 2 to 3 leaves. After this stage no considerable growth occurred. The plants remained in a depressed growth stage, and the growth cessation continued by the time of regrowth.

The growth cessation was confirmed by determining RGR of the plants. During the second and the third 10 DAT RGR of the plants treated with benzsulfuron was much lower than that of the untreated control (Table 2). There was no great difference in the RGR between the application rates of benzsulfuron. However, a great increase in RGR occurred during the fourth 10 DAT when benzsulfuron was applied at 17 g/ha, whereas no considerable change in RGR was observed between the third and the fourth 10 DAT benzsulfuron was treated at 34 and 51 g/ha.

RGR represents the efficiency of the plant as producer of new material Blackman, 1919 cited in (1). Therefore, decrease in the RGR indicates that the plants treated with benzsulfuron is being suppressed in producing new dry material. The suppression may be attributed to decrease in leaf area and efficiency of whole plant as an assimilating system (1). On the other hand, increase in the RGR may result from regrowth of the depressed plants.

Regrowth of the depressed plant was recognized by a new leaf being produced between the old leaves. When benzsulfuron was applied at 17 and 51 g/ha, first regrowth was observed 30 and 80 DAT, respectively (Table 3). Percent regrowth increased as DAT increased. The lower the application rate, the faster and the more was the regrowth. The regrowth was closely related with the residual activity of benzsulfuron on *S. pygmaea*. There was no change in number of roots by 60 DAT when benzsulfuron was applied at 51 g/ha, but the number of roots started to increase from 70 DAT (Fig. 2). This was the time when residual activity of benzsulfuron decreased (Fig. 3). When planting of tubers was done at 10-day intervals after the application of benzsulfuron, growth inhibition as measured by number of roots did not occur by 60 DAT. However, the plants grown from the tuber planted 60 days after benzsulfuron treatment were not greatly inhibited as compared with the untreated control. These results indicate that regrowth of the depressed plant depend on the residual activity of benzsulfuron which is directly related with the application rate.

In rice field, benzsulfuron treated at 51 g/ha would be effective for controlling *S. pygmaea*. Although regrowth of the depressed plant occurs 80 DAT, the regrowing plants would not affect the growth of rice. At the time of regrowth of the depressed plants, rice plants are tall enough to take an advantage for competing with *S. pygmaea* being regrown.

Aside from the effect of benzsulfuron on regrowth of the depressed *S. pygmaea*, additional question arises about growth of the plant after the regrowth. When the regrown plant was harvested 150 days after benzsulfuron treatment, there were new offshoots and tubers formed. The new tubers were not sprouted as long as they were connected to the parent plant. When the new tubers were separated and planted, however, they were capable of proliferation and production of new plants (data not presented). This indicates that benzsulfuron carryover does not occur in new plants originated from the depressed plant.

**Physiological activity of the depressed *Sagittaria pygmaea*** During the period of *S. pygmaea* remaining in a depressed growth stage, the tubers were biologically viable (Table 4). When tubers obtained from the depressed plant 10 DAT were placed in solutions of TTC, these solutions absorbed light as much as solutions from tubers of untreated plants. At 35 DAT no tuber was found in the untreated plant, whereas there was a biologically viable tuber in the depressed plant treated at 17 g/ha. As the regrowth was occurred and preceded, the tuber reserves were being consumed and finally depleted. A similar effect was found 100 DAT in tuber obtained from the plant treated at 51 g/ha. Tuber viability lasted for 130 days when benzsulfuron was applied at 85 g/ha. This indicates that although growth of *S. pygmaea* was ceased by benzsulfuron, the tuber remains biologically viable before the depressed plant regrows.

Respiratory activity of the depressed *S. pygmaea* was evident following benzsulfuron treatment (Table 5). The respiration in leaf and root was slightly greater in the treated plant than the untreated plant both 20 and 50 DAT. However, the reverse was observed in respiration of the tuber 20 DAT. At 50 DAT tubers remained after benzsulfuron treatment continued to respire. There was no great difference in respiration of the depressed plant between the application rates. On the other hand, leaves in the depressed plant were able to photosynthesize (Fig. 3). Assimilation of $^{14}C$ in the leaves of the depressed plant treated at the rate of 51 g/ha benzsulfuron 20 and 50 DAT reached about 64% and 66% of the untreated plant, respectively. This decrease was due probably to either in photosynthetic rate or decrease in leaf area as an assimilating system or both. The photosynthetic assimilation increased as the

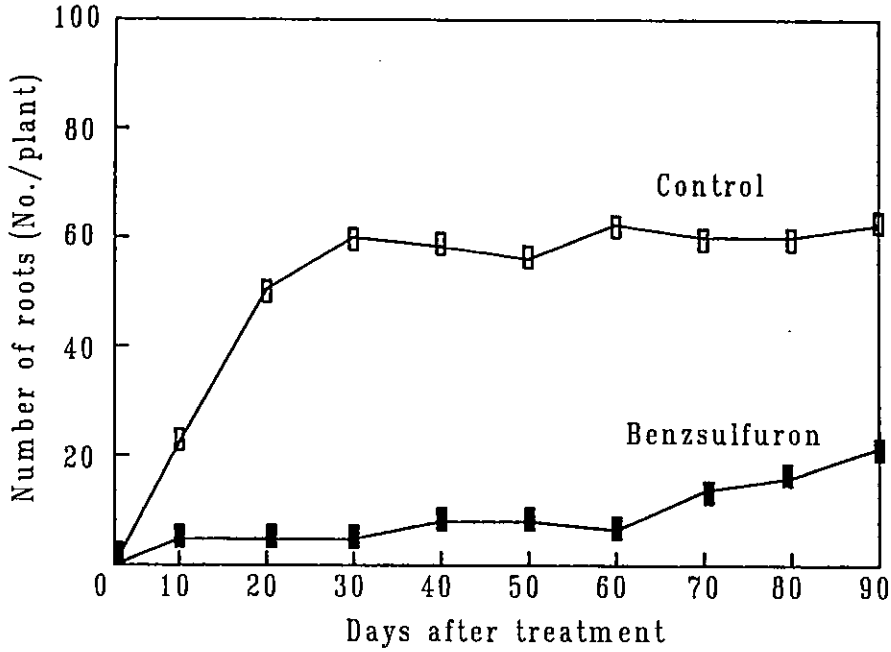


Fig. 1. Effect of benzsulfuron on root growth of Sagittaria pygmaea.

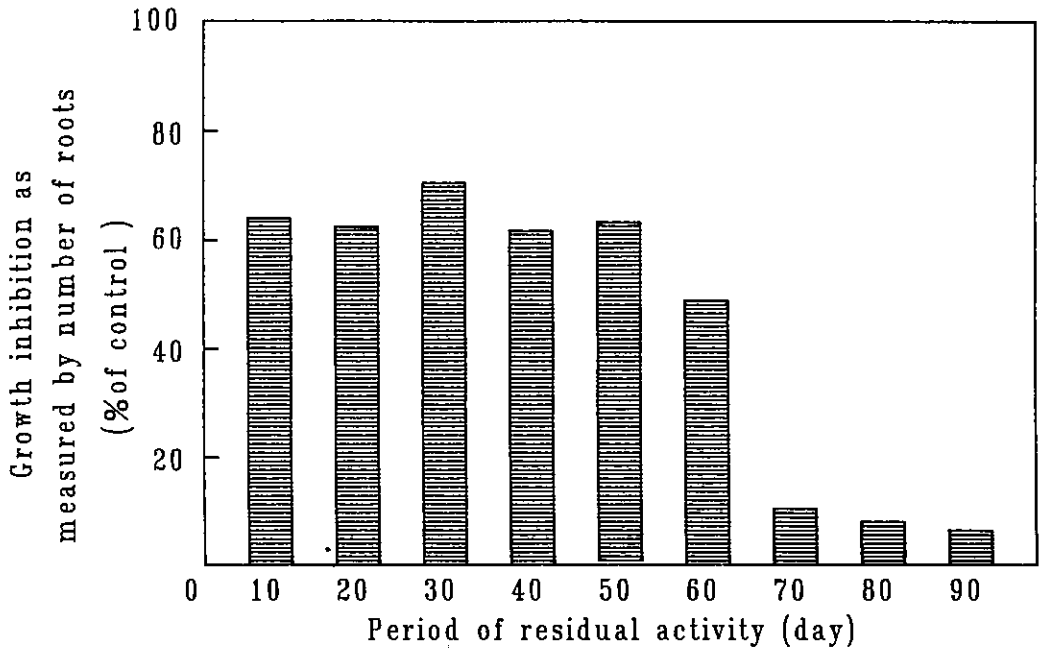


Fig. 2. Growth inhibition of Sagittaria pygmaea as affected by residual activity of benzsulfuron. At 10-day intervals after application of 51 g/ha benzsulfuron tubers were planted and allowed to grow for 10 days before harvesting.

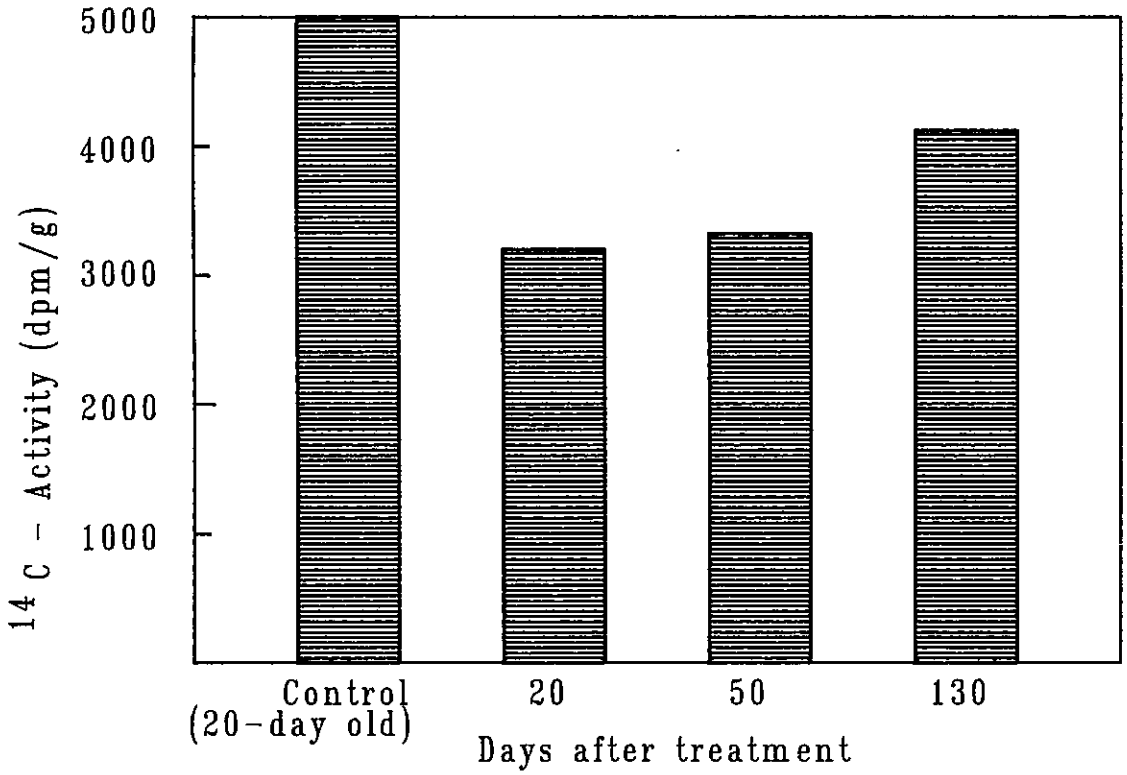


Fig. 3. Assimilation of $^{14}$C in leaves at various times after application of benzsulfuron Sagittaria pygmaea.

regrowth preceded. At 130 DAT the new leaf obtained from the plant being regrown assimilated greater $^{14}C$ than those leaves of the depressed plant.

Benzsulfuron treatment of *S. pygmaea* not only caused increase in respiration compared with untreated controls, but also resulted in decrease in photosynthesis. These effects might result in decrease in RGR during the growth retardation as show in Table 2. Although the leaves in the depressed *S. pygmaea* continues to photosynthesize, the assimilates may not be enough for normal growth. As long as there is residual activity of benzsulfuron, the weed remains in a depressed growth stage.

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# RESIDUES AND DISSIPATION OF THREE MAJOR HERBICIDES, BUTACHLOR, CHLOMETHOXYNIL AND BENTHIOCARB IN PADDY FIELDS IN TAIWAN

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## ABSTRACT

Field experiments on the residues and dissipation status of the herbicides in paddy fields were carried out at Kaohsiung District Agricultural Improvement Station, Pintung (tropical zone), and Taoyuan District Agricultural Improvement Station, Taoyuan (subtropical zone). Three most popular herbicides used for the control of weeds in paddy fields in Taiwan, are butachlor [*N*-(Butoxymethyl)-2- chloro-2',6'- diethylacetanilide], benthiocarb (thiobencarb) [*S*- (4-Chlorobenzyl)- *N*,*N*- diethyl- thiol-carbamate] and chlomethoxynil (2,4- Dichlorophenyl- 3-methoxy- 4- nitrophenyl ether). In this experiment, these herbicides were used alone as well as mixing the two herbicides at the recommended rates. Paddy water and surface soils (0-6 cm) were analyzed at the designed period for the 1st and 2nd crops. As it was expected, the residues of the herbicides in the paddy water decreased as time elapsed and in the 2nd crop it showed higher dissipation rate than that of the 1st crop. The residue levels were correlated with the water solubility of the herbicides falling in the order of benthiocarb > butachlor > chlomethoxynil. Except the residue data of the 1st crop in Kaohsiung district, dissipation of the herbicides in the soils followed the first order kinetics with DT-50 (50% disappearance time) ranging from 0.28 to 10.61 days and DT-90 (90% disappearance time) ranging from 19.63 to 42.66 days depending upon the herbicides and 1st or 2nd crop. At Taoyuan district, benthiocarb was found to be more persistent than chlomethoxynil and butachlor was the least.

## INTRODUCTION

Herbicides play a very important role in the present rice production. In Asian countries, rice is mainly cultivated by the method of transplanting in paddy fields. The most popular herbicides used for the control of weeds of transplanted rice in paddy fields in Taiwan were butachlor, chlomethoxynil and benthiocarb (thiobencarb). Among these three herbicides, butachlor was used more extensively and it could be applied alone or mixed with the other two herbicides.

Investigations related to the behavior of these herbicides in the environment have already been carried out by several investigations. These include photodegradation (3,7), microbial degradation (4,6,12) and dissipation in paddy water and soils (1,5) for butachlor, photodegradation (8, 11), disappearance in irrigation water (18), degradation in soils (9,14,17) and fate in the model ecosystem (2) for benthiocarb, fate in the model ecosystem (13) and degradation in soils (15,16) for chlomethoxynil. However, information regarding the residues

and dissipation of these three herbicides in fields, especially in Taiwan, is limited. In order to pursue further for the safety and accurate use of these herbicides, a comparison of their fate in fields would be very important.

## MATERIALS AND METHODS

Pure butachlor of 99.2%, benthiocarb of 100% and chlomethoxynil of 99.9% purity were obtained from Monsanto Co., U.S.A., Kumiai Chemical Industry Co., Ltd., Japan and Ishihara Sangyo Co., Ltd., Japan, respectively. In the field experiments, the herbicides of five different formulations were used namely: 1) 5% butachlor granule, 2) 10% benthiocarb granule, 3) 7% chlomethoxylnil granule, 4) 2.5% butachlor mixed with 3.5% chlomethoxynil granule, 5) 3% butachlor mixed with 5% benthiocarb granule. All granular type herbicides were commercial products formulated in Taiwan and obtained from the market. Some characteristics of the two soils from Kaohsiung and Taoyuan in this experiment were analyzed and found to be the clay loam, with clay 28.56 and 28.56%, silt 27.40 and 28.00%, sand 44.04 and 43.4%, organic matter 2.53 and 2.94%, and had a pH of 5.99 and 5.31, respectively.

The field experiments were performed during 1st and 2nd crops at Kaohsiung District Agricultural Improvement Station, Pintung, and Taoyuan District Agricultural Improvement Station, Taoyuan Prefecture, Taiwan, respectively. The herbicides were applied 3 days after the transplanting which was performed on Jan. 27 for the 1st crop and June 30 for the 2nd crop at Kaohsiung District and Mar. 10 for the 1st crop and Aug. 7, 1986 for the 2nd crop at Taoyuan District. According to the design of randomized complete block, each crop had 5 treatments and 4 replications and therefore had a total of 20 test plots. Each plot was 10 $m^2$ (4 m x 2.5 m) in area and treated with the recommended rate of 30 Kg commercial product per hectare. The water and soil samples were collected before 1 day and 0, 1, 2, 4, 8, 16, and 32 days after the application of the herbicides. Additional sampling at 64 days after the treatment in the 2nd crop was also made. Sampling was made after about 1 hr of the application on 0 day. Each 125 ml paddy water from the plot of the same treatment was collected and mixed to make a total of 500 ml. At the same time, soils from three points collected from surface 0-6 cm were mixed to serve as the testing sample at every plot. The water and soil samples were stored in a refrigerator with temperature below 4°C and analyzed for the residues of herbicides as quick as possible.

Five grams of NaCl was dissolved in 500 ml of water sample and then extracted with 3 portions of each 100 ml benzene. After dehydration with 8 g anhydrous $Na_2SO_4$, the benzene extracts were evaporated to dryness and the herbicide was washed by n-hexane to a volume of 10 ml and then analyzed by ECD-GC. The soil sample (about 50 g) in 250 ml flask was extracted with three 50 ml portions of acetone on a shaker at 200 rpm for 30, 15 and 15 min, respectively. Acetone extracts were separated from soil sample by a centrifuge at 3,000 rpm for 10 min. It was then evaporated on a rotary evaporator below 40°C to a volume of about 8 ml. After adding 10% NaCl solution to obtain a final concentration of 1% NaCl, it was then extracted with 3 portions of each 40 ml benzene. The residue was analyzed in the same manner as in the case of water sample

ECD gas chromatography was performed through the experiment with Hitachi Gas Chromatograph model 663-50. The glass column (2 m x 3 mm I.D.) with 3% OV-1 on 80/100 mesh Chromosorb WHP

Table 1. The recoveries and detectable limits of the three herbicides.

| Herbicide | Recovery | | Detectable limit | |
|---|---|---|---|---|
| | Water | Soil | Water | Soil |
| | -------- % --------- | | ------- ppb -------- | |
| Butachlor | 98.7 | 88.7 | 0.01 | 0.10 |
| Benthiocarb | 101.7 | 85.1 | 0.60 | 10.00 |
| Chlomethoxynil | 95.8 | 87.7 | 0.01 | 0.08 |

Table 2. Residues of the herbicides in water samples.

| Sampling time | Treatment 1 | 2 | 3 | 4 | | 5 | |
|---|---|---|---|---|---|---|---|
| | Buta. | Benth. | Chlome. | Buta.+ | Chlome. | Buta.+ | Benth. |
| days | ---------- ppb ---------- | | | | | | |
| 1st crop at Taoyuan District: | | | | | | | |
| -1 | ND[1] | ND | ND | ND | ND | ND | ND |
| 0 | 121.52 | 389.33 | 63.88 | 244.18 | 11.69 | 212.16 | 470.47 |
| 1 | 202.17 | 1040.25 | 2.84 | 102.28 | 2.23 | 275.42 | 372.77 |
| 2 | 233.53 | 415.87 | 5.75 | 173.89 | 9.34 | 217.40 | 514.54 |
| 4 | 120.21 | 272.92 | 5.68 | 27.99 | 4.88 | 50.97 | 264.09 |
| 8 | 26.03 | 150.30 | 2.00 | 6.38 | 2.60 | 16.69 | 114.50 |
| 16 | 14.99 | 96.32 | 1.12 | 1.99 | 1.55 | 5.22 | 72.08 |
| 32 | 2.86 | 44.68 | 0.39 | 1.18 | 0.16 | 0.60 | 39.33 |
| 1st crop at Kaohsiung District: | | | | | | | |
| -1 | ND | ND | ND | ND | ND | ND | ND |
| 0 | 254.21 | 622.50 | 21.38 | 161.55 | 9.76 | 155.42 | 532.21 |
| 1 | 167.15 | 1021.70 | 9.67 | 252.81 | 12.33 | 99.37 | 622.38 |
| 2 | 111.15 | 978.20 | 3.54 | 93.55 | 5.38 | 25.74 | 601.60 |
| 4 | 94.84 | 514.60 | 2.04 | 88.77 | 2.70 | 44.67 | 674.00 |
| 8 | 38.80 | 205.10 | 1.30 | 15.34 | 1.54 | 43.72 | 416.00 |
| 16 | 9.54 | 95.60 | 1.57 | 1.17 | 0.53 | 8.57 | 102.22 |
| 32 | 1.75 | 29.40 | 0.64 | 0.96 | 0.84 | 1.06 | 47.83 |

continued

| Sampling time | Treatment 1 | 2 | 3 | 4 | | 5 | |
|---|---|---|---|---|---|---|---|
| | Buta. | Benth. | Chlome. | Buta.+ | Chlome. | Buta.+ | Benth. |
| days | ppb | | | | | | |
| 2nd crop at Taoyuan District: | | | | | | | |
| -1 | ND | ND | ND | ND | ND | ND | ND |
| 0 | 413.59 | 2192.29 | 12.90 | 730.92 | 78.74 | 563.74 | 573.56 |
| 1 | 72.62 | 246.18 | 14.92 | 76.07 | 19.42 | 91.25 | 173.13 |
| 2 | 30.03 | 82.82 | 3.96 | 17.00 | 6.75 | 20.05 | 64.94 |
| 4 | 12.23 | 50.40 | 0.96 | 6.61 | 2.37 | 8.93 | 47.90 |
| 8 | 6.10 | 5.60 | 0.66 | 1.41 | 1.06 | 1.59 | 5.53 |
| 16 | 2.83 | 7.70 | 0.85 | 1.29 | 0.15 | 2.41 | 6.37 |
| 32 | 0.54 | 5.30 | 0.21 | 0.15 | 0.04 | 0.51 | ND |
| 64 | ND | ND | ND | ND | ND | ND | ND |
| 2nd crop at Kaohsiung District: | | | | | | | |
| -1 | ND | ND | ND | ND | ND | ND | ND |
| 0 | 246.13 | 1343.50 | 21.38 | 161.55 | 19.72 | 289.41 | 191.61 |
| 1 | 167.15 | 1021.70 | 9.67 | 252.81 | 12.33 | 111.64 | 216.55 |
| 2 | 157.44 | 643.50 | 8.86 | 179.50 | 12.04 | 75.41 | 206.55 |
| 4 | 63.88 | 720.20 | 5.74 | 38.36 | 5.64 | 95.82 | 80.57 |
| 8 | 11.35 | 133.95 | 0.93 | 9.77 | 0.73 | 14.31 | 13.99 |
| 16 | 0.50 | 10.72 | 0.70 | ND | 0.17 | ND | ND |
| 32 | ND | ND | 0.23 | ND | ND | ND | ND |
| 64 | ND | ND | ND | ND | ND | ND | ND |

1 ND: Butachlor 0.01 ppb; Benthiocarb 0.60 ppb; Chlomethoxynil 0.01 ppb.

Table 3. The meteorological information of the trial locations.

| Location | Crop | Period | Temperature Max. | Min. | Ave. | Rainfall |
|---|---|---|---|---|---|---|
| | | | ------- °C ------- | | | mm |
| Kaohsiung District | 1st | 1/27-2/28 | - | - | 19.0 | 31.2 |
| | 2nd | 6/30-9/2 | 36.3 | 22.1 | 29.0 | 481.2 |
| Taoyuan District | 1st | 3/10-4/11 | 28.4 | 11.6 | 18.7 | 414.8 |
| | 2nd | 8/7-10/10 | 36.4 | 18.0 | 26.9 | 494.9 |

were employed for analysis of butachlor and chlomethoxynil. For the analysis of benthiocarb, the liquid phase of the packing material was replaced by 3% OV-17. Operating temperature were as follows : injection port, 250°C; detector, 280°C; column, 215°C for analysis of butachlor and benthiocarb, 225°C for analysis of chlomethoxynil, respectively. Nitrogen was used as a carrier gas.

## RESULTS AND DISCUSSION

The recoveries and detectable limits of the three herbicides in water and soils are shown in Table 1. The recoveries of the herbicides were more than 95% in water and 85% in soil. From their higher recoveries and lower detectable limits, the methods of analysis in this study were found to be satisfactory.

Table 2 showed the residues of the herbicides in the paddy water. The maximum concentrations of 2,192.29 ppb of benthiocarb, 563.74 ppb of butachlor and 78.74 ppb of chlomethoxynil were detected in 0 day from the 2nd crop at Taoyuan District. Substantially, the residues of herbicides in every treatment decreased as time elapsed. No detectable residues were found in the samples 64 days after the application in the 2nd crop. However, at the 2nd crop it showed higher dissipation rate than that of the 1st crop. This could be explained by the fact that a hot weather was noted in the 2nd crop whereas a relatively cooler weather was observed during the 1st crop as shown in the meteorological information of the trial locations (Table 3). In paddy field, herbicides were dissolved into paddy water after granular preparation was applied. Therefore, the water solubility of the respective herbicide probably influenced the concentration of herbicide in the paddy water. The solubility of benthiocarb, butachlor and chlomethoxynil in water was approximately 30 (20°C), 23 (24°C) and 0.3 ppm (15°C), respectively. As it was expected, the residue levels of the herbicides in the paddy water were correlated with the water solubility of the herbicides falling in the order of benthiocarb butachlor chlomethoxynil. As shown in Table 4, the coefficient variations of the four replication ranged from 7 to 95 %. In order to simplify the procedure of analysis, four soil samples from the same treatment were mixed to make one sample before detecting in the 2nd crop. The residues of the herbicides in soils decreased as time elapsed and it was found to be similar to the cases of the paddy water (Tables 4 and 5). To compare the dissipation rate of the herbicides in soils, the logarithms of the herbicidal concentration remaining (% of the concentration at 0 day) have been plotted against time. The significant coefficient of the relationship obtained indicated that the dissipation followed the first-order rate law. From the slopes of the lines, DT-50 (50% disappearance time) and DT-90 (90% disappearance time) for the different treatments were calculated (Table 6). Except the residue data of the 1st crop in Kaohsiung Distirict, dissipation of the herbicides in the soils followed the first order kinetics with DT-50 ranging from 0.28 to 10.16 days and DT-90 ranging from 19.63 to 42.66 days depending upon the herbicides and the 1st or 2nd crop. The longer persistence of the herbicides in soil in the 1st crop as compared to the 2nd crop may be attributed to the response of the different climatic conditions between the two crops. Among the three herbicides, the difference in dissipation rate in soils was not very remarkable. At Taoyuan District, benthiocarb was found to be more persistent than chlomethoxynil and butachlor was the least. From the above

Table 4. Residues of herbicides in the soil samples at the 1st crop.

| Sampling time CV(%)[2] | Treatment 1 Buta. | 2 Benth. | 3 Chlome. | 4 Buta.+ Chlome. | | 5 Buta.+ Benth. | |
|---|---|---|---|---|---|---|---|
| days | ppm | | | | | | |
| Taoyuan District: | | | | | | | |
| -1 | ND[1] | ND | ND | ND | ND | ND | ND |
| 0 | 0.860 | 1.42 | 1.416 | 1.110 | 0.844 | 0.992 | 1.04 |
| CV | 72 | 40 | 47 | 41 | 64 | 66 | 39 |
| 1 | 0.970 | 1.58 | 0.981 | 0.862 | 0.652 | 0.844 | 1.24 |
| CV | 79 | 53 | 14 | 48 | 29 | 37 | 46 |
| 2 | 0.770 | 1.15 | 0.804 | 0.577 | 0.552 | 0.698 | 0.73 |
| CV | 61 | 57 | 24 | 49 | 54 | 56 | 36 |
| 4 | 0.476 | 0.74 | 0.553 | 0.350 | 0.388 | 0.421 | 0.58 |
| CV | 42 | 39 | 11 | 40 | 39 | 64 | 35 |
| 8 | 0.308 | 0.60 | 0.476 | 1.154 | 0.197 | 0.176 | 0.29 |
| CV | 45 | 54 | 23 | 78 | 46 | 45 | 27 |
| 16 | 0.332 | 0.50 | 0.406 | 0.158 | 0.183 | 0.202 | 0.24 |
| CV | 45 | 24 | 7 | 76 | 55 | 40 | 30 |
| 32 | 0.134 | 0.28 | 0.220 | 0.126 | 0.116 | 0.100 | 0.19 |
| CV | 22 | 58 | 14 | 95 | 34 | 20 | 41 |
| Kaohsiung District: | | | | | | | |
| -1 | ND | ND | ND | ND | ND | ND | ND |
| 0 | 0.601 | 2.20 | 2.014 | 0.469 | 1.348 | 0.512 | 1.59 |
| CV | 67 | 31 | 39 | 70 | 25 | 64 | 34 |
| 1 | 0.237 | 2.03 | 1.684 | 0.132 | 1.193 | 0.208 | 1.31 |
| CV | 34 | 39 | 29 | 30 | 24 | 63 | 38 |
| 2 | 0.187 | 2.24 | 2.027 | 0.147 | 1.060 | 0.206 | 1.06 |
| CV | 11 | 21 | 40 | 27 | 49 | 87 | 21 |
| 4 | 0.176 | 2.22 | 2.212 | 0.119 | 1.022 | 0.120 | 1.18 |
| CV | 28 | 26 | 24 | 42 | 39 | 58 | 19 |
| 8 | 0.179 | 1.69 | 1.401 | 0.113 | 0.917 | 0.087 | 0.91 |
| CV | 34 | 18 | 26 | 35 | 36 | 23 | 10 |
| 16 | 0.150 | 1.46 | 1.494 | 0.118 | 1.110 | 0.062 | 0.70 |
| CV | 47 | 17 | 17 | 42 | 51 | 32 | 31 |
| 32 | 0.150 | 1.48 | 1.674 | 0.101 | 0.812 | 0.057 | 0.59 |
| CV | 40 | 28 | 33 | 30 | 55 | 70 | 37 |

1 ND: Butachlor 0.0001 ppm; Benthiocarb 0.01 ppm; Chlomethoxynil 0.00008 ppm.

2 CV(%): CV is the coefficient of variation.

CV(%) = (standard deviation/average) x 100.

Table 5. Residues of herbicides in the soil samples at the 2nd crop.

| Sampling time | Treatment 1 | 2 | 3 | 4 | | 5 | |
|---|---|---|---|---|---|---|---|
| | Buta. | Benth. | Chlome. | Buta.+ | Chlome. | Buta.+ | Benth. |
| days | ppm | | | | | | |
| Taoyuan District: | | | | | | | |
| -1 | ND[1] | ND | ND | ND | ND | ND | ND |
| 0 | 1.487 | 0.57 | 0.844 | 1.276 | 0.783 | 1.219 | 0.45 |
| 1 | 1.184 | 0.48 | 0.633 | 0.835 | 0.693 | 0.830 | 0.41 |
| 2 | 0.965 | 0.36 | 0.511 | 0.713 | 0.447 | 0.606 | 0.29 |
| 4 | 0.489 | 0.38 | 0.437 | 0.506 | 0.368 | 0.459 | 0.18 |
| 8 | 0.213 | 0.23 | 0.211 | 0.308 | 0.355 | 0.393 | 0.19 |
| 16 | 0.161 | 0.14 | 0.196 | 0.139 | 0.245 | 0.167 | 0.13 |
| 32 | 0.088 | 0.05 | 0.076 | 0.094 | 0.043 | 0.045 | 0.02 |
| 64 | 0.017 | ND | 0.007 | ND | ND | 0.004 | ND |
| Kaohsiung District: | | | | | | | |
| -1 | ND | ND | ND | ND | ND | ND | ND |
| 0 | 0.870 | 1.96 | 1.348 | 1.094 | 0.850 | 0.507 | 0.61 |
| 1 | 0.661 | 1.09 | 1.449 | 0.921 | 0.827 | 0.521 | 0.52 |
| 2 | 0.716 | 0.92 | 0.954 | 0.757 | 0.437 | 0.264 | 0.45 |
| 4 | 0.521 | 1.07 | 1.248 | 0.872 | 0.268 | 0.254 | 0.30 |
| 8 | 0.341 | 0.48 | 0.649 | 0.563 | 0.084 | 0.190 | 0.19 |
| 16 | 0.057 | 0.18 | 0.249 | 0.103 | 0.082 | 0.065 | 0.16 |
| 32 | 0.004 | ND | 0.144 | 0.017 | 0.063 | 0.004 | 0.04 |
| 64 | 0.003 | ND | 0.007 | 0.010 | 0.006 | 0.003 | ND |

1 ND: Butachlor 0.0001 ppm ; Benthiocarb 0.01 ppm ; Chlomethoxynil 0.00008 ppm.

Table 6. Time of disappearance for the herbicides in the soils.

| Treat. | Herbicides | 1st crop | | | 2nd crop | | |
|---|---|---|---|---|---|---|---|
| | | DT-50 | DT-90 | r | DT-50 | DT-90 | r |
| | | ----- days ----- | | | ----- days ----- | | |
| Taoyuan District: | | | | | | | |
| 1 | Butachlor | 10.08 | 32.25 | **0.9313 | 1.00 | 25.96 | **0.9145 |
| 2 | Benthiocarb | 10.61 | 42.66 | **0.9277 | 7.24 | 30.14 | **0.9970 |
| 3 | Chlomethoxynil | 5.42 | 38.42 | **0.9111 | 5.18 | 28.39 | **0.9882 |
| 4 | Butachlor | 1.35 | 27.38 | *0.8020 | 2.17 | 26.56 | **0.9717 |
| | Chlomethoxynil | 5.09 | 33.02 | **0.8903 | 6.42 | 29.17 | **0.9885 |
| 5 | Butachlor | 4.51 | 28.22 | **0.8842 | 3.08 | 22.32 | **0.9924 |
| | Benthiocarb | 8.11 | 37.52 | *0.8551 | 5.71 | 28.11 | **0.9794 |
| Kasohsiung District: | | | | | | | |
| 1 | Bubachlor | - | 62.70 | 0.559 | 3.01 | 19.63 | **0.9261 |
| 2 | Benthiocarb | 46.71 | 160.66 | *0.8441 | 0.28 | 21.70 | **0.9111 |
| 3 | Chlomethoxyni | 97.60 | 351.83 | 0.4279 | 8.44 | 28.48 | **0.9918 |
| 4 | Butachlor | - | 58.38 | 0.5060 | 4.84 | 24.67 | **0.9356 |
| | Chlomethoxynil | 50.12 | 198.59 | 0.7521 | 0.62 | 23.57 | **0.9311 |
| 5 | Butachlor | - | 27.12 | *0.7851 | 3.40 | 21.46 | **0.9394 |
| | Benthiocarb | 17.31 | 75.95 | **0.9122 | 6.14 | 29.20 | **0.9905 |

1 5% level of significance.

2 1% level of significance.

evidences in this study, only benthiocarb showed a very slight potentiality to have an impact on the environment, if any, among the three major herbicides used in paddy fields in Taiwan.

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# BEHAVIOR OF QUINCLORAC IN SOILS, RESULTS OF BIOASSAYS

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## ABSTRACT

Studies were made to examine the behavior of quinclorac in soils, particular under flooded conditions, using bioassays with *Echinochloa crus-galli* and rice as indicator plants. Quinclorac is taken up by *Echinochloa* and rice plants mainly via the root system but also by the leaves. The uptake is very rapid, e.g. a root dip treatment of 10 minutes at 5 ppm provided already some apparent effect. Tests in six different Japanese paddy soils showed that the compound is only lightly bound to the soil fraction. The mobility is lower in heavy soils and in soils with a high organic matter content. The fastest control of *Echinochloa crus-galli* was achieved when quinclorac compound was moved into the root zone by a leaching treatment. Therefore, best control of ECHCG will be achieved in fields that allow some water percolation.

## INTRODUCTION

At the previous 10th APWSS conference in Thailand the outstanding and specific activity of quinclorac against *Echinochloa* species and the high selectivity on rice plants has been reported (1, 2). Quinclorac is presently under development in Japan for the use in transplanted paddy rice cultivation and further in seeded rice under upland or paddy condition.

The herbicide is available as granule or sprayable formulation. In flooded fields the granules are easily dissolved and distribution of the active material is very even.

Waterflow and soil characteristics may influence the behaviour of herbicide under practical application conditions. Therefore, it is of great importants to know about the behavior of the chemical in the field in soil and water.

This report, based on several model tests, will explain the possible behavior and action of quinclorac for *Echinochloa* control in paddy fields.

## MATERIALS AND METHODS

**Chemical**

Common name : quinclorac
Chemical name : 3,7-dichloro-8-quinoline carboxylic acid
Code name : BAS 514 H
Formulations : BAS 51400 H 50% WP, BAS 51405 H 1% granular, BAS 51406 H 1% granular

**Test plants**

Rice variety : "Koshihikari" and *Echinochloa crus-galli* (ECHCG). All trials have been carried out with 3 replications.

**Petri dish test** Rice and ECHCG seeds were dip-treated in a benomyl + thiram solution (5% for 10 minutes) for disinfection and then soaked in water for 1 day.

Filter paper was used for set 1 and a 1 cm layer of disinfected soil for set 2. 20 ml rsp. 60 ml of chemical solution was added to each petri dish prior to seeding of rice and ECHCG. The dishes were kept under laboratory conditions at 25°C. The plant growth was visually evaluated.

**Vertical movement in soil (modified JAPR (Japan association for advancement of phyto-regulators) method)** Specially designed acryl vessels of 10 cm x 10 cm x 20 cm with horizontal sleds at one cm intervals on 3 inside walls and a small hole in the bottom were used. The fourth wall of the vessel is detachable. The hole was plugged with a gauze stopper to allow a two-way water movement. Ten cm of fine sieved paddy field soil was filled on top of a 3 cm sand layer. The vessel was placed inside a waterfilled pan. The water depth inside the vessel was adjusted to 3 cm after paddling the top 5 cm of soil. At 24 hours after chemical application, a leaching treatment of 3 cm/day for 3 days was made by lowering the water level of the outer pan. Soil columns were then separated horizontally into 2 cm layers. Each layer was placed into a plastic cup and ECHCG seeds were added. The plant growth was visually evaluated.

**Lateral distribution** ECHCG plants were grown up to 1.5 leaf-stage in four rows, forming a star-shaped pattern, in a 35 cm x 58 cm pan. Granular formulations for the total area were applied only to the center of the pan. Throughout the trial period the water depth was maintained either at soil surface level (water saturated) or at 3 cm height by subsoil irrigation. The effect on ECHCG and the effectively controlled area was visually evaluated.

**Influence of leaching on the efficacy** Wagner pots (1/5000 acre) were filled with 3 liter of soil. Twelve days before the application ECHCG was seeded and rice seedlings were transplanted at 2 plants/hill and 2 hills/plot. The water level was adjusted to 3 cm before the application and maintained at 1 cm after the leaching treatment. Leaching was done by inserting an injection needle through a rubber stopper at the bottom of the pot.

1. Draining all water from the pot

Quinclorac at 0.1 and 0.3 kg/ha a.i. was applied at two days after transplanting when rice and ECHCG were at the 3 leaf-stage. Starting at 1,6,24 and 46 hours after the application , respectively, all water from the pots was leached out from the bottom. At 24 hours after leaching start the pots were refilled with water. The effect was visually evaluated.

2. Leaching of 3 cm/day for 3 days

At 2.5 leaf-stage of ECHCG and 2.8 leaf-stage of rice, quinclorac at 0.1, 0.2 and 0.3 kg/ha a.i. was applied. The leaching treatment was started at 1, 6 and 24 hours after application. The leaching amount was measured by reducing the water depth from 4 cm to 1 cm and adding up again to 4 cm. A total of 9 cm leaching over 3 days was made. Control plots without leaching were also included. The effect was visually evaluated.

**Dipping treatment** Rice and ECHCG seedlings were raised separately. At the 2.5 leaf-stage of ECHCG and 3 leaf-stage of rice, the roots of the seedlings were carefully washed and 10 plants were used for each plot. Roots or leaves of the prepared seedlings were dipped either for 10 minutes, 1 hour or 24 hours into chemical solution of 0.5, 5 or 50 ppm. Seedlings were planted separately in plastic cups either with or without a prior washing, as listed in the following design.

10 minutes root dipping----->wash---->transplant
1 hour root dipping----->wash---->transplant
24 hours root dipping----->wash---->transplant
10 minutes leaf dipping----->wash---->transplant
1 hour leaf dipping----->wash---->transplant
10 minutes leaf dipping-----> transplant
The effect was visually evaluated.

**Wash-out through a 10 cm soil layer** Paddy soil was collected from six different locations in Japan. The air-dried and sieved material was filled and compacted (not paddled) into 1/2000 acre sized Wagner pots. All soils were prepared in the same way. Pots were then flooded and the water level was adjusted to 3 cm. At 24 hours after flooding quinclorac at 0.5 kg ai/ha was applied. The leaching treatment was started 24 hours after application.

Leaching was made by draining the water from the bottom of the pots, 3 cm at a time and repeated 15 times to a total of 45 cm. The water level was reduced each time from 3 cm to 0 cm within about 10 minutes. Water refilling was made by overhead sprinkling from a watering can. Leachates for each 3 cm wash-out were kept separately and supplied regularly to ECHCG plants at 1 leaf-stage, grown in plastic cups. The evaluation was made visually.

ECHCG was also sown to each soil after the leaching treatment in order to check the amount of biologically available residual quinclorac in the treated pots.

Soils used

| | origin | type | organic matter |
|---|---|---|---|
| 1. | Kagoshima | sandy loam | 2-3 % |
| 2. | Yamaguchi | sandy loam | 2.5 % |
| 3. | Okayama | loam | 3.5 % |
| 4. | Ebina | light clay | 5.2 % |
| 5. | Ushiku | light clay | 15.7 % |
| 6. | Ryugasaki | clay loam | 1.97 % |

## RESULTS AND DISCUSSION

**Petri dish test** There was no great difference in unit activity between quinclorac and benthiocarb in the petri dish test when plants were grown on filter paper. Both compounds achieved nearly 100 % control at a concentration of 1 ppm. However, using soil instead of filter paper, quinclorac showed a 10 times higher activity on ECHCG than benthiocarb. In fact, the efficacy of benthiocarb was reduced to 1/10 by the presence of soil, whereas quinclorac hardly lost any efficacy. The attachment to soil particles is low (Fig. 1).

**Vertical movement and lateral distribution** Because the vertical translocation test was made with high dosages (0.4 kg and 0.6 kg ai/ha), quinclorac was moved and could be detected in the bio-assay. Though most of the applied chemicals stayed in the top 10 cm soil layer in the heavy soil, some amount was carried through the 10 cm layer of Ebina light soil (Fig. 2).

The lateral distribution of quinclorac was superior to mefenacet. In 1 cm flooded condition, quinclorac gave 90 % control of ECHCG on all the plot area whereas mefenacet achieved 42.5 %. Under water-saturated condition, quinclorac at 0.3 kg ai/ha gave 90 % control on 16.5 % of the plot area compared to 2.5 % with mefenacet at 1.2 kg ai/ha. (Table 1)

**Influence of leaching on the weed control** In Figs. 3 and 4 the influence of leaching on ECHCG control is shown when the leaching treatment was made in a time course after the quinclorac application. Since there was no crop injury the selectivity values are not mentioned.

The effect of quinclorac appeared faster the sooner the leaching treatment was started after application. This fact was confirmed in two different leaching regimes. As it is explained by a following study, quinclorac is more rapidly taken up via roots than via the leaves. Quinclorac was moved down into the root zone by the leaching treatment and thus was faster taken up than without leaching. There was no loss of efficacy by giving a leaching treatment. The percolation start early after application brought about the strongest initial effect on ECHCG.

**Dipping treatment** Root dipping provided a higher degree of efficacy than the foliage dipping when the same concentrations and dipping periods are compared. The difference was particulary evident after a 10 minutes dip. (Fig. 5). A root dipping treatment for 10 minutes in a 5 ppm quinclorac solution gave already an apparent effect, whereas the tenfold higher rate was required for a leaf dip. The 10 minutes root dipping treatment in a 50 ppm solution provided total control of ECHCG. At a concentration of 0.5 ppm an exposure period of 24 hours root dipping achieved about 90 % control.

The uptake of quinclorac via root or foliage was considerably faster than that of the reference product benthiocarb at 50 ppm (Fig. 6).

The rapid root uptake by plants can be considered one of the main reasons for the consistent high effect of quinclorac.

**Wash-out of quinclorac from soils** In spite of a 45 cm water column leaching treatment, some amount of quinclorac still stayed in the 10 cm layer in the Ushiku and Ebina soils and 40 % and 10 % ECHCG control, respectively, was achieved. There was no activity on ECHCG found in the other soils (Table 2).

Leachates applied to ECHCG started to be effective from the 3-6 cm level, except for the Okayama soil, showing an activity already in the 0-3 cm column. In the Ushiku soil the downward movement was somewhat delayed. Quinclorac was washed out to non-detectable levels from the Okayama, Yamaguchi and Kagoshima soils with 36-39 cm or 39-42 cm water columns. The leachate from Ushiku soil still showed relatively high activity on ECHCG even with a 42-45 cm column. Some activity was found at 42-45 cm level also with Ebina and Ryugasaki soils.

The wash-out pattern of quinclorac from Ushiku soil shows a little lower peak and a more gentle declining slope (Fig. 7).

Ushiku soil contains 15.7% organic matter, the highest among the tested soil, and is followed by Ebina soil containing 5.2% organic matter. The organic matter content does play an important role for movement of quinclorac in soils. But the Ryugasaki soil, which showed the third slowest release of quinclorac, contains the lowest rate of organic matter. Thus the organic matter contents alone does not explain the release of quinclorac if we look at soil types, Ryugasaki soil belongs to clay loams whereas Okayama soil is a loam and Kagoshima and Yamaguchi soils are sandy loams. The results of the vertical movement test showed less movement

Table 1. Lateral distribution of BAS 514.06H in water saturated soil and in 1 cm deep flooded condition.

| Treatment | kg ai/ha | Water saturated soil condition | | Water depth 1cm | |
|---|---|---|---|---|---|
| | | 90% contr. area in % | Effective distance in cm | 90% contr. area in % | Effective distance in cm |
| BAS 514 06H | 0.2 | 8.0 | 6.0 | 100.0 | 29(all) |
| | 0.3 | 16.5 | 8.5 | 100.0 | 29(all) |
| NIN-801 | 1.2 | 2.5 | 2.5 | 42.5 | 15.5 |

Table 2. Wash-out of BAS 514 06 H through 10 cm soil; effect of residual quinclorac on ECHCG after a 45 cm water leaching treatment.

| Soil | ECHCG control in % |
|---|---|
| Kagoshima | 0 |
| Yamaguchi | 0 |
| Okayama | 0 |
| Ebina | 10 |
| Ushiku | 40 |
| Ryugasaki | 0 |

of quinclorac in heavy soil. Also the soil particle size therefore could be an influencing factor to the attachment of quinclorac to soils.

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# EFFECT OF LAND PREPARATION ON WEEDS AND YIELD OF SUGARCANE

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## ABSTRACT

The effective of various cultivation technics combined with promising herbicides in sugarcane field had carried out during July 1985, to April 1986, at Rayong Field Crops Research Center. The experimental area was comprised of *Digitaria* spp., *Brachiaria reptans* (L.) Gard. et Hubb., *Cyperus rotundus* L. and *Euphorbia geniculata* Orteg. etc. Sugarcane variety F-140 was used and laid out in simple trial with 4 treatment. All of treatments were ploughed as the same level but there were different in timing of harrowing, herbicide application and hand weeding. The results showed that 16.3-52.5% of newly emerged weeds could be decreased in the treatments which were kept ploughing soil in sunny condition for two weeks, thereafter dragged the pieces of perennial weeds (mostly 70% of purple nutsedge) to the edge of the field before planting the cane-setts whereas yielded 53.1 tons/ha and 52.5 tons/ha obtained from each that followed by monthly hand weeding and herbicide application respectively. In the case of incomplete land preparation, hand weeding treatment and the treatment which was applied with atrazine plus ametryn at the rate of 2.5 + 2.5 kg ai/ha gave moderately weed control. Farmer field trial was also studied for comparing expense and yield production.

## INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the economic crops. It is usually growing for sugar mills. There are 0.54 million hectares planted sugarcane in 1986-1987. Weeds competed with sugarcane for water and mineral nutrients, cane productions are normally impacted by weeds, particularly in the early phase of growing. The yield reduction range from a little to complete crop losses depending upon the time of infestation, weed composition and management practices. Promkam et al. (2) reported that the critical period of weed competition in sugarcane was the first 4 months, discontinuing weeding during the first two months was the most critical and caused yield losses over 40%. Govindra and Singh (1) noted that *Cyperus rotundus* L. and other narrowleaf weeds reduced cane yields by about 45%. Clean weeding increases cane yields for the first 120 days of crop growth. Actually, cultivation in sugarcane field is quite different from other crop growing, planted cane requires frequent cultivation for increasing growth and development. By principal, land preparation is one method of weed control. An appropriate soil preparation not only provides soil conditions favourable for cane growth and

development but it is also to get ready weed free conditions at planting time and increase efficiency of herbicides which will be applied later. At present, average cane yield production remained 29.5-28.8 tons/ha., this might due to improper cultivation and pest management particularly, weed control practices that most of growers still prefer to use conventional method such as dry season ploughing, only one or two passes in the same day and hand hoeing 2-3 times until the cane canopy is closed-in. Too early ploughing caused certain weed seeds and stem portions of perennials to germinate and establish before planting time. Suwanarak (3) showed that highest yield obtained from the treatment which was operated by using suitable implements for good soil preparation and uprooting of rhizomes and stem segments of perennial noxious weed, these were dragged to the edge of the field before planting the cane-setts, herbicides were then applied as pre-emergence and post-emergence. It might be noted that the number of weeds particularly, perennials declined as the number of preplanting harrowings increased. Therefore, desirable of cultivation for better weed control should be developed and transfered to the extensionists and growers. The objectives of these studies were: 1. to compare the effect of different land preparation methods on weed control, growth and yield of sugarcane. 2. to compare sugarcane yield production between appropriate weed management and conventional practices.

## MATERIALS AND METHODS

The first experiment was carried out at Rayong Field Crops Research Center, Rayong Province, eastern of Bangkok during July 1985 to April 1986. The soil type was sandy clay loam. Seed pieces with 2-3 buds of sugarcane variety F-140 were planted with a planting space of 0.50-1.30 meters in a plot size of 15.0-42.0 meters. Carbofuran insecticide at the rate of 62.5 kg/ha and fertilizer (15-15-15) at the rate of 312.5 kg/ha were applied at planting and when cane was 3 months old.

Land preparation was started in July, all treatments were subsoiled. Ploughing operations were made by using a tractor drawn disc plow to a depth of 15-20 inches. Specific details of each plot could be divided into four categories as follows:

1. Harrowed at two weeks after ploughed, then planted the cane-setts and applied atrazine plus ametryn at the rate 2.5 + 2.5 kg ai/ha.
2. Ploughed, immediately planted and applied atrazine plus ametryn at the rate of 2.5 + 2.5 kg ai/ha.
3. Harrowed at two weeks after ploughed, then planted and monthly hand weeding until fourth month.
4. Ploughed, immediately planted and monthly hand weeding until fourth month.

In treatment 1 and 3, spring tooth harrow was used for uprooting of rhizomes then dragged newly emerged weeds out of the plots.

Weed control evaluation by visual scoring and weighing dry weeds were taken at 2, 3, 4 and 5 months after planting. Sugarcane yield production, plant height and quality of sugar content were also recorded.

The second experiment aimed to compare yields, expenses and profit per unit area between integrated weed management and farmer conventional practices. They were set up in irrigated area of Kamphaeg-Saen farmer field, Nakorn-Pathom Province, the western of Bangkok, during May,

1986 to April, 1987. The soil was a loamy clay consisting of major weeds as *Echinochloa colonum* (L.) Link., *Leptochloa chinensis* Nees., *Commelina bengalensis* L., *Cenchrus echinatus* L., *Amaranthus spinosus* L. and *Tribulus terrestris* L. etc. Each plot was designed into the area of 0.8 hectare and planted with cane variety F-156.

The method of integrated weed management was started in early rainy season, the land was ploughed into a depth of about 30 cm, then it was subsoiled to a greater depth and harrowed. Furrows and fertilization (16-16-16) at the rate of 312.5 kg/ha were made before planting.

Atrazine at the rate of 3.0 kg/ha was applied over the furrows after watered. Cultivation using tractor drawn plow for hilling weeds between rows was done at 30 days after planting. Spot hand weeding along the cane rows and small tractor drawn spring tooth harrow for elimination newly emerged weeds between rows at 45 days after planting. Off-barring, 60 days after planting, fertilization with $(NH_4)_2SO_4$ at the rate of 125 kg/ha and then hilling up. A second hilling up by using machine hillers attached with spring tooth harrow to eradicate further weed growth was done at 90 day after planting.

Farmer plot was undertaken by conventional practices. The soil was ploughed and furrowed. Fertilizer (16-16-16) at the rate of 312.5 kg/ha was applied in the furrow prior to planting and then followed by atrazine at the rate of 3.0 kg ai/ha. Second fertilization was made at 90 days after planting. Monthly hand weeding during the end of second month until the fourth month.

An assessment in each plot was carried out by measuring growth, tillering and cane yield production at harvesting. Total costs and profit per unit area were also recorded.

## RESULTS AND DISCUSSION

In 1985-1986, the result from the experiment which was carried out in Rayong Field Crops Research Center revealed that most of weed species occuring in those areas were composed of narrow-leaves namely: *Digitaria adscendense* (HBK) Henr., *Bachiaria reptans* (L.) Gard. et Hubb., *Cyperus rotundus* L., *Echinochloa colonum* (L.) Link., and *Dactyloctenium aegyptium* Willd etc, major broad-leaves were *Euphorbia geniculata* Orteg., *Hydyotis biflora* (L.) Lam. and *Trianthema portulacastrum* L. Degree of weed control in each plot based on visual rating scale and weighing dry weeds were shown in Tables 1 and 2. It was found that, the treatments which were ploughed, exposed the soil out by sunshine for 2 weeks, harrowed and pulled newly emerged weeds and pieces of some perennials (mostly 70% was purple nutsedge) and then planted the cane-setts followed by applied atrazine + ametryn at the rate of 2.5 + 2.5 kg ai/ha gave better weed control than the plots which the soil were ploughed and immediately planted. According to atrazine plus ametryn gave slightly control perennials which heavy infested in those areas, therefore good soil preparation combined with monthly hand weeding showed slightly better controlling in weeds by nearly the same height when measured at 3 and 9 months after planting (Table 3). Only ploughing and monthly hand weeding could not control either annuals and perennials because of subsequent emerged weeds much more than other plots. This caused to reduce crop growth and cane yield. In Table 4, highest yield was 53.1 tons/ha obtained from the well soil preparation plot and followed with monthly hand weeding for the first four months. However,it was not different in yield when comparing to the same soil preparation but followed with herbicide application. Besides these, non harrowing and immediately planted gave lower yield in both treatments which were followed with herbicide application or monthly hand weeding. Due to the efficacy for weed

Table 1. Weed control rating and dry weed weight on different methods of land preparation. Rayong Province, 1985-1986.

| Treatment | Weed control rating[1] | | | | Dry weight of weeds ($gm/m^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2[2] | 3 | 4 | 5 | 2 | 3 | 4 | 5 |
| ploughing + harrowing[3] + herbicides | 4.0 | 3.5 | 2.8 | 2.5 | 262.0 | 123.5 | 217.0 | 234.0 |
| ploughing + herbicides | 3.5 | 3.5 | 2.5 | 2.5 | 253.2 | 104.6 | 239.6 | 279.7 |
| ploughing + harrowing[3] + monthly hand weeding | 4.5 | 4.0 | 4.0 | 3.0 | 185.5 | 81.1 | 62.3 | 123.9 |
| ploughing + monthly hand weeding | 3.0 | 4.0 | 3.0 | 2.0 | 209.1 | 82.6 | 80.5 | 261.0 |

1 weed control rating 1 = no control, 5 = completely control
2 months after planting
3 harrowing at 2 weeks after ploughing

Table 2. Relative dry weed weight of each species at 5 months after planting. Rayong Province, 1985-1986.

| Treatments | Weed Species | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Digitaria adscendense* | *Brachiaria reptans* | *Cyperus rotundus* | *Echinochloa colonum* | *Euphorbia geniculata* | Others | Total Weed weight (gm/m ) |
| ploughing + harrowing[2] + herbicides | 29.6 | 68.1 | 24.3 | 16.9 | 54.2 | 40.9 | 234.0 |
| ploughing + herbicides | 21.4 | 31.9 | 161.5 | 9.4 | 14.2 | 41.3 | 279.7 |
| ploughing + harrowing[2] + monthly hand weeding | 16.5 | 12.7 | 26.9 | 4.2 | 8.5 | 55.1 | 123.9 |
| ploughing + monthly hand weeding | 41.3 | 14.1 | 118.7 | 11.6 | 21.4 | 53.9 | 261.0 |

1 weeds concluded of *Hydyotis biflora*(L.) Lam., *Tridax procumbens* L. *Cyperus compressus* L. and *Amaranthus viridis* L.
2 harrowing at 2 weeks after ploughing

Table 3. Effect of land preparation on plant height. Rayong Province, 1985-1986.

| Treatment | Plant Height[1] (cm) | | |
|---|---|---|---|
| | 2 months[2] | 3 months | 9 months |
| ploughing + harrowing[3] + herbicides | 28.8 | 48.4 | 236.8 |
| ploughing + herbicides | 30.4 | 51.7 | 208.7 |
| ploughing + harrowing + monthly hand weeding | 26.5 | 48.5 | 235.6 |
| ploughing + monthly hand weeding | 24.7 | 43.1 | 176.8 |

1 plant height measured from ground to the first dewlap
2 months after planting
3 harrowing at 2 weeks after ploughing

Table 4. Effect of land preparation on tillering, yield and quality of sugarcane at harvesting (10 months after planting). Rayong Province, 1985-1986.

| Treatments | tillers/ha | Yield | | C.C.S. |
|---|---|---|---|---|
| | | tons/ha | as % of best yield | |
| ploughing + harrowing[1] + herbicides | 60900 | 52.5 | 98.9 | 12.4 |
| ploughing + herbicides | 46350 | 37.5 | 70.6 | 14.9 |
| ploughing + harrowing[1] + monthyl hand weeding | 61950 | 53.1 | 100.0 | 15.2 |
| ploughing + monthly hand weeding | 41100 | 32.5 | 61.2 | 14.4 |

1 harrowing at 2 weeks after ploughing

control was poor, therefore decreasing in yield was over 29.5%. It was important to note that, the mechanical weed control using tractor to plough and expose the soil out by sunshine can destroy some rhizomes of perennials but incompletely control on escapable portions which become to be the subsequent problem weeds. Therefore, herbicide application and hand weeding gave nearly weed control and yield.

The result from Kamphaeng-Saen farmer field showed in Tables 5 and 6. Atrazine at the rate of 3.0 kg ai/ha gave good weed control for 4-5 weeks. The plot of integrated weed management (IWM plot) which was used machine cultivators for weeding, off-barring and hilling up since the end of second month until the cane-canopy were closed-in could control weed quite well, furthermore growth and yield in this plot was higher than in farmer plot. Eventhough, due to an appropriate soil preparation before planting caused higher investment than farmer plot, but yield of 90.63 and 51.25 tons/ha were obtained in IWM plot and farmer plot respectively. At that time, the cane was sold at a price of US$ 14.23/ton. The IWM plot got US$ 1289.66/ha, while the farmer plot got US$ 729.29/ha. Comparing to their profit were US$ 560.73/ha and US$68.88/ha respectively. It might be concluded that mechanical operation at 2-3 leaves stage of cane growth and to repeat this process until 3-4 months, this gave the cane developed into a sturdy young stool and well developed root system. Furthermore, it would be served to control weeds during the early vegetative period. Even though sugarcane can grow on heavy or light soils but they must be deep and good drainage. In this case, subsoiling is needed to improve soil structure. Nowaday, particularly in large scale farms, frequent weeding by tractor drawn cultivators are used and combined with single spray of certain promising herbicides. These are usually enough for weed control throughout growing season and gave higher yield production. However, manual weeding is still propularily used during the dry season especially in small scale farms but it requires many labours and increase wages particularly, unfavourable weather condition of wet season, it could not do in time, so hand weeding is inefficient. Due to these reasons, in farmer plots which were conducted during rainy season, yield was lower whereas weeding cost was higher than integrated weed management plot by 39.38 tons/ha and US$ 65.97/ha, respectively.

## CONCLUSION

Weed control in sugarcane required both mechanically and with herbicides. The careful land preparation necessary for planting and gave a good control of weeds. Soil should be ploughed and exposed for 2 weeks, then harrowed before planted, could reduced weed infestation 16.3-52.5%, growth and yield were also higher than that from an improper soil preparation plots.

In farmer field trial, the integrated weed management by means of subsoiling, ploughing, harrowing and made soil surface into a fine tilth. The cane-setts were planted, then that atrazine at the rate of 3.0 kg ai/ha was applied over the furrows after watered. These operation could control weeds nearly 90% at least 4-6 weeks. Thereafter, off-barring and hilling-up by cultivators should be practiced 1-2 times before cane-leaves were closed-in. This method gave excellent weed control and yield of 90.63 tons/ha whereas conventional method actually consisting of pre-emergence herbicide and monthly hand weeding during the first four months gave yield of 51.25 tons/ha. Furthermore, total costs of manual weeding was higher than mechanical weed control approximately US$ 65.97/ha.

Table 5. Comparison of investment costs for planting in integrated weed management (IWM) and farmer plots as Nakorn - Pathom Province, 1986-1987.

| Items | Costs (US$/ha) | |
|---|---|---|
| | IWM[1] plot | Farmer plot |
| subsoiling | 23.15 | - |
| ploughing + harrowing | 92.59 | 92.59 |
| mechanical weeding | 23.15 | - |
| manual weeding | 8.10 | 97.22 |
| herbicides + application | 18.98 | 18.98 |
| planting | 15.05 | 37.04 |
| fertilizer | 89.35 | 151.39 |
| others[2] | 458.56 | 263.19 |
| Total costs | 728.93 | 660.41 |

1 IWM = Integrated weed management

2 costs for fungicide (triadimefon), harvesting, loading and truck

Table 6. Comparison of plant height, tillering, yield, income and profit for plant cane in integrated weed management and farmer plots at Nakorn - Pathom Province, 1986-1987.

| Items | IWM[1] plot | Farmer plot |
|---|---|---|
| plant height (cm) | 289.50 | 241.40 |
| tillering (tiller/ha) | 55400.00 | 46556.25 |
| C.C.S. | 13.75 | 13.22 |
| Yield (tons/ha) | 90.63 | 51.25 |
| Total costs (US$/ha) | 728.93 | 660.41 |
| income[2] (US$/ha) | 1289.66 | 729.29 |
| profit (US$/ha) | 560.73 | 68.88 |

1 IWM = Integrated weed management

2 Value of the yield at a price of US$ 14.23/ton

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# KILL AND REGROWTH FROM RHIZOME FRAGMENTS OF *IMPERATA CYLINDRICA* (L.) RAEUSCHEK AFTER FOLIAR APPLICATION OF GLYPHOSATE AND IMAZAPYR

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## ABSTRACT

An experiment was performed to assess the effects of glyphosate and imazapyr on the regrowth of rhizome buds at five and eight days foliar application. The treatments were an untreated control, glyphosate 2.88 kg ae/ha, imazapyr at 0.50 kg ae/ha and imazapyr at 0.75 kg ae/ha. After the rhizomes were sampled from the field, the apical shoot was excised and the rhizomes were divided into 4-noded sections in order to stimulate bud growth and development. Both glyphosate and imazapyr caused a drastic reduction in the length of the leafy regrowth from the excised apical end at five days after foliar application. At eight days after application, regrowth was nil for both herbicides. Glyphosate caused a reduction in the number and total length of secondary rhizomes and shoots for the 5-days batches of rhizomes. Imazapyr was more effective, and it completely suppressed the development of rhizome buds so that there was an absence of bud development from rhizomes sampled five or eight days after foliar spraying.

## INTRODUCTION

*Imperata cylindrica* (L.) Raeuschel or *lalang* is one of the ten most important weeds of plantation crops in Malaysia. During the last decade, glyphosate, a translocated herbicide, has been used extensively to control the weed. More recently, another translocated herbicide, imazapyr, has been reported to provide long lasting control of this noxious weed (1, 11). However, little is known of the regenerative potential of the rhizome buds after the foliar application of imazapyr. Knowledge of the rapidity to kill rhizomes can help the practical planter to make decisions on post-spraying operating like rotavation, clearing, burning, slashing or fertilizer application.

The main objective of this experiment is to determine how fast glyphosate and imazapyr kills the rhizomes of the weed.

## MATERIALS AND METHODS

**Experimental site** The site in MARDI Research Station was colonized uniformly by *I. cylindrica* for about 10 years. The soil was peat which had an organic matter of 85 per cent and a pH of 3.8. The area was well drained throughout the experimental period. The weed, 1.4 m in height, was in its vegetative stage at the time of spraying. The density of the weed was 130-190 shoots/$m^2$ and approximately 5 percent of these shoots were brown in colour.

**Design and treatments** A randomised complete block design with an untreated control (no weeding, A0), and three chemical treatments (A1 : glyphosate at 2.88 kg ae/ha, A2 : Imazapyr 0.50 kg ae/ha and A3, imazapyr 0.75 kg ae/ha) were employed. Plot size was 5 m x 8 m, but only the inner 4 m x 7 m was used for evaluation of treatment effects. The gap between the plots was 1.7 m and this area was cleared manually.

**Application of herbicides** The herbicides Roundup (A1) and Assault 250 A (A2 or A3) were mixed with tap water and sprayed in 450 litres/ha using a knapsack sprayer fitted with a fan jet (5/64 inch nozzle). Application was performed on 19th, November, 1986 on a slightly cloudy day. A very slight drizzle occurred approximately 45 minutes after spraying both herbicides and it lasted three minutes, but one hour after spraying the weather was dry and sunny. The later, weather lasted at least 3 hours and no rain occurred during the subsequent 20 hours.

**Sampling of rhizomes** Each field plot was divided into two sub-plots for sampling of shoots and rhizomes at 5 and 8 days after spraying. From each subplot, 10 shoot-rhizome systems were sampled but only five of similar rhizome length, width and number of nodes were selected. The apical shoot was excised, and the scale leaves of the rhizome were removed. The initial numbers of nodes/rhizome and the length of the rhizomes were recorded. Each rhizome was fragmented into 4-noded sections because an earlier study has shown that 4-noded sections had a higher percentage of sprouting success than 1 or 2-noded sections (4). These sections were placed in a row in large plastic trays lined with moist filter paper (Whatman no. 1), and covered with polythene in order to preserve the humidity. All axillary rhizomes and shoots which were present of the start of the experiment were excised, leaving behind rhizome buds.

**Assessment of treatment effects**

**Apical regrowth** At 20 days after sectioning, the length of the apical regrowth was measured from the excised end to the tip of the new shoot.

**Secondary regrowth** Some buds grew out and developed into shoots or rhizomes exceeding 5 mm in length. Their number and length were recorded at 20 days after sectioning.

**Decay of primary rhizomes and buds** During the 30 days period observations were carried out on the colour of rhizome sections and the buds which did not grow out.

**Percentage control of the weed** In the field plots, the percentage control of the weed was recorded at four months after spraying.

## RESULTS

**Initial number of nodes and length of primary rhizomes** Results showed that there were non-significant differences in the number of nodes and length of the rhizomes at the start of the experiments for both batches of rhizomes (Table 1).

**Length of apical regrowth** Both glyphosate and imazapyr caused a drastic reduction in the length of the apical regrowth at 20 days after sectioning (Table 1). At five days after herbicide treatment, rhizomes from the untreated control had a length of 47.5 cm compared with 6.4cm (glyphosate), 2.1 cm (imazapyr at 0.5 kg) and 1.9 cm (imazapyr at 1.0 kg). Differences among chemical treatments were however not significant. At eight days after herbicide treatment, only rhizomes from untreated control showed regrowth and there was no regrowth from rhizomes sampled from herbicide treated plots (Table 1).

Table 1. Initial number of nodees and length of primary rhimary rhizomes, length of apical regrowth, and number and total length of rhizomes and shoots per primary rhizome.

| Treatments (dosages in kg ae/ha) | Mean initial number of nodes per primary rhizome | Mean initial length per primary rhizome (cm) | Mean length of apical regrowth at 20 days (mm) | Mean number of secondary rhizomes and shoots per primary rhizome at 20 days | Mean total length of secondary rhizomes and shoots rhizome at 20 dats (mm) |
|---|---|---|---|---|---|
| First batch | | | | | |
| A0 : Untreated control | 30.6 | 43.5 | 47.5 a | 3.7 (2.2) a | 136.7 (11.5) a |
| A1 : Glyphosate at 2.88 kg | 32.9 | 45.4 | 6.4 b | 0.7 (1.4) b | 6.1 (2.7) b |
| A2 : Imazapyr at 0.50 kg | 31.9 | 45.8 | 2.1 b | 0 (1.0) c | 0 (1.0) b |
| A3 : Imazapyr at 0.75 kg | 31.4 | 44.9 | 1.9 b | 0 (1.0) c | 0 (1.0) b |
| 5% L.S.D. | n.s. | n.s. | * | (*) | (*) |
| Second batch | | | | | |
| A0 : Untreated control | 34.3 | 51.7 | 53.0 | 3.6 (2.2) a | 172.9 (11.8) a |
| A1 : Glyphosate at 2.88 kg | 34.9 | 51.8 | 0 | 0.4 (1.2) b | 2.9 (1.8) b |
| A2 : Imazapyr at 0.50 kg | 33.5 | 48.9 | 0 | 0 (1.0) b | 0 (1.0) b |
| A3 : Imazapyr at 0.75 kg | 33.1 | 49.5 | 0 | 0 (1.0) b | 0 (1.0) b |
| 5% L.S.D. | n.s. | n.s. | n.a. | (*) | (*) |

First batch sampled 5 days after field application
Second batch sampled 8 days field application
Values in breckets are transformed (x + 1)
n.s. = not-significant;
* = significant at P=0.05
n.a. = not analysed
Values followed by the same letter in the same column are not significantly different

**Number of secondary rhizomes and shoots that sprouted at 20 days after sectioning** At five days after herbicide treatment, the first batch of rhizomes from the untreated control had an average of 3.7 secondary rhizomes and shoots per primary rhizome compared with 0.7 per primary rhizome for glyphosate treatment (Table 1). In contrast, imazapyr treatment resulted in absence of bud sprouting for both batches of rhizomes. Both glyphosate and imazapyr caused a significant reduction in bud sprouting.

**Total length of secondary rhizomes and shoots at 20 days after sectioning** For the first batch of rhizomes, both herbicides caused a significant reduction in the total length of secondary sprouts. Table 1 shows that the total length was 136.7 cm for the untreated control compared with 6.1 cm in glyphosate treatment. The same trend was observed for the second batch of rhizomes, i.e. both glyphosate and imazapyr caused a significant reduction in apical regrowth when compared with the untreated control.

**Observations on rhizome sections** During the initial 20 days period, some rhizome sections turned green or purple. After 20 days, some sections started to decay i.e. turned soft and brown or black. At the conclusion of the experiment i.e. 20 days after sectioning, some buds on sections obtained from control plots as well as plots sprayed with glyphosate or imazapyr were white or pale yellow or slightly brown indicating that they might still be viable; apparently they did not grow to lengths beyond 5 mm.

Many buds and secondary rhizomes from rhizome sections that were obtained from plots treated with imazapyr were soft and brown at 20 days after sectioning. However, some buds and secondary rhizomes from rhizome sections that were obtained from plots treated with glyphosate were soft and brown at the same period.

**Control of *I. cylindrica* in the field** At four months after application, both glyphosate and imazapyr caused a significant reduction in the control when compared with untreated plots (Table 2). Differences between plots treated with glyphosate and imazapyr were significant but there was no significant difference in the percentage control at the two dosages of imazapyr.

Table 2. Percentage of *I. cylindrica* by glyphosate and imazapyr in the field at months after application.

| Treatment | % Control |
|---|---|
| A0 Untreated | 9.3 a[3] |
| A1 Glyphosate at 2.88 kg ae/ha[1] | 88.0 b |
| A2 Imazapyr at 0.50 kg ae/ha[2] | 97.3 c |
| A3 Imazapyr at 0.75 kg ae/ha[2] | 99.5 c |
| s.e. of a treatment mean | 0.707 |

1 Average of 10 new green shoots/plot.

2 Absence of green shoots in all the plots. Some mother shoots were partially-green.

3 Values followed by the same letter are not differ significantly.

## DISCUSSION

Three main findings have emerged from the present investigations. First, apical regrowth from the cut end of the primary shoot was completely suppressed by both glyphosate and imazapyr at 8 days after field spraying. It is interesting to note that at 8 days after foliar application of both herbicides only slight chlorosis or scorching of leaves was detected, and yet both herbicides have dramatic effects on the elongation of the apical shoot. Glyphosate is known to suppress leaf elongation within four days (4) while imazapyr stopped the growth of plants within hours after treatment (9).

Second, none of the buds in rhizomes sections obtained from plots treated with imazapyr grew to lengths beyond 5 mm, indicating that the herbicide and/or its metabolites had caused cessation of growth of meristematic cells. another possibility is that imazapyr could have caused bud dormancy but this appears unlikely since many buds showed decay. The herbicide is known to inhibit the synthesis of DNA in corn within eight hours after treatment (10). Within 5 days of foliar application of imazapyr on *I. cylindrica*, secondary growth on its rhizomes was inhibited.

A similar trend was observed for rhizome for rhizome samples from glyphosate treated plots. However, there was still some growth of buds on rhizome sections from plots at 5 or 8 days after the foliar application of glyphosate, but elongation was drastically reduced. The section tests confirmed the viability of some of the buds but fewer buds per rhizome developed. Previous studies have shown similar results (4, 5).

Third, the extensive decay of buds and sections from imazapyr treated plots suggested basipetal translocation of the herbicide. Fine et al. (3) has reported that imazapyr is readily absorbed by the foliage and roots and is translocated rapidly throughout the plant and to the underground organs, thus preventing regrowth. The data on Table 1 showed that there was no regrowth from rhizome sections from imazapyr treated plots. Table 2 shows that the control in field plots was 97.3 to 99.5% four months after imazapyr treatment and this confirmed the effectiveness of imazapyr in preventing regrowth (6).

Glyphosate caused a drastic reduction in secondary bud growth and development and it appears that its effect is more pronounced at eight days than at five days after foliar spraying (Table 1). Since glyphosate is translocated preferentialy to meristematic regions (2,8) it is likely that the developing buds of the rhizome sections received the glyphosate and/or its metabolites, resulting in the death of some buds (4). The kill of buds is related to the concentration of glyphosate in the buds and it may well be that some buds had a sub-lethal concentration of glyphosate and were therefore able to develop further. This would account for the regrowth of *I. cylindrica* in the field plots four months after spraying (Table 2).

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# CONTROL OF *IMPERATA CYLINDERICA* USING ROUNDUP HERBICIDE- MIST BLOWER APPLICATION TECHNIQUE

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## ABSTRACT

Roundup® herbicide, a post-emergence product containing 36% glyphosate as isopropylamine (IPA), was sprayed on *Imperata cylindrica* using a Maruyama mist blower. The results at 180 DAT showed that Roundup at 0.72-1.08 kg ae/ha with either 10 or 20 L/ha application volume provided 90% *Imperata cylindrica* control. At the recommended rate of 2.16 kg ae/ha using conventional application technique of 650 L/ha spray volume, the control was also 90%. The injury of 2 1/2-year old oil palm or rubber where experiments were conducted was not observed indicating that spray drift was not a problem. The Maruyama mist blower can deliver a very fine uniform spray droplet to the target leaf surface with options of LV application.

## INTRODUCTION

The hard-to-kill tropical *Imperata cylindrica* is well distributed nation-wide in the crop growing area, water reservoir, waste land and along the roadside. To control this weed, using Roundup, a post-emergence herbicide containing 36% ae isopropylamine (IPA) salt of glyphosate, proved to be the most economical means. The normal recommended rate for complete control is 2.16 kg ae/ha with 625 L/ha application volume. The conventional volume of 625 L/ha, which was later on called the high volume (HV) spray, is considered an improper way of spraying Roundup. HV method consumes too much water and could create herbicide run-off from leaf surface. The low volume (LV) spray of 10-250 l/ha application volume is introduced instead, using several kinds of LV technique/applicators. This paper provides information on the use of Roundup for *Imperata cylindrica* control with mist blower capable of delivering water volume of 10-20 L/ha.

## MATERIALS AND METHODS

The studies were conducted in 2 1/2-year old rubber and oil palm in Southern Thailand from January 1986 to March 1987. For rubber, the experiments were laid out in the randomized complete block design with three replications having 120 sq in. plot size for each treatment. For oil palm, the nonreplicated trial was conducted in 140 sq m. treatment plot size each.

The rates of Roundup (from Monsanto Agricultural Company) ranges from 0.72-2.16 kg ae/ha applied with either HV or LV application method. The HV technique was done by the use of knapsack sprayer with flood jet nozzle (red polijet) delivering water volume of 625 L/ha whereas that of LV was executed through mist blower (Maruyama MD 300). The latter with modified spray nozzle having orifice size of 0.5 and 0.8 mm consumes water volume of 10 and 20 L/ha respectively.

The spray of Roundup was done onto the healthy *Imperata cylindrica* of 1-1.5 m in height and of 150-200 plants/$m^2$ in density, after which the control performance was assessed monthly. The evaluation was terminated for the reasons that either the plot was fully takenover by the germination of broadleaf weeds or by the strong encroachment of the adjacent legume cover crop.

## RESULTS AND DISCUSSION

The performance of Roundup for *Imperata cylindrica* control using mist blower was shown in Table 1. The results of this dry season trial showed that Roundup at all tested rates of 0.72, 1.08 and 1.44 kg ae/ha with either 10 or 20 L/ha application volume provided excellent control (86-96%) at 180 days after spraying. The performance of the 20 L/ha application volume slightly better than that of the 10 L/ha as far as the product efficacy and consistency are concerned. The slightly superior of the former is presumable due to a better spray coverage.

A good control by Roundup applied through mist blower even at rate lower than that of normal recommendation (2.16 kg ae/ha) could have resulted from two possibilities. The first one could arise as a fine uniform droplets and low water volume consumed by mist blower creates no run-off, in general or no herbicide-waste, in particular, from leaf surface. The second was that the more concentrate surfactant solution could enhance the absorption of Roundup by leaf. Besides, the prolonged dry weather after Roundup application, which was done in late rainy season, could be one of those factors affecting the control performance.

The results in Table 2 were likely to support the above idea. Roundup 0.72-1.08 kg ae/ha sprayed in the middle of rainy season provided control only 45-68% at 180 DAT, much lower than those obtained in the dry season. At the recommended rate of 1.44 kg ae/ha with 20 L/ha spray volume, the control was 78% at 180 DAT compared to 96% control of the same but conducted in the dry season. For HV spray, Roundup 2.16 kg ae/ha showed 88% control while a lower rate of 1.44 kg ae/ha provided only 67% control (Table 2).

The outcome from dry and wet season experiments in rubber reveal the fact that the control of Roundup on *Imperata cylindrica* varies considerably according to season. Regardless of other factor influencing herbicide activities, Roundup applied in late rainy season or early dry season provides better control than that of same ae rate but applied in the early or middle of rainy season. This can verify as to how Roundup 1.44 kg ae/ha provided good control on *Imperata cylindrica* shall the product be sprayed at certain period of time.

In oil palm, the results from wet season are quite different from those obtained in rubber. Roundup 0.72-1.44 kg ae/ha with 10 L/ha spray volume showed 80-90% control at 90 DAT (Table 3). For HV application method, Roundup 1.08-2.16 kg ae/ha provided 90-100% control. A good control by Roundup (even at low rates) with either LV or HV spraying methods could attribute from a lower density of weed infestation compared to that in rubber. The unprecedented drought period after spraying could be one of many reasons for makig good control.

Table 1. The control performance of Roundup on *Imperata cylindrica* using mist blower of 10 L/ha vs 20 L/ha application volume (rubber, dry season).

| Roundup Rate Kg ae/ha | Application Volume L/ha | % Control at (Days After Spraying) 15 | 60 | 120 | 180 |
|---|---|---|---|---|---|
| 0.72 | 10 | 73 | 87 | 80 | 86 |
| 1.08 | 10 | 83 | 98 | 94 | 92 |
| 1.44 | 10 | 93 | 98 | 96 | 95 |
| 0.72 | 20 | 85 | 90 | 80 | 91 |
| 1.08 | 20 | 90 | 95 | 96 | 96 |
| 1.44 | 20 | 93 | 100 | 96 | 96 |

Table 2. The control performance of Roundup on *Imperata cylindrica* using LV of 20 L/ha volume vs HV of 625 L/ha (rubber, wet season).

| Roundup Rate Kg ae/ha | Application Volume L/ha | % Control at (Days After Spraying) 15 | 60 | 120 | 180 |
|---|---|---|---|---|---|
| 0.72 | 20 | 70 | 72 | 57 | 45 |
| 1.08 | 20 | 75 | 78 | 72 | 68 |
| 1.44 | 20 | 95 | 88 | 84 | 78 |
| 1.44 | 625 | 85 | 77 | 77 | 67 |
| 2.16 | 625 | 97 | 92 | 90 | 88 |

Table 3. The control performance of Roundup on *Imperata cylindrica* using LV of 10 L/ha volume vs HV of 625 L/ha (oil palm, wet season).

| Roundup Rate Kg ae/ha | Application Volume L/ha | % Control at (Days After Spraying) 30 | 60 | 90 |
|---|---|---|---|---|
| 0.72 | 10 | 80 | 90 | 80 |
| 1.08 | 10 | 90 | 100 | 90 |
| 1.44 | 10 | 85 | 95 | 90 |
| 1.08 | 625 | 70 | 90 | 90 |
| 1.44 | 625 | 85 | 95 | 95 |
| 2.16 | 625 | 90 | 100 | 100 |

As far as crop injury is concerned, we could not detect any in both crops when the application was made in such a way to avoid direct contact. However, applying Roundup with mist blower should be conducted with caution, according to preliminary results not reported here. Uneven or spotty controls were sometimes evident. Presumably, it arose from misconducting of spraying practice. Workers should be intensively trained to ensure maximum benefit of Roundup mist blower application technique for *Imperata cylindrica* control.

## CONCLUSION

1. Roundup sprayed with mist blower is efficaceous. The 20 L/ha spray volume resulted in a better control than 10 L/ha.
2. Roundup spray conducted in late rainy season normally provided better control than that conducted in the middle of rainy season.
3. Roundup at recommended rate of 2.16 kg ae/ha with HV spray can be reduced to 1.44 kg ae/ha with LV spray but still provide effective control.
4. Proper application of Roundup using mist blower is unlikely to cause any obvious crop injury.
5. Although the mist blower is an alternative means of LV equipment, practice for use must be fulfilled to ensure maximum benefit.

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# THE EFFECTS OF TILLAGE AND WEEDING SYSTEM ON WEED CONTROL AND THE YIELDS OF FOUR HIGHLAND VEGETABLES

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## ABSTRACT

Highland vegetable growing methods in Indonesia use very intensive tillage or land preparation and manual weeding. Based on the introduction of zero-tillage and minimum tillage systems in the upland food crop production as an effort to converge soil and moisture, an experiment to adopt the system for highland vegetables has been conducted in the growing centre Lembang, West Java, in 1986. Tomato, cabbage, chinese cabbage, and red chili have been tested in the trial. Two methods of land preparation combined with herbicide application without or with manual weeding, have been used with a split-plot design. Results revealed that the zero-tillage system with glyphosate pre-planting application produced equal yields of tomato, cabbage, chinese cabbage and red chili to the conventional full tillage, followed by 2 manual weedings or application of alachlor before planting. These preliminary studies show the possibility of zero-tillage system for highland vegetables by application of glyphosate to control weeds before planting, without reducing yield.

## INTRODUCTION

Tomato (*Lycopersicum esculentum*), cabbage (*Brassica oleraceae*), chinese cabbage (*Brassica chinensis*) and red chili (*Capsicumm annum*) are the vegetables commonly grown in the cool highland area of Indonesia. Most of the area are located in volcanic soil with rolling topography or in steep slopes (4).

The method of land preparation is totally by intensive manual hoeing followed by 2 manual weedings after planting, to control the weeds and to loosen the soil. By these methods, the surface erosion is very severe, eventhough sometimes the cultivated fields are narrowly terraced. As a consequence of this method the fertility of soil has been decreased very fast so that the farmers have to provide heavy manuring and fertilization in order to maintain the production level (1).

The total mandays used for the growing vegetables are very high. In the 1983/1984 survey by the Department of Agriculture, they found the total manday requirements as follows : tomato 348, cabbage 361, chinese cabbage 164 and the red chili is 611 mandays per hectare. The main portion of the mandays are used for land preparation and weedings (5).

To solve this problem, the zero tillage system which has been proven to have benefit in food crop production in reducing manday need and conserving soil and moisture could be used in vegetable growing (2, 6). To prove this hypothesis a trial was conducted in Lembang Horticultural Station, West Java during wet season from January to April 1986.

## MATERIALS AND METHODS

A split-plot designed experiment was conducted at the Horticultural Research Station Margahayu, Lembang, West Java for the preliminary studies of zero tillage for vegetable growings. The main plots were; (1), Zero tillage (no tillage) prepared by the application of glyphosate at 1.44 kg ae/ha to control the weeds before planting (30 DBP), followed by 2 manual weedings at 20 and 40 DAP. The crop seedlings were planted at 30 DAP. (2), Zero tillage (no tillage) by glyphosate at 1.44 kg ae/ha without manual weeding follow ups. (3), Full tillage (traditional 2 hoeings plus 1 harrowing), followed by alachlor application at 4 DBP. (4), Full tillage (as no. 3) followed by 2 manual weedings at 20 and 40 DAP.

As the sub-plots 4 vegetables, tomato, cabbage, chinese cabbage and red chili were planted with 3 replications. Sub-plot size was 7.5 X 10 sq metres each with the plant population as follows ; tomato 275, cabbage 240, chinese cabbage 300 and red chili 480 plants/plot. 35,750;31,200;39,000;62,400 plants/ha.

The crops were fertilized with 30 MT of manuring (cow dung), 150 kg of N, 100 kg of $P_2O_5$ and 100 kg of $K_2$ O. To generate data we assessed the percentage of weed coverage at 45 and 60 DAT, fresh weight of weeds at 60 DAT in kg/plot, and the yield of each crop at harvest (kg fresh weight/ha).

## RESULTS AND DISCUSSION

The main weeds which existed before planting were *Galensoga parvifolia*, *Ageratum conizoides*, *Borreria alata* or *laevis* and *Cynodon dactylon*. The total weed coverage was 100% and 30 cm in average height at 0 DAT.

Weed converage assessment at 45 DAP of all crops (sub-plots) revealed that no tillage system followed by 2 manual weedings, provided equal weed control to the full traditional tillage, either in combination with alachlor application or with 2 manual weeding follow ups. No tillage without weeding, provided less weed control. However, at 60 DAT all treatments showed different weed coverage significantly. The best control was by full tillage followed by 2 manual weedings (Table 1 ). No tillage only, caused the biggest mean weed coverage (75 %).

Assessment of fresh weight of weeds revealed that the lowest weight was provided by the full tillage + alachlor. No tillage without weeding caused the highest weight level (Table 2).

It is clear that the effects of no tillage followed by 2 manunal weedings is equal to the traditional full tillage followed by 2 maunal weedings as the standard practice.

Now look at treatment effects on the vegetable production on tomato and red chili, all 4 treatments did not give significant differences in yield, but no tillage on cabbage and chinese cabbage significantly caused the lowest yields (Table 3). Those three other treatments provided equal yields of the cabbages. It means that these crops really need weeding either manually or chemically with a pre-emergence herbicide.

Table 1. Effects of land preparation method and weeding system on weed growth of vegetable production.

| Treatment | Mean Weed Coverage (%) At 45 DAP[1] | | | | |
|---|---|---|---|---|---|
| | Tomato | Cabbage | Ch. Cabbage | Red Chili | Mean of 4 Crops |
| 1. No Tillage fb 2 manual weedings | 18.5 a[2] | 22.6 a | 21.2 a | 26.5 a | 22.2 a |
| 2. No Tillage, no weeding | 61.2 d | 71.5 c | 60.0 c | 68.8 c | 65.4 b |
| 3. Full Tillage + Alachlor | 37.2 c | 35.2 b | 35.2 b | 50.0 b | 39.4 a |
| 4. Full Tillage fb 2 manual weeding | 28.8 b | 19.2 a | 23.8 a | 23.8 a | 24.1 a |
| | Mean Weed Coverage (%) At 60 DAP | | | | |
| 1. No Tillage fb 2 manual weedings | 45.0 b | 35.2 a | 46.9 b | 37.2 a | 41.0 b |
| 2. No Tillage, no weeding | 71.5 d | 75.2 c | 77.0 c | 77.0 c | 75.2 d |
| 3. Full Tillage + Alachlor | 59.0 c | 56.8 b | 46.9 b | 68.8 b | 57.9 c |
| 4. Full Tillage fb 2 manual weeding | 37.2 a | 34.2 a | 37.2 a | 33.0 a | 35.4 a |

1 DAP = Days After Planting

2 Small letters : similar letters means no significant differences.

Table 2. The effects of land preparation method and weeding system on the weed growth at harvest time.

| Treatment | Fresh Weight of Weeds - Kg/Plot | | | | |
|---|---|---|---|---|---|
| | Tomato | Cabbage | Ch. Cabbage | Red Chili | Mean |
| 1. No Tillage fb 2 manual weedings | 222.3 | 25.0 | 15.6 | 13.6 | 69.1 |
| 2. No Tillage, no weeding | 139.3 | 157.0 | 158.6 | 14.5 | 117.3 |
| 3. Full Tillage + Alachlor | 30.8 | 93.8 | 18.8 | 13.3 | 39.1 |
| 4. Full Tillage fb 2 manual weeding | 144.3 | 71.6 | 11.3 | 14.0 | 60.3 |

Table 3. Effects of land preparation method and weeding system on the vegetable production.

| Treatment | Fresh Weight of Weeds - Kg/Plot | | | | |
|---|---|---|---|---|---|
| | Tomato | Cabbage | Ch. Cabbage | Red Chili | Mean |
| 1. No Till fb 2 manual weedings | 6733 a | 13066 b | 24687 b | 2234 a | 11680 |
| 2. No Tillage, no weeding | 6733 a | 933 a | 7154 a | 2322 a | 4285 |
| 3. Full Tillage + Alachlor | 4106 a | 9866 b | 23106 b | 1787 a | 9716 |
| 4. Full Tillage fb 2 manual weeding | 6053 a | 12686 b | 23243 b | 2128 a | 11022 |

By looking at the average yield of all tested vegetable crops, no yield reduction was encountered by the no-tillage-method as long as the weeding and other crop maintenance are followed as required. Beside that, no-tillage method has the major advantages of conserving soil and moisture and reducing the labour need.

## CONCLUSION AND RECOMMENDATION

The trial results lead us to conclude as follows: Vegetable production in highland area could be accomplished without heavy land preparation as now practiced in Indonesia. They can be grown with no tillage without reducing the yield as long as the maintenance includes weedings with pre-emergence herbicide or manually.

To reconfirm the results, large scale evaluation is required in the centre of the production area.

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# RICE YIELD LOSSES CAUSED BY TRANSPLANTED *ECHINOCHLOA GLABRESCENS* AND POSSIBLE CONTROL METHODS

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## ABSTRACT

Seedlings of *Echinochloa* spp. are often transplanted into the field together with rice (*Oryza sativa* L.) seedlings because they are morphologically similar. When *Echinochloa glabrescens* Munro ex Hook. f. that was transplanted with rice was not controlled, yield losses ranged from 7%, (when 5% of the rice hills were infested with the weed) to 87% (when 50% were infested). Manual weeding of transplanted weed seedlings is laborious, time-consuming, ineffective, and impracticable. Quinclorac (3,7-dichloro-8-quinoline carboxylic acid) was the most promising herbicide for controlling transplanted *E. glabrescens*. Quinclorac, quinclorac + bensulfuron (methyl 2-[[(4,6- dimethoxy pyrimidin-2-yl) aminocarbonyl] aminosulfonyl methyl] benzoate), and quinclorac + thiobencarb (S-[(4-chlorophenyl) methyl] diethyl carbamothioate) were most effective in controlling transplanted *E. glabrescens* and other weed species that emerged after the rice had been transplanted.

## INTRODUCTION

Seedlings of grasses such as those of *Echinochloa* species cannot be easily distinguished from rice seedlings during the early growth stages. Most often they are transplanted into field with the rice seedlings (3, 6, 9) even by the most observant farmers (11). Tang and Wu (10) reported that 7.7% of the barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv. spp. *hispidula* (Retz.) Honda # ECHCG] growing in rice fields was introduced from seedling nurseries during transplanting.

Transplanted weeds are highly competitive (2, 9), difficult to control by hand weeding, and cannot be controlled by selective herbicides (7). In a pot experiment with four rice seedlings per hill, Jiang (2) reported yield reductions of 48% when one barnyardgrass plant competed with four rice seedlings, 65% when two plants competed, and 71% when three plants competed with the rice seedlings. In a farmer's field, Rao and Moody (9) found that when 29% of the hills were infested with *E. glabrescens*, there was a 22% yield loss.

This study was conducted to a) quantify the yield losses caused by the transplanting of *E. glabrescens* with rice, b) determine the effect of the age of transplanted *E. glabrescens* seedlings on their competetiveness against rice and on the performance of selective herbicides, and c) determine if *E. glabrescens* transplanted with rice, and other weeds occurring in the field, can be controlled with herbicides following transplanting.

## MATERIALS AND METHODS

Three experiments were conducted at the experimental farm of the International Rice Research Institute (IRRI) in 1986 and 1987. Land preparation consisted of one plowing and two harrowings in Experiments 1 and 3 and two plowings and three harrowings in Experiment 2, all at 1 week intervals. Twenty-day-old rice (cultivar IR62) seedlings were transplanted at a 20 x 20 cm spacing. Three rice seedlings, or two rice seedlings and one weed seedling were transplanted per hill.

Urea was applied at the rate of 100 kg N/ha. 50% basal and the remainder at panicle initiation. For insect pest control, 1.5% kg ai/ha monocrotophos (dimethyl *cis*-1- methyl-2-methyl- carbamoylvinyl phosphate) was sprayed as needed.

Grain yield, adjusted to 14% moisture, was determined from a 6 $m^2$ sample area in the center of each plot and is expressed in t/ha.

Details of each of the experiments follow.

**Experiment 1. Effect of different levels of weed infestation on crop yield** A randomized complete block design with four replications was used. There were nine weed infestation levels ranging from 0 to 50%. Plot size was 5 x 5 m. Weeds other than the transplanted *E. glabrescens* were removed by frequent hand weedings. *E. glabrescens* was sampled from a 1 $m^2$ sampling area 30, 60, and 90 days after transplanting (DAT) and at harvest, dried at 80°C for 48h, and weighed.

**Experiment 2. Control of weed seedlings of different ages** A split-plot design replicated four times was used. *E. glabrescens* seedlings (10, 15, and 20 days old) were assigned to the main plots and different weed control treatments to the subplots (see Fig. 1). The subplot size was 4 x 4 m. The level of infestation of *E. glabrescens* in all the plots except those maintained weed free was 30%. Weeds other than the transplanted *E. glabrescens* were removed by frequent hand weedings. The dry weight of *E. glabrescens* was determined 60 DAT using two 0.5 x 0.5 m quadrats per plot.

**Experiment 3. Control of transplanted *E. glabrescens* and other weed species** A randomized complete block design was used. There were fifteen treatments (see Fig. 2) and four replications. Plot size was 4 x 5 m. *E. glabrescens* was transplanted into 25% of the hills in all plots, except the weedy check, in which weeds were allowed to grow naturally, and the weed free plots. Weeds were sampled 60 DAT using a 1 x 1 m quadrat. They were separated into *E. glabrescens* growing in the rice hills and other weeds (grasses, broadleaves, and sedges), dried at 80°C for 48h, and weighed.

## RESULTS AND DISCUSSION

**Experiment 1** At all sampling times weed weight increased with percentage infestation. The dry weight of *E. glabrescens* increased up to 90 DAT when 5 to 40% of the hills were infested (Fig. 3). When 50% of the hills were infested, maximum weed dry weight was reached at 60 DAT and then it declined probably because of intraspecific competition. In all cases, the greatest increase in dry weight occurred between 30 and 60 DAT. At all infestation levels weed weight declined from 90 to 110 DAT due to senescence.

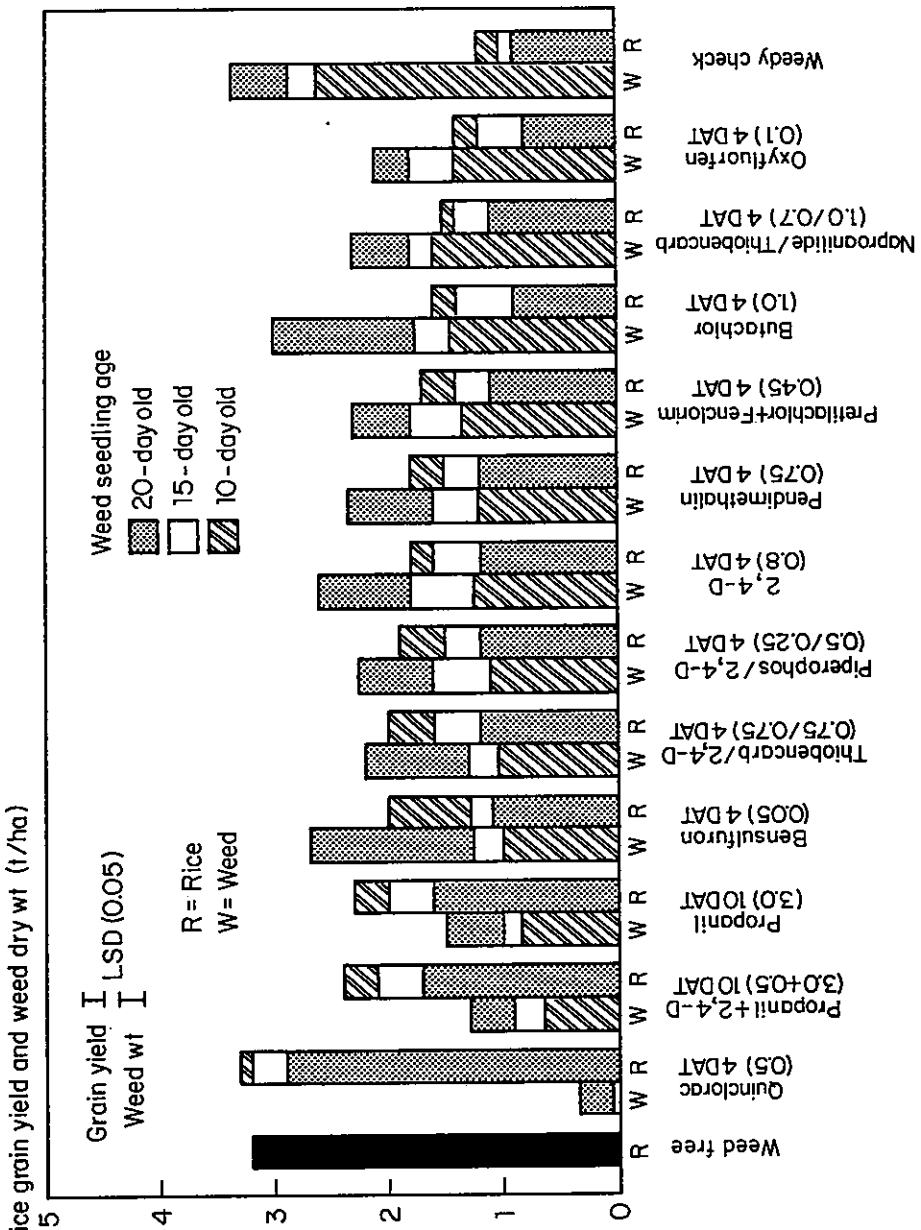


Figure 1. Effect of age of transplanted Echinochloa glabrescens and different weed control treatments on weed dry weight 60 days after transplanting (DAT) and rice grain yield. IRRI, 1986 late wet season.

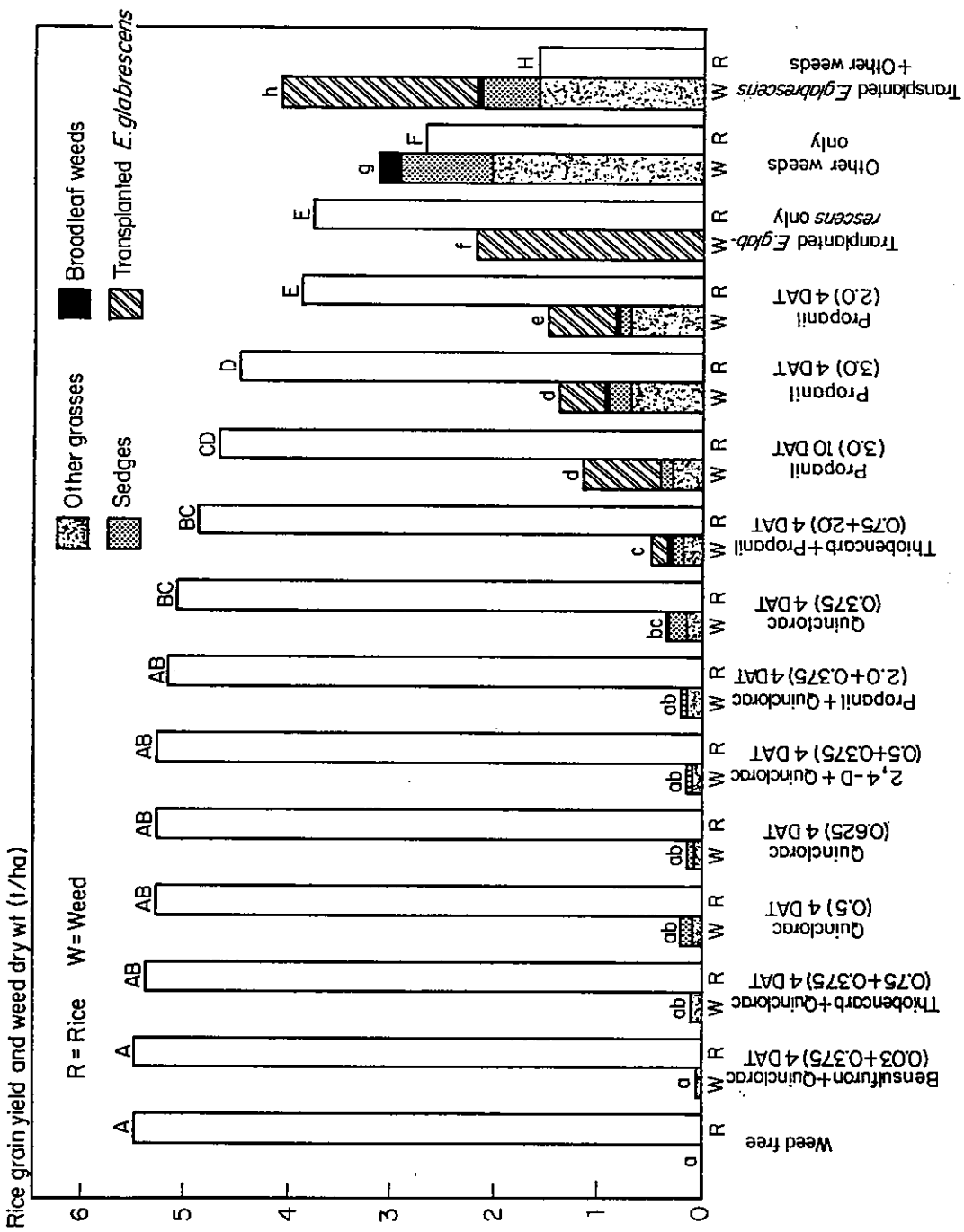


Figure 2. Weed weight and rice grain yield as affected by different weed control treatments. IRRI, 1987 dry season. Means followed by a common letter are not significantly different at the 5% level by DMRT.

Weed weight at all sampling times was negatively correlated with rice grain yield (Fig. 4). Yield loss ranged from 7% when 5% of the rice hills were infested with *E. glabrescens*, to 87% when 50% infested. For every 100 kg dry weight of *E. glabrescens* at 90 DAT, a loss of 52 to 108 kg grain yield/ha was observed, depending on the degree of weed infestation. Catizone (as cited in 1) reported 46 to 72 kg/ha rice yield loss for each 100 kg of *Echinochloa* dry matter. Jiang (2) found that yield loss due to transplanted *E. crus-galli* was caused by reduction in the number of effective panicles/hill, filled grains/panicle, and weight of grains/panicle.

**Experiment 2** All the herbicides reduced transplanted *E. glabrescens* dry weight significantly when compared to the weedy check. Degree of control decreased with increased age of transplanted weed seedlings. Quinclorac was the most effective herbicide (Fig. 1). It killed all the 10-day-old seedlings of *E. glabrescens* and reduced the dry weight of the 15-day-old seedlings by 99% and the 20-day-old seedlings by 90%.

The next most effective herbicides were propanil [*N*-(3,4- dichloro phenyl) propanamide], which reduced weed dry weight by 56%, and propanil + 2,4-D [(2,4-dichloro phenoxy) acetic acid] which reduced weed dry weight by 60%, the other herbicide treatments were relatively ineffective causing 52% or less reduction in the dry weight of transplanted *E. glabrescens*.

A highly significant negative correlation ($y=2.801 - 0.717X$, $r=-0.88^{**}$) was observed between weed dry weight and rice grain yield. Irrespective of the age of the weed seedlings, the highest grain yields were obtained when quinclorac was applied. The next highest grain yields were obtained with propanil and propanil + 2,4-D.

Lower grain yields and greater yield losses due to transplanted *E. glabrescens* were recorded in this experiment than in Experiment 1 at the same infestation level. This might have been due to drought stress that occurred at different times during this experiment.

**Experiment 3** *E. glabrescens*, smallflower umbrellasedge (*Cyperus difformis* L. # CYPDI), globe fingerush [*Fimbristylis miliacea* (L.) Vahl # FIMMI], and monochoria [*Monochoria vaginalis* (Burm. f.) Presl # MOOVA] were the major weeds that emerged after transplanting.

A number of herbicides and their combinations controlled transplanted *E. glabrescens* and the other weed species that emerged after transplanting (Fig. 2). The best weed control was obtained with quinclorac + bensulfuron. Other treatments that were nearly as effective as quinclorac + bensulfuron were thiobencarb + quinclorac, quinclorac (0.5), quinclorac (0.625), quinalorac + 2,4-D, and quinclorac + propanil. The higher efficacy of quinclorac in controlling transplanted *E. glabrescens* in this experiment compared to Experiment 2 may have been due to a water level of 2-5 cm maintained throughout crop growth in this experiment. In Experiment 2 it was difficult to keep the field flooded. The best results are obtained with quinclorac under flooded conditions(4).

A highly significant negative correlation was observed between weed dry weight and rice grain yield ($y=5.494 - 0.886X$, $r=-0.98^{**}$). The highest grain yield with a herbicide treatment was obtained with quinclorac + bensulfuron (Fig. 2). Other treatments in which yields were not significantly lower than the weed free check were quinclorac (0.5), quinclorac (0.625), quinclorac + 2,4-D, and quinclorac + propanil.

Yield losses due to transplanted *E. glabrescens* in this experiment, which was conducted in the dry season, were less than those observed in Experiment 1, which was conducted in the wet season. Moody (5) reported that weeds caused greater yield losses in the wet season.

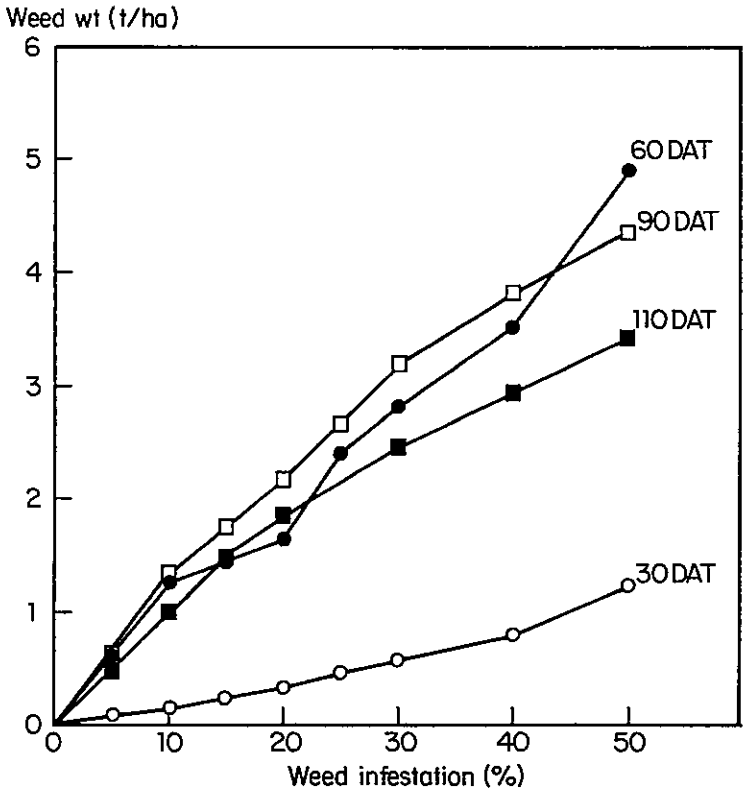


Figure 3. Dry weight of transplanted Echinochloa glabrescens at different times after transplanting at varying levels of infestation of the weed. IRRI, 1986 wet season. DAT = days after transplanting.

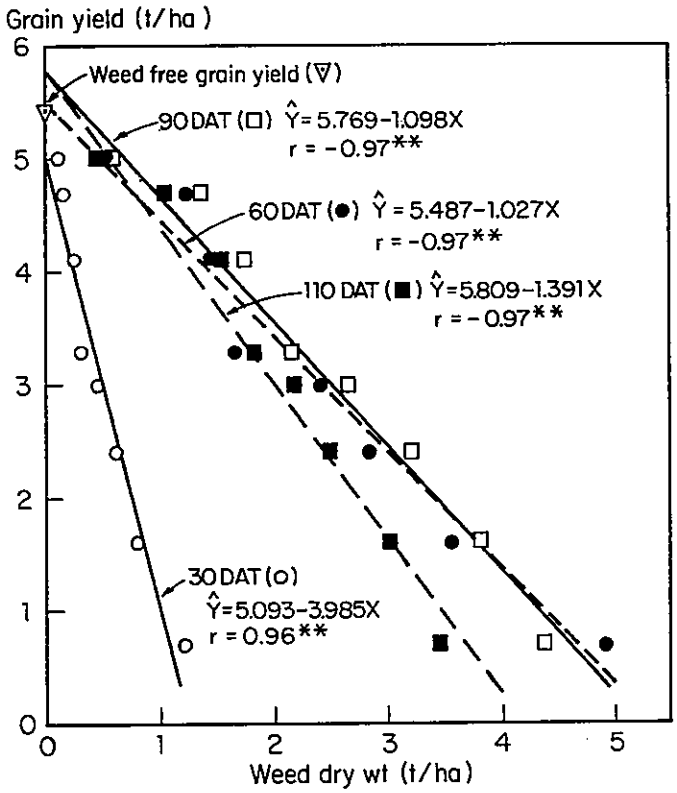


Figure 4. Relationship between dry weight of transplanted Echinochloa glabrescens at different times after transplanting and rice grain yield. IRRI, 1986 wet season. DAT = days after transplanting.

Transplanted weeds are highly competitive and difficult to control by hand weeding because of the difficulty in distinguishing them from rice. We have observed less than 40% of transplanted *E. glabrescens* seedlings were removed when hand weeding was done 20 DAT.

Both the rice and the weeds will be uprooted, if transplanted *E. glabrescens* is removed by hand pulling when it reaches a stage that it can be differentiated from rice. Cutting the weeds is time consuming and provides only partial control.

Preventing the transplanting of weed seedlings by controlling them in seedling nurseries (7, 8) or by using herbicides to control them after transplanting appear to be the only practical control methods. It appears that quinclorac applied alone or in combination with other herbicides can control transplanted *E. glabrescens* and other weed species that emerge after transplanting.

## ACKNOWLEDGEMENT

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# CONTROL OF BROADLEAF WEEDS WITH FLUROXYPYR IN SUGARCANE AND GRAIN SORGHUM IN NORTHERN NEW SOUTH WALES AND QUEENSLAND, AUSTRALIA

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## ABSTRACT

Fluroxypyr (STARANE* Trade mark of the Dow Chemical Co.) as the 1 - methyl heptyl ester was tested against broadleaf weeds in sugarcane and grain sorghum crops between 1982 and 1986. Fluroxypyr 150-300 g ae/ha gave excellant control of noogoora burr (*Xanthium pungens*), bathurst burr (*X. spinosum*), common thornapple (*Datura stramonium*), fierce thornapple (*D. ferox*), giant sensetive plant (*Mimosa invisa*), prickly African cucumber (*Cucumis metuliferus*), stinking passionflower (*Passiflora foetida*), phasey bean (*Macroptilium lathyroides*), spiny headed sida (*Sida acuta*), pink burr (*Urena lobata*), centro (*Centrosema pubescens*), lablab (*Lab lab purpureans*), blue billygoat weed (*Ageratum houstonianum*), annual ground cherry (*Physalis angulata*), wild gooseberry (*P. minima*), caltrop (*Tribulus terrestris*), pigweed (*Portulaca oleracea*), mintweed (*Salvia reflexa*) volunteer sunflowers (*Helianthus annuus*), and sesbania pea (*Sesbania cannabina*), Balsum pear (*Momordica charantia*), Swamp weed (*Melochia corchifolia*) and cowpea (*Vigna unguiculata*) and 400-600 g/ha controlled milkweed (*Euphorbia heterophylla*), large caltrop and bladder ketmia (*Hibiscus trionum*). Vines of the Convolvulaceae familly which include bellvine (*Ipomoea plebeia*), morning glory (*I. purpurea*), star of bethlehem (*I. quamoclit*), red convolvulus (*I. hederifolia*) and pink convolvulus (*I. triloba*) were only moderately susceptible to fluroxypyr 300 g/ha but were controlled with tank mixtures of fluroxypyr 200 g + 2,4-D 500 g/ha. Giant spider flowers (*Cleome hasslerana*), squareweed (*Spermacoce latifolia*), Amaranthus species (*Amaranthus cruentus* and *A. macrocarpus*) and giant pigweed (*Trianthema portulacastrum*), were not controlled by fluroxpyr at the rates tested. Tank mixtures of fluroxypyr 150 g + atrazine (750-1000) g/ha controlled *Amaranthus* spp. and Giant pigweed, increased the rate of brownout of all species and gave some pre-emergence control of thornapples. All of the above treatments were selective to sugarcane (*Saccharum officinarum*), grain sorghum (*Sorghum bicolor*).

## INTRODUCTION

Sugarcane and grain sorghum are two of the most important crops grown in the summer rainfall areas of Queensland and Northern New South Wales. Sugarcane cultivated on the humid tropical and sub-tropical coastal regions, is Australia's third most important crop (by value) after wheat and barley, while grain sorghum, grown further inland in the sub-humid region, is the most important of the summer cereal crops and accounts for nearly 10 percent of the annual global sorghum traded (7).

For many years phenoxy-based herbicides have provided cheap and efffective post-emergent control of broadleaf weeds in sugarcane and sorghum. However, in grain sorghum 2,4-D dicamba and picloram sometimes have impaired root development, (3, 4, 9-12), caused leaf curling (4, 10) affected grain weight, grain number and tillering (3, 4, 8-13) and delayed flowering (Walker, pers. comm.). Consequently grain sorghum yield reduction, of up to 40%, following post-emergent spraying with 2,4-D and 2,4-D/picloram has occurred (8). There has also been increased opposition to use of 2,4-D and 2,4,5-T in sorghum and sugarcane due to damage to nearby sensitive crops, together with human health concerns associated with their use.

Trial work in Australia between 1978 and 1982 confirmed selectivity of fluroxypyr to wheat previously reported from Europe (1, 2, 17) and demonstrated efficacy on *Polygonum* spp. In 1982 the field research programme was expanded to include weed control in grain sorghum and sugarcane. This paper reports results of weed control and crop selectivity in these crops.

## MATERIALS AND METHODS

Three formulations of fluroxypyr as 1-methyl heptyl ester were tested in sugarcane and grain sorghum field trials. These were EF381 (250 g/L), AF172 (200 g/L) and AF177, (300 g/L, STARANE* Selective Herbicide).

Fluroxypyr was tested in small plot trials using randomised complete block designs with three to four replications. Each plot was 20 $m^2$ in sorghum and varied between 30 and 40 $m^2$ (depending upon row spacing) in sugarcane.

In sorghum the herbicides were sprayed over the top of the crop using a propane operated sprayer fitted with a boom and 50 cm spaced 110 degree flat fan nozzles calibrated to apply 100 L/ha. In sugarcane the herbicides were applied as directed inter-row sprays using a hand held lance calibrated to apply 150 to 400 L/ha.

Trials were rated two to six weeks after application using visual assessment methods or line transect counts, and ratings were expressed percentage control. Yields were only recorded in sorghum trials, in which grain was harvested with a Hege 125.

## RESULTS AND DISCUSSION

**Weed control** Percentage control of weeds in grain sorghum and sugarcane are presented in Tables 1 and 2 respectively. Number of trials from which each mean is derived are shown in square brackets and coefficient of variation (cv) are shown in parenthesis. Fluroxypyr, 150 to 300g/ha gave excellent control of a number of important weeds including (*Datura* spp., *Physalis* spp., *Xanthium* spp., *Helianthus annus*, *Ageratum houstonianum*, *Tribulus terrestris*, *Mimosa invisa*, *Centrosema pubescena*, *Macroptillum lathyroides*, *Cucumis metuliferus*, *Passiflora foetida* and *Sida acuta*).

In sorghum the most important weed was *Datura*, due to the nil tolerance which has been set for *Datura* seed contamination in grain. Fluroxypyr gave excellent post-emergent control of *Datura* (Table 1) but gave no residual control of new germinations which has been a feature of picloram + 2,4-D formulations (70 + 280 g/ha), used as a standard. Tank mixing of fluroxypyr with atrazine (150 + 1000 g/ha) controlled existing plants and gave season long control of new seedlings without affecting crop selectivity (Walker, pers. comm.). The fluroxypyr atrazine

Table 1. Control of broadleaf weeds with Fluroxpyr in sorghum crops in Queensland and Nthn New South Wales 1983-1986.

| Treatment | Weed species / Rate g/ha | Mean Percentage Control | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Datura* spp. | *Physalis* spp. | *Xanthium pungens* | *Helianthus annuus* | *Sesbania cannabina* | *Hibiscus trionum* | *Salvia reflexa* | *Tribulus terrestris* | *Amaranthus cruentus* | *Amaranthus macrocarpus* | *Portulaca oleraceae* | *Trianthema portulacastrum* | *Commelina benghalensis* |
| Fluroxypyr | 100 | 85 [3] | 60 [1] | 100 [3] | – | – | 22 [4] | 30 [1] | 43 [6] | 20 [3] | 0 [3] | 50 [2] | – | – |
| Fluroxypyr | 150 | 93 (10) [7] | 98 [4] | 98 (5) [6] | 60 [2] | 81 [2] | 25 (34) [7] | 75 [2] | 60 (69) [10] | 20 [4] | 5 [8] | 83 [3] | 70 [4] | 26 [1] |
| Fluroxypyr | 200 | 94 (10) [9] | 100 [4] | 93 (16) [7] | 97 [3] | – | 28 (87) [8] | 80 [2] | 74 (42) [16] | 43 (63) [4] | 13 (118 [9] | 83 [3] | – | 20 [1] |
| Fluroxypyr | 300 | 89 (12) [8] | 100 [4] | 99 (4) [7] | 98 [4] | 96 [2] | 37 (92) [7] | 75 [2] | 81 (27) [14] | 58 (41) [4] | 23 (101 [10] | 100 [1] | 84 [4] | – |
| Fluroxypyr and Atrazine | 150 + 150 | 97 (4) [5] | 100 [3] | 87 [3] | 83 [1] | – | 54 (59) [6] | 56 [1] | 69 (39) [6] | 62 (44) [4] | 57 (54) [3] | 80 [2] | – | 46 [1] |
| Fluroxypyr and Atrazine | 150 + 1000 | 96 (5) [5] | 100 [5] | 96 (9) [5] | 60 [2] | 100 [2] | 83 (20) [7] | 85 [1] | 96 (8) [7] | 86 (30) [5] | 92 (13) [7] | – | 99 [4] | 92 [1] |
| Atrazine | 1000 | 73 [4] | 97 [3] | 13 [3] | 10 [1] | 86 [2] | 19 [6] | 56 [1] | 40 [4] | 75 (53) [6] | 57 (67) [3] | – | 93 [4] | 66 [1] |
| Picloram + 2,4-D and Atrazine | 35 + 140 + 1000 | 98 [4] | 100 [4] | 98 [4] | 70 [1] | 100 [2] | 47 (69) [6] | 80 | – | 90 [2] | 100 [4] | | 98 [4] | 92 [1] |
| Picloram + 2,4-D | 70 + 280 | 85 (22) [6] | 100 [4] | 100 [4] | 90 [1] | 99 [2] | 44 (66) [6] | 67 [2] | 80 [2] | 92 (10) [4] | 37 (66) [5] | – | 75 [4] | 46 [1] |
| Untreated | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 | 0 |
| Plant size/growth stage | | Cotyledon to 12 leaves | 4-20c tall | 3-150cm tall Cotyledon | 15-45cm tall | 5-12cm tall | 4-28cm tall | 12-15cm tall | 5-50cm diam | 8-20cm high | 8-20cm diam | 7-15cm diam | 2-15cm diam | 6cm 1-6 leave |

[Number of trials]
(Co-efficient of variation %)

Table 2. Control of vines and broadleaf weeds with Fluroxypyr in sugar cane 1983-1986

| Treatment | Weed species / Rate g/ha | Mean Percentage Control | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Passiflora foetida* | *Mimosa Invisa* | *Cucumis metuliferus* | *Ipomoea* spp. | *Ageratum houstonianum* | *Euphorbia heterophylla* | *Spermacoece latifolia* | *Lablab purpurens* | *Centrosema pubescens* | *Macroptilium lathroides* | *Sida acuta* | *Urena lobata* |
| Fluroxypyr | 200 | 96 [3] | 100 [1] | 99 [1] | 100 [2] | 99 [3] | 56 [4] | 0 [1] | | | 99 [1] | 100 [1] | |
| Fluroxypyr | 250 | 88 [7] (14) | 88 [3] | 99 [1] | 82 [8] (20) | 90 [4] | | 17 [3] | 70 [1] | 80 [2] | 100 [1] | 100 [1] | 50 [1] |
| Fluroxypyr | 300 | 90 [2]* | 94 [2]* | | 25 [1]* | | 52 [6] (64) | | | | | | |
| Fluroxypyr | 500 | 92 [6] (11) | 92 [3] | 100 [1] | 85 [6] (22) | 100 [3] | | 21 [3] | | 80 [1] | 99 [1] | 99 [1] | 60 [1] |
| Fluroxypyr | 600 | 95 [1]* | | | | | 90 [4] | | | | | | |
| Triclopyr | 480 | 40 [3] | 89 [2] | | | 99 [3] | | 62 [2] | | | 100 [1] | 100 [1] | |
| Triclopyr | 960 | 42 [2] | 92 [2] | | | | | | | | | | |
| 2,4,5-T | 1500 - 1680 | 58 [3] | 84 [2] | | | | | | | | | | |
| 2,4-D (Ester) | 500 | 69 [3] | | 99 [1] | 97 [3] | 98 [3] | | | | 100 [1] | 96 [1] | | 100 [1] |
| | | | | | 87 [2] | 85 [2] | | 45 [2] | 70 [1] | 100 [1] | 100 [1] | 100 [1] | |
| 2,4-D + 2,4,5-T | 600 + 600 | 73 [2] | | | | | | | | | | | |
| 2,4-D + 2,4,5-T | 840 + 840 | | 90 [5] (8) | | | | | | | | | | |
| Fluroxypyr + 2,4-D (E) | 200 + 500 | 99 [4] | | 100 [1] | 100 [2] | 100 [3] | | 40 [1] | | | 100 [1] | 100 [1] | |
| Fluroxypyr + 2,4-D (A) | 200 + 500 | 99 [3] | | 93 [1] | 100 [2] | 100 [2] | 83 [4] | 65 [2] | | | 100 [1] | 100 [1] | |
| Fluroxypyr + 2,4-D (A) | 250 + 500 | 68 [3]* | 100 [1]* | | 99 [4] | | | | | 100 [1] | | | 100 [1] |
| Fluroxypyr + 2,4-D (A) | 300 + 500 | 83 [7]* | 67 [3]* | | 95 [3] | | | | | | | | |
| Fluroxypyr + Atrazine | 450 + 2000 | | | | | | 100 [3] | | | | | | |
| 2,4,5-T + metribuzin | 600 + 700 | | | | | | 95 [2] | | | | | | |
| Picloram + 2,4-D | 225 + 900 | | | | | | 98 [5] | | | | | | |
| Plant size/growth stage | | 100-200cm fruit and flowers. 5-600cm (late post) | 10-80cm up to 20 leaves 200-300cm late with flowers | 50-200cm flowers | 20-200cm 300cm late flowers | 3-10cm 40cm flowers | 20cm to 100cm flowers | 20-100cm 100cm flowers | | | 30-100cm | 100-150cm flowers | |

* Aerially applied.
(E) = Ester formulations

tank mix also controlled *Amaranthus* spp., *Trianthema portulacastrum*, *Hibiscus trionum* and *Commelina benghalensis* (species tolerant to fluroxypyr alone), increased the rate of necrosis, improved the reliability of atrazine (post-emergent) under dry conditions and gave better control of larger plants. In sugarcane *Ipomoea* spp. the most ubiquitous of the vines species were readily controlled by 2,4-D or MCPA but were only moderately susceptible to fluroxypyr 300 g/ha. *Mimosa invisa*, a declared noxious weed, is confined to the wet tropical region of Australia around Tully and Innisfail and is widespread in sugarcane crops of the Pacific Islands and Papua New Guinea. It is usually controlled with mixtures of 2,4-D and 2,4,5-T (840 + 840 g/ha) but was more susceptible to 2,4,5-T than 2,4-D. *Passiflora foetida* was also more susceptible to 2,4,5-T (500 g/ha) than 2,4-D. Control was variable when it was treated as an established plant or under dry conditions. Fluroxypyr 250-300 g/ha gave excellent control of *Mimosa* and *Passiflora* in the small plot trials, but was more variable when applied from aircraft. *Ipomoea* species except *Ipomoea triloba* appeared to be relatively susceptible to fluroxypyr (300 g/ha) in small plot trials but were not well controlled when fluroxypyr was applied by aircraft. The addition of 2,4-D (500 g/ha) to fluroxypyr gave better control of *Ipomoea* spp. than fluroxypyr alone, without affecting the control of other vine species or crop selectivity.

*Euphorbia heterophylla* is a relatively new weed that has been found in small areas from Bundaberg to Cairns (5). Control has been achieved on seedlings with 2,4,5-T (600 g/ha) and on mature plants with 2,4,5-T + metribuzin (600 + 700 g/ha) respectively (16). Fluroxypyr, 600 g/ha controlled plants at all growth stages.

**Adjuvants** No adjuvants were added to the fluroxypyr treatments in either sugarcane or sorghum. Non-ionic surfactants and refined petroleum oils added to the tank mixes of fluroxypyr and atrazine. They had little effect on weed control but were observed to increase phytotoxicity and reduced grain yield.

**Grain sorghum selectivity** Grain sorghum variety trials conducted under weed-free conditions have demonstrated that phytotoxicity was influenced by growth stage (3, 12, 13), rate of herbicide applied (3, 12), climatic (environment) conditions (3, 10, 13, 17) and genotype (3, 8, 11, 12, 17). The greatest and least predictable influence on yield appears to be genotype/environmental interaction (8, 10, 13, 17).

Work conducted in Central Queensland between 1974 and 1977 (14) demonstrating yield reductions of up to 40% resulted in the withdrawal of 2,4-D, picloram + 2,4-D and dicamba from the Department of Primary Industries sorghum weed control recommendations. The same herbicides are still recommended for weed control in sorghum in Northern N. S. W. and S. E. Queensland where temperatures at application appear to be lower (13) and where fewer cases of damage have been recorded.

To assess the impact of genotype and environmental factors on selectivity, fluroxypyr was tested on a number of commercially important grain sorghum cultivars at sites in Central and south east Queensland.

In Central Queensland Walker (pers. comm.), demonstrated that fluroxypyr (300 g/ha) was more selective to five cultivars than either 2,4-D (500 g/ha) or picloram + 2,4-D (70 & 280 g/ha) which both significantly delayed flowering and reduced grain yield, while in South East Queensland Campbell (6) tested fluroxypyr (300 and 600 g/ha) and 2,4-D amine (500 g/ha) formulations on two grain sorghum cultivars at four growth stages and showed that only the

earliest application (21 days after planting) of each herbicide affected plant growth. Both herbicide inhibited secondary root development causing lodging. Fluroxypyr (at both rates) was less damaging than 2,4-D.

Agrisearch Pty. Ltd. have tested fluroxypyr (300-600 g/ha) under weed free conditions, in six multi-variety grain sorghum trials, (Central and Southern Queensland), over four seasons (1983 and 1986). When applied as a topical broadcast spray to grain sorghum plants at the 6-8 leaf growth stage, (with secondary roots present), fluroxypyr was completely selective to all cultivars.

**Sugarcane selectivity** The Bureau of Sugar Experiment Stations have conducted multi-variety screens at Tully Research Station since 1984. Fluroxypyr 250, 500 and 1000 g/ha, 2,4-D sodium salt (3700 g/ha), MCPA amine (5000 g/ha), 2,4-D amine (2200 g/ha) 2,4-D butyl ester (1400 g/ha) and 2,4-D butyl + 2,4,5-T ethyl esters (420 + 420 + and 840 + 840 g/ha) were applied to ten sugarcane varieties, H56, Q96, Q107, Q113, Q115, Q117, Q119, Q122, Q124, and Triton as late post-emergent applications when the cane was tillered and 30 to 60 cm tall. MCPA, 2,4-D sodium salt and 2,4-D butyl ester were the most phytotoxic formulations causing typical hormone bending in the stems of the variety H56 (the most sensitive variety) and modest bending in the varieties Q96 and Q107. Fluroxypyr 250 and 500 g/ha did not affect any of the varieties and caused only slight but acceptable twisting at 1000 g/ha (Williams pers. comm.).

These data demonstrate that fluroxpyr offers excellent control of a narrow range of important broadleaf weeds that have previously been controlled by 2,4-D or 2,4,5-T and greater crop safety than 2,4-D. Fluroxypyr was compatible with a number of other herbicides 2,4-D, MCPA and atrazine was tank-mixed with these products to control a broader specturm of weed species.

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# GROUND APPLICATIONS OF CERTAIN HERBICIDES FOR CONTROL OF CATCLAW MIMOSA (*MIMOSA PIGRA* L.)

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## ABSTRACT

Studies on the control of *Minosa pigra* L., MIMPI (6), were conducted in Chiang Mai, Thailand from mid-1986 to early 1987. Experiments were conducted on both young mimosa plants (aged 3 months) and mature plants (aged 13 months). Comparative treatments were made using glyphosate, [N-(phosphonomethyl) glycine], dicamba (3,6-dichloro-2-methoxybenzoic acid), and triclopyr {[(3,5,6- trichloro-2-pyridinyl) oxy] acetic acid} at varying rates. The results obtained 4 months after application showed that glyphosate at 4.5 kg ae/ha and dicamba or triclopyr at 3.0 kg ae/ha provided satisfactory control of young mimosa plants. Minimum commercial acceptable control of mature plants was observed at twice (2X) the equivalent rates required to control young plants. Both dicamba and triclopyr showed additional soil residual control of mimosa seed growth in comparison to glyphosate treatments.

## INTRODUCTION

Catclaw mimosa is one of the most troublesome weeds in Thailand and also one of the most difficult species to control. In general, over the past six years, it has been determined that the response to herbicides is closely related to proper application, timing and growth characteristics of the plant. Younger plants, or resprout, are more sensitive to foliar applied herbicides than mature plants and require less application volume for effective control.

Glyphosate, dicamba and triclopyr are three of the most effective herbicides in the local market which can potentially be used to control catclaw mimosa (3, 4). The objective of this study was to identify the optimum rates of glyphosate, dicamba and triclopyr which can effectively be used to control catclaw mimosa at various growth stages using conventional ground spray equipment.

## MATERIALS AND METHODS

Separate experiments were conducted using young and mature catclaw mimosa plants. Young plants consisted of 3-month old resprouts rising from previously cut stumps. The plants were 2 m high with basal stems beginning to turn in color. Mature test plants were also selected from previously cut stumps, but with 13 months regrowth of 3.5 to 4 m stem length.

Table 1. Effects of three herbicides on the young catclaw mimosa plants aged 3 months.

| Herbicide | Rate of Application | | Weed control, DAA[1] | | | | Seed germ. control, DAA | | |
|---|---|---|---|---|---|---|---|---|---|
| | Product, L/ha | kg ae/ha | 30 | 60 | 90 | 120 | 60 | 90 | 120 |
| | | | rating scale[2] | | | | | | |
| Glyphosate | 6.25 | 2.25 | 3.5 | 3.0 | 2.5 | 2.0 | 3.3 | 2.1 | 1.0 |
| | 12.50 | 4.50 | 4.0 | 4.5 | 4.8 | 4.0 | 3.3 | 2.1 | 1.0 |
| Dicamba | 6.25 | 3.00 | 4.5 | 4.8 | 5.0 | 4.8 | 5.0 | 5.0 | 4.8 |
| | 12.50 | 6.00 | 4.5 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 4.9 |
| Triclopyr | 6.25 | 3.00 | 4.0 | 4.5 | 4.5 | 4.0 | 5.0 | 4.9 | 4.7 |
| | 12.50 | 6.00 | 4.5 | 4.8 | 5.0 | 4.9 | 5.0 | 4.9 | 4.8 |
| Untreated | - | - | 1.0 | 1.0 | 1.0 | 1.0 | 2.8 | 2.6 | 2.5 |

1 DAA = days after application

2 1 = no weed control or no seed germination control and 5 = complete weed control or complete seed germination control.

Table 2. Effect of certain herbicides on the mature catclaw mimosa plants aged 13 months.

| Herbicide | Rate of Application | | Weed control, DAA[1] | | | | Seed germ. Control, DAA | | |
|---|---|---|---|---|---|---|---|---|---|
| | Product, L/ha | kg ae/ha | 30 | 60 | 90 | 120 | 60 | 90 | 120 |
| | | | rating scale[2] | | | | | | |
| Glyphosate | 12.5 | 4.5 | 3.5 | 3.5 | 2.5 | 2.0 | 4.0 | 2.0 | 1.0 |
| | 25.0 | 9.0 | 4.3 | 4.1 | 4.0 | 4.0 | 4.0 | 1.9 | 1.0 |
| | 50.0 | 18.0 | 4.3 | 5.0 | 5.0 | 5.0 | 4.0 | 1.8 | 1.0 |
| Dicamba | 12.5 | 6.0 | 4.5 | 5.0 | 4.8 | 4.4 | 5.0 | 4.8 | 4.5 |
| | 25.0 | 12.0 | 4.5 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 4.8 |
| Triclopyr | 12.5 | 6.0 | 4.3 | 4.1 | 4.1 | 4.0 | 5.0 | 4.6 | 3.5 |
| | 25.0 | 12.0 | 4.5 | 4.5 | 4.8 | 4.8 | 5.0 | 4.8 | 4.7 |
| Untreated | - | - | 1.0 | 1.0 | 1.0 | 1.0 | 2.5 | 2.5 | 2.5 |

1 DAA = days after application

2 1 = no weed control or no seed germination control and 5 = complete weed control or complete seed germination control.

Field plots were arranged in a randomized complete block design with three replications. Each treatment plot measured 6.6 x 15 m or approximately 100 $m^2$. Treatments of glyphosate ranged between 2.25-18.0 kg ae/ha, and for dicamba and triclopyr between 3.0-12.0 kg ae/ha. All herbicide applications were made using a hand gun fitted with a jet nozzle and high powered pump at a pressure of 30 $kg/cm^3$. Application volumes of 1250 l/ha were used for treating young plants, and 2500 l/ha for treating mature plants.

Efficacy and inhibition of seed germination ratings were made at 30-day intervals after treatment using a 1-5 visual rating system. One (1) equalled no injury or no seed germination control and a rating of 5 indicated all plants or germinating seeds were completely controlled by the herbicide application.

## RESULTS AND DISCUSSION

Within the rate range tested all herbicides were active against control of catclaw mimosa (Tables 1 and 2). Lower rates of dicamba (3.0 kg ae/ha) showed a higher level of control of both young and mature plants 120 days after application (DAA) than did either glyphosate (4.5 kg ar/ha) or triclopyr (3.0 kg ae/ha). Control of young plants was less for glyphosate (4.5 kg ae/ha) than for applications made at the same timing with dicamba (3.0 kg ae/ha). The data suggests that higher application rates of glyphosate (18.0 kg ae/ha) are needed for more satisfactory control of catclaw mimosa than with both dicamba or triclopyr. Among the three herbicides tested dicamba was more effective against catclaw mimosa than either glyphosate or triclopyr which is in disagreement with previous reports (1, 2, 4, 5) that the performances of those three herbicides are more or less similar.

Both dicamba and triclopyr showed excellent soil residual control (120 DAA) of germinating catclaw mimosa from seed, in comparison to the level of control observed with glyphosate. This is understandable in that glyphosate is known to be inactive as a soil herbicide. Repeat application of glyphosate inevitably will be necessary, in comparison to dicamba or triclopyr, to control seedling growth.

In doing spraying business, costs of expense are probably the main concern. Based on current local price of product per liter which dicamba (containing 4 lb ae per gallon of product) costs 350, triclopyr (also 4 lb ae per gallon product )750, and glyphosate (3 lb ae per gallon product)350, it can be seen that dicamba costs the least at equal level of efficacy. Triclopyr and dicamba had not much problem with mixing water and rainface period after application which usually are better made in rainy season. It should be known that any application which is not perfect, reapplication must be made and it will cost more money. So far, according to our opinion after six years of experience in controlling catclaw mimosa as a business, dicamba seems to give the best compromise.

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# EFFECT OF APPLICATION TIMING AND RESIDUAL PERIOD OF LONDAX ON MAIN PADDY WEEDS IN TAIWAN

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## ABSTRACT

Londax, with 0.4 and 0.5 kg/ha, was tested to control *Ammannia baccifera*, *Ammannia multiflora*, *Rotala indica*, *Lindernia anagallis* var. *verbenefolia*, *Lindernia pyxidaria*, *Sagittaria trifolia*, *Sagittaria pygmaea*, *Monochoria vaginalis*, *Cyperus difformis*, *Scirpus juncoides*, *Scirpus planiculmis* and *Echinochloa crus-galli*, main paddy weeds in Taiwan, at 0, 5, 10, 15 and 20 days after seeding (DAS) and also on residual effect at 0, 5, 10, 15 and 20 days after application (DAA). The results showed most tested weeds even at 20 DAS were controlled thoroughly by londax at both rates , but at 0.4 kg/ha, only inhibiting growth of *E. crus-galli* and *S. planiculmis*. Residual effect revealed most tested weeds species were controlled up to 20 DAA, however , *E. crus-galli*, *S. trifolia*, *S. juncoides* and *S. planiculmis* only could be controlled at 0-10 DAA even though at 0.5 kg/ha.

## INTRODUCTION

In Taiwan, transplanting method is the prevalent ways of planting rice. And multi-site field experimental data indicated that whole season weed competition resulted in mean yield losses of 16% for transplanted rice (1). In general, *Ammannia baccifera*, *Ammannia multiflora*, *Rotala indica*, *Lindernia anagallis* var. *verbenefolia*, *Lindernia pyxidaria*, *Sagittaria trifolia*, *Sagittaria pygmaea*, *Monochoria vaginalis*, *Cyperus difformis*, *Scirpus juncoides* and *Echinochloa crus-galli* are predominant in Taiwan's paddy fields (1). However, *S. trifolia* and *S. pygmaea* became more seriously recent years, which might be related to continuous application of herbicides during the past years (5). *Scirpus planiculmis* was distributed commonly in Chunghwa and Yunlin districts. Transplanting is usually carried out 1-3 days after final land preparation by farmers of Taiwan. Herbicides, as butachlor, benthiocarb and chlomethoxynil, used at this stage to control annual and perennial weeds that propagate by seeds. The requirement of different application time for some herbicides at different seasons in a special feature in the practical usage of herbicides in Taiwan, because weeds emerge and grow faster under high temperature (2). In most areas where *S. trifolia* and *S. pygmaea* are predominant, the secondary herbicide application is now practiced. Foliage treatments of bentazon is recommended at 30-40 and 20-30 days after transplanting for the first and second rice crop, respectively. Herbicides applied twice would increase production cost, thus herbicides with longer residue are necessary to be introduced into Taiwan.

Londax ( methyl 2-[[[[[(4,6- dimethoxy pyrimidin-2-yl) amino] carbonyl] amino] sulfonyl] methyl] benzoate ) is a new broad spectrum herbicide for paddy rice. It, the active ingredient, is highly effective for control of most annual and perennial broadleaf weeds and sedges, but is less effective on gramineous species, including barnyard grass (8). The mode of action of londax is to inhibit cell division and cell growth by blocking the bio-synthesis of the essential amino acids, valine and isoleucine (8). The objectives of this study were focused on application timing and residual effect to confirm the sufficiency of weed control of londax.

## MATERIALS AND METHODS

Seeds, with over 50% germinant percentage of *A. baccifera*, *A. multiflora*, *R. indica*, *L. anagallis* var. *verbenefolia*, *L. pyxidaria*, *S. trifolia*, *M. vaginalis*, *C. difformis*, *S. juncoides* and *E. crus-galli*, were collected. Tubers freshly of 1, 0.15 and 1 g per unit weight were collected and used for the vegetative propagation of *S. pygmaea* and *S. planiculmis*, respectively.

In July of 1987, 1/2,000 acre Wagner's pots (diam., 27 cm; depth, 30 cm ) were used. Sandy loam soil, had a pH of 6.8 and contained 1.5% organic matter were collected from adjacent research-plot areas, was fumigated with methyl bromide at 112 kg/ha to kill existing weed seeds. Soil prepared by mixing with N, P and K fertilizer ( N : $P_2O_5$ : KCl=3 : 1.5 : 1.5 g/pot ) was placed in each pot. Thirty seeds and five tubers of each tested species were placed in a pot. Then londax was applied with 0.4 and 0.5 kg/ha (0.002 and 0.0025 g/pot, respectively at 0, 5, 10, 15 and 20 days after seeding (DAS) or planting. Another test was to apply londax at both doses the same rates, then to seed or plant each species at 0, 10, 15 and 20 days after application (DAA). Six pots of each treatment were as replication. Pots were frequently watered to 2-3 cm over the soil surface throughout the test period, but to keep actually soil in flooded condition about 5 cm water depth within 10 days after application. Weed control was evaluated by comparing plant counts of untreated pots with those in herbicide treated pots at 15 and 30 days after londax application. At 30 days after londax application, all weeds in each pot were harvested and recorded plant height, leaf number and fresh weight to determine the effect of herbicide on seedling growth under different stages of tested weed species. Possible residual effects of this herbicide on weed control were determined using the same parameters as mentioned above at 15 and 30 days after weed seeding.

## RESULTS AND DISCUSSION

**Effect of application timing of londax on paddy weeds control** The results of paddy weeds control with londax applied at different time after weed seeding were shown in Table 1. Data presented were weeds counts at 15 days after londax application. It was found *S. difformis* and *L. pyxidaria* were controlled thorocghly at 0, 5, 10, 15 and 20 DAS within 15 days after londax application at 0.4 and 0.5 kg/ha dosage. The control of *S. juncoides*, *M. vaginalis*, *R. indica* and *A. multiflora* were progressively less effective at 15 and 20 DAS with londax at both rates. *S. trifolia* and *S. pygmaea*, however, showed more sensitive at 15 and 20 DAS than at 0, 5, and 10 DAS to londax at higher rate. The better control in *E. crus-galli* and *S. planiculmis* often obtained at 0 and 5 DAS by londax at any tested rates. The results shown in Tables 2 and 3 were

Table 1. Paddy weeds control with londax applied at 0.4 and 0.5 kg/ha 0, 5, 10, 15, and 20 DAS. Data presented were weed counts at 15 days after herbicides application.

| Weed[2] | Dosage ( kg/ha ) 0.4 DAS | | | | | 0.5 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 0 | 5 | 10 | 15 | 20 |
| | % [1] | | | | | | | | | |
| *Echinochloa crus-galli* | 85 | 50 | 40 | 0 | 0 | 100 | 85 | 67 | 0 | 0 |
| *Sagittaria pygmaea* | 0 | 0 | 0 | 50 | 50 | 0 | 0 | 0 | 50 | 75 |
| *Sagittaria trifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 80 |
| *Scirpus planiculmis* | 75 | 100 | 0 | 0 | 0 | 75 | 100 | 40 | 0 | 0 |
| *Scirpus juncoides* | 100 | 100 | 90 | 75 | 70 | 100 | 100 | 100 | 100 | 95 |
| *Monochoria vaginalis* | 100 | 100 | 100 | 100 | 65 | 100 | 100 | 100 | 100 | 100 |
| *Rotala indica* | 100 | 100 | 100 | 70 | 50 | 100 | 100 | 100 | 100 | 75 |
| *Ammannia multiflora* | 100 | 100 | 100 | 80 | 50 | 100 | 100 | 100 | 100 | 50 |
| *Cyperus difformis* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Lindernia pyxidaria* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

1 " % " indicated the control percentage.

2 *Lindernia anagallis* var. *verbenefolia* and *Ammannia baccifera* did not germinate at the day of investigation.

Table 2. Paddy weeds control with londax applied at 0.4 and 0.5 kg/ha[1].

| Weed | parameter[3] | Dosage ( kg/ha ) 0.4 DAS 15 | 0.4 DAS 20 | 0.5 DAS 15 | 0.5 DAS 20 |
|---|---|---|---|---|---|
| | | ---------- %[2] ---------- | | | |
| *Scirpus* | WC | 100 | 75 | 100 | 88 |
| *juncoides* | PH | 100 | 69 | 100 | 88 |
| | TN | 100 | 100 | 100 | 100 |
| | LN | 100 | 0 | 100 | 40 |
| | FW | 100 | 75 | 100 | 100 |
| *Monochoria* | WC | 100 | 75 | 100 | 100 |
| *vaginalis* | PH | 100 | 69 | 100 | 100 |
| | TN | — | — | — | — |
| | LN | 100 | 72 | 100 | 100 |
| | FW | 100 | 90 | 100 | 100 |
| *Rotala* | WC | 100 | 50 | 100 | 100 |
| *indica* | PH | 100 | 75 | 100 | 100 |
| | TN | 100 | 90 | 100 | 100 |
| | LN | 100 | 96 | 100 | 100 |
| | FW | 100 | 99 | 100 | 100 |
| *Lindernia* | WC | 100 | 70 | 100 | 100 |
| *pyxidaria* | PH | 100 | 85 | 100 | 100 |
| | TN | 100 | 100 | 100 | 100 |
| | LN | 100 | 55 | 100 | 100 |
| | FW | 100 | 90 | 100 | 100 |

1 Investigation at 30 days after londax application.

2 % indicated the control (suppression) percentage.

3 WC: weed counts, PH: plant height, TN: tiller number, LN: leaf number, FW: fresh weight.

the data of paddy weeds control with londax applied at different time after weeds seeding and investigated at 30 days after londax application. Basing on weed counts, *C. difformis*, *L. anagallis* var. *verbenefolia*, *A. baccifera* and *A. multiflora* were still controlled to 100% even though at 20 DAS. The control percentage of *S. juncoides*, *M. vaginalis*, *R. indica* and *L. pyxidaria* were decreased slightly to 50-75% at 20 DAS at 0.4 kg/ha (Table 2). Londax had better control to *S. pygmaea* at any tested time at both rates (Table 3). The control of *S. trifolia* by londax was more better at higher rate than at lower rate. The control percentage of *E. crus-galli* and *S. planiculmis* were not over 50% at 15 and 20 DAS. The inhibition of londax to plant height and leaf number of weed species showed the similar trend to decrease at 15 and 20 DAS except *S. trifolia* and *S. pygmaea*. The plant height and leaf number in *E. crus-galli* and *S. planiculmis* were inhibited over 75% by londax, but the growth rate of *E. crus-galli* was not suppressed markedly at 10, 15 and 20 DAS by londax of 0.4 kg/ha dosage. The inhibition of plant height was more significantly than that of leaf number in *S. trifolia* and *S. pygmaea*. Only the tiller number ( or stem number ) in *E. crus-galli* and *S. planiculmis* were not controlled effectively by londax at 0.4 kg/ha. But there was no tillers in *M. vaginalis* at the time of investigation. According to the suppression in fresh weight, most treatments achieved to 100% only except *E. crus-galli* with londax at 0.4 kg/ha only about 50% at 15 and 20 DAS.

To conclude the results of londax to paddy weeds control at different application timing: the efficiency of londax to *C. difformis*, *A. baccifera*, *A. multiflora* and *L. anagallis* var. *verbenefolia* were faster than other species: and they were eradicated up to 20 DAS. It was related to the fast and uniform germination of these species (7). *S. juncoides*, *M. vaginalis*, *R. indica* and *L. pyxidaria* needed longer period to germinate (6), there had many different growth stages of the same weed in the population. The reason resulted in the higher suppression in fresh weight than in plant counts was some of these weeds occurred with younger seedlings at 15 and 20 DAS. *S. trifolia* and *S. pygmaea* germinated very slowly (3), they could not absorb enough herbicide until at 10 DAS. So the suppression of fresh weight of these two species were over 95% at 20 DAS. While the growth rate of *E. crus-galli* and *S. planiculmis* were restricted to 90% ( based on fresh weight ) at 20 DAS except *E. crus-galli* control with londax at 0.4 kg/ha. Although grasses were generally less susceptible to londax (8), the growth of grasses were still inhibited significantly by it.

**Residual activity of londax on paddy weeds control** The period after londax application was longer, the effect on *S. planiculmis* and *S. juncoides* control was less effective (Table 4). But *S. planiculims* control with londax at 0.5 kg/ha was still 100% at 20 DAA. The control percentage of *S. pygmaea* achieved to 100% at 10 DAA. *E. crus-galli* and *S. trifolia* were not be observed any control effect at 15 days after weed seeding. And some species did not germinate at the investigating time.

Data presented in Table 5 were the residual activity of londax on paddy weeds as shown by % control of weed counts and % suppression of plant height, tiller number, leaf number, and fresh weight at days after weed seeding which were made at 0, 5, 10, 15 and 20 days after londax application. respectively. The % control of weed counts of *C. difformis*, *R. indica*, *L. pyxidaria*, *A. baccifera*, *A. multiflora* and *L. anagallis* var. verbenefolia were 100% up to 20 DAA. *E. crus-galli*, *M. vaginalis* and *S. juncoides* control beceme poorer at longer period after londax application. The weed counts of *S. pygmaea* and *S. planiculmis* were not controlled markedly at different DAA. *S. trifolia* was not controlled by londax under all treatments. The

Table 3. Paddy weeds control with londax applied at 0.4 and 0.5 kg/ha[1].

| Weed | parameter[3] | Dosage ( kg/ha ) 0.4 DAS 0 | 5 | 10 | 15 | 20 | 0.5 DAS 0 | 5 | 10 | 15 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % [2] | | | | | | | | | |
| *Echinochloa crus-galli* | WC | 85 | 65 | 50 | 45 | 0 | 100 | 100 | 100 | 50 | 0 |
| | PH | 85 | 88 | 50 | 20 | 5 | 100 | 100 | 100 | 90 | 88 |
| | TN | 90 | 87 | 75 | 12 | 0 | 100 | 100 | 100 | 100 | 100 |
| | LN | 80 | 85 | 23 | 0 | 0 | 100 | 100 | 100 | 82 | 75 |
| | FW | 85 | 90 | 85 | 62 | 32 | 100 | 100 | 100 | 95 | 95 |
| *Sagittaria pygmaca* | WC | 80 | 90 | 92 | 95 | 95 | 95 | 100 | 100 | 100 | 100 |
| | PH | 70 | 90 | 90 | 92 | 91 | 95 | 100 | 100 | 100 | 100 |
| | TN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | LN | 50 | 85 | 87 | 87 | 87 | 95 | 100 | 100 | 100 | 100 |
| | FW | 95 | 96 | 96 | 96 | 96 | 96 | 100 | 100 | 100 | 100 |
| *Sagittaria trifolia* | WC | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 75 | 75 | 90 |
| | PH | 90 | 80 | 95 | 97 | 95 | 90 | 80 | 100 | 97 | 95 |
| | TN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | LN | 0 | 25 | 25 | 50 | 70 | 25 | 50 | 85 | 87 | 85 |
| | FW | 97 | 97 | 97 | 92 | 97 | 97 | 97 | 97 | 97 | 97 |
| *Scirpus planicalmis* | WC | 50 | 50 | 50 | 0 | 0 | 90 | 100 | 100 | 42 | 25 |
| | PH | 90 | 95 | 95 | 80 | 80 | 90 | 100 | 100 | 90 | 90 |
| | TN | 75 | 90 | 80 | 0 | 0 | 75 | 100 | 100 | 100 | 100 |
| | LN | 80 | 85 | 75 | 77 | 75 | 86 | 90 | 90 | 90 | 85 |
| | FW | 97 | 97 | 97 | 95 | 90 | 100 | 100 | 100 | 97 | 90 |

1 Investigation at 30 days after londax application.

2 % indicated the control (suppression) percentage.

3 WC: weed counts, PH: plant height, TN: tiller number, LN: leaf number, FW: fresh weight.

Table 4. Residual activity of londax on paddy weeds as shown by weeds counts at 10 days after weed seeding which was wade at 0, 5, 10, 15 and 20 davs after londax application respectively[1].

| Weed | Dosage ( kg/ha ) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.4 DAA | | | | | 0.5 DAA | | | | |
| | 0 | 5 | 10 | 15 | 20 | 0 | 5 | 10 | 15 | 20 |
| | ---------- %[2] ---------- | | | | | | | | | |
| *Echinochloa crus-galli* | 15 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 |
| *Scirpus juncoides* | 70 | 80 | 50 | 50 | 30 | 100 | 70 | 65 | 50 | 50 |
| *Sagittaria pygmaea* | 0 | 100 | 72 | 70 | 67 | 32 | 100 | 87 | 82 | 77 |
| *Scirpus planiculmis* | 100 | 90 | 77 | 70 | 70 | 100 | 100 | 100 | 100 | 100 |
| *Sagittaria trifolia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Monochoria vaginalis* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Rotala indica* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Cyperus difformis* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

1 *Ammannic baccifera* , *A. multiflora* , *Lindernia anagallis* var. *verbenefolia* and *L. pyxidaria* did not germinate at the day of invesigation.

2 " % " indicated the control percentage.

Table 5. Residual activity of londax on paddy weeds at 30 days after weed seeding which was made at 0, 5, 10, 15 and 20 days after londax application respectively.

| Weed | parameter[2] | Dosage ( kg/ha ) 0.4 DAA | | | | | 0.5 DAA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 | 0 | 5 | 10 | 15 | 20 |
| | | % [1] | | | | | | | | | |
| *Echinochloa crus-galli* | WC | 90 | 70 | 32 | 0 | 0 | 100 | 70 | 50 | 0 | 0 |
| | PH | 50 | 60 | 60 | 72 | 20 | 90 | 65 | 57 | 80 | 60 |
| | TN | 80 | 97 | 90 | 80 | 20 | 87 | 95 | 90 | 100 | 80 |
| | LN | 80 | 85 | 24 | 0 | 0 | 100 | 100 | 100 | 82 | 75 |
| | FW | 85 | 90 | 85 | 62 | 32 | 100 | 100 | 100 | 90 | 85 |
| *Scirpus juncoides* | WC | 100 | 90 | 72 | 0 | 0 | 100 | 100 | 90 | 12 | 0 |
| | PH | 100 | 95 | 70 | 45 | 50 | 100 | 95 | 80 | 55 | 50 |
| | TN | —[3] | — | — | — | — | — | — | — | — | — |
| | LN | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 40 |
| | FW | 100 | 100 | 100 | 100 | 75 | 100 | 100 | 100 | 100 | 100 |
| *Monochoria vaginalis* | WC | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 100 | 50 | 0 |
| | PH | 100 | 100 | 100 | 95 | 65 | 100 | 100 | 100 | 95 | 70 |
| | TN | — | — | — | — | — | — | — | — | — | — |
| | LN | 100 | 100 | 100 | 100 | 70 | 100 | 100 | 100 | 100 | 100 |
| | FW | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 |
| *Sagittaria pygmaea* | WC | 80 | 90 | 90 | 65 | 52 | 100 | 80 | 77 | 70 | 67 |
| | PH | 70 | 72 | 45 | 57 | 60 | 97 | 82 | 72 | 57 | 60 |
| | TN | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | LN | 50 | 85 | 87 | 87 | 87 | 90 | 100 | 100 | 100 | 100 |
| | FW | 97 | 97 | 97 | 97 | 97 | 97 | 100 | 100 | 100 | 100 |
| *Sagittaria trifolia* | WC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | PH | 90 | 75 | 75 | 82 | 62 | 90 | 80 | 80 | 82 | 70 |
| | TN | — | — | — | — | — | — | — | — | — | — |
| | LN | 0 | 25 | 25 | 50 | 70 | 25 | 50 | 87 | 87 | 85 |
| | FW | 97 | 97 | 97 | 95 | 97 | 97 | 97 | 97 | 97 | 97 |
| *Scirpus planiculmis* | WC | 50 | 72 | 67 | 67 | 50 | 90 | 90 | 67 | 67 | 50 |
| | PH | 90 | 77 | 75 | 10 | 0 | 100 | 100 | 95 | 47 | 42 |
| | TN | 75 | 80 | 82 | 50 | 0 | 75 | 100 | 100 | 100 | 50 |
| | LN | 85 | 80 | 75 | 77 | 75 | 87 | 90 | 90 | 90 | 87 |
| | FW | 97 | 97 | 97 | 95 | 90 | 99 | 100 | 100 | 97 | 95 |

1 " % " indicated the control (or suppression) percentage.

2 WC: weed counts, PH: plant height, TN: tiller number, LN: leaf number, FW: fresh weight.

3 " - " showed these weeds did not germinate at the day of investigation.

inhibition in plant height and leaf number also indicated most tested weeds species would grow restrictedly at 0-20 DAA. Data of fresh weight appeared londax could inhibit growth rates of most tested species at 0-20 DAA. But there had different degree within each species. Londax had excellent control to *E. crus-galli*, *S. juncoides* and *S. pygmaea* at 0-10 DAA at both rates, however, it could control other tested weed species at 0-20 DAA. The period of germination may be the most important factor to efficiency of londax (2, 4).

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# HALOXYFOP EE FOR PERENNIAL GRASS CONTROL IN JAPAN

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## ABSTRACT

Haloxyfop (CLINCHER$^R$ 20EC) as the ethoxyethyl ester has been evaluated for the control of the perennial problem grasses *Miscanthus sinensis*, *Phragmites communis*, and *Imperata cylindrica* in Japan. Haloxyfop EE ester has given excellent long term control of 1 to 1.5 meter tall *Phragmites communis* at 0.5 kg ai/ha, 1 to 1.5 meter tall *Miscanthus sinensis* at 0.75 to 1.0 kg ai/ha and 0.6-1.0 meter tall *Imperata cylindrica* at 1.0 to 1.5 kg ai/ha. Applications made during growth after seedhead emergence of *Imperata cy cylindrica* gave superior herbicidal activity compared to applications made prior to seedhead emergence. Haloxyfop ethoxyethyl ester was found to be compatible in combination with the broadleaf herbicides clopyralid and fluroxypyr but reduced grass activity was evident when tank mixed with MCPA and triclopyr.

## INTRODUCTION

Haloxyfop also know as DOWCO*453 is a new selective post-emergence grass herbicide (1, 2, 3). Twenty percent of haloxyfop ethoxyethyl (EE) ester is developed as CLINCHER$^R$ 20 EC for industrial vegetation control use in Japan.

*Miscanthus sinensis*, *Phragmites communis* and *Imperata cylindrica* are troublesome perennial grasses in Japan. The purpose of the present work is to study the herbicidal activity of CLINCHER 20EC on them and the compatibility in combination with the broadleaf herbicides, fluroxypyr, clopyralid, triclopyr and MCPA for general weed control.

## MATERIALS AND METHODS

Field trials were conducted at Gotemba in Shizuoka in 1985 and 1986. Soil type was volcanic ash soil. Trials were done in a randomized complete block design with treatments replicated three times. Application volume was fixed at 1 kiloliter per hectare. All chemical applications were made with a compressed air shoulder sprayer with a single nozzle lance, with a cone type (0.5 mm orifice) that could deliver a swath 30 cm wide if held 30 cm above the weed tops.

*Phragmites communis* was applied at 1.0 to 1.5 meter tall on July 16, 1985 with each plot measuring 3 x 3 square meters.

*Miscanthus sinensis* was applied at 1.0 to 1.5 meter tall on June 11, 1985 and for second trial at 0.5 to 1.0 meter tall on June 27, 1986 with 3 x 3 sq. m. plots. *Imperata cylindrica* was applied prior to seedhead emergence, 0.2 to 0.5 meter tall on June 10, 1986 and during growth after seedhead emergence, 0.6 to 1.0 meter tall on July 9, 1986 with 2 x 5 sq. m. plots.

Chemicals applied are Haloxyfop: CLINCHER 20EC, 20% w/w of haloxyfop ethoxyethyl ester; Haloxyfop + Fulroxypyr (1:1): XGA-2027, 100g/L of haloxyfop EE ester + 100g/L of fluroxypur 1-methylheptyl ester; Haloxyfop + Fluroxypyr (1:1.5): XGA-2028, 100g/L of haloxyfop EE ester + 150g/L of fluroxypyr MHE ester; Haloxyfop + Clopyralid (1:1): XGA-2045, 100g/L of haloxyfop EE ester + 100g/L of clopyralid; Haloxyfop + Triclopyr: tank mixed CLINCHER 20EC and 30% w/w of triclopyr amine; Haloxyfop + MCPA: tank mixed CLINCHER 20EC and 19.5% of MCPA; and Glyphosate: Roundup, 40% of glyphosate.

Assessments of grass control were made at one month intervals up to three months, and twelve months after treatment to observe regeneration rate.

Control ratings were based on visual score of percentage kill and a rating scale of 1-100 were used, 0 = indicating no kill and 100 = complete kill.

## RESULTS AND DISCUSSION

***Phragmites* control** Haloxyfop EE applied at 0.5 kg ai/ha gave excellent control of *Phragmites communis* (Table 1). Maximum control occurred at 3 months after treatment. When the rate was increased to 0.75 and 1.0 kg ai/ha the control level was increased and the speed accelerated. Haloxyfop EE suppressed the regeneration of *Phragmites* in next season very well at all the rates tested. Comparable levels of *Phragmites* control are achieved with haloxyfop EE at 0.5 kg ai/ha to glyphosate 3.0 kg ai/ha. Combination with broadleaf herbicide, fluroxypyr accelerated the brownout of *Phragmites*. Combination with broadleaf herbicide, clopyralid did not affect the herbicidal activity of haloxyfop on *Phragmites*.

***Miscanthus* control** To achieve satisfactory control of *Miscanthus* 0.75 kg ai/ha of haloxyfop EE was required (Tables 2 and 3). Comparable levels of *Miscanthus* control are achieved with haloxyfop EE at 1.0 kg ai/ha to glyphosate at 3.0 kg ai/ha. Haloxyhop EE at 1.0 kg ai/ha significantly suppressed the regeneration of *Miscanthus* in next season. The combination with fluroxypyr and clopyralid did not affect the herbicidal activity haloxyfop EE on *Miscanthus*, but haloxyfop EE reduced grass activity when tank mixed with MCPA and triclopyr. They did not show any suppression against the regeneration.

***Imperata* control** Haloxyfop EE at 1.0 kg ai/ha have excellent control of *Imperata cylindrica*, when it was applied during growth after seedhead emergence. Application made during growth after seedhead emergence of *Imperata* gave superior herbicidal activity compared to applications made prior to seedhead emergence. Haloxyfop EE suppressed regeneration of *Imperata* in next season satisfactorily at 1.5 kg ai/ha and completely at 2.0 kg ai/ha. Comparable levels of *Imperata* control are achieved with haloxyfop EE at 2.0 kg ai/ha to glyphosate at 3.0 kg ai/ha. The combination with fluroxypyr did not affect the herbicidal activity of haloxyfop on *Imperata* (Table 4).

Haloxyfop EE was found to be required 1.0 to 1.5 kg ai/ha to achieve excellent control of perennial grass and to be compatible in combination with the broadleaf herbicides fluroxypyr and clopyralid, but reduced grass activity was evident when tank mixed with MCPA and triclopyr.

Table 1. Comparative efficacy of haloxyfop alone and in combination with fluroxypy and clopyralid on *Phragmites communis* - 1985.

| Treatments | | % Control Months After Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | kg ai/ha | 0.3 | 0.7 | 1 | 2 | 3 | 12 |
| Haloxyfop | 0.5 | 20 | 50 | 60 | 77 | 90 | 85 |
| Haloxyfop | 0.75 | 20 | 57 | 63 | 83 | 90 | 85 |
| Haloxyfop | 1.0 | 33 | 77 | 80 | 100 | 100 | 90 |
| Haloxyfop+Fluroxypyr | 0.38+0.57 | 20 | 63 | 70 | 97 | 100 | 80 |
| Haloxyfop+Fluroxypyr | 0.5+0.75 | 33 | 70 | 73 | 97 | 97 | 80 |
| Haloxyfop+Fluroxypyr | 0.75+1.13 | 57 | 87 | 90 | 100 | 100 | 80 |
| Haloxyfop+Clopyralid | 0.38+0.38 | 27 | 60 | 63 | 87 | 87 | 80 |
| Haloxyfop+Clopyralid | 0.5+0.5 | 20 | 57 | 60 | 83 | 87 | 80 |
| Haloxyfop+Clopyralid | 0.75+0.75 | 27 | 63 | 63 | 90 | 90 | 90 |
| Glyphoste | 3.0 | 15 | 20 | 27 | 73 | 90 | 90 |
| Untreated | - | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2. Comparative efficacy of haloxyfop alone and in combination with fluroxypyr and clopyralid on *Miscanthus sinensis* - 1985.

| Treatments | | % Control Months After Treatment | | | |
|---|---|---|---|---|---|
| | kg ai/ha | 1 | 2 | 3 | 12 |
| Haloxyfop | 0.5 | 70 | 73 | 67 | 67 |
| Haloxyfop | 0.75 | 77 | 80 | 75 | 75 |
| Haloxyfop | 1.0 | 80 | 83 | 83 | 83 |
| Haloxyfop+Fluroxypyr | 0.38+0.57 | 60 | 60 | 50 | 47 |
| Haloxyfop+Fluroxypyr | 0.50+0.75 | 80 | 70 | 60 | 50 |
| Haloxyfop+Fluroxypyr | 0.75+1.13 | 83 | 70 | 70 | 67 |
| Haloxyfop+Clopyralid | 0.38+0.38 | 60 | 60 | 50 | 47 |
| Haloxyfop+Clopyralid | 0.5+0.5 | 80 | 70 | 50 | 40 |
| Haloxyfop+Clopyralid | 0.75+0.75 | 83 | 70 | 60 | 57 |
| Glyphosate | 3.0 | 45 | 73 | 80 | 90 |
| Untreated | - | 0 | 0 | 0 | 0 |

Table 3. Comparative efficacy of haloxyfop alone and in combination with fluroxypyr, triclopyr and MCPA on *Miscanthus sinensis* - 1986.

| Treatments | | % Control Months After Treatment | | | |
|---|---|---|---|---|---|
| | kg ai/ha | 1 | 2 | 3 | 12 |
| Haloxyfop | 0.75 | 80 | 80 | 83 | 53 |
| Haloxyfop | 1.0 | 80 | 90 | 90 | 60 |
| Haloxyfop+Fluroxypyr | 0.75+0.75 | 80 | 83 | 80 | 55 |
| Haloxyfop+Fluroxypyr | 1.0+1.0 | 83 | 87 | 83 | 60 |
| Haloxyfop+Triclopyr | 1.0+4.0 | 67 | 67 | 57 | 10 |
| Haloxyfop+MCPA | 1.0+4.0 | 70 | 77 | 60 | 0 |
| Glyphosate | 3.0 | 73 | 83 | 90 | 73 |
| Untreated | - | 0 | 0 | 0 | 0 |

Table 4. Comparative efficacy of haloxyfop alone and in combination with fluroxypyr on *Imperata cylindrica* at different stages, before and after seedhead emergence - 1986.

| Treatments | kg ai/ha | % Control Months After Treatment | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 12 |
| Applied prior to seedhead emergence | | | | | |
| Haloxyfop | 0.75 | 70 | 60 | 50 | - |
| Haloxyfop | 1.0 | 60 | 85 | 78 | - |
| Haloxyfop | 1.5 | 67 | 85 | 88 | - |
| Haloxyfop | 2.0 | 82 | 93 | 97 | - |
| Haloxyfop+Fluroxypyr | 0.75+0.75 | 70 | 60 | 50 | - |
| Haloxyfop+Fluroxypyr | 1.0+1.0 | 70 | 70 | 70 | - |
| Haloxyfop+Fluroxypyr | 1.5+1.5 | 70 | 80 | 90 | - |
| Glyphosate | 3.0 | 96 | 97 | 91 | - |
| Untreated | - | 0 | 0 | 0 | - |
| Applied during growth after seedhead emergence | | | | | |
| Haloxyfop | 0.75 | 73 | 77 | 88 | 50 |
| Haloxyfop | 1.0 | 77 | 90 | 83 | 63 |
| Haloxyfop | 1.5 | 77 | 90 | 87 | 73 |
| Haloxyfop | 2.0 | 80 | 100 | 100 | 100 |
| Haloxyfop+Fluroxypyr | 0.75+0.75 | 77 | 87 | 87 | 50 |
| Haloxyfop+Fluroxypyr | 1.0+1.0 | 80 | 90 | 87 | 63 |
| Haloxyfop+Fluroxypyr | 1.5+1.5 | 80 | 90 | 87 | 67 |
| Glyphosate | 3.0 | 100 | 100 | 100 | 97 |
| Untreated | - | 0 | 0 | 0 | 0 |

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# WEED CONTROL BY HERBICIDES IN BEET ROOT (*BETA VULGARIS* L.)

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## ABSTRACT

For beet root (*Beta vulgaris* L.), butachlor [N - (butoxymethyl) 2-chloro-2', 6'-diethyl acetanilide] at 1.5 and 2.0 kg, alachlor [2-chlofo-2', 6'-diethyl-N- (methoxy methyl) acetanilide] at 2.0 kg and fluchloralin [N-(2 chloro-ethyl) 2-, 6 dinitro-N-propyl-4-(trifluoro-methyl) analine] at 1.0 kg ai/ha were found to be suitable herbicides as measured by the reduced dry matter production of weeds on the 60th day. *Digitaria marginata* Link, (the major monocot weed) was controlled effectively by all the three herbicides and *Euphorbia* spp. by alachlor. None of the herbicidal treatments had any adverse effect on the yield of beet root.

## INTRODUCTION

Beet root (*Beta vulgaris* L.) is an important commercial root crop and belongs to the family Chenopodiaceae. Unlike Western countries where beet root is grown mostly for sugar production and table purpose, in India it is grown as a vegetable. It is cheap compared to other vegetables and is a rich source of carbohydrate. This crop thrives well in cool season and requires light friable soil for best growth. It also needs good amount of fertilizers and frequent irrigation (6). This situation also encourages good growth of broad spectrum of monocot and dicot weeds. Beets require 2-4 weed free weeks after 50% emergence to prevent yield losses (17). Season long competition can produce an yield loss of 30-95% (5, 11, 13).

Conventional method of handweeding being labour intensive has rendered the cultivation of beet roots highly uneconomical for the commercial grower. Weed control by using herbicides in beet root has been tried by many workers in different parts of the world. The most commonly used herbicides were pyrazon (1, 3, 5, 7, 9), phenmedipham (1, 2, 8, 9, 12, 16), chloridazon (12, 13, 14, 16), lenacil (7, 8, 15) and metamitron (15). Phenmedipham and metamitron have been used mostly as post emergent sprays, chloridazon and lenacil as pre emergent sprays and pyrazon as both pre and post emergent sprays.

The objective of the present study was to evaluate some new herbicides for their comparative performance in controlling weeds in beet root and also to workout the economics of using such herbicides.

## MATERIALS AND METHODS

The two trials on the control and weeds in beet root were laid out during the years 1984-85 at the experimental farm of the Indian Institute of Horticulture Research, Bangalore. The treatments were common to both the trials and they included alachlor and butachlor at 1.5 and 2.0 kg and fluchloralin at 0.75 and 1.0 kg ai/ha. The control plots were hand weeded.

Tractor (Mitsubishi) was used to plough the land. Firstly, disc ploughing was done to a depth of 30 cm followed by disc harrowing for breaking clods. Lastly, cultivator was used for levelling the soil. The soil was sandy clay loam belonging to Ivarkandapura series and belonged to the family of Aquic Haplustalf. The pH of the soil was 6.8 to 7.0, organic carbon, 0.63%, available phosphorus, 6.9 kg/ha, available potassium, 122 kg/ha and total nitrogen, 0.041%. The cation exchange capacity was 11.3 meg/100 g of soil.

Alachlor and butachlor were sprayed pre-emergent to weeds one day after sowing of beet root seeds. Being a volatile compound, fluchloralin was sprayed in plots which had enough moisture in the soil to prevent evaporation of the herbicide from the surface. Maruti foot sprayer, 8 meter long delivery hose with PF 3 BA spray lance (cost of each equipment RS.482) was used to spray the plots. Spray mixture of 2100 ml was necessary to cover 21 $m^2$ area of the plot (i.e., 1000 litres/hectare). Channel irrigation was done and borewell water was used for this purpose.

Three handweedings were carried out at intervals of 20 days in both the trials. The plot sizes were 7.0 x 3.0 m (It took 32 minutes to weed out each plot by one women labour using khurpi, a small hand operated implement), plant and row distance was 15 and 60 cm respectively in both the trials. The statistical design followed for both the trials was randomized block design with three replications. The sowing date for trial I was 7-6-1984 and trial II, 5-1-1985. The harvesting date for trial I was 6-9-1984 and trial II, 26-3-1985.

The average rainfall during June through September, 1984 was respectively, 79.5, 150.3, 54.4 and 101.4 mm and January through March, 1985 was 0.0, 0.0 and 0.3 mm. The mean maximum and minimum temperatures for 1984 varied 21.0-30.7°C (Max) and 10.3-21.5°C (Min) and 1985 between 21.0-31.3°C (Max) and 12.3-18.8°C (Min).

Germination of the crop seed and yield of the crop and percentage of monocot to dicot weeds/$m^2$ (date presented for trial II only), percentage of monocot and dicot weed species/$m^2$ (data presented for trial II only) and dry weight of weeds/0.5 $m^2$ were taken as parameters for assessing herbicide efficacy in beet root. As trial I revealed some very interesting trends in the control of different weed species, by herbicides, a detailed study of weeds was taken up in trial II.

The predominant weeds associated with beet root crop were the monocotyledonous *Digitaria marginate* Link., *Brachiaria erusiformis* (Sm) Griseb., *Eriochloa polystachya* H.B. et K., *Paspalidium germinatum* Stapf., *Setaria glauca* P. Beauv., *Cynodon dactylon* Pers. and *Cyperus* spp. and dicotyledonous *Lagasca mollis* Car., *Oldenlandia affinis* (Roem Schultz) Dc., *Urena lobata* L., *Aeschenomene indica* L., *Euphorbia* spp., *Ageratum mexicanum* L., *Cyanotis axillaris* Roem and Schultz and *Commelina benghalenis* L.

## RESULTS

**Effect of herbicides on the dry matter production of weeds** In beet root trial I (Table 1), butachlor at 1.5 and 2.0 kg ai/ha and fluchloralin at 1.0 kg ai/ha and in trial II (Table 1), all the treatments except fluchloralin 0.75 kg ai/ha gave effective control of weeds as measured by the reduced dry matter production of weeds on the 60th day.

**Effect of herbicides on the percentage of monocot to dicot weeds (Beet root trial II)** Except for alachlor 1.5 kg ai/ha, the rest of the treatments controlled monocots better than dicots as indicated by the per cent presence of monocot to dicot weeds / $m^2$ on 60th days (Table 2).

The major monocot weeds *Digitaria marginate* and *Brachiaria erusiformis* were controlled very effectively by alachlor 2.0 kg ai/ha and butachlor and fluchloralin at both the concentrations as measured by the per cent of total weeds/$m^2$ on the 60th day (Table 3). The percentage of *Erichloa* was more in alachlor, 1.5 and butachlor, 2.0 kg ai/ha when compared to hand weeded control. Best control of this weed was seen in fluchloralin, 1.0 kg ai/ha. Alachlor, 2.0 kg, butachlor, 1.5 kg and fluchloralin 0.75 kg ai/ha could not control *Cynodon dactylon* well. *Paspalidium germinatum* was controlled very effectively even at the lower concentration of butachlor and fluchloralin (Table 3).

None of the treatments was effective in controlling the major dicot weed *Lagasca mollis*. In fact the percentage growth of this weed was much more in treatments compared to handweeded control (Table 4). *Urena lobate* was suppressed by alachlor 2.0 kg and butachlor at both the concentrations. Fluchloralin was ineffective against this weed. *Oldenlandia affinis* outgrew in alachlor and butachlor 2.0 kg and fluchloralin at both the concentration when compared to handweeded control. Butachlor and fluchloralin even at the lower concentration controlled *Mollugo pentaphylla*. Alachlor did not allow *Euphorbia* spp. to come up. Compared to alachlor or butachlor, fluchloralin was less effective in controlling *Bidens pilosa* and *Ageratum mexicanum* (Table 4).

**Effect of herbicides on the yield of beet root** In trial I, butachlor at both the concentration increased the yield significantly when compared to handweeded control and also the rest of the treatments (Table 1). In trial II, the yield in treatments was on par with the handweeded control (Table 1).

**Economics of chemical weed control trials** For calculation of economics of herbicidal trials in beet root, one handweeding/crop, 50 labour/ha, RS.12.75/labour was kept constant. All the herbicidal treatments were found to be more economical than the handweeded control (Table 5).

## DISCUSSION

In our trials on herbicidal weed control in beet root, alachlor, butachlor and fluchloralin as preemergent treatments gave effective control of weeds upto a period of 60 days under sandy loam soil conditions. The previous research indicates that these three herbicides have not been tried at all in beet root. The only preemergent herbicides that were in vogue in this crop were chloridazon (14, 16) and lenacil (8, 15). Phenmedipham (8, 12, 16) and metomitron (15) although were good postemergent herbicides for beet root, could not give the desired level of weed control to avoid crop losses as the weeds grew initially for 10-15 days before the herbicides were applied to them. As mentioned earlier beet foot requires at least two to three weeks of initial weed free period to avoid yield losses (17).

Table 1. Effect of herbicides on the dry matter production of weeds and yield of beet root[1]

| Treatment | Rate[2] | Trial I | | Trial II | |
|---|---|---|---|---|---|
| | | Dry wt. of weeds[3] | yield[4] | Dry wt. of weeds[3] | yield[4] |
| H.W. Control[5] | - | 266.7 a | 9.7 c | 300.0 a | 10.0 |
| Alachlor | 1.50 | 186.7 b | 9.5 c | 136.7cde | 9.6 |
| Alachlor | 2.00 | 183.3 ab | 9.2 c | 60.0e | 9.6 |
| Butachlor | 1.50 | 133.3 bc | 16.3 a | 200.0bc | 11.3 |
| Butachlor | 2.00 | 116.7bc | 15.7 ab | 133.3 cde | 13.0 |
| Fluchloralin | 0.75 | 176.7 abc | 11.2 abc | 290.0 ab | 8.8 |
| Fluchloralin | 1.00 | 143.3 bc | 8.3 c | 196.7 cd | 9.0 |
| | | | | | NS[6] |

1 Means followed by the same superscript letters within each column are not sigificantly different from each other. Test has been done using L. S. D. (Students 't' - test).

2 Kilogram active ingredient per hectare.

3 Expressed in grams of weeds/0.5m$^2$ on 60th day.

4 Expressed as kg/plot.

5 Handweeding carried out three times at 20 days interval.

6 Not significantly different.

Table 2. Effect of herbicidal treatments on monocot and dicot weeds (Beet root trial II).

| Treatment | Monocot weed[1] | Dicot weed[1] |
|---|---|---|
| Handweeded control | 85.0 | 15.0 |
| Alachlor 1.5 kg ai/ha | 80.0 | 20.0 |
| Alanclor 2.0 kg ai/ha | 46.6 | 53.3 |
| Butachlor 1.5 kg ai/ha | 36.6 | 63.3 |
| Butachlor 2.0 kg ai/ha | 38.3 | 61.6 |
| Fluchloralin 0.75 kg ai/ha | 28.3 | 71.6 |
| Fluchloralin 1.0 kg ai/ha | 26.6 | 63.3 |

1 Expressed as % of total weeds/m$^2$ on 60th day.

Table 3. Effect of herbicide treatments on the major monocot weed species (Beet root trial II)[1]

| Treatment (kg ai/ha) | *Dilgtaria marginata* | *Brachiaria efusiformis* | *Eriochloa polystachyal* | *Paspalidium germinatum* | *Cyperus* spp. | *Cynodon dactylon* | *Panicum repens* |
|---|---|---|---|---|---|---|---|
| H.W. Control | 38.3 | 31.6 | 23.3 | 6.3 | 0.3 | 0.0 | 0.0 |
| Alachlor 1.5 kg ai/ha | 4.3 | 15.0 | 41.6 | 7.0 | 2.0 | 0.0 | 0.0 |
| Alachlor 2.0 kg ai/ha | 0.0 | 0.0 | 15.0 | 5.6 | 0.0 | 20.0 | 6.0 |
| Butachlor 1.5 kg ai/ha | 0.0 | 0.0 | 28.3 | 0.0 | 0.0 | 13.3 | 0.0 |
| Butachlor 2.0 kg ai/ha | 0.0 | 0.0 | 31.6 | 0.0 | 2.6 | 4.0 | 0.0 |
| Fluchloralin 0.75 kg ai/ha | 0.0 | 6.3 | 17.6 | 0.0 | 1.0 | 4.3 | 0.0 |
| Fluchloralin 1.0 kg ai/ha | 0.0 | 0.0 | 3.3 | 0.0 | 3.3 | 20.0 | 0.0 |

1 Expressed as % of total weeds/$m^2$ on 60th day.

Table 4. Effect of herbicide treatments on the major dicot weed species (Beet root trial II)[1]

| Treatment | | | *Lagasca mollis* | *Urena lobata* | *Mollugo pemtaphylla* | *Oldenlandia affihis* | *Commelina* spp. | *Cyanotis axillaris* | *Euphorbia* spp. | *Ageratum mexicanum* | *Bidens pilosa* | *Acanthospermum hispidum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H.W. Control | | | 3.5 | 3.3 | 2.5 | 0.6 | 0.6 | 0.2 | 0.3 | 0.0 | 0.0 | 0.0 |
| Alachlor | 1.5 | kg ai/ha | 8.0 | 18.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Alachlor | 2.0 | kg ai/ha | 32.6 | 0.0 | 3.3 | 14.5 | 0.0 | 2.3 | 0.0 | 0.0 | 0.0 | 0.2 |
| Butachlor | 1.5 | kg ai/ha | 56.5 | 0.0 | 0.0 | 1.3 | 0.2 | 2.6 | 2.6 | 0.0 | 0.0 | 0.0 |
| Butachlor | 2.0 | kg ai/ha | 31.6 | 0.0 | 0.0 | 22.0 | 0.0 | 0.0 | 7.3 | 0.0 | 0.0 | 0.0 |
| Fluchloralin | 0.75 | kg ai/ha | 20.0 | 8.3 | 0.0 | 30.0 | 1.0 | 0.6 | 1.0 | 6.6 | 3.3 | 0.6 |
| Fluchloralin | 1.0 | kg ai/ha | 15.0 | 15.0 | 0.0 | 18.3 | 0.0 | 2.3 | 2.0 | 3.3 | 1.6 | 0.6 |

1 Expressed as % of total weeds/$m^2$on 60th day.

Table 5. Net profit obtained in beet root due to herbicide treatments.

| Herbicide | Rate[1] | Duration of weed control[2] | Net profit[3] |
|---|---|---|---|
| Alachlor | 1.5 | 45 | 280 |
| Butachlor | 1.5 | 45 | 280 |
| Fluchloralin | 1.0 | 60 | 250 |

1 Kilogram active ingredient per hectare.

2 Expressed in days.

3 Expressed in rupees per hectare over the handweeded control.

In our study *Digitaria marginata* (major monocot weed) (Table 3) was controlled very effectively by all herbicides and *Euphorbia* spp. by alachlor (Table 4). Davis et al (4) got 6-8 weeks control of annual broadleaved weeds in beet root when they treated their fields with HCS-3438 at 2-4 lb/ac as pre-emergent sprays on organic soil.

In the present trial none of the herbicides tested had any adverse effect on the yield of beet root. A net profit of RS 280/ha over the handweeded control was obtained in beet root when alachlor or butachlor was used at 1.5 kg ai/ha and RS 250 with fluchloralin at 1.0 kg ai/ha. Senior et al (10) got increased yields of beet root with post emergence application of metomitron 3.5 kg/ha applied at the two true leaf stage. Petrov (8) found geksiluer at 2.0-2.4 kg/ha as preemergent treatment economical for beet root. Bhan et al (1) got maximum beet root yield with pyrazon, 3.0 kg plus phenmedipham 2.0 kg/ha when compared to other treatments.

The herbicides, alachlor, butachlor and fluchloralin which proved effective and economical for beet root in the present investigation has great potential for use in countries (especially USA where beet root is grown on large scale) having similar edaphic and climatic condition mentioned in our trials.

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# THRESHOLD LEVEL OF WEED CONTROL IN SOYBEAN CROP FOR SMALL FARMERS

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## ABSTRACT

A series of experiment was conducted in pot as well as in field at BIOTROP from August 1986 - August 1987 to study the competition between soybean (*Glycine max* L. Merr) and weeds. The pot experiment was carried out in accordance with those developed by de Wit (1960) and Spitters and van den Bergh (1982), while field experiments were carried out with vegetation analysis as that developed by Numata (1971). In the subsequent analysis it was assumed that RSO (Relative Space Occupation) as developed by Spitters and van den Bergh (1982) indicated how far the natural resources had been utilized by either soybean or weeds. This assumption was further extended that SDR (Summed Dominance Ratio) which incorporated dry weight as one of its component represented the utilization of natural resources. It was found that in this experimental condition, soybean SDR of 70% at 3 MAP represented the threshold level of SDR for soybean which corresponded to weed SDR of 30% at 3 weeks after planting.

## INTRODUCTION

In 1973 Indonesia still exported soybean about 36000 ton, but since 1975 she has been importing the increasing amount of soybean and it costed US$ 140 million in 1985 (6).

The projected national consumption during the IVth Five Year Development Plan has been increasing from year to year. It has been apparent also that the realization of the projected program has been short of the plan; it is therefore imperative that more works be done with greater attention for soybean production through both extensification and intensification. Intensification programs refer to activities to increase yield per unit area such as a) the use of high yielding varieties, b) improved cultural technique, c) fertilizer application, d) improved irrigation , e) integrated pest management, also f) improved harvesting, storing and processing; while extensification involves the expansion of areas planted with soybean. Areas which are available for expansion are unfortunately, those classified as marginal land (1, and Sudjadi and Satari, 1986). Some of those areas are dominated by alang-alang (*Imperata cylindrica* (L.) Beauv.). This type of area has been given a high priority for agricultural development (5) through transmigration.

Although recently government has been stimulating the establishment of big agricultural firms, most of soybean productions are currently still done by small farmers. This paper reports research findings based on small farm operation.

## MATERIALS AND METHODS

**Pot experiments** The pot experiments were carried out from March - August 1987 at BIOTROP, Bogor, Indonesia. Plastic pots of 5 l capacity were filled up with soil (latosol), sieved and fertilized with TSP (60 kg $P_2O_5$/ha), urea (45 kg/ha), $K_2SO_4$ (50 kg $K_2O_5$/ha).

For experiment 1, short tropical variety of soybean c.v. Willis was planted in monoculture on those pots at densities of 2, 4, 6 and 8 plants/pot. Similarly *Bidens pilosa* L. was also planted in monoculture at densities of 3, 6, 9 and 12 plants/pot. Those plants were harvested twice i.e. 3 and 13 weeks after planting. They were replicated 4 x in completely randomized design. The plants were kept free of pests and diseases, and watered daily.

At 3 week harvest, and RSO (Relative Space Occupation) were calculated from total dry weight of both soybean and *B. pilosa* according to spitters and van den Bergh (1982). Grain yield of soybean was recorded from 13 week harvest.

For experiment 2, treatment arrangements following the method developed by de Wit (9) were set up. These treatments consisted of soybean densities of 2 and 4 plants/pot and *B. pilosa* at 3 and 6 plants/pot planted in monoculture and a mixture of 2 plant soybean + 3 plants of *B. pilosa*/pot. These treatments were replicated 6 x in a completely randomized design. The plants were harvested at 3 weeks after planting and crowding coefficient was calculated.

For experiment 3, the treatments were arranged factorially and randomized completely. The first factor was soybean density consisting of 3 levels i.e. 2, 4 and 6 plants/pot; while the second factor was competing mixture of *B. pilosa* at 0, 3, 6 and 9 plants/pot. The treatment were replicated 3x. The plants were kept free of pests and diseases and watered daily. The grain yield was recorded from harvest at the end of the experiment at 90 days after planting.

**Field experiments** Two field experiments were carried out, i.e. one experiment was done on areas previously dominated by alang-alang (*Imperata cylindrica* L. Beauv.), and another one on upland area non-alang-alang.

The first experiment was done at BIOTROP, Bogor, Indonesia from August 1986 - March 1987 on latosol soil which was grown by alang-alang for the last 16 months. The plots measured 4 x 5 $m^2$ with pathway of 1 m wide. The experimental design was split-plot, the main plot was alang-alang control consisting of 4 methods i.e. imazapyr (2.0 kg ai/ha), glyphosate (2.5 kg ai/ha), glufosinate (3.0 kg ai/ha) and manual control, while sub-plot was time of soybean planting, i.e. 1, 2 and 3 months after alang-alang control.

Soybean variety was Americana planted at 40 x 20 cm by dibbling the seed. The plots were fertilized with TSP (60 kg $P_2O_5$/ha), urea (45 kg N/ha) and $K_2SO_4$ ( 50 kg $K_2O_5$/ha), given in strip along the dibbling line. To prevent seedling damage from *Agromyza phaseoli* Furadan 36 was utilized. Weed control was done manually after calculation of SDR (Summed Dominance Ratio) (3). Weeds estimated through quadrat sampling of 0.5 x 0.5 m twice in each plot, at 3 weeks after planting. The plants were sprayed with Azodrin at 2 cc/l and harvest was done at 120 days after planting.

The second experiment was also carried out at BIOTROP from February - June, 1987. The experimental design was split-plot, the main plot was planting distance consisting of three different planting distances i.e. 30 x 10, 40 x 10 and 40 x 20 cm; while subplot was weed control consisting of four different methods of weed control i.e. alachlor (1.4 kg ai/ha);

manual (at 3 and 6 weeks after planting), alachlor (1.4 kg ai/ha) followed by manual weeding at 6 weeks after planting and weedy check.

The soybean variety used was Willis. Weed condition was estimated using quadrat measuring 50 x 50 cm. Weed density, coverage and dry weight of weeds and soybean were sampled at 3 and 6 weeks after planting. The plants were maintained well and harvested 90 days after planting.

## RESULTS AND DISCUSSION

**Pot experiment** The dry weight of soybean and *B. pilosa* at 3 weeks were analysed according to the model developed by Spitters and van den Bergh (7).

$$\theta = \left(\frac{\beta z}{\beta z + 1}\right)\Omega \quad ; \quad RSO = \frac{\theta}{\Omega}$$

where :

$\theta$ = dry weight; $\beta$ = space occupied by a single plant growing alone; z = density; $\Omega$ = maximum dry weight which may be obtained at high plant density.

From simple calculations (Figs. 1, 2, 3) the values of , and RSO were obtained (Table 1).

The soybean population at 4 plants/pot is approximately equivalent to planting distance of 30 x 10 cm in the field. It leaves approximately 28% for weeds to explore. At a density of 2 plants/pot which is approximately equivalent to planting distance of 40 x 20 cm leaves a greater space for weed to explore; it means a greater risk that yield may be reduced by competition. When soybean plants were kept free of competition from weeds (*B. pilosa*), the grain yield was not different at those densities of 2, 4, 6 and 8 plants/pot (Table 2).

It seems that those plastic pots were too small to facilitate greater grain yield in the higher plant density. Although at 3 weeks after planting marked differences were observed in term of RSO and leaf number, but at the end of the experiment the grain yield was not different with different in planting densities; law of constant final yield seems to operate in this experiment (4).

However when soybean plants were allowed to compete against different weed densities the grain yield showed different responses where low density of soybean (2 plants/pot) was more sensitive to competition (Table 3).

The reduction of yield due to the presence of *B. pilosa* (3 plants/pot) was shown where the soybean density was 2 plants/pot, but *B. pilosa* at 3 plants/pot could be tolerated by soybean at 4 or 6 plants/pot; it seems that soybean density of 2 plants/pot was sensitive to weed competition.

When nature of the competition was studied through de Wit method (9) based on the dry weight of soybean and *B. pilosa* at 3 weeks after planting (Fig.5), showed that crowding coefficient of soybean (3 plants/pot) was 1 (one); the relative reduction of soybean and *B. pilosa* dry weight were similar.

The grain production of soybean may be though of as affected by the synthesis of production potential which is determined during the early growth, represented by the number of productive node, ultimately number of pods, and the capacity of soybean to realize the production potential through filling up pods into grains.

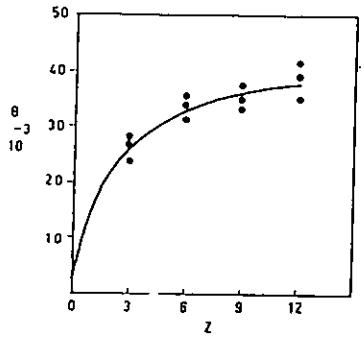


Fig.1. Hyperbolic relationship θ vs z. B. pilosa, 3 WAP.

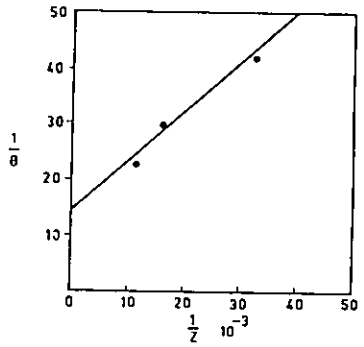


Fig.2. Linear relationship 1/θ vs 1/2. B. pilosa, 3 WAP.

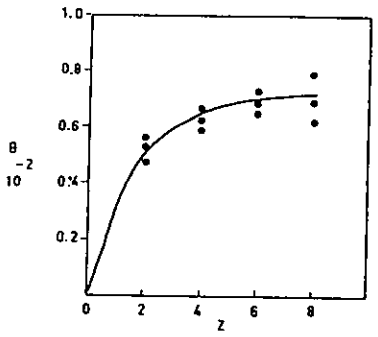


Fig.3. Hyperbolic relationship θ vs z. soybean, 3 WAP

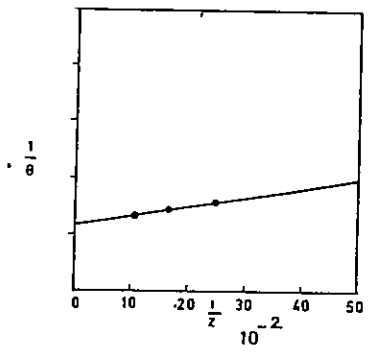


Fig.4. Linear relationship 1/θ vs 1/2. soybean, 3 WAP

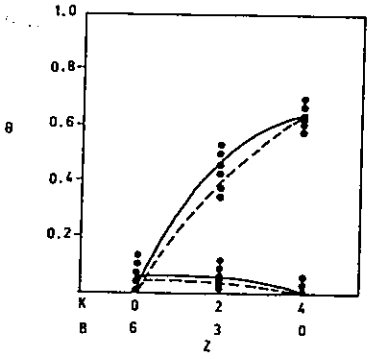


Fig.5. Graphical representation of θ vs z of soybean (K) and B. pilosa (B), in monoculture (——), and mixture (----).

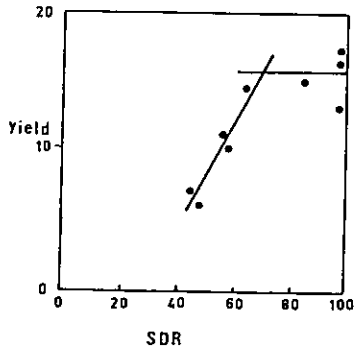


Fig.6. The relationship between soybean SDR 3 WAP and yield (qt/ha), SDR of 70% indicated threshold level.

Table 1. The values of , and RSO of soybean and *B. pilosa* at 3 weeks after planting.

| Species | | | RSO (%) at density | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Soybean | 0.65 | 0.97 | 56.50 | 66.10 | 72.20 | 76.50 | 79.60 | 82.00 | 83.90 | 85.40 |
| *B. pilosa* | 0.02 | 0.07 | 8.10 | 4.50 | 6.00 | 7.90 | 8.80 | 10.10 | 13.80 | 15.30 |

Table 2. Grain yield of soybean (gr/pot), RSO, and leaf number (no./pot) at 3 weeks after planting at various densities.

| Items | Soybean densities (Plants/pot) | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| RSO (3 WAP) | 56.50 | 72.20 | 79.20 | 83.90 |
| Leaf Number | $8^{a1}$ | $15^{b}$ | $23^{c}$ | $25^{c}$ |
| Grain yield (gr/pot) | 9.6 | 9.8 | 10.2 | 9.5 |

1 Numbers in row followed by different letter indicate statistical difference (p 0.05)

Table 3. Grain yield of soybean (g/pot) at various densities growing together with weed at various densities.

| Soybean densities | Weed densities | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| 2 | $8.9^{efl}$ | $6.7^{ed}$ | $5.3^{b}$ | $3.5^{a}$ |
| 4 | $9.8^{f}$ | $9.7^{f}$ | $7.6^{de}$ | $6.1^{bcd}$ |
| 6 | $9.9^{f}$ | $8.6^{af}$ | $6.9^{cd}$ | $5.5^{bc}$ |

1 Numbers followed by different letter indicate statistical difference (p 0.05)

When the population is low, the production potential can high if kept free of weed competition, but normally low, and the presence of weed will affect the capacity to develop high production potential, it seems that soybean density is very important in this aspect. Soybean density capable of giving RSO around 70% which is equivalent to planting density of 30 x 10 cm for this variety (Wills) seems to offer a better chance of high yield. When the population is too high, the high production potential may be achieved easily, but most of the resources have been utilized to form high production potential , and less utilized for realizing the potency, and only a small proportion of the production potential could be realized.

**Field experiments** The results of alang-alang control prior to soybean planting was shown in Table 4.

The development symptom of imazapyr (2.0 kg ai/ha) damage was slow; one month after spraying imazapyr (2.0 kg ai/ha), alang-alang showed only about 20% damage while that of glufosinate showed practically total kill. However 3 months later the regrowth under glufosinate treatment reached about 50% already, while the damage of imazapyr was still progressing and to some extent so was that under glyphosate treatment; the regrowth of alang-alang under manual cultivation was quite considerable already about 70%.

The establishment of soybean was good and weed control was done manually. The grain yield was shown in Table 5.

The soybean growth was quite different from those reported (8), where soybean (c.v. ORBA) suffered a heavy phytotoxicity from the residual activity of imazapyr (1.0 kg ai/ha) when experiment was done in pot. In this experiment, no phytotoxicity of soybean was observed, even 1 month after spraying of imazapyr at 2.0 kg ai/ha.

However the grain yields were very low and when the value of SDR'S (at 3 weeks) were consulted their values were around 40.1 - 42.5%; and these were very low also.

It seems that low SDR values are indicative of low grain yield. Another result of practical significance was that zero tillage technique could be utilized to establish soybean crop in alang-alang field following application of appropriate herbicide.

The use of SDR which incorporated dry weight as one of its component to predict the grain yield of soybean was further demonstrated in the second experiment. The complete values of SDR at 3 and 6 WAP were presented in Appendix I and II.

The calculation of SDR at 3 weeks after planting, followed by the appropriate statistical analysis was useful to evaluate the efficacy of weed control (Table 6).

The application of alachlor (1.4 kg ai/ha) as pre-emergence herbicide was successful in increasing the SDR value of soybean, or reducing the SDR value of weeds (Table 7). Treatment No. 3 was effectively similar to treatment 1 (control, since SDR's values were calculated before weeding at 3 weeks after planting; while treatment 3 was similar to treatment 4 since both received pre-emergence treatment of alachlor 1.4 kg ai/ha.

The values of SDR at 6 weeks after planting was shown in Table 7 also showed clearly how weed control treatment affect the values of SDR.

The condition of soybean under treatment 2 had been improving in term of soybean SDR, it increased from 47.4 at 3 WAP to 63.4 at 6 WAP. This was so because soybean under treatment 2 was weeded at 3 WAP, while soybean under treatment 1., 3 and 4 remained unchange.

These weed control treatments were reflected also in the growth performance and yield of soybean (Table 8).

Table 4. Percentage of damage of alang-alang prior to soybean planting.

| Treatments | Months after treatment | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Imazapyr (20 kg ai/ha) | 18.3 | 30.0 | 69.3 |
| Glyphosate (2.5 kg ai/ha) | 30.0 | 86.0 | 88.3 |
| Glufosinate (3.0 kg ai/ha) | 99.3 | 84.3 | 48.3 |
| Manual | 81.7 | 60.0 | 31.0 |

Table 5. The pods and grain yield of soybean c.v. Americana as affected by alang-alang control.

| Treatments | Pod number (No./plant) | Grain yield (kg/ha) |
|---|---|---|
| Imazapyr (20 kg ai/ha) | 21.1[bl] | 746.7 |
| Glyphosate (2.5 kg ai/ha) | 19.6[ab] | 760.0 |
| Glufosinate (30 kg ai/ha) | 15.6[a] | 680.0 |
| Manual | 15.4[a] | 696.7 |
| | | (NS) |

1 Number in column followed by different letter indicate statistical difference (p 0.05).

Table 6. The average value of SDR's of soybean calculated at 3 weeks after planting as affected by various treatments.

| Treatment | SDR of soybean |
|---|---|
| Control (weedy check) | 48.2[al] |
| Manual weeding 2 x (at 3 & 6 WAP) | 47.4[a] |
| Alachlor (1.4 kg ai/ha) followed by manual 6 WAP | 65.3[b] |
| Alachlor (1.4 kg ai/ha) | 70.9[b] |

1 Numbers followed by different letter indicate statistical difference (p 0.05).

Table 7. The average value of SDR of soybean calculated at 6 WAP as affected by various treatments.

| No. | Treatments | SDR values |
|---|---|---|
| 1. | Control | 49.7[a1] |
| 2. | Manual weeding 2x | 63.4[b] |
| 3. | Alachlor (1.4 kg ai/ha) followed by manual 6 WAP | 64.7[b] |
| 4. | Alachlor (1.4 kg ai/ha) | 64.1[b] |

1 Numbers followed by different letter indicate statistical difference (p 0.05).

Table 8. The effect of weed control on the growth performance and yield of soybean (c.v. Willis).

| No. | Weed Control | Height (cm) 9 WAP | Leaf Area Index 9 WAP | Pod number 9 WAP | Filled pods harvest | Grain yield ton/ha |
|---|---|---|---|---|---|---|
| 1. | Weed check | 45.8[a1] | 3.1[a] | 11.9[a] | 10.3[a] | 0.8[a] |
| 2. | Manual weeding (at 3 and 6 WAP) | 56.7[b] | 5.2[b] | 16.7[ab] | 15.8[b] | 1.5[bc] |
| 3. | Alachlor (1.5 kg ai/ha) f.b. manual 6 WAP | 58.7[b] | 5.9[b] | 19.4[b] | 18.4[b] | 1.6[c] |
| 4. | Alachlor (1.5 kg ai/ha) | 56.9[b] | 5.2[b] | 18.5[b] | 17.0[b] | 1.3[b] |

1 Numbers in column followed by different letter indicate statistical difference at (p 0.005).

Appendix 1. Analysis of vegetation in the field experiment 3 weeks after planting(SDR).

| Species | Treatments[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $A_1B_0$ | $A_1B_1$ | $A_1B_1$ | $A_1B_1$ | $A_2B_0$ | $A_2B_1$ | $A_2B_2$ | $A_2B_3$ | $A_3B_0$ | $A_3B_1$ | $A_3B_2$ | $A_3B_3$ |
| 1. Soybean (*Glycine max* L. Merill.) | 52.6 | 53.5 | 72.4 | 74.9 | 50.2 | 49.3 | 68.7 | 75.3 | 41.8 | 39.4 | 54.7 | 62.6 |
| 2. *Boreria alata* | 24.7 | 27.9 | 13.8 | 8.7 | 23.9 | 21.8 | 17.2 | 13.2 | 31.6 | 16.8 | 16.5 | 15.7 |
| 3. *Ageratum conyzoides* L. | 10.5 | 10.6 | 2.6 | 4.3 | 8.3 | 8.6 | 1.1 | 3.5 | 5.6 | 11.9 | 5.5 | 5.5 |
| 4. *Eleutheranthea ruderalis* (Sch.) Bip. | 1.9 | 0.6 | 3.2 | 3.4 | 4 | 7.1 | 5.1 | 3.6 | 4.3 | 6.7 | 11.2 | 6.9 |
| 5. *Phyllanthus niruri* L. | 2.3 | 3.7 | 0.6 | 0.7 | 3.8 | 3.6 | | | 4.8 | 6.8 | 1.9 | 1.9 |
| 6. *Porophyllum ruderalle* | 0.3 | 0.5 | | | 0.2 | 1 | 1.1 | 2.4 | 0.6 | | | 0.9 |
| 7. *Himosa oudica* | 0.6 | | 0.6 | | | | 0.3 | | 2.2 | | | 1 |
| 8. *Cleome permum* DC. | 0.3 | 0.3 | | | 0.6 | | | | 0.3 | 3.3 | 1.8 | 1.8 |
| 9. *Oxalis corniculata* L. | 0.2 | 0.1 | 0.9 | | 0.4 | | 0.3 | | | 1.6 | | |
| 10. *Digitaria ciliaris* (Retz.) Koel. | 4.9 | 1 | 1.9 | 0.3 | 5.3 | 4.4 | 0.9 | 1 | 0.5 | 1.9 | | |
| 11. *Imperata cylindrica* (L.) Beauv. | 0.7 | 0.5 | 1.4 | 3.1 | | 1.8 | | 0.6 | 3.1 | 3.8 | | |
| 12. *Celosin argantea* L. | 0.8 | | | 2.9 | 0.8 | | | | | 0.3 | | |
| 13. *Trida orocumbens* L. | | 0.4 | | | 0.1 | | | | | | | |
| 14. *Erechtites valerianifolia* | | 0.6 | | 1 | | 0.8 | | 0.3 | 1 | 2.2 | 1.2 | 1.7 |
| 15. *Panicum reoens* | | | 2.5 | | 1 | | 2.9 | | | 3.4 | | 1.5 |
| 16. *Commelina benghalis* L. | | | | 0.6 | | 0.3 | 0.5 | | | | | |
| 17. *Bidens pilosa* L. | | | | | 0.5 | | 2 | | 0.5 | 0.2 | 1.9 | |
| 18. *Croton hirtus* | | | | | 0.4 | | | | 2.4 | | | |
| 19. *Polygala paniculata* | | | | | 0.2 | | | | | 0.4 | | |
| 20. *Emilia sonchifolia* (L.) DC ex thite | | | | | | 10.2 | | 1 | 0.5 | | 0.6 | 1.3 |
| 21. *Eleusine indica* (L) Gaertn. | | | | | | | | | | | | |
| 22. *Cyperus rotundus* L. | | | | | | 0.3 | | | 0.7 | | 3.7 | |
| 23. *Cyperus iria* L. | | | | | | | | | | | | |
| 24. *Cynodon dactylon* (L.) Pers. | | | | | | | | | | 2.1 | | |
| 25. *Amaranthus* spp. | | | | | | | | | | 0.8 | | |
| 26. *Eupatorium odoratum* | | | | | | | | | | | 0.6 | |

1. $\lambda_1$= Planting distance 30 x 10 cm
   $\lambda_2$= Planting distance 40 x 10 cm
   $\lambda_3$= Planting distance 40 x 20 cm

   $B_0$= weedy check
   $B_1$= manual weeding(3 WAP)
   $B_2$= alachlor(1.4 kg ai/ha) + 6 WPA
   $B_3$= alachlor(1.4 kg)

Appendix II. Analysis of vegetation in field experiment 6 weeks after planting(SDR).

| Species | Treatments[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $A_1B_0$ | $A_1B_1$ | $A_1B_2$ | $A_1B_3$ | $A_2B_0$ | $A_2B_1$ | $A_2B_2$ | $A_2B_3$ | $A_3B_0$ | $A_3B_1$ | $A_3B_2$ | $A_3B_3$ |
| 1. Soybean (*Glycine max* L. Merill.) | 57.1 | 68.6 | 68.2 | 69 | 46 | 61 | 64.5 | 66.3 | 40.7 | 60.7 | 61.5 | 56.4 |
| 2. *Boreria alata* | 24 | 10.7 | 19.2 | 18.3 | 31.3 | 13.5 | 13 | 15.3 | 30 | 12.7 | 18.4 | 12.3 |
| 3. *Ageratum conyzoides* L. | 5.9 | 6.3 | 6.4 | 4.9 | 6.7 | 6.3 | 7.3 | 4 | 11.9 | 8 | 2.9 | 4.6 |
| 4. *Eleutheranthera ruderalis* (Sch.) Bip. | 1.6 | 1.3 | 2.3 | 1.3 | 2.9 | 2.3 | 6 | 3.2 | 1.9 | 2.3 | 3.8 | 7.4 |
| 5. *Phyllanthus niruri* L. | 1.2 | 0.5 | 0.9 | 1.8 | 1.5 | 2 | 1.7 | 1.2 | 3.7 | 2 | 0.3 | 4.7 |
| 6. *Porophyllum ruderalle* | 0.2 | - | | | | 0.4 | | 0.5 | 0.6 | 0.7 | 0.3 | 0.5 |
| 7. *Cleome rutidosnermum* | 0.3 | 0.4 | | | 0.2 | | | 0.2 | 1 | 0.3 | | 0.5 |
| 8. *Oxalis corniculata* L. | 0.4 | - | 0.5 | 0.4 | 0.2 | 0.3 | 0.7 | 0.8 | 0.1 | 1.6 | 0.2 | 0.8 |
| 9. *Digitaria ciliaris* (Retz.) Koel. | 6.8 | 2.4 | 0.8 | 1 | 9.2 | 4.9 | 2.4 | 5.2 | 6.9 | 3.5 | 10.3 | 3.8 |
| 10. *Mimosa invisa* L. | 0.6 | 2.1 | 0.4 | | | 0.2 | 0.6 | | 0.3 | 0.4 | | 0.3 |
| 11. *Sida rhobifolia* L. | 0.8 | - | | | | 0.4 | 0.4 | | | | | |
| 12. *Celosin argantea* L. | 0.6 | - | | | | | | 0.8 | | | | |
| 13. *Imperata cylindrica* (L.) Beauv. | 0.2 | 1.3 | 0.5 | 1.8 | 0.6 | 0.9 | | | 1.9 | 0.8 | 0.9 | 3.6 |
| 14. *Erechtites valerianifolia* | 0.2 | 0.2 | | | | | 0.5 | | 0.4 | 1.6 | | 0.6 |
| 15. *Tridax orocumbens* L. | - | 0.4 | | | 0.2 | | 0.3 | | | 0.2 | | |
| 16. *Cyperus iria* L. | - | 1.2 | | | | | | | | | | |
| 17. *Euohorbia hotorophylla* L. | - | 0.3 | | | 0.2 | | | | | | | |
| 18. *Cyperus rotundus* L. | - | 2.4 | | 0.3 | 0.5 | 6.5 | | 0.9 | | 1.4 | 1.5 | 0.8 |
| 19. *Paspalum conjugatum* Berg. | - | - | 1.4 | | | | | | | 0.4 | | 1.7 |
| 20. *Polygala paniculata* | - | - | | 0.3 | | | | | | | | |
| 21. *Bidens pilosa* L. | - | - | | | | 0.3 | | 0.4 | | | | |
| 22. *Emilia sonchifolia* (L.) DC. ex thite | - | - | | | 0.2 | | 0.4 | 0.8 | 0.3 | | | |
| 23. *Stachytarpheta jamaicensis* (L.) | - | - | | | | | 0.2 | | | | | |
| 24. *Panicum reoens* L. | - | 1.3 | | | | 0.4 | 0.8 | 0.5 | | | | |
| 25. *Eleusine indica* (L.) Gaertn. | | | | | | 0.9 | | | | 3.2 | | 0.8 |
| 26. *Cynodon dactylon* (L.) Pers. | - | - | - | - | - | - | 0.4 | | | | | |
| 27. *Themeda arguens* | | | | | | | 0.8 | | | | | |
| 28. *Commelina benghalensis* | | | | | | | | | | | | 0.9 |

1 See Appendix II.

Weed controls increased soybean height, Leaf Area Index (up to 6 WAP) pod number, filled pods and grain yield (Table 8), of particular interest was the effect of weed control on pod number. Early control of weeds by application of preemergence herbicide set high production potential (treatment 3 and 4) of soybean in the form of pod number. manual weed control at 3 WAP remedied the production potential of soybean (treatment 2).

It was rather unfortunate that this cultivar shed its leaves very early, since LAT dropped drastically at 8 weeks after planting, so the capacity to realize the high production potential was much reduced.

While alachlor (1.4 kg ai/ha) and manual weeding were sufficient to support high production potential of soybean they were not good enough to facilitate the realization of the production potential. This was improved by further manual cultivation at 6 WAP as shown by treatment 3 which produced the highest grain yield of 1.6 ton/ha.

Inspection of SDR values of soybean at 3 WAP and grain yield showed an interesting relationship (Fig. 6).

Values of SDR between 40 - 70% was linearly correlated with grain yield; where the increasing value of SDR also indicated the increasing grain yield; however when SDR values were above 70% were not followed by increasing grain yield. It seems that value of soybean SDR at 3 WAP of 70% can be taken as a threshold level for weed control. It bears practical implication since it can be utilized as an indicator whether soybean crop is good enough in term of its crop-weed competition. Values lower than 70% indicates that this soybean crop suffers much competition from weeds and weed control is badly needed.

The control should be done quickly, to facilitate soybean crop to recover and set its optimum production potential. This should be done not later than its critical period (2) and in this experiment 3 WAP was sufficient, later than 6 weeks after planting will not be useful since at this time, for soybean c.v. Willis started to shed some of its leaf. It is obvious then that early weed control is beneficial and the application of preemergence herbicide is usually better than manual weeding at 3 WAP, from the point of view of grain yield.

From the results of the series of experiment above it is concluded that soybean SDR of 70% measured 3 WAP, may be taken as a threshold level, below which the grain yield will be less. This threshold level may not coincide with the economic threshold level as discussed by Coussen (1980) because the arguments used to arrive at the value has not included the necessary economic consideration. However for small farmers, where the opportunity to find another job is limited, if not nil, it is more important to convert the available to something consumable or marketable, and weeding works will be a good option. It is in this context, the writer suggest the application this threshold level of weed control.

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# CASE STUDIES ON AERIAL HERBICIDE APPLICATIONS ON PASTURES IN HAWAII

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## ABSTRACT

Case studies on aerial herbicide applications on three ranches are being conducted in Hawaii. This report covers the cost of aerial applications on each ranch. An aerial application was 1/3 the cost of a ground application. Moreover, aerial applications over large target areas can be completed in timely manner, whereas the much slower ground applications frequently cannot. Because of the expense of the aircraft, it was critical that the ground support be highly efficient.

## INTRODUCTION

Aerial herbicide application on pastures offer several advantages over ground application. Because aircraft can cover large areas in a short time, application costs per acre are low. Furthermore the long term costs of weeds and weed control are reduced:

1. Each cycle of herbicide application is completed when applied by air. in contrast, ground applications may be hampered by weather, equipment breakdown, worker absence, or the press of other duties. This can result in unrestrained weed growth and reduced forage yield. As the brush grows larger, herbicide applications become more difficult, further slowing weed control operations until eventually, the pasture has degenerated into brushland.
2. Steep, remote, or densely vegetated terrain, which are difficult or impossible to spray with ground rigs, can be readily treated from the air.
3. Aerial applicaton are typically more precise than ground applications, particularly if the ground spray crews are poorly trained, or if they are working on difficult terrain.
4. Quick removal of the brush canopy allows forage production increases immediately rather than incrementally with the much slower ground application.

Aerial application does have some disadvantages:

1. The cost of the operation is borne initially, rather than spread over the year as it is with ground application.
2. Aerial spraying may not be feasible in close proximity to homes and farms.

Although the advantages of aerial herbicide application on pasture land *vis-a-vis* ground applications are clear, convincing ranchers of that is difficult, given the large initial investment that is required. In order to develop the cases for aerial application, case studies were initiated on ranches that had adopted aerial application.

## PROCEDURE

Three ranches, designated H, D, and N, participated. Each rancher financed his own operation.

**Ranch H** Ranch H was located on the eastern and windward side of the island of Maui. The average annual rainfall was 2540 mm, more or less uniformly distributed throughout the year (1). The primary brush species in the target area was *Psidium guajava* L., the most common pasture weed in Hawaii (6, 7). Most of the stands were 3-7 m tall and so densely populated so as to hinder equipment movement. Although Ranch H had a ground application program in place, that program could not keep up with the guava growth.

**Ranch D** Ranch D was also located on the windward side of east Maui. The ranch had an average rainfall of 2743 mm, more or less uniformly distributed throughout the year (1). The major brush problem on Ranch D was *Ardesia humulis* Vahl, a small tree from Sri Lanka (7) that forms stands so dense as to be impenetrable to a man on foot. *A. humulis* appears to be a serious potential pest of humid lands. Fortunately it seems to be confined to the eastern tip of Maui.

**Ranch N** Ranch N was on the windward side of west Maui. This area was drier, with an average annual rainfall of 1016 mm of rainfall, more or less uniformly distributed over the year (1). The major brush species here was *Schinus terebinthifolius* Raddi, a small tree native to Brazil (7).

## RESULTS

**Ranch H** Fortunately guava was susceptible to 2,4-D (2, 3), a very inexpensive herbicide. However, control requires 2 or 3 applications in annual treatments (4). The ground application program in place was too slow to keep up with the problem and was expensive (Table 1).

The first application of 2.2 kg 2,4-D ae/ha at Ranch H was made in August 1985. A second identical application was made in July 1986.

After each application, the guava plants were severely defoliated but they began to recover after 6 months. These responses were consistent with experiments conducted earlier (3, 4). A third application was intended. However, the new owners of Ranch H abandoned the project. They believed that brush could be controlled by intensive grazing management.

The cost of the first Ranch H aerial application was 1/3 that of ground applications, even though the first aerial operation was rather inefficient because of poor ground equipment and an inexperienced ground crew (Table 1). Moreover, this assumed that the entire infested area could be covered by ground equipment within a year which was not the case.

The greatest economy was made on labor, such that even with the added cost of aircraft rental, and disregarding equipment and fuel costs of ground spraying, almost $60/ha was saved.

Table 1. Costs of ground and aerial applications of 2,4-D on Ranch H.

| | Ground | Aerial[1] |
|---|---|---|
| Volume-rate (l/ha) | 1307 | 93 |
| Area sprayed (ha/day) | 2.1 | 137 (7 hr) |
| Area sprayed (ha/hr) | 0.3 | 20 |
| 2,4-D applied (kg/ha) | 2.9 | 2.2 |
| Cost: | | |
| Herbicide ($/ha @ $3.17/L) | 12.82 | 9.88 |
| Labor ($/ha @ $10/hr) | 76 | 1.97[2] |
| Surfactant ($/ha @ 1.66/l) | 11.16 | 0.77 |
| Aircraft ($/ha) $325/hr) [3] | 0 | 18.26 |
| Total cost ($/ha) | 99.98 | 30.88 |

1 Data for application of August 1985

2 Calculation includes 6 man-hr preparation and clean-up time.

3 Calibration includes 0.7 hr ferrying time.

Table 2. Actual and theoretical costs of aerial application of 2,4-D on Ranch D.

| | Actual[1] | Theoretical |
|---|---|---|
| Volume-rate (l/ha) | 93 | - |
| Area sprayed (ha) | 115 | - |
| Time to spray (hr) | 9 | 4.0 |
| Area sprayed (ha/hr) | 14 | 29 |
| 2,4-D rate (kg/ha) | 4.5 | - |
| Cost: | | |
| Herbicide ($/ha @ 3.17/l) | 19.76 | 19.76 |
| Surfactant ($/ha @ 1.66/l) | 0.77 | 0.77 |
| Labor ($/ha @ $10/hr)[2] | 2.86 | 1.56 |
| Aircraft ($/ha @375/hr)[3] | 31.63 | 15.32 |
| Total Cost | 55.02 | 37.41 |

1 Water supply restricted by storm damage resulted in slow reloading. Spraying time extended from theoretical 6 hr to 11 hr.

2 Calculation includes 6 man-hr preparation and clean-up time.

3 Calculation includes 0.7 hr ferrying time. Table 3. Cost of aerial application of picloram on Ranch N.

Table 3. Costs of aerial application of picloram on Ranch N.

| | |
|---|---|
| Volume-rate (l/ha) | 93 |
| Area sprayed (ha) | 65 (2.25 hr) |
| Area sprayed (ha/hr) | 29 |
| Picloram applied (kg/ha) | 0.7[1] |
| Cost: | |
| Herbicide ($/ha @26.46/l) | 77.26 |
| Surfactant ($/ha @ 1.66/l) | 0.77 |
| Labor ($/ha @ $10/hr)[2] | 2.00 |
| Aircraft ($/ha @ 375/hr)[3] | 17.30 |
| Total Cost ($/ha) | 102.44 |

1 49 ha at 0.56 kg/ha, 16 hr @ 1.1 kg/ha

2 Calculation includes 4 man-hr preparation and cleanup time.

3 Calculation includes 0.7 hr ferry time.

Since the aerial application was made at a volume-rate of only 93 l/ha in contrast to 1307 l/ha for ground applications, and since surfactant use is a function of the volume-rate, much less surfactant was required for the aerial application. Hence, there was a savings of over $10.00/ha on surfactant costs.

Theoretically there should be no difference in herbicide costs between aerial and ground application. But in fact, overdosing is typical with ground application because of poor mobility in dense brush and poorly trained personnel. The ground crew on Ranch H was just recently trained so their application techniques were relatively precise. Even so, there was about 30% overdosing equivalent to nearly $3.00/ha. Overdosage in the range of 6-fold is not unusual, and even 13-fold had been encountered. Therefore the precision of aerial application is a significant economic and environmental advantage from this standpoint.

**Ranch D** The aerial application of 4.4 kg 2,4-D ae/ha at Ranch D in November 1986 was severely hampered by recent storm damage to the water system of the ranch. Water flow was so severely restricted that reloading of the aircraft took as long as 15 min rather than the one minute it should have. Because of this, the application took 8 hr, rather than the 4 hr expected, which inflated the aircraft and labor costs (Table 2).

*A. humilis* was quickly defoliated but by 7 mo was refoliating. A followup application is planned for September when rainfall is expected to ease.

The extended length of time to complete the herbicide application resulted in a cost of $55.02/ha rather than the theoretical $37.41/ha. The difference between the theoretical Ranch D cost and the cost at Ranch H was because Ranch D used a higher rate of 2,4-D.

**Ranch N** Because *S. terebinthifolius* was not sensitive to 2,4-D (5), Rancher N used picloram (3) which was a more expensive herbicide. Most of the area was treated at 0.56 kg/ha. A few acres were treated at 1.12 kg/ha for an average of 0.70 kg/ha. The higher herbicide cost accounted for most the higher cost at Ranch N *vis-a-vis* ranches H & D (Table 3). On the other hand, prior experience with *S. terebinthifolius* indicated that a single picloram treatment would

suffice. Repeat treatments would probably not be necessary. Also, because Ranch N was smaller than Ranches H & D, the unit aircraft rental cost was higher. In all cases, the aircraft charge included travel to and from its home base. Although aerial costs were higher than that at the other ranches, ground application would probably be higher because a large part of Ranch N was very steep.

## CONCLUSION

Aerial herbicide application was much more economical than ground application. Labor costs dominated in ground application. In contrast, because of the rapidity with which aircraft covered large areas, labor costs were greatly reduced in aerial applications. Furthermore, because of the lower volumes utilized in aerial applications, surfactant costs were also greatly reduced. However, the ground support must be efficient to avoid wasting aircraft time on the ground.

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# CLUSTER BEAN - A POSSIBLE HERBICIDAL SOURCE FOR MANAGING CHINA DODDER

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## ABSTRACT

Eighteen valuable vegetable crops of this area were chosen for study of the host-parasite relationship with reference to china dodder, *Cuscuta chinensis* Lam., a complete stem parasite or a phloem parasite. They are *Amaranthus viridis* L. (Amaranthaceae), *Cucumis sativus* L., *Lagenaria siceraria* (Molina) Standley, *Luffa acutangula* (L.) Roxb., *Momordica charantia* L., *Trichosanthes cucumerina* L. (Cucurbitaceae), *Abelmoschus esculentus* (L.) Moench, *Hibiscus cannabinus* L., (Green cultivar), *Hibiscus cannabinus* L. (Red cultivar) (Malvaceae), *Canavalia gladiata* (Jacq.) DC., *Cyamopsis teragonoloba* (L.) Taub., *Dolichos lablab* L., *Vigna mungo* (L.) Hepper, *Vigna radiata* (L.) Wilczek, *Vigna trilobata* (L.) Verdc. (Papilionaceae), *Capsicum frutescens* L., *Lycopersicon lycopersicum* (L.) Karsten, *Solanum melongena* L. (Solanaceae). The hosts showed varying grades of interaction regarding the ensuing parameters examined: 1. Stimulation of parasite seed germination by host, 2. the potentiality of the germinated seedling to infect the susceptible host, 3. permitting the unrestricted growth of parasite by the host, 4. potentiality of any one of these hosts to inhibit parasite's ramification, if any. All the vegetable hosts induced germination in the otherwise dormant seeds of china dodder except *Dolichos lablab*, *Hibiscus cannabinus* (Green cultivar) and *Canavalia gladiata*. *Hibiscus cannabinus* (Red cultivar), *Lycopersicon lycopersicum*, *Lagenaria siceraria* , *Momordica charantia*, *Trichosanthes cucumerina* induced germination but not susceptible to seedling infection. Except in *Canavalia gladiata* all other exposed parts of the crops were infected and the parasite established haustoria. But exceptionally *Cyamopsis tetragonoloba* (cluster bean) alone showed induction of stunted growth, abnormal dwarfing, premature cessation of branching and degeneration of flowers in the parasite. The parasite on cluster bean managed to survive with a pseudodichotomous gracillarioid habit. A check is inflicted on its unmanageable, profuse ramification from one host to the other. Secondary branches from the adjacent hosts were found to infect cluster bean shoots and soon attain the dwarfing trait and the same was found to extend back to the parasite on hosts from which it originated, Thus, parasitising cluster bean appears to be end for china dodder. This situation suggests a phloem transmitted active principle from cluster bean to china dodder which needs to be further investigated for its potential source as a bioherbicide. The details of the data have been discussed.

## INTRODUCTION

There are reports of dodders parasitising vegetable crops. Karapetyan (4) no losses caused by *Cuscuta campestris* in vegetable crops. Agricaltura Italiana (2) dr to the attack of *Cuscuta* sp. on *Vicia faba*, potato and sugar beet. Narayana Reddy severe infestation of *C. chinensis* on onion seedlings. Yang (11) recorded t *chinensis* had a wide range of hosts including several vegetables.

Awatigeri et al. (1) reported the attack of *C. chinensis* on chillies and b Hutchison (3) experimented with dodder control measures in tomato fields. Narayana made some suggestions to manage dodders on pulses in tropical areas. Some vegetabl dodder infection in Turkey (8). So there is a need for a search of suitable vegeta resist or trap dodders.

China dodder is a fast spreading menace in vegetable and pulse crops of District, A. P., South India. There is no basic information on germination infestation and ramification capacity of *Cuscuta chinensis* on vegetable crops o Incidentally, cluster bean has been found to inhibit the growth of china dodder. like *Chenopodium* sp. also resist dodder growth (Pers. discussion with Dawson, 1987 This aspect also forms contents of this paper.

## MATERIALS AND METHODS

China dodder seeds used for study were collected on 24-4-1985 from green gr Krishna Dt., A. P. infected with parasite. They were cleaned, dried and stored ir glass stoppered bottles. Fresh seed, one sample each of a cultivar, of 17 vegeta were obtained from seed corporation on 27-7-1986.

Earthenware pots of uniform size were filled with field soil and cattle manure we 3:1 ratio. On 3-8-1986, three vegetable seeds of each cultivar sown at 1 to 2 cm c centre of each pot. An interspace of about 2 cm was maintained between one seed and Ten dodder seeds with an interspace of 1-1.5 cm were sown in a circle around th seeds. Pots were watered from the rim carefully to keep seed alignment intact til germinated. A control pot with the china dodder seeds alone and without the vegetabl also maintained throughout the experiment. Stimulation of germination of untreated c seeds was looked for. When they failed to germinate scarified seeds were used for i In instances where the seedling failed to establish on the host, infestation was a using vegetative branches of dodder. Pots were watered twice a day. Germination b hosts and parasite were tabulated (Table 1).

Germination of dodder seeds in pots was keenly observed throughout the period of Observation in respect of the following parameters were recorded: 1. Stimulation of of parasite seed by host. 2. The potentiality of the germinated parasite seedling to susceptible host. 3. Whether unrestricted growth of parasite on the host pre Potentiality of anyone of these hosts to inhibit the parasite ramification. The dat (Table 2).

Table 1. Germination behaviour of vegetable cultivar and china dodder seeds.

| Sl. No. | Vegetable hosts[1] | No. of days required for germination | | No. of dodder seeds germinated per ten |
|---|---|---|---|---|
| | | host | dodder | |
| | Amaranthaceae | | | |
| 1. | *Amaranthus viridis* | 4 | 14 | 2 |
| | Cucurbitaceae | | | |
| 2. | *Cucumis sativus* | 1 | 2 | 7 |
| 3. | *Lagenaria siceraria* | 7 | 12 | 3 |
| 4. | *Luffa acutangula* | 3 | 14 | 1 |
| 5. | *Momordica charantia* | 4 | 17 | 2 |
| 6. | *Trichosanthes cucumerina* | 4 | - | - |
| | Malvaceae | | | |
| 7. | *Abelmoschus esculentus* | 2 | 19 | 1 |
| 8. | *Hibiscus cannabinus* (Green cultivar) | 2 | - | - |
| 9. | *Hibiscus cannabinus* (Red cultivar) | 4 | 10 | 1 |
| | Papilionaceae | | | |
| 10. | *Canavalia gladiata* | 4 | 30 | 1 |
| 11. | *Cyamopsis tetragonoloba* | 3 | 13 | 4 |
| 12. | *Dolichos lablab* | 3 | - | - |
| 13. | *Vigna mungo* | 3 | 12 | 3 |
| 14. | *Vigna radiata* | 3 | 11 | 4 |
| 15. | *Vigna trilobata* | 4 | 11 | 2 |
| | Solanaceae | | | |
| 16. | *Capsicum frutescens* | 4 | 12 | 1 |
| 17. | *Lycopersicon lycopersicum* | 3 | 19 | 2 |
| 18. | *Solanum melongena* | 3 | 13 | 1 |

1 Seed of vegetables were sown on 3-8-'86.

Table 2. Relation of host - parasite behaviour

| Sl. No. | Vegetable hosts | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| | Amaranthaceae | | | | |
| 1. | *Amaranthus viridis* | + | + | + | - |
| | Cucurbitaceae | | | | |
| 2. | *Cucumis sativus* | + | + | + | - |
| 3. | *Lagenaria siceraria* | + | - | + | - |
| 4. | *Luffa acutangula* | + | + | + | - |
| 5. | *Momordica charantia* | + | - | + | - |
| 6. | *Trichosanthes cucumerina* | + | - | + | - |
| | Malvaceae | | | | |
| 7. | *Abelmoschus esculentus* | + | + | + | - |
| 8. | *Hibiscus cannabinus* (Green cultivar) | - | + | + | - |
| 9. | *Hibiscus cannabinus* (Red cultivar) | + | - | + | - |
| | Papilionaceae | | | | |
| 10. | *Canavalia gladiata* | + | - | - | - |
| 11. | *Cyamopsis tetragonoloba* | + | + | + | + |
| 12. | *Dolichos lablab* | - | - | + | - |
| 13. | *Vigna mungo* | + | + | + | - |
| 14. | *Vigna radiata* | + | + | + | - |
| 15. | *Vigna trilobata* | + | + | + | - |
| | Solanaceae | | | | |
| 16. | *Capsicum frutescens* | + | + | + | - |
| 17. | *Lycopersicon lycopersicum* | + | - | + | - |
| 18. | *Solanum melongena* | + | + | + | - |

Parameters:

1 Stimulation of parasite seed germination by host.

2 The potentiality of the germinated seedling to infect the susceptible host.

3 Permitting the unrestricted growth of parasite by the host.

4 Potentiality of any one of these hosts to inhibit parasites ramification.

No dodder seed in the control germinated. All vegetable seeds germinated within four days except *Lagenaria siceraria* which took seven days (Table 1). Parasite seeds germinated only after host seeds germinated. Except *Dolichos lablab* and *Hibiscus cannabinus* (green cultivar) all others induced germination in china dodder (Table 2). This indicates the need of host stimulus for dodder seed to germinate. Though equal number of seeds with equal distance from host seeds were maintained, there is variation in parasite germination. The induction of germination occurred within 1 to 25 days and the number of seeds germinated varied from 1-7 with different hosts. Hence, not only the intensity of stimulus but also the degree of seed response to stimulus varied from host to host. *Cucumis sativus* induced 70% germination. Though it is attacked by parasite vigorously, this can first be grown in fields to induce germination of dodder and deplete the seed bank in the field. However soon after attack is established, *Cucumis sativus* is to be completely cleared before the land can be put to future use. Ten to twenty days is recommended here for this operation. Susceptibility to infection varied with host and stage of parasite (Table 2). Tsivion (10) stated that tomatoes and beans are resistant because of hypersensitive reaction to parasitisation: Such may be true for seedling infestation to *Canavalia gladiata* , *Dolichos lablab*, *Hibiscus cannabinus* (Red cultivar) *Lagenaria siceraria*, *Lycopersicon lycopersicum*, *Momordica charantia*, *Trichosanthes cucumerina* and also for vegetative branches in *Canavalia gladiata*. *Cyamopsis tetragonoloba* (cluster bean) also got infected by dodder. But it soon induced stunted growth, abnormal dwarfing, premature cessation of growth and degeneration of flowers if produced. Vegetative branches of dodder from the adjacent hosts infect cluster bean and soon attain dwarfing trait with pseudodichotamous gracillarioid habit. The inhibitory principle originating in cluster bean then further gets circulated through the parasite's branches. Parasitising stems, leaves or fruits of cluster bean appears to be end for china dodder. According to Tsivion (10) *C. campestris* cannot succeed in establishing haustorial connection with some bean hosts. Cluster bean permits connection but inhibitory active principle is transmitted to china dodder. This needs further investigation for its potential source as a bioherbicide. Herbicidal management of dodder is a very tough proposition due to the parasite forming a canopy on the crop. Useful results were obtained when cluster bean was raised as mixed crop with other susceptible crops for eg: green gram (9). Cluster bean can be employed as an alternate crop to reduce seed bank, and as a border crop to check invasion of china dodder. A solution to dodder control lies in a) inducing suicidal germination of dodder seed to reduce the seed bank, b) in identifying a bioherbicide from such species that trap the spread of dodder - cluster bean could be a source; c) in growing resistant varieties of vegetable crops in dodder-prone areas, d) interculture of trap crops/catchcrops.

Dodder management programme for future should come on these inexpensive lines keeping in view the farmer's purse in developing countries.

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# A SUSTAINABLE SYSTEM OF ANNUAL CROPPING

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## ABSTRACT

Using squash (*Cucurbita maxima*) as a topical example, a system of cropping year-by-year on the same land is described. The system integrates current knowledge of plant density, herbicide development and minimal cultivation techniques. After preparing the land in beds, 1.5 m wide, every second bed is sown as a nine-row strip of a green crop (oats, *Avena sativa*). This green crop gives wind protection for the squash which is sown in the alternate beds. It also provides useful cover of which would otherwise be bare in the early stages of development of the squash crop. In succeeding years, the oats and squash positions are swapped. Both are sown with minimal cultivation. The importance of accurate and uniform squash sowing depth is discussed and chemical weed control options are compared.

## INTRODUCTION

The profitability of annual cropping, particularly vegetables, mainly depends on almost continuous use of land. First class land is a valuable resource and the grower is under continuing pressure to maximise yields and achieve a good economic return. Longterm, the key to success is to accomplish this and yet preserve the structure of the soil to safeguard its ongoing productivity. In New Zealand, this means avoidance of excessive cultivation, combined with judicious use of herbicides for weed control. Also, for high-value crops, providing protection from the wind is invariably worthwhile.

The present decade has seen particular squash, *Cucurbita maxima* "Buttercup", develop into a multi-million dollar crop for the Japanese market (4). It is climatically suited to a number of districts and serves as an appropriate example for the concept of a sustainable husbandry system with reduced cultivation, chemical weed control and wind protection. This is the alternate bed system described in this paper.

## CULTURAL PREREQUISITES

Soil preparation, seedbed technology and short-term wind protection are important features of this production system.

The maintenance of good structure is vital in continuously cropped soil. When preparing for sowing it is desirable to minimise heavy tractor cultivation operations and trafficking, consistent with the need to make seedbeds, apply fertilisers and sprays and gain access for

harvesting. Minimum cultivation techniques have been tested on numerous crops including those in the family Cucurbitaceae (2). In experiments over two seasons better early growth of both gherkins and pumpkins was obtained in uncultivated seedbeds, prepared from pasture by chemical desiccation and then sown with a chisel-coulter seed drill. Subsequently the best yields were usually obtained from no-cultivation treatments.

Squash has a large seed, approximately 14 mm long and 180 mg in weight, and the plant grows rapidly. In a soil temperature of 18°C it germinates quickly and seedlings emerge in six or seven days.

In free-draining loam soils, sowing depths are usually between 20 and 40 mm. In our experience under good soil conditions, both depths of sowing achieve greater than 90% emergence and result in similar yields. However, if conditions are unusually wet, deep sowing may result in reduced emergence. For example, at Levin Research Centre in 1985-86, 94% emergence was obtained from 20 mm depth but only 69% from 40 mm.

Work in New Zealand to date has shown that a density in the range 1.0 to 1.5 plants/$m^2$ usually results in fruit yields between 20 and 25 tonnes/ha (Fig. 1). There is no clear difference in yield from rows spaced 1.5 m and 3.0 m apart at similar overall densities.

Squash seedlings and young plants are quite sensitive to wind damage and a windy spell can damage foliage and cause delays in maturity of several weeks (R. J. Wood, pers. comm.). A quick-growing, cheap, green crop such as oats, maize or ryegrass has been grown on minimal cultivation areas to preserve soil structure, and provide wind protection; it also reduces weed growth. The green crop is killed by desiccation with a suitable herbicide before it can offer any competition to the cash crop.

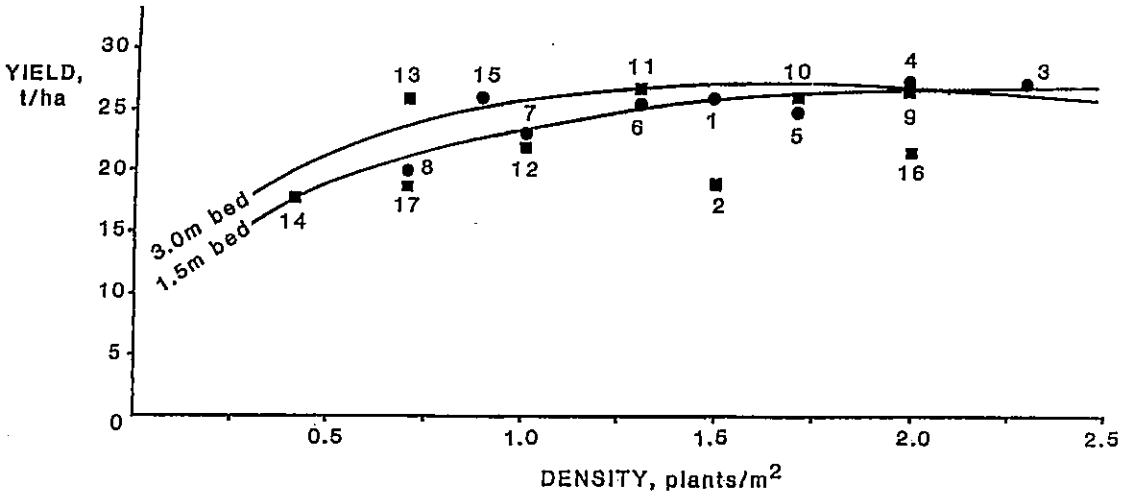
## THE ALTERNATE BED SYSTEM

A sequence of operations is outlined in Table 1. When setting up for the first time, land is prepared in time to sow oats in early October in alternate beds 1.5 m wide. In subsequent years this green crop would be sown with minimal cultivation by direct-drilling into the beds previously occupied by the squash cash crop.

Sowing in early October allows the oats time to grow and provide good coverage and usually attain a height of 0.4-0.5 m by the time the intervening squash beds are sown in mid-December. If necessary weeds can be controlled in the oats by spraying a suitable herbicide, for example ioxynil 0.6 kg/ha, in late October.

Two options are available to terminate growth of the oats at the end of their useful life as shelter and before they shed seed. If the oats have grown sufficiently, up until the time of squash emergence, a non-selective herbicide such as glyphosate can be applied. Or, if the oats have grown slowly and a further few weeks development could provide better shelter they can be sprayed. At any time before the running-stem growth phase of squash plants, a selective herbicide such as fluazifop-butyl could be used.

The degree of cultivation needed for the squash seedbed will depend on soil type and on the kind of sowing equipment available. In any case, the seeds should be sown accurately at a depth of 30 mm and 250 to 300 mm apart. In rows 3.0 m apart overall plant density is then 1.3 to 1.1 per $m^2$.




Fitted curves for beds 3.0m wide ■
and 1.5m wide ●

Source(unpublished data): 1,2 Wilson, G. J., Horticultural Research Station, Pukekohe, N. Z.
3-14 Douglas, J. A., Ruakura Agricultural Research Centre, Hamilton, N. Z.
15-17 Geelen, J. A., J. Wattie Canneries, Hastings, N. Z.

Figure 1. Yields of squash at different plant densities.

Figure 2. Alternate bed system, showing herbicide-treated squash seedbeds sheltered by strips of green crop oats.

Table 1. Alternate bed system - proposed sequence of operations.

| Period | Gree crop bed, oats or Cash crop bed, squash | Operation |
|---|---|---|
| July-Sept. | Oats and squash | Prepare land in beds 1.5m wide (see text) |
| early Oct . | Oats | Harrow |
| early Oct. | Oats | Sow, 9 rows at 15 cm per bed |
| end Oct. | Oats | If necessary, spray post-emergence herbicide against annual weeds, e. g. ioxynil |
| mid Nov. | Squash | Apply fertiliser, cultivate as necessary |
| mid Dec. | Squash | Prepare final seedbed |
| mid Dec. | Squash | Sow, 1 row per bed, seeds 25-30 cm apart |
| mid Dec. + 1 day | Squash | Spray post-sowing residual herbicides, see Table 2 |
| At date of sowing squash, decide on one of two options: | | |
| Either | | |
| mid Dec. | Oats | Spray non-selective herbicide to kill oats, e. g. glyphosate |
| or, | | |
| mid Dec. + 2-3 weeks | Oats | Spray selective herbicide to kill oats, e. g. fluazifop-buthy |

Table 2. Weed control and sqush yield after post - sowing application of herbicide mixtures based on cyanazine and methazole.

| Herbicide and rate kg/ha ai | Weed control, % of no-herbicide treatment | Total yield, tonnes/ha |
|---|---|---|
| cyanazine 0.8 | 17 | 24.1 |
| cyanazine 0.5 + alachlor 1.2 | 12 | 24.8 |
| cyanazine 0.8 + alachlor 1.2 | 10 | 23.9 |
| cyanazine 0.5 + chloramben 3.0 | 13 | 23.7 |
| cyanazine 0.8 + chloramben 3.0 | 9 | 24.3 |
| methazole 1.0 | 56 | 24.0 |
| methazole 0.7 + alachlor 1.2 | 6 | 26.1 |
| methazole 1.0 + alachlor 1.2 | 11 | 23.8 |
| methazole 0.7 + chloramben 3.0 | 11 | 25.0 |
| methazole 1.0 + chloramben 3.0 | 12 | 23.7 |
| cyanazine 0.5 + methazole 0.7 | 12 | 23.4 |
| no-herbicide treatment[1] | 100 (= 1214 weeds/$m^2$ | 20.9 |
| LSD 5% for any herbicide compared with no-herbicide treatment (CV = 9.0%) | | 2.3 |

1 Weed counts recorded two weeks after crop emergence, thereafter hand-hoed.

Good control of weeds is necessary for squash growing, particularly in the early stages of growth. The crop is particularly sensitive to herbicides and few are in general use. However, under closely prescribed conditions of seed placement, herbicide rate and spray timing marginally safe herbicides can be used. For example, cyanazine has been successfully utilised in this way (3). Further to this work, a number of combinations of herbicides, based on tolerated rates of cyanazine and also methazole have been tested (Table 2). Both alachlor and chloramben provided useful improvements in weed control when the main species occurring were *Digitaria sanguinalis, Portulaca oleracea, Solanum nigrum* and *Amaranthus powellii*. The herbicides were applied the day after sowing. When weed counts had been recorded two weeks after crop emergence, the no-herbicide treatment was periodically hoed, as required, to prevent weed growth. Other treatments received light hoeing when necessary, to maintain weed control.

All herbicides substantially reduced the initial weed population. The lesser success of the single methazole treatments is attributed to the resistance of *Digitaria sanguinalis* to this herbicide. The yield from every herbicide treatment was significantly higher than that of the no-herbicide treatment. As weeds were removed at the small seedling stage, before competition could be of any consequence, this difference in yield is explained by the detrimental effects of hoeing, especially mechanical damage, on the herbicide-free treatment.

## DISCUSSION

Experimentation in the past few seasons on different aspects of squash production suggests that the needs to (a) use land continuously, (b) to preserve good soil structure (c) to control weeds and (d) to provide wind protection can all be satisfied in one co-ordinated growing system similar to that described. The suggested innovations integrate current knowledge of plant density, herbicide development and minimal cultivation techniques.

Further work is needed on all these aspects. The harm caused by wind is commonly observed but poorly documented. Research is needed to quantify the benefits of wind protection, in terms of quicker crop growth and maturity when green crops are used as described in this paper. It is recognised also that the green crop itself could be cut and utilised for animal feed.

Weed problems in most market gardening crops are countered by both chemical and mechanical means. Both have their limitations and the present studies show that even in a sensitive crop herbicides can sometimes be utilised with less damage than hoeing.

After two decades of steadily increasing use of herbicides and of powered tractor implements there is no evidence of a general decline in weed populations in cultivated land. In the long term, the most hopeful method of alleviating annual weed pressures is by developing techniques of minimal soil disturbance, to discourage germination, combined with prudent use of herbicides. However, near-elimination of cultivation is a radical step which needs more grower experience yet. The compromise of the alternate bed system proposed in this paper allows normal cultivation in the area of the crop plant row but minimal soil disturbance elsewhere. If the normal and minimal cultivation areas are alternated in successive sowings the frequency of cultivation is effectively halved over the years with likely improvement in soil structure and long term productivity.

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# SCREENING FOR VARIETAL RESISTANCE TO BUTACHLOR AND ITS INHERITANCE IN RICE

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## ABSTRACT

Two-3 leaf stage seedlings of 6 rice varieties were transplanted in plastic case (22 x 15.5 x 6 cm) filled with fine sand. Different dosages of butachlor were treated just before transplanting with butachlor EC. The rice seedlings were grown under 20°C and 30°C in growth cabinets as well as under green house. There was no significant difference in the response of rice varieties to butachlor under different temperature conditions. The optimum dosage of butachlor for screening of resistance appeared to be 30-40 kg ai/ha under green house. Rice varieties and lines in total of 329 were screened for butachlor resistance at 30 kg ai/ha under green house. Seventeen varieties were found to be moderately resistant to butachlor. Mode of inheritance of resistance to butachlor was studied with $F_2$ plants of 3 crosses between resistant and susceptible rice varieties. The resistance of Kwanakbyo and IR 667-98 was found to be controlled by a single recessive gene.

## INTRODUCTION

Application of herbicide has become an essential practice for rice production in Korea. The risk of crop injury by a herbicide usually increases as its treated acreage increases since the herbicide is exposed to greater variety of growing conditions such as soil types, growing season (temperature), application time, status of rice seedling, transplanting method, rice varieties, etc.

Many studies and attempts have been made in order to minimize risk of the crop injury by herbicides in the area of development of new safer herbicide or mixtures and improvement of formulation besides other aspects of cultural practices and application methods.

On the other hand, in the recent years it is also known that the studies have been conducted by many scientists to develop crop varieties that are resistant to herbicides. Search for genetic material through a large scale screening will be the first approach to initiate a program to develop the herbicide resistance in crop varieties.

Difference in the response of crop plants to herbicides have been noted and cultivars of many crops have been screened for herbicide resistance (4, 7, 18, 19).

Ichizen (10) and Lee (12) have reported varietal difference of rice in response to herbicides and physiological differences among rice cultivars to simetryne was studied by Ishizuka et al. (11). Most of the studies have been dealing only with a few rice varieties and

screening trials with large number of rice varieties have not been reported for resistance to a herbicide.

Studies were carried out to clarify the inheritance of herbicide resistance and a single recessive (6, 8, 14). or dominant (2, 16) gene was identified. Other studies also indicated that the inheritance of herbicide resistance was cytoplasmic (17) or quantitative (3, 5, 15). A series of studies were conducted to identify rice varieties that are resistant to a herbicide and to clarify the mode of inheritance of the resistance in several cross combinations. Butachlor was selected for these studies since it is the most widely used herbicide in Korea.

## MATERIALS AND METHODS

In order to establish a screening method for resistance to butachlor, 6 rice cultivars were tested and those were Tetep, Tadukan (Indica), Jinheung, Akibare (Japonica), Tongil and Yushin (Indica x Japonica). Plastic case (22 cm x 15.5 cm x 6 cm) was filled with fine sand and treated with butachlor EC at the rate of 0, 10, 20, 30, 40 and 50 kg ai/ha with 3 replications. Ten seedlings at 2-3 leaf stage of each rice varieties were transplanted right after the butachlor treatment for each replication. The same experiment was conducted under 20°C and 30°C in growth cabinet and under green house (from May 31, 1984), respectively to compare the influence of temperature to the development of crop injury symptom. Visual observations were made for rating the phytotoxicity on 0-5 scale (5 being complete-kill and 0 being no effect) with one week interval.

For the experiment to screen resistant rice varieties to butachlor, plastic case (53 cm x 40 cm x 9 cm) was filled with fine sand and 30 kg ai/ha of butachlor EC was treated on May 27, 1985. Twenty seedlings at 2-3 leaf stage for each of 392 varieties were transplanted and placed under green house. Observations were made in the same way as the above.

In order to make genetic analysis on the inheritance of resistance to butachlor, 3 crosses (Kwangmyungbyo (S) x Kwanakbyo (R), Kwanakbyo (R) x Namyangbyo (S) and Samseongbyo (S) x IR 667-98 (R) were made in 1985 and $F_1$ plants were grown during 1985-1986 winter to obtain $F_2$ seeds. $F_1$ and $F_2$ plants as well as parent varieties were treated with the same methods as screening experiment on July 14, 1986 and visual observations were made 4 weeks after treatment on an individual plant basis. Rating of 0-2 was regarded resistant and 3-5 susceptible.

For all the experiments above, ammonium sulfate was applied before transplanting at the rate of 200 kg/ha.

## RESULTS AND DISCUSSION

Table 1 shows the mean response of 6 rice varieties to different dosages of butachlor under different growing conditions. Observations were made at 7, 14, and 21 days after treatment (DAT). Phytotoxicity of butachlor to rice seedling increased as the period of exposure to butachlor was extended as well as the rate of butachlor was increased. However, there was no significant difference in phytotoxicity under different temperature conditions.

It was reported that absorption and translocation of a herbicide in rice plant would be increased under higher temperature (11, 13) and the phytotoxicity was accelerated by higher temperature (1, 12). It seems that the results showed very little effect of temperature since

Table 1. Mean response of six rice varieties to different dosages of butachlor under 20°C , 30° C, and green house conditions[1].

| Condition | Dose | 7 DAT | | 14 DAT | | 21 DAT | |
|---|---|---|---|---|---|---|---|
| | | Lethal Percent | Injury[1] Rating | Lethal Percent | Injury Rating | Lethal Percent | Injury Rating |
| | kg ai/ha | % | | % | | % | |
| 20°C | 0 | 2 | 0.0 | 15 | 0.0 | 15 | 0.0 |
| | 10 | 11 | 2.3 | 58 | 3.7 | 72 | 4.2 |
| | 20 | 10 | 1.7 | 62 | 3.7 | 77 | 4.2 |
| | 30 | 11 | 1.5 | 48 | 3.2 | 68 | 4.0 |
| | 40 | 17 | 2.5 | 47 | 3.5 | 74 | 4.3 |
| | 50 | 17 | 2.2 | 58 | 3.7 | 78 | 4.3 |
| 30°C | 0 | 2 | 0.0 | 15 | 0.0 | 15 | 0.0 |
| | 10 | 3 | 1.8 | 24 | 3.0 | 73 | 4.7 |
| | 20 | 2 | 2.0 | 21 | 3.3 | 68 | 4.5 |
| | 30 | 3 | 2.2 | 28 | 3.2 | 84 | 4.8 |
| | 40 | 8 | 2.7 | 40 | 3.7 | 99 | 5.0 |
| | 50 | 30 | 3.2 | 63 | 4.5 | 100 | 5.0 |
| Green | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| house | 10 | 0 | 2.0 | 0 | 2.0 | 11 | 3.2 |
| | 20 | 0 | 2.0 | 2 | 2.0 | 10 | 3.3 |
| | 30 | 3 | 2.3 | 6 | 2.8 | 35 | 4.5 |
| | 40 | 8 | 3.0 | 12 | 3.5 | 42 | 4.7 |
| | 50 | 10 | 3.3 | 12 | 4.0 | 42 | 5.0 |

1 Visual injury rating
0 : No injury ; 5 : Completely killed

Table 2. Frequency distribution of rice varieties in crop injury at different observation timings.

| Injury rating | 7 DAT | 14 DAT | 21 DAT |
|---|---|---|---|
| 0 | | | |
| 1 | 1 | | |
| 2 | 128 | 1 | |
| 3 | 218 | 107 | 47 |
| 4 | 45 | 253 | 212 |
| 5 | | 31 | 133 |
| Total | 392 | 392 | 392 |

Table 3. List of moderately resistant rice varieties to butachlor.

| | |
|---|---|
| Chialbyo | HP 748-1-2-1-13-1 |
| Kwanakbyo | HP 904-3-1-13-1 |
| Mochakhu 1 | HP 744-2-1 |
| Khumal | HP 745-2-1-13-1 |
| Hunan 62 | WX 123-163-45-7-1 |
| Kaohsiung 68 | IR 667-98 |
| Samlibji | IR 747 |
| Bananue local D | Zhy-Lian-Ai-Yun-Nam |
| KH 1001 | |

Table 4. Segregation of resistant and susceptible plants to butachlor in $F_2$ populations of 3 crosses.

| Cross Combination | $F_2$ Plant | | $X^2(1:3)$ | P |
|---|---|---|---|---|
| | Resistant | Susceptible | | |
| Kwangmyungbyo(s) /Kwanakbyo(R) | 48 | 149 | 0.042 | 0.90-0.75 |
| Kwanakbyo(R) /Namyangbyo(S) | 32 | 129 | 2.255 | 0.25-0.10 |
| Samseongbyo(S) /IR 667-98(R) | 47 | 157 | 0.418 | 0.75-0.50 |

Note: Response of $F_1$ plants to butachlor showed susceptibility in all 3 cross combinations.

the lowest dosage of butachlor was even more than 5 times higher than recommended rate and at a very early stage of rice growth. Green house condition appears to be more ideal to evaluate the resistance of rice to butachlor compared to 20°C or 30°C constant temperature conditions under which very little response was observed to the rates of butachlor. Therefore, it was concluded that appropriate conditions for evaluation of resistance to butachlor would be under green house at butachlor dosage of 30-40 kg ai/ha with observation at 21 days after treatment or later.

No varietal difference was noted in the response of rice seedlings to different growing conditions. Rice cultivars and advanced lines of total in 392 were tested to screen resistance to butachlor at 30 kg ai/ha under green house. Frequency distribution of the rice varieties for each visual rating of resistance at 3 observations is summarized in Table 2.

Phytotoxicity of butachlor to rice varieties was increased as the period of exposure to butachlor was extended. Among all tested, 47 varieties or 12% showed moderate resistance (visual rating at 3) to butachlor at 21 DAT. Seventeen varieties were found to show continuous resistance to butachlor after 21 DAT and those varieties are described in Table 3. There were clear differences in varietal response to butachlor although no varieties were found to be highly resistant to butachlor.

Three crosses were made between resistant (R) and susceptible (S) varieties (Kwangmyungbyo(S)/Kwanakbyo(R), Kwanakbyo(R)/Namyangbyo(S), and Samseongbyo(S)/IR 667-98(R) to butachlor based on the result of the above screening test in order to clarify its mode of inheritance of the resistance. The segregation of $F_2$ population in the 3 crosses is shown in Table 4. $F_1$ plants of all the cross combinations showed high susceptibility to butachlor. In $F_2$ population, the segregation ratio of resistant and susceptible plants fit very well to 1:3 ratio. It may, therefore, be concluded that the resistance of Kwanakbyo and IR 667-98 to butachlor was controlled by a single recessive gene and the same type of inheritance was reported in other crops with different herbicides (6, 8, 14).

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# INTER-SPECIFIC COMPETITION BETWEEN PADDY RICE AND BULRUSH *SCIRPUS JUNCOIDES* ROXB. AS AFFECTED BY DIFFERENT SEASONS OF RICE TRANSPLANTING

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## ABSTRACT

Through the mono- and mixed-culture of paddy rice with bulrush at 3 different transplanting seasons, the inter-specific competition effects were confirmed. The plant height of bulrush became shorter while the height longer with delaying the seasons. The ratio in shoot number of rice to that of bulrush was nearly constant as about 1 : 3 regardless of any seasonal difference, while the actual shoot numbers became less by delaying the transplanting and the total shoot number of both experimented plant species showed more or less increase in mixed stand than in both pure stands. The dry matter weight in both species were decreased by delaying the transplanting seasons, and the tendency of decrements in dry matter production were more remarkable in bulrush than in rice. In a word, by delaying the transplanting seasons, the advance in competition ability for dry matter production was detected rather in bulrush, and for shoot number security was in rice, respectively.

## INTRODUCTION

Recently in Korea, the succession in weed species may have been drastically developed by the continuous use of a herbicide, by change in crop variety, early transplanting using of machine and younger crop seedlings, and lowering of paddy land utility in winter season, etc. (1, 2). On this respect, the proportion of perennial weeds and sedge species were increased (4, 5) and the efficient control methods became difficult to establish according to differential growth of crop and weed species in seasons, and inter-specific competition for different components. Even many researchers (7) have studied on the weed competition performances under different cropping seasons, there were not enough on bulrush, and practically lack of it in Korea. The present investigation was undertaken to bridge the gap.

## MATERIALS AND METHODS

Three different seasonal transplanting plots; namely early transplanting at May 1, normal transplanting at June 1, and late transplanting at July 1, were established. For every seasonal transplanting, pure standing of bulrush or rice with density of 21 plants per $m^2$ for each, and mixed standing of both bulrush and rice together with 21 plants each per $m^2$ were prepared within three replications. Standard methods to manage paddy field from Chonnam ORD were occupied to carry out the experiments at the University Exp. Farm. At heading stage of experimented paddy

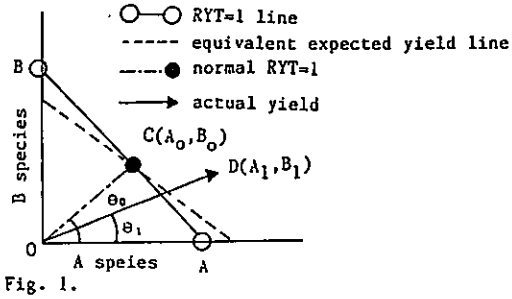


Fig. 1.

$A_0$: Yield of A species in normal RYT=1

$B_0$: Yield of B species in normal RYT=1

$A_1$: Actual yield of A species in mixture with B species

$B_1$: Actual yield of B species in mixture with A species

$\tan\theta_0=B_0/A_0$, $\tan\theta_1=B_1/A_1$, $ACR=(\theta_0-\theta_1)/\theta_0$

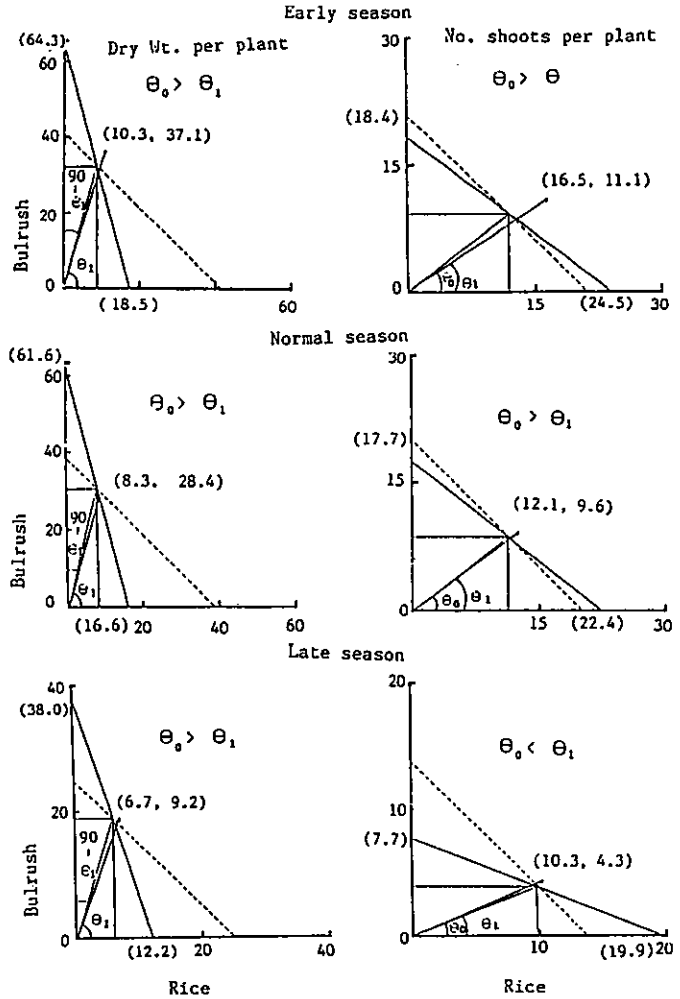


Fig. 2 Expected equivalent yield(---), relative yield total (——), actual yield(—→) in number of shoots (or tillers) per plant in binary association of bulrush and rice at different cropping seasons.

Table 1. Variation in growth traits of rice and bulrush as affected by their interspecific competition under the different rice cropping seasons.

| Cropping season | Plant height(cm) | | | No. shoots/plant | | | Shoot D.Wt.(g/plant) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pure (P) | Mixed (M) | M/P | Pure (P) | Mixed (M) | M/P | Pure (P) | Mixed (M) | M/P |
| Early | | | | | | | | | |
| Rice | 77.0 | 78.3[1] | 1.017 | 18.5 | 10.3 | 0.557 | 24.2 | 16.5 | 0.682 |
| Bulrush | 73.0 | 75.1 | 1.029 | 64.3 | 37.1 | 0.577 | 18.4 | 11.1 | 0.603 |
| Standard | | | | | | | | | |
| Rice | 78.7 | 82.0 | 1.042 | 16.3 | 8.3 | 0.509 | 22.4 | 12.1 | 0.540 |
| Bulrush | 73.3 | 76.5 | 1.044 | 61.6 | 27.4 | 0.461 | 17.7 | 9.6 | 0.542 |
| Late | | | | | | | | | |
| Rice | 88.7 | 84.7 | 0.955 | 12.2 | 6.7 | 0.549 | 19.9 | 10.3 | 0.518 |
| Bulrush | 65.3 | 70.2 | 1.075 | 38.0 | 19.2 | 0.505 | 7.7 | 4.3 | 0.558 |
| Mean | | | | | | | | | |
| Rice | 81.5 | 81.7 | 1.002 | 15.7 | 8.4 | 0.535 | 22.2 | 13.0 | 0.586 |
| Bulrush | 70.5 | 73.9 | 1.048 | 54.6 | 28.2 | 0.516 | 14.6 | 8.3 | 0.568 |
| LSD 0.05 | | | | | | | | | |
| Rice | 8.3 | NS | - | 3.1 | 3.3 | - | 4.1 | 5.3 | - |
| Bulrush | 5.2 | 4.8 | - | 14.4 | 7.6 | - | 7.8 | NS | - |

1 Mixed column in rice and bulrush indicate the rice with bulrush and bulrush with rice, alternatively.

Table 2. Variations in relative yield total(RYT), aggressivity(AGRS), and competition ability(CA) in number of shoots and dry matter weights per $m^2$ under binary association of bulrush and rice at different cropping seasons.

| Cropping season | No. shoots/$m^2$ | | | Dry Wt. /$m^2$ | | |
|---|---|---|---|---|---|---|
| | RYT | AGRS | CA | RYT | AGRS | CA |
| Early | 0.567 | -0.010 | -0.565 | 0.643 | 0.040 | 0.196 |
| Standard | 0.485 | 0.024 | -0.549 | 0.541 | -0.001 | 0.115 |
| Late | 0.529 | 0.020 | -0.483 | 0.538 | -0.020 | 0.411 |

RYT = 1/2(Yij/Yii + Yji/Yjj), AGRS = 1/2(Yij/Yii - Yji/Yjj), and CA = (Yi - Yj)/(Yi + Yj), where, i : rice, and j : bulrush, respectively.

rice, plant species were harvested together to assess the respective growth performances in height, shoot number and dry matter production. Growth data were reanalyzed into relative yield total(RYT), aggressivity(AGRS), competition ability(CA) and aggressivity in component ratio of species(ACR). Computing of those indice are as follows and also illustrated in Fig. 1.
RYT = 1/2(Yij/Yii + Yji/Yjj); AGRS = 1/2(Yij/Yii - Yji/Yjj); CA = (Yi - Yj)/(Yi + Yj); ACR(aggressivity in component ratio of species)

## RESULTS AND DISCUSSION

Plant height of both experimented plant species has been a little bit higher by standing at binary association than at pure stand each. Because of the plant height are related with lighting condition, bulrush which is less grown by delaying the cropping season would be less efficient species than rice. However the number of shoots per plant in mixed stand was resulted as less than 60% of that in pure stand. Biologically, rice has less tillers per plant than bulrush, whereas the shoot number of bulrush were markedly reduced by delaying the transplanting seasons. Also in dry matter production of individual plant, the reduction rate of both experimented plant species at mixed stand was resulted almost similar level of each pure stand. However the gradual reduction by delaying the transplanting was rather severe in rice (0.682 - 0.518) than in bulrush (0.603 - 0.558). (Table 1)

By use of these growth data, some of competition indice were calculated as RYT, AGRS, and CA. RYT values as less than 1.0 indicate the natural feasibility of inter-specific competition between both experimented plant species. On the other hand, by delaying the transplanting seasons, aggressivity value of rice has rather improved in securing of tiller number, and rather adversely developed in dry matter production than bulrush. This result indicates that the delaying of competition seasons has resulted for bulrush to improve in dry matter production, and to adversely develop in shoot number securing, respectively (Table 2).

The analysis of aggressivity in component ratio of species(ACR) was made to integrate the above indice indicating the property of inter-specific competition between two plant species (Fig. 2).

Through in all cropping seasons, the shoot number per plant in pure stand was appeared relatively much in bulrush and biologically less in rice. However in mixed stand by delaying the competition seasons, the generating function in shoot number has rather improved in rice than in bulrush. And, inversely to above tendency in shoot number, dry matter production as delaying the seasons has rather improved in bulrush than in rice. On the other hand, total production in dry matter weights of both plant species was not increased while the advancement in each component of competition ability was achieved as the above results.

As a conclusion, bulrush may have been evaluated as a less inter-specifical competing weed to rice, especially in delaying situation of cropping seasons. Most of those results were evidently caused due to their relative growth habits in multiple mixture as well as binary association.

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# INTER- AND INTRA-SPECIFIC COMPETITION OF BULRUSH *SCIRPUS JUNCOIDES* ROXB. WITH PADDY RICE AS AFFECTED BY DIFFERENT DENSITY OF BOTH PLANT SPECIES

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## ABSTRACT

Intra-specific competition : According to the increment of bulrush density, the number of shoots of bulrush per a given area was consistently increased in both site pure and binary stand with rice. However, the influence of inter-specific competition from rice to the individual shoot number of bulrush was gradually reduced and substituted by intra-specific competition due to the increase in its own density. The rate of dry matter production of bulrush per a given area has been rather high at mixed standing with rice than at monostanding. This was caused by the reasonable increase or maintenance in equivalent dry matter production to mono-standing. The evidence was assured at the competitive inter-relationship between both plant species (RYT _ 1.0), and the competition ability of bulrush at the mixed association was rather reliable in the shoot number security (CA 0), while unreliable in dry matter weight production (CA 0), respectively. Inter-specific competition : Even under a given density of bulrush with the fluctuating density of rice plant, the competition potentials of bulrush could not affect the rice in both sites shoot number security and dry matter production per plant. However, the retardation in shoot number security of bulrush was severe as the increment of rice density, and especially the dry matter production of bulrush was critically suppressed until the rice density at 3 hills per pot.

## INTRODUCTION

Bulrush, *Scirpus juncoides* Roxb, is an important paddy weed increasing recently and normally known as an annual species propagated by seeds. Iwasaki et al. (1980) at Japan, and Guh and Huh (2) in Korea has reported on the inter-specific competition of bulrush with paddy rice or other weed species, and the other studies on the ecology of bulrush were also publicated (4, 5, 6, 7). The inter-relationships between two plant species growing in binary association generally known to express not only simple competition but also complexed and gradual connections (3). On this respects, the present investigation was undertaken to recognize the principal properties in inter-specific competition between bulrush and rice, and intra-specific competition of bulrush under the incessantly changing situation in newly developed crop variety and its plant type.

## MATERIALS AND METHODS

The experiments were achieved under two processes, i.e. the trial of intra-specific competition and the trial of inter-specific competition of bulrush with paddy rice. The growing performances of bulrush from the mono-culture was compared with that from binary associated culture with paddy rice. The growth of experimented plant species were compared with pot (1/2000a Wagner pot) trials, recording dry matter weights, tiller numbers, and plant heights at the heading stage of rice.

**Intra-specific competition** The growth performances of bulrush with various densities as 1, 5, 10, 15 and 30 plants per pot in mono-standing was compared with those in binary associated standing with rice which was fixed as 4 hills per pot.

**Inter-specific competition** The growth performances of bulrush at a fixed density as 4 plants per pot in mono-standing were compared with those in binary associated standings of rice at various densities as 1, 2, 3, 4 and 5 hills per pot.

**Equations** For comparing the variabilities in competition function, the growth perfomances were described again by computing of following equations:

RYT(relative yield total) = $1/2(Yij/Yii + Yji/Yjj)$

AGRS(aggresivity) = $1/2(Yij/Yii - Yji/Yjj)$

CA(competition ability) = $(\bar{Y}i - \bar{Y}j)/(\bar{Y}i + \bar{Y}j)$

## RESULTS AND DISCUSSION

**Intra-specific competition** By addition of bulrush density 1 to 30 plants per pot, the shoot number of bulrush per pot at mono-standing was quadratically increased from 72 up to 255, and that per plant was inversely decreased from 72 down to 8.5. On the other hand at binary association, even occupying the pot space by 55 to 37 tillers of rice per pot, number of bulrush shoots per plant did not show any more significant differences from that of mono-standing. This result may has been caused by the lack of tiller number per fixed rice plants which are not naturally numerous enough unlike a bulrush. Such a result also indicates that the number of bulrush shoots per pot in binary association is rather affected by inter-specific competition with rice (Fig. 1).

The component analysis of competition loss in number shoot of bulrush showed also reasonably similar tendency as the above. Namely, the competition loss in shoot number of bulrush at binary association with rice, as increasing of bulrush densities, was principally caused by inter-specific site in lower density of bulrush, while by intra-specific in more or less higher densities (Fig. 2).

The intra-specific competition loss of bulrush was measured also in site of dry matter production. Dry matter weights of mono-stood bulrush was quadratically increased from 32 up to 76 g per pot while per plant decreased down 2.5g, respectively. However in binary association, the total dry matter production per pot of both plant species were duplicated unlike the tillering aspect. This results may caused by rice plant which may generate the prominent dry matter even in binary association with heavily increments of bulrush densities. This results also indicate that the dry matter production of individual bulrush plant can almost be generated independently upon the inter-specific competition with rice (Fig. 3).

Such a description was also graphically presented as the componential comparison of competition loss in dry matter production of bulrush plant (Fig. 4). Namely, the main

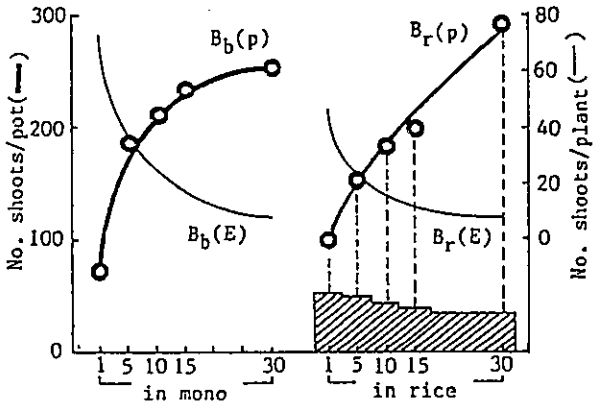


Fig. 1. Variations in number of shoots of bulrush in mono- and binary association in rice (▨: $R_{b(P)}$). $B_{b(P)}$, $B_{b(E)}$, $B_{r(P)}$, and $B_{r(E)}$ indicate the shoot No. of bulrush in mono (per pot), in mono (per plant), in rice (per pot), and in rice (per plant), respectively.

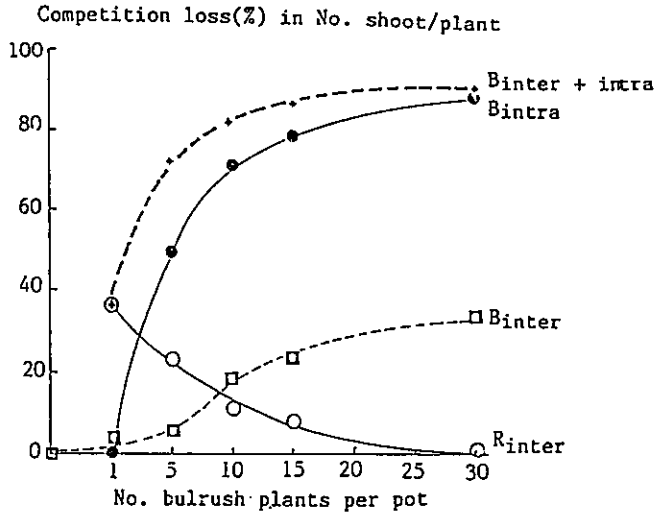


Fig. 2 Comparison of inter - and intra - specific competition loss (%) in number of bulrush shoots per plant as affected by various densities of bulrush [B, R, inter, and intra indicate the bulrûch rice, inter - specific and intraspecific competition respectively]

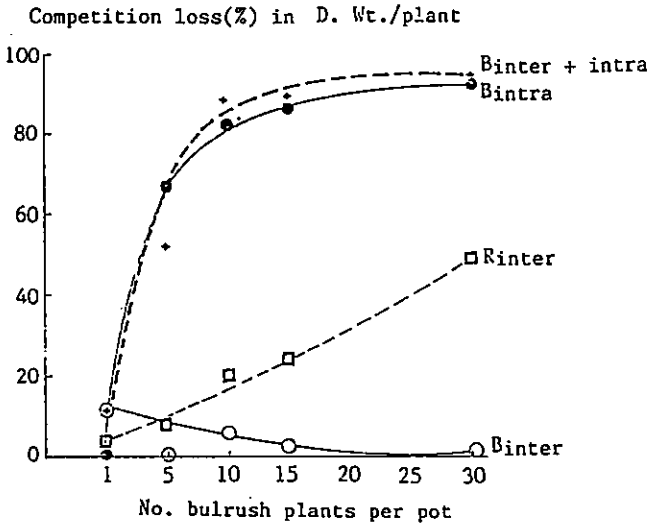


Fig. 3 Comparison of inter - and intra - specific competition loss (%) in dry matter weights of bulrush shoots per plant as affected by various densities of bulrus. [Abb. : refer to Fig.]

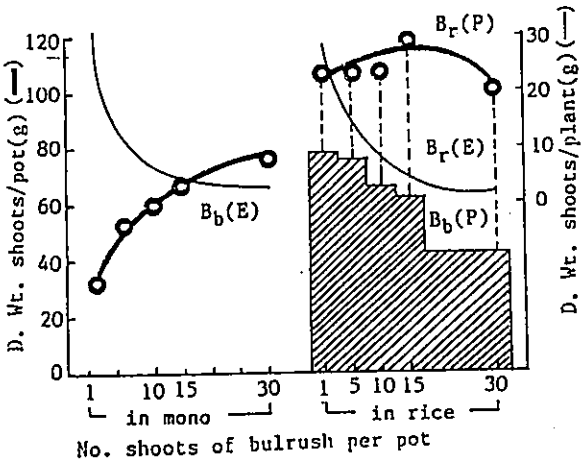


Fig. 4. Variations in dry weight of shoots of bulrush in mono- and binary association in rice ( : $R_{b(p)}$). Abb.: refer to Fig. 1.

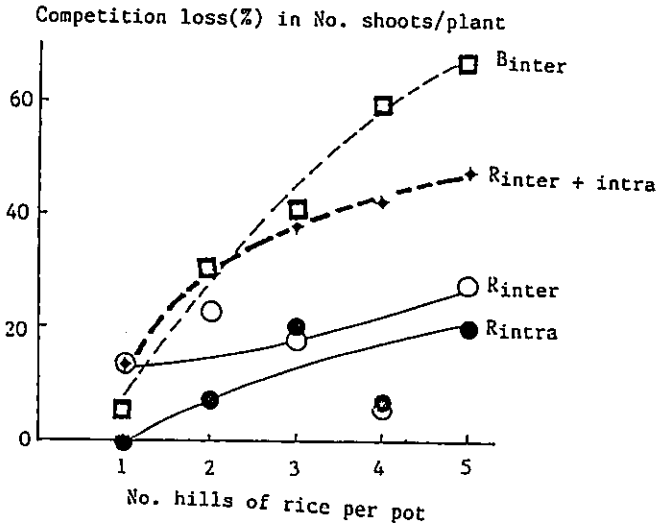


Fig. 5 Comparison of inter and intra - specific competition loss (%) in number of bulrush shoots per plant as affected by densities of rice. (Abb. : refer to Fig. 2)

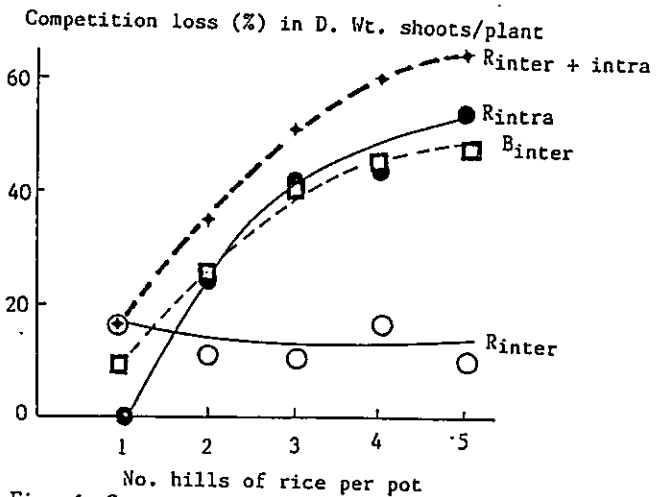


Fig. 6 Comparison of inter - and intra - specific competition loss (%) in dry matter weight of bulrush per plant as affected by densities of rice. [Abb. : refer to Fig. 2]

competition loss in dry matter of bulrush at binary association with rice was principally caused by intra-specific competition due to increments of bulrush its own density. Especially at more or less higher densities of bulrush than 15 plants per pot, no noticeable inter-specific competition effects on the dry matter formation of individual bulrush was detected.

To estimate the variabilities in productive inter-relationships, difference in aggressive nature in binary association comparing with that in mono-standing, and dominant competition ability in binary association was analyzed by indices of RYT (10), AGRS (8), and CA (1) (Table 1). Less value than 1.0 in RYT at both shoot number and dry weight of bulrush per pot indicate rather competitive productive relationships between bulrush and rice in binary association than that of the cooperative or independent. On the other hand, the adaptive ability to binary association comparing with mono-standing of both plant species were described as AGRS, and bulrush was resulted as less aggressive than rice. By addition of bulrush densities, AGRS of bulrush became gradually aggressive to the situation of under binary association. However, there was natural dominance of bulrush to form relatively prominent shoots number and that of rice in dry matter productions, respectively.

**Inter-specific competition** Fixed density of bulrush plants (4 plants/pot) were compared in binary association with various densities of rice as 0, 1, 2, 3, 4 and 5 hills per pot. By increments of rice density per pot, the competition loss in shoot number bulrush was drastically increased. This loss of bulrush has been little bit severe with additional intra-specific competition with rice (ca. 5%). The results indicate that the number of bulrush shoots per plant in binary association should be principally reduced by inter-specific competition with rice, especially in gradual increment of densities. (Fig. 5). Therefore, even the bulrush is naturally dominant to form its own shoots in binary association with rice, the feasibility of drastic loss of shoot number of bulrush by increments of rice density would be evidently concluded.

However, in dry matter sites, the competition loss of bulrush plant was drastically increased up to 3 hills of rice per pot, and thereover the inter-specific loss was alleviated. And rice was inter-specifically competed with bulrush at almost constant rate (less than 20%) and principally competed due to own density increments. This result indicates that rice plant are more naturally dominant in dry matter production than bulrush, and the increment of rice density was so powerful to cause the competition losses of bulrush in inter-specific site and rice in intra-specific site, together (Fig. 6).

As a results of competition ability analysis, RYT values showed gradual decreasing by addition of rice densities per pot. However most RYT value was basically less than 1.0, which indicate no other than competition relations between two experimented plant species in binary association. Also, generally negative value of bulrush in AGRS suggest for the nature of bulrush to be relatively less adaptive to binary association with rice. On the other hand, the competition ability (CA) has presented the dominance in shoot number security for bulrush, but that in dry matter production for rice, respectively (Table 2).

As a conclusion, bulrush comparing with rice has relatively small size and get a dominant growing characters at more or less the lower canopy of the binary association with rice. On this respect, even though the rice plant took a dominant place in dry matter production at binary association, while the bulrush take it in shoot number security, respectively. And the relative increment of bulrush density in binary association would rather cause more losses in

Table 1. Variations on relative yield total (RYT), aggressivity (AGRS), and competition ability (CA) in number of shoots and dry matter weights of differently densed bulrush per pot under binary association with rice plants (4 hills per pot, fixed)[1].

| Bulrush density (plants/pot) | No. shoots per pot | | | Dry Wt. per pot | | |
|---|---|---|---|---|---|---|
| | RYT | AGRS | CA | RYT | AGRS | CA |
| 1 | 0.802 | -0.163 | -0.071 | 0.898 | -0.054 | -0.486 |
| 5 | 0.747 | -0.199 | 0.325 | 0.750 | -0.165 | -0.415 |
| 10 | 0.728 | -0.091 | 0.500 | 0.742 | -0.064 | -0.245 |
| 15 | 0.713 | -0.069 | 0.563 | 0.796 | 0.040 | -0.051 |
| 30 | 0.813 | 0.140 | 0.736 | 0.638 | 0.126 | 0.160 |

1 PYT : Relative yield total = 1/2(Yij/Yii + Yji/Yjj), AGRS : aggressivity = 1/2(Yji/Yii - Yji/Yjj), and CA : competition ability = (Yi - Yj)/(Yi + Yj), where, i : bulrush, and j : rice, respectively.

Table 2. Variations in relative yield total(RYT), aggressivity(AGRS), and competition ability(CA) in number of shoots and dry metter weights of bulrush per pot(4 plants per pot, fixed) under binary association with differently densed rice plants[1].

| Rice density (hills/pot) | No. shoots per pot | | | Dry Wt. per pot | | |
|---|---|---|---|---|---|---|
| | RYT | AGRS | CA | RYT | AGRS | CA |
| 1 | 1.107 | 0.150 | 0.862 | 0.898 | 0.060 | 0.361 |
| 2 | 0.806 | 0.056 | 0.722 | 0.828 | -0.045 | 0.059 |
| 3 | 0.757 | -0.022 | 0.597 | 0.715 | -0.092 | -0.113 |
| 4 | 0.563 | -0.073 | 0.358 | 0.656 | -0.076 | -0.200 |
| 5 | 0.520 | -0.109 | 0.228 | 0.675 | -0.124 | -0.276 |

1 Calculation formular : refer to Table 1.

shoot number of bulrush by the intra-specific competition, and that of rice density cause mainly severe losses in dry matter production of bulrush by the inter-specific competition with rice.

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# THE EFFECTS OF IMAZAPYR ON OIL PALM FRUITING

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## ABSTRACT

The imidazolinone herbicide imazapyr is under active development in Malaysia, Indonesia and Thailand for the control of lalang (*Imperata cylindrica*) and for general weed control in plantation crops. In order to determine the effects of imazapyr on the fruiting behaviour of oil palms, a replicated field trial using effective doses of imazapyr for lalang control was established in 6-years-old oil palms in Johore, Malaysia, in January 1986. This paper presents the results of the trial. Application of imazapyr at doses up to 1.5 kg ae/ha had no detectable effect on fruit bunch production, fresh fruit bunch (FFB) yield, percentage parthenocarpic fruit or pollen viability. The visual symptoms associated with chemically induced parthenocarpy were not observed following the use of imazapyr.

## INTRODUCTION

The herbicide imazapyr, (Fig. 1) is a member of the imidazolinones, a new class of compounds discovered by American Cyanamid Company. It has both pre- and post-emergence activity against a broad spectrum of weeds, and is in commercial use in a number of countries, including the United States and the United Kingdom, for industrial weed control and brush control under the trademark ARSENAL herbicide. It is also being used on rubber and oil palm in Latin America.

**Imazapyr**

Chemical Names: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazoline-2-yl)nicotinic acid (IUPAC)
2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo)-1H-imidazol-2-yl-3-pyridinecarboxylic acid (CA)

Chemical Family: Imidazolinone

Structural Formula:

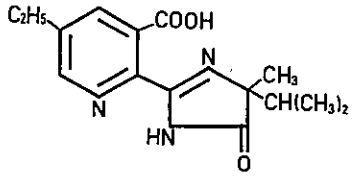


Figure 1.

In Southeast Asia imazapyr has been under development for several years for weed control in rubber and oil palm under the trademark ASSAULT herbicide. It has shown particular promise for the control of lalang (*Imperata cylindrica*) and also for extended control of other grass species, e. g. *Paspalum* and *Ottochloa* (2, 3, 6, 7, 8).

Studies have been conducted to determine whether the use of imazapyr for weed control in oil palm leads to herbicide residues in the fruit. In a study carried out in Johore, Malaysia, in 1983 no detectable residues of imazapyr were found in pericarp or kernels of oil palm fruits from palms treated four times at the anticipated commercial use rate of 0.75 kg ae/ha (Acid equivalent (ae) refers to the amount of active ingredient in terms of the parent acid (4)) over a period of nine months. Samples in this study were taken immediately after the final herbicide application and 50 days afterwards (1).

In a similar study carried out in Costa Rica in 1983, seven-year-old palms were treated with single applications of imazapyr at up to 2.0 kg ae/ha, and fruits were sampled at 15, 30, 60 and 90 days after application. No detectable residues of imazapyr were found at any of the sampling intervals(1).

These studies indicated that translocation of imazapyr to the fruit bunches of the oil palm was not occurring in detectable quantities. To complement this information a field study was established in Johore, Malaysia, in 1986 to determine whether application of imazapyr would affect fruit bunch yield and incidence of parthenocarpy. Observations on pollen viability were also made.

## MATERIALS AND METHODS

The study was designed as a randomized block field trial with four replications and six treatments.

Plots comprised 10 palms, and untreated guard rows were left between plots. The palms were D X P and were six years old at the time of treatment in January 1986.

Herbicide treatments comprised three levels of imazapyr (0.375, 0.75 and 1.50 kg ae/ha), two standards (glyphosate at 2.16 kg ae/ha and paraquat + diuron at 0.42 + 0.42 kg ai/ha) and an untreated control. All herbicide treatments were applied under the palms in a continuous sprayed strip 4 metres wide covering both palm circles and inter-row areas. On 22nd January 1986, treatments were applied in 400 litres of water per hectare using an Oxford Precision Sprayer. There was no rain on the day of spraying.

Numbers and weights of fresh fruit bunches (FFB) and numbers of male inflorescences were recorded for all palms in the trial, starting on 1st February 1986. Bunches from all treatments were also sampled at random for analysis over the period of 4th April to 17th September 1986. The bunch analysis included calculation of percentage parthenocarpic fruit by weight.

From 21st to 28th April 1986 samples of pollen were taken from male inflorescences, and germination tests were conducted to determine percentage of viable pollen. Approximately 500 pollen grains per inflorescence were counted following germination in sucrose solution.

On 20th September 1986 all plots were resprayed with the same herbicide treatments. Recording of FFB numbers and weights and sampling for bunch analysis were continued until April 1987. Further pollen samples were taken for viability tests from December 1986 to March 1987.

Table 1. Yield of oil palm fresh fruit bunches following Imazapyr treatment.

| Treatment | Dose (kg ae or ai/ah) | Mean Yield Per 10-Palm Plot | | | |
|---|---|---|---|---|---|
| | | Period 1 | | Period 2 | |
| | | FFB Number | FFB Weight (kg) | FFB Number | FFB Weight(kg) |
| Imazapyr | 0.375 | 44.7 | 488.5 | 36.7 | 496.1 |
| Imazapyr | 0.75 | 39.5 | 531.7 | 37.2 | 491.6 |
| Imazapyr | 1.5 | 44.2 | 571.4 | 38.7 | 522.4 |
| Glyphosate | 2.16 | 40.0 | 466.0 | 38.7 | 507.4 |
| Paraquat+Diuron | 0.42+0.42 | 38.2 | 459.4 | 39.0 | 518.0 |
| Untreated | | 42.5 | 539.2 | 33.7 | 458.6 |
| L.S.D. (P<Q.05) | | 11.8 | 154.8 | 7.46 | 109.8 |

Period 1 = 1st February to 17th September 1986
Period 2 = 26th September 1986 to 29th April 1987

Table 2. Incidence of parthenocarpy following Imazapyr treatment.

| Treatment | Dose(kg ae or ai/ha) | 4th Apr. -17th Sep. 1986 | | | 26th Sep. 29th Apr. 1987 | | |
|---|---|---|---|---|---|---|---|
| | | Number of Samples | Meat % Parthenocarpic Fruit (By Weight) | | Number of Samples Sampled | Mean % Parthenocarpic Fruit (By Weight) | |
| | | | Untrans | Arcsine | | Untrans | Arcsine |
| Imazapyr | 0.375 | 31 | 4.28 | 0.198 | 32 | 2.54 | 0.151 |
| Imazapyr | 0.75 | 36 | 6.04 | 0.226 | 31 | 1.54 | 0.116 |
| Imazapyr | 1.5 | 35 | 3.81 | 0.184 | 33 | 2.25 | 0.141 |
| Glyphosate | 2.16 | 29 | 6.10 | 0.224 | 33 | 3.66 | 0.161 |
| Paraquat + Diuton | 0.42+0.42 | 26 | 6.23 | 0.234 | 39 | 2.94 | 0.155 |
| Untreated | | 26 | 6.44 | 0.233 | 30 | 3.51 | 0.174 |
| L.S.D (p<0.05) | | | | 0.051 | | | 0.036 |

Table 3. Pollen viability following Imazapyr treatment.

| Treatment | Dose(kg ae or ai/ha) | April 1986. | | | December 1986 - March 1987 | | |
|---|---|---|---|---|---|---|---|
| | | Number Pollen Samples | Mean % Viable Pollen | | Number Pollen Sampled | Mean % Viable Pollen | |
| | | | Untrans | Arcsine | | Untrans | Arcsine |
| Imazapyr | 0.375 | -[1] | - | - | 14 | 91.1 | 1.284 |
| Imazapyr | 0.75 | 5 | 66.6 | 0.764 | 12 | 90.7 | 1.272 |
| Imazapyr | 1.5 | 4 | 70.3 | 0.824 | 16 | 89.9 | 1.262 |
| Glyphosate | 2.16 | 3 | 62.4 | 0.678 | 19 | 88.5 | 1.249 |
| Paraquat + Diuron | 0.42+0.42 | 6 | 60.5 | 0.685 | 10 | 86.0 | 1.200 |
| Untreated | | 4 | 42.2 | 0.453 | 9 | 92.2 | 1.297 |
| L.S.D. ($p<0.05$) | | | | 0.448 | | | 0.092 |

1 No pollen samples were available from palms treated with imazapyr at 0.375 kg ae/ha at the first sampling. From each sample approximately 500 pollen grains were observed.

Table 4. Male inflorescence production and apparent sex ratios following Imazapyr treatment.

| Treatment | Dose(kg ae or ai/ha) | 1st Feb. -17th Sep. 1986 | | | 25th Sep. 1986 -27 Apr. 1987 | | |
|---|---|---|---|---|---|---|---|
| | | Mean Male Inflorescence Per 10 Palms | Mean FFB No. | Apparent Sex Ratio (% Female) | Mean Male Inflorescence Per 10 Palms | Mean FFB No. | Apparent Sex Ratio (% Female) |
| Imazapyr | 0.375 | 9.5 | 44.7 | 82.5 | 24.7 | 36.7 | 59.7 |
| Imazapyr | 0.75 | 12.5 | 39.5 | 76.0 | 25.0 | 37.2 | 59.8 |
| Imazapyr | 1.5 | 12.2 | 44.2 | 78.3 | 26.0 | 38.7 | 59.8 |
| Glyphosate | 2.16 | 8.0 | 40.0 | 83.3 | 26.2 | 38.7 | 59.6 |
| Paraquat + Diruon | 0.42+0.42 | 10.5 | 38.2 | 78.5 | 23.5 | 39.0 | 62.4 |
| Untreated | | 9.7 | 42.5 | 81.3 | 28.2 | 33.7 | 54.3 |

## RESULTS

Results of the experiment are presented in Tables 1 to 4. Results are presented separately for two periods - 1st February to 17th September 1986 (between the first and second applications) and 26th September 1986 to 29th April 1987 (after the second application).

No significant differences were observed between the untreated control and any of the herbicide treatments in respect of fresh fruit bunch numbers or weight (Table 1).

Incidence of parthenocarpic fruit was unaffected by treatment during the first period of observation. In the second period a statistically significant reduction in incidence of parthenocarpy was recorded in palms treated with imazapyr at 0.75 kg ae/ha when compared with the controls. Other chemical treatments showed non-significant reductions (Table 2).

The imazapyr treatments had no significant effect on pollen viability. In the second observation period pollen viability for palms treated with paraquat + diuron was reduced when compared with the control; this reduction was just significant at the level and is of doubtful meaning particularly as a non-significant increase in pollen viability was recorded for this treatment in the earlier period (Table 3).

Comparison of male inflorescence production with FFB production permitted the calculation of apparent sex ratios at harvest. There was no indication that the herbicide treatments had any effect on apparent sex ratio over the period of observation (Table 4). Sexual differentiation, however, would have taken place prior to herbicide treatment(5), so these observations indicate only that the treatments do not lead to later abortion of inflorescences of either sex.

Post-treatment observations were made on the trial plots every four weeks. No effects of the herbicide treatments on foliar growth or colouration or on development of male or female inflorescences were detected in these observations.

In an observation strip adjacent to the trial area, sprayed by the estate cooperator on 22nd February 1986 with glyphosate + picloram (0.35 kg ae/ha + 0.045 kg ai/ha), several palms with abnormally developed stigmata were observed on 25th March 1986. The stigmata were darker in colour than those on untreated palms at the same stage of development and did not open in the normal way. Careful observation of the main trial area indicated that this effect did not occur on any other treated plots (imazapyr, glyphosate or paraquat + diuron).

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# POTENTIAL ALLELOPAHTIC SUBSTANCES IDENTIFIED FROM ANNUAL CROP STRAWS

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## ABSTRACT

Phenolic acids, fatty acids and organic acids were extracted from crop straws such as barley, wheat and rye, and were analyzed by GLC. Aqueous and alcohol extracts from these straws exhibited marked inhibitory effects on the growth of lettuce seedlings, showing barley wheat rye in order of inhibition. The presence of 12 simple free phenolic acids in the straws was confirmed and among them, ferulic acid present in about 20% or more in each straw was the predominant phenolic compound, followed by *p*-coumaric, sinapic, protocatechuic and caffeic acids etc.. Polyphenols such as scopoletin and rutin were present in the amount of 3.65 and 2.74 ppm, respectively, in barley straw. Further, barley straw contained 3.31 mg/g of total fatty acids and 3.8 mg/g of organic acids present in markedly higher amounts than those of wheat and rye straws. Phenolic substances together with fatty acid like linoleic acid and organic acid like malate seem to be potential allelopathic substances which exert strong inhibitory effects on the germination and growth of lettuce.

## INTRODUCTION

Chemical interference with plant growth is termed allelopathy which has been identified as an important plant interference in plant communities (2, 18, 19). Numerous researchers have reported plant growth inhibition after plant residues were incorporated in soil (2, 5, 14, 21). Allelopathy has been reported in many crop species such as wheat (8, 12, 23), barley (11, 15), rye (2, 3, 12), corn (8), sorghum (8, 21), sunflower (13) plant and in many weed species (20).

There are a number of reports on the use of crops such as barley, wheat and rye as allelopathic potentials. Residues of fall planted, spring-killed rye significantly reduced total weed biomass when compared to controls with no residues (2). Barley used as a smother crop might be successful at eliminating weeds because it inhibited seed germination and the growth of selected plant species (15). Wheat also seems to have allelopathic potential on many weed-seed germination and seedling growth (12, 23). Further Kwak and Kim (11), Kwon and Kim (12) reported that aqueous extracts of barley, wheat and rye residues inhibited germination and growth of paddy and upland weeds, especially effective on paddy weed such as on *Potamogeton distinctus*.

The concept that some crop plants may be inhibitory to the certain weed has been receiving a great attention in the search for alternative weed-control method. One way is to apply residues

of allelopathic weeds or crop plants as mulches or plant an allelopathic crop in a rotational sequence and to allow the residues to remain in the fields (17). Another approach is to utilize a rotational crop in a cropping sequence which may not be harvested to provide toxicity to weeds by exudation or upon decay of it's residues (17).

Phenolic compounds in plant residues have been intensively studied as allelopathic substances as the potential of protective agent against plants, insects and fungi (4, 16, 20). It is known that these substances are inhibitory to respiration, phosphorylation and enzyme activities such as malate and succinate dehydrogenase. In addition, many other secondary metabolites have been estimated to be biologically active (1, 20).

It has been known to some Korean farmers that application of barley residues to paddy field or planting of barley as a rotational sequence in paddy field is somewhat effective on controlling paddy weed, *Potamogeton distinctus* (9). This work was focused to identify potential allelopathic substances from annual crop residues such as barley, wheat and rye.

## MATERIALS AND METHODS

**Alcohol extract and germination test** Alcohol (70% MeOH) extracts of crop straws such as barley (*Hordeum vulgare* var. *hexastichon*), wheat (*Triticum aestivum*) and rye (*Secale cereale*) were made from dry shoot of them harvested at heading stage. One hundred ml of 70% MeOH solution was added to each of 10g dry materials and kept at 25°C for 48 hrs. The filtrate was evaporated and filled up to 100ml. The extracts were diluted to 2.0, 5.0 and 10.0%. Three selected species such as *Lactuca sativa*, *Echinochloa crusgalli* and *Oryza sativa* were used for assaying the presence of allelopathic substances. Twenty seeds of three species were seeded on the filter paper in petri dish with three replications. Ten ml of each concentration was added to each petri dish. Experiment was conducted at growth chamber (temp.: 20°C, light intensity: 3,000 lux, photoperiod: 16 hrs). Germination rates were observed at 7 days after incubation of each crop straws.

**Isolation and identification of phenolic compounds** Extraction of phenolic compounds was made by the methods of Kuwastuka and Shindo (10). Fifteen g of each dried sample was mixed 1 liter of methanolic sodium hydroxide (MeOH: 0.1N NaOH=7:3) for 48 hrs, respectively. Crude extracts were filtered and the filtrate was adjusted to pH 7.0 with HCl, concentrated to about 300ml at 40-45°C. Aqueous phase was acidified to pH 2 with HCl and again extracted with ether. Then extracts were completely dried. Finally 0.3ml of TMS (trimethylsilylacetamide, 25% solution in acetonitrile) was added to dried residues, and it was allowed to stand for 3 minutes in water bath at below 60°C and 2ul was injected into the gas chromatograph. Further, the extraction of polyphenols was performed by HPLC by the methods of Snook (22).

**Identification by gas chromatography** The analysis of various extracts was performed on a Pye Unicam gas chromatograph equipped with a flame ionization detector, and glass column, 1.5 x 4mm (inner diameter), packed with chromosorb W (100-120 mesh) coated with 5% silicon SE 30. The flow rate of carrier gas (nitrogen) was 30ml/min.. The temperature of injector and detector were maintained at 270°C and 280°C, respectively. The column temperature was programmed at the rate of 5°C/min. from 130°C to 250°C.

**Isolation and identification of fatty and organic acids** Fatty organic acids were extracted by the method of Court and Hendel (6). One hundred ml of MeOH containing glutaric acid (ISTD) 50mg

and 7.2ml $H_2SO_4$ were added to 10g of the dried sample of each crop straw and then shaked for 24 hrs. Crude extracts were filtered with Toyo 5B. and the filtrate was extracted with 10ml of chloroform four times and then injected into the gas chromatograph. A Pye Unicam gas chromatograph was used for analysis with glass column, 2.7m x 4mm (inner diameter), packed with chromosorb W (100-120 mesh) coated with 5% Silar 10C. The temperature of injector and detector were maintained at 230°C and 250°C, respectively. The column temperature was programmed at the rate of 8°C/min. from 90°C to 230°C.

## RESULTS AND DISCUSSION

Alcohol extracts from three crop straws markedly inhibited the germination of lettuce seeds (*Lactuca sativa*) as the concentrations increased from 2 to 10% showing inhibition of 100, 100 and 80% at 10% (W/V) of barley, wheat and rye straws, respectively (Table 1). The highest germination inhibition was detected in the extracts of barley under varied concentrations. However, the negligible effects were observed in rice and barnyard-grass seeds germination, indicating that sensitivity of plant species to these extracts differed, depending upon testing plant species. These results suggest that extracts from these crop straws contain some potential inhibitory substances effective on the germination of the testing plants. The similar observations were made by in the earlier reports of Kwak and Kim (11), Kwon and Kim (12), although their results showed much lower inhibitory effects on germination than the present results.

Nine phenolic acids such as salicylic, vanillic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, tannic + gallic, ferulic, caffeic, sinapic and phlorglucinol were analyzed from barley, wheat and rye straws by GLC. In addition, syringic was only observed in barley and wheat straws, *p*-chlorobenzoic only in rye and catechol only in barley straw. Among the phenolic acids detected, the predominant phenolic acid seemed to be ferulic, followed by sinapic, protocatechuic and caffeic acids in three different crop straws (Table 2). Five to seven additional phenolic acids in this study were detected by GLC compared to the earlier reports analyzed by paper chromatography done by Kwak and Kim (11), and Kwon and Kim (12) who reported phenolic acids such as *p*-coumnric, *p*-hydroxybenzoic, ferulic, vanillic acids in barley, wheat and rye straws. Composition and quantity of phenolic acids differ by plant species used and analytical method employed. However, it is clear that these three crop straws contain a large number of phenolic acids which may be related to allelopathic effect.

Polyphenols which were not detected by GLC were analyzed HPLC. Polyphenols such as chlorogenic, scopoletin, rutin and keamperolglycoside were detected in barley straw, rutin in wheat, and scopletin and rutin in rye straw. These indicates that among three crops straws barley had the highest number and content of phenolic compounds (Table 3). Scopoletin and rutin seem to be the major polyphenols in these three crops. Barley straws contained 3.65 ppm of scopoletin and 2.74 ppm of rutin which were present in highest amounts among three crop straws.

This can be suggested that the highest inhibitory effect extracted by alcohol extracts of barley may be related to phenolic compounds in both present phenolic acids and polyphenols. There are a number of reports that phenolic compounds and their derivatives can be the chemical sources of allelopathy (7, 16, 20). Further, Woo and Kim (24) evaluated the inhibitory effects of authentic acids on the germination of lettuce seed. They reported that several authentic

Table 1. Percent germination of testing plants as effected by alcohol extracts[1] of crop straws.

| Crops | Conc. (%) | *Lactuca sativa* 2[2] | 5 | 10 | UC[3] | *Oryza sativa* 2 | 5 | 10 | UC | *Echinochloa crusgalli* 2 | 5 | 10 | UC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ---------- % [4] ---------- | | | | | | | | | | | |
| Barley | | 65.0 | 0.0 | 0.0 | 100 | 98.3 | 100 | 30.0 | 100 | 98.3 | 30.0 | 0.0 | 100 |
| Wheat | | 98.3 | 21.7 | 0.0 | 100 | 100.0 | 100 | 58.3 | 100 | 100.0 | 93.3 | 56.7 | 100 |
| Rye | | 100.0 | 30.0 | 20.0 | 100 | 98.3 | 100 | 100.0 | 100 | 100.0 | 88.3 | 55.0 | 100 |

1 Extracted with 70% methanol solution.

2 Percent concentration of extracts (w/v).

3 Untreated control.

4 Determined at the 7 days after incubation.

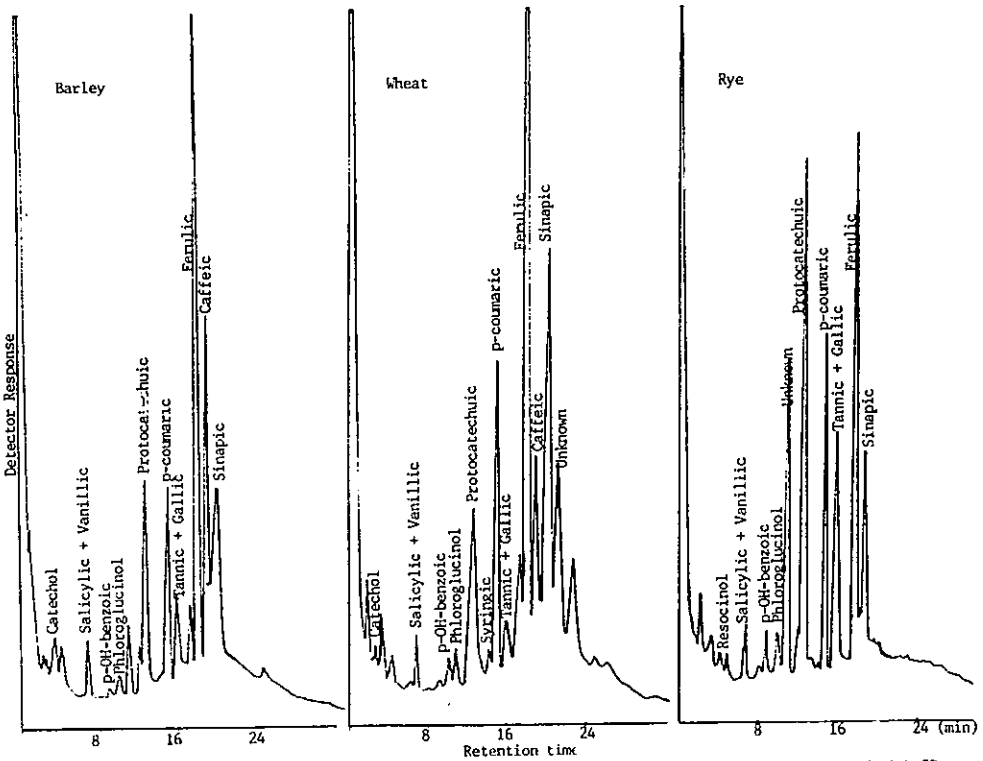


Figure 1. GLC chromatogram of the TMS derivatives of phenolic compounds(1.5m X 4mm glass column packed with 5% SE-30 on 100-120 mesh Chromosorb W)

Table 2. Constitution of phenolic compounds identified from different crop straws.

| | Phenolic compounds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crops | Catechol | p-chloro-benzoic | Salicylic +Vanillic | p-hydroxy-benzoic | Phloro-glucinol | Protoca-techuic | Syringic | p-coumaric | Tannic +Gallic | Ferulic | Caffeic | Sinapic |
| Barley | 1.05 | - | 1.65 | 0.95 | 0.84 | 6.95 | 0.70 | 6.72 | 3.65 | 23.44 | 8.92 | 15.92 |
| Wheat | - | - | 1.00 | 0.39 | 0.88 | 7.88 | 0.33 | 7.32 | 3.32 | 25.48 | 6.62 | 14.88 |
| Rye | - | 0.73 | 1.85 | 1.79 | 2.32 | 16.82 | - | 10.32 | 9.76 | 19.00 | 10.39 | 1.56 |

1 Percent of total GLC analyzed phenolic compounds.

Table 3. Polyphenol contents extracted from crop straws[1].

| Crops | Chlorogenic | Scopoletin | Rutin | Keamperol glycoside |
|---|---|---|---|---|
| | | --------- ppm --------- | | |
| Barley | 0.92 | 3.65 | 2.74 | 0.21 |
| Wheat | - | - | 0.23 | - |
| Rye | - | 1.38 | 1.80 | - |

1 Polyphenol contents were determined by HPLC.

Table 4. Fatty acids of crop straws as analyzed by GLC.

| | Fatty acids | | | | | | |
|---|---|---|---|---|---|---|---|
| Crops | Palmitic | Stearic | Oleic | Linoleic | Linolenic | Total | /U[1] |
| | | --------- mg/g ---------- | | | | | |
| Barley | 1.01 | 0.13 | 0.09 | 0.34 | 1.74 | 3.31 | 0.02 |
| Wheat | 0.77 | 0.10 | 0.08 | 0.29 | 1.44 | 2.68 | 0.48 |
| Rye | 0.73 | 0.16 | trace | 0.27 | 0.61 | 1.77 | 1.01 |

1 A ratio of total saturated / total unsaturated fatty acids.

Table 5. Organic acids of crop straws as determined by GLC.

| | Organic acids | | | | | |
|---|---|---|---|---|---|---|
| Crops | Oxalic | Fumaric | Succinic | Malic | Citric | Total |
| | | ---------- mg/g ---------- | | | | |
| Barley | 0.16 | trace | trace | 2.67 | 0.97 | 3.80 |
| Wheat | 0.21 | 0.15 | 0.35 | 0.72 | 0.42 | 1.85 |
| Rye | 0.14 | trace | trace | 0.96 | 0.55 | 1.65 |

phenolic acids inhibited lettuce seed germination, particularly, ferulic acid which is the most dominant phenolic compound obtained from these three crop residues completely inhibited seed germination of lettuce at $10^{-3}$M. The major phenolics suggested in the above of this study exhibited the considerable inhibition of lettuce seed germination. Our results and other's reports are coincided that phenolic and their derivatives can exhibit the strong inhibitory effects on seed germination.

Five fatty acids such as palmitic, stearic, oleic, linoleic and linolenic, five organic acids like oxalic, fumaric, succinic, malic and citric acid, were detected by GLC in barley, wheat and rye straws (Tables 4, 5). Barley straw contained higher quantity of fatty acids, 3.31mg/g and organic acids 3.80mg/g than those of wheat and rye. Among the fatty acids determined, linolenic acid was a major fatty acid present in 1.74mg/g, representing about 52% of total fatty acids. In addition, the ration of total saturated over unsaturated fatty acids was significantly lower in barley straw ( s/us = 0.02) than those of wheat ( s/us = 0.48) and rye ( s/us = 1.01). Alsaadawi (1) reported that they isolated nine fatty acids from *Polygonum aviculare* and soil under *Polygonum* stands, and these fatty acids significantly inhibited growth of bermudagrass seedlings even in the low concentration of 5 ppm. Malic acid among organic acids was present in the highest amount, representing more than 90% of total organic acid in barley. Thus it can be again suggested that linolenic and malic acids may be two important nonvolatile acids relating to allelopathic effect exhibited by barley straw.

Based on result and observations, the following suggestions can be made. The highest inhibitory effect was observed in barley straw, followed by wheat and rye straw. Phenolic compounds including polyphenols detected from three crop straws can play an important role in exhibition of allelopathic effect. Further, fatty and organic acids may be also related to allelopathic effects exerted by these three crops. It is postulated that this study reconfirms the earlier work of Kim et al. (9) in the effectiveness of barley straw application to control a paddy weed, *Potamogeton distinctus*.

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# CONTROL OF *IMPERATA CYLINDRICA* AND *ECHINOCHLOA CRUS-GALLI* : AN APPROACH TO IMPROVING THE PERFORMANCE OF IMAZAPYR IN VERY LOW VOLUME SPRAY APPLICATION

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## ABSTRACT

The influence of formulation and method of application on the biological activity of imazapyr (Arsenal, Assault) was investigated using *Imperata cylindrica* (L.) Raeuschel and *Echinochloa crus-galli* (L.) Beauv. as test species. The individual variables studied included spray production and dispersal, spray retention and coverage and foliar uptake and translocation. The phytotoxic action of imazapyr was affected by the concentration of the active ingredient, the ethylene oxide of the linear alcohol surfactant added, the concentration of the surfactant in the spray solution and the type of spray equipment used. High concentrations of active ingredient and/or surfactant caused a reduction in herbicide activity. Under the conditions of the experiments the spinning disc applicator gave uneven spray deposition leading to inadequate coverage of the plants.

## INTRODUCTION

In a move to relieve the demand for arable land on Java, the Indonesian Government have adopted a Transmigration Programme. This involves resettling rural families on the sparsely-populated Outer Islands of Sumatra, Kalimantan and Irial Java. The vegetation of these islands consists of rain forest, swamp areas and tropical grassland savannah which is dominated by the perennial species *Imperata cylindrica* (L.) Raeuschel (alang-alang).

*I. cylindrica* grows to a height of 2 metres and possesses long, coarse, flat leaf-blades which burn very easily and are only palatable for cattle for a few weeks after sprouting. Its tough, extensively-creeping rhizome system enables the plant to spread and establish itself even in soils of very low fertility . Long distance dispersal and colonisation is achieved by means of numerous, light, airborne seeds. Inhibition of growth of crops such as rice and maize has been observed in the presence of roots and rhizomes of *I. cylindrcia* and whilst protecting the soil against erosion, the rhizomes impede the percolation of rain water into the ground (4).

To prevent destruction of the valuable rain forests, much of the land which has been available for resettlement schemes in Indonesia has been *I. cylindrica* savannah. The transmigrant farmers, who have only simple tools, are not physically capable of eradicating the grass. The underground rhizome system of *I. cylindrica* is so that the labour force of a whole

family is barely sufficient to keep one hectare of land weed-free during the year. Since the rhizomes are protected undergroud, burning the vegetation gives rise to pure stands of *I. cylindrica* know as 'fire climax' (3). Clearing the land of *I. cylindrica* is essential if small-scale farmers are to be brought above subsistence level. In recent years there has been an increase in the use of translocated herbicides for the control of *I. cylindrica* due to their ability to destroy viable rhizome buds, hence preventing regrowth. Sustained control of *I. cylindrica* has been demonstrated in field trials in Indonesia with post-emergence, medium volume (350-600 l/ha) hydraulic spray applications of the American Cyanamid compound imazapyr - a rapidly-absorbed, phloem-mobile, imidazolinone herbicide showing broad-spectrum activity (1). Results of early work in Indonesia outlined considerable potential for reductions in costs of active ingredients, water transport and labour using very low volume controlled droplet application (9). However, decreased phytotoxic action of imazapyr against *I. cylindrica* when sprayed at 20 litres/ha using the 'Micron herbi' spinning disc was found in the field in Sumatra (2).

This study was designed to investigate whether the reduced activity of imazapyr at very low volume rates was due to inappropriate formulation, to inadequacies in the spraying equipment or to a combination of the two. At the same time, the objective was to determine which of the three linear alcohol surfactants tested would promote the biological effect of imazapyr to the greatest extent in *I. cylindrica*, the optimum ratio and concentration of surfactant and active ingredient in the spray solution as well as the importance of controlling herbicide placement for full exploitation of the potential of the phytotoxic molecule. To simplify the study, the spraying operation was considered as a series of separate events: the production and dispersal of the spray, spray retention and distribution on the plant and uptake and translocation of the herbicide within the plant tissue. Experimental detail was established using the tropical annual grass *Echinochloa crus-galli*, which is a serious weed of rice. This species was chosen because of the ease, speed and uniformity of germination of its seeds under glasshouse conditions and because of the comparison it provides with the perennial test species, *I. cylindrica*.

## MATERIALS AND METHODS

**Plant propagation** *I. cylindrica* was propagated from 8 cm rhizome fragments in a glasshouse maintained at 30-35°C during the day and 18-20°C at night. An average R. H. of 60% was recorded. Quartz-halogen supplementary lighting was used to ensure a minimum day length of 14 hours. Plants used for experimental purposes had a minimum of 5 leaves and a well-developed rhizome system. *E. crus-galli* was propagated from seed under similar glasshouse conditions and treated at the 4-5 leaf stage.

**Spray application of herbicide solutions** Three nonionic, $C_{13}$-$C_{15}$ linear alcohol surfactants (ICI SYNPERONIC A series) with an average content of 2, 7 and 20 ethylene oxide (EO) residues, respectively, (hereafter referred to as A2, A7, and A20), were added to solutions of the isopropylamine salt of imazapyr without surfactant, to give a range of herbicide and surfactant concentrations. The length of the EO chain affects the pyhsical properties of the surfactant; thus, A2 is a clear liquid with poor water solubility, A7 ia a viscous, opaque liquid with intermediate water solubility and A20 is a hard white solid with good water solubility. The

doses of imazapyr applied were equivalent to 0.35 and 0.175 kg active ingredient (ai)/ha for *I. cylindrica* and *E. crus-galli*, respectively. Surfactant concentrations used ranged from 0.1 to 5% w/v in the final spray volume. The different formulations of imazapyr were applied to the plants in a laboratory spraying cabinet at volume rates equivalent to 200 and 20 litres (l)/ha, using a 'Teejet' LP8002 hydraulic nozzle (pressure = 2 bar) and a 'Micron Herbi' spinning disc (p.d. = 9 v; 1.4 mm red restrictor), respectively. The biological effect of the spray treatments was quantitatively assessed (8). Herbicide retention and distribution on the plant were measured colorimetrically using Lissamine Red as a tracer (8). An Optomax Image-Analyser was used to measure leaf area. Droplet diameter and droplet number per unit area were measured using the magnesium oxide slide method (5).

**Microdroplet application** Lissamine Red was added to the formulations used in the spray trials and the resulting solutions applied to the test species as 1 l droplets using a 50 l syringe with a dispenser attachment. The area of the dried deposit was measured and used as an indication of the free acid (specific activity = 44 Ci/mg) was included in solutions for uptake and translocation studies. For studies of the effect of the EO content and concentration of the surfactants on uptake and translocation the solutions were applied 10 cm from the tip of mature leaves as three 1 l droplets as described above. The influence of droplet size on uptake was measured by applying the herbicide as eighty 200 m or ten 400 m droplets using the WRO single droplet applicator (6). Uptake of imazapyr was quantified at five time intervals between 0 and 72 hours after application using the cellulose acetate stripping technique followed by liquid scintillation counting (7). Translocation of imazapyr was assessed qualitatively by autoradiographic techniques; full experimental details are recorded in Townson (8).

## RESULTS

**Biological activity** Symptoms of phytotoxicity appeared within 3-4 days after application in *E. crus-galli* and 6-10 days in *I. cylindrica* as a progressive development of purple coloration of the leaves from the tip, moving basipetally along the leaf lamina. This was followed shortly by yellowing margins, chlorosis and finally necrosis. Severe contact scorch was apparent on the control plants sprayed with 5 % w/v surfactant alone. The most rapid death of foliage was noted for plants treated with imazapyr and 0.25 % w/v A7 at 200 l/ha. No regrowth was observed. In *I. cylindrica* symptoms of damage appeared earlier when A20 rather than A2 was included in the spray solution, whereas the reverse was true for *E. crus-galli* (data not shown). The A7 surfactant improved herbicidal activity more than A2 and A20 in both test species at medium and very low volume rates. At spray volumes of 200 l/ha, a surfactant concentration of 0.25 % w/v was found to be optimum but at 20 l/ha the greatest phytotoxicity occurred with a surfactant concentration of 0.5 % w/v. Reduced activity of imazapyr resulted from the use of surfactant concentrations above 1.0 % and 2.5 % w/v in medium and very low volume applications respectively. In general, the variability of response and time to death of plants were greater with the spinning disc treatments than with those with the hydraulic system (Table 1).

**Spray production and dispersal**

Table 1. Phytotoxicity of formulations of imazapyr at 0.35 kg ai $ha^{-1}$ to *I. cylindrica*.

| Volume rate (l/ha) | EO content of surfactant | Rate of surfactant (% w/v) | % Damage after 6 days[1] | Time to death (days) |
|---|---|---|---|---|
| Effect of EO content of surfactant | | | | |
| 200 | 2 | 0.25 | 6 (2) | 20 (1) |
| 200 | 7 | 0.25 | 42 (4) | 18 (1) |
| 200 | 20 | 0.25 | 10 (2) | 25 (2) |
| 20 | 2 | 0.25 | 8 (6) | 32 (2) |
| 20 | 7 | 0.25 | 11 (3) | 26 (2) |
| 20 | 20 | 0.25 | 7 (2) | 45 (3) |
| Effect of rate of surfactant | | | | |
| 200 | 7 | 0.10 | 8 (1) | 22 (2) |
| 200 | 7 | 0.25 | 42 (4) | 18 (1) |
| 200 | 7 | 0.50 | 22 (6) | 19 (1) |
| 200 | 7 | 1.00 | 11 (2) | 20 (2) |
| 20 | 7 | 0.25 | 11 (3) | 26 (2) |
| 20 | 7 | 0.50 | 16 (5) | 23 (1) |
| 20 | 7 | 1.00 | 5 (2) | 31 (2) |
| 20 | 7 | 2.50 | 12 (4) | 40 (2) |
| 20 | 7 | 5.00 | 6 (2) | 39 (3) |
| Control | | | % damage 45 days after treatment. | |
| No surfactant | | | | |
| 200 | - | - | 78 (4) | |
| 20 | - | - | 77 (12) | |
| No imazapyr | | | | |
| 200 | 2 | 5 | 66 (6) | |
| 200 | 7 | 5 | 63 (7) | |
| 200 | 20 | 5 | 70 (10) | |
| No surfactant | | | | |
| 200 | - | - | 78 (4) | |
| 20 | - | - | 77 (12) | |
| No imazapyr | | | | |
| 200 | 2 | 5 | 66 (6) | |
| 200 | 7 | 5 | 63 (7) | |
| 200 | 20 | 5 | 70 (10) | |

Values are means of 3 replicates (± S. E. for 2 d. f.)

1 % Damage assessed on a quantitative scale from 0 (no phytotoxic symptoms) to 100 (dead).

Fig. 1. Distribution of spray: (A) ACROSS the swath of a 'Micron Herbi' spinning disc; (B) ALONG the swath of a 'Micron Herbi' spinning disc- and (C) ALONG the swath of a 'Teejet' LP8002 hydraulic nozzle.

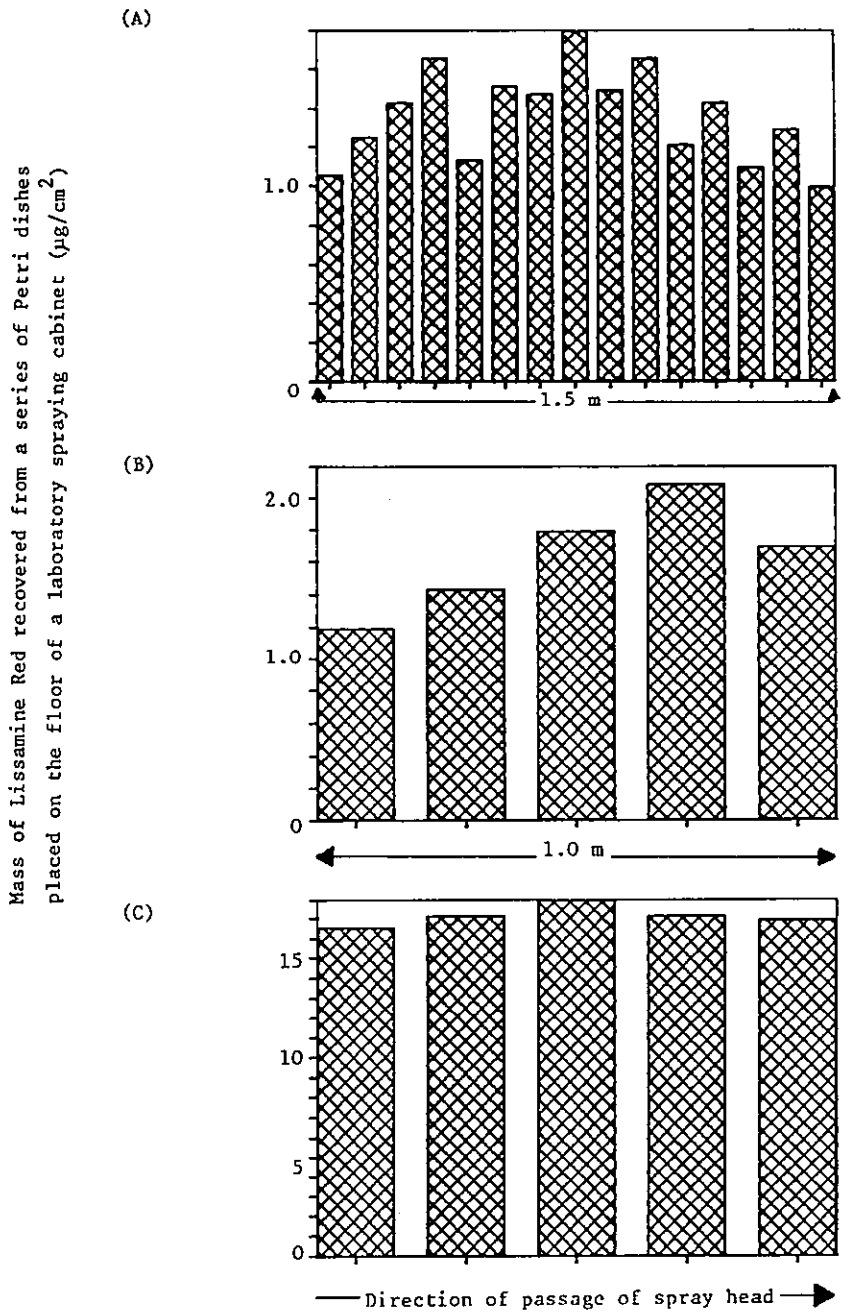


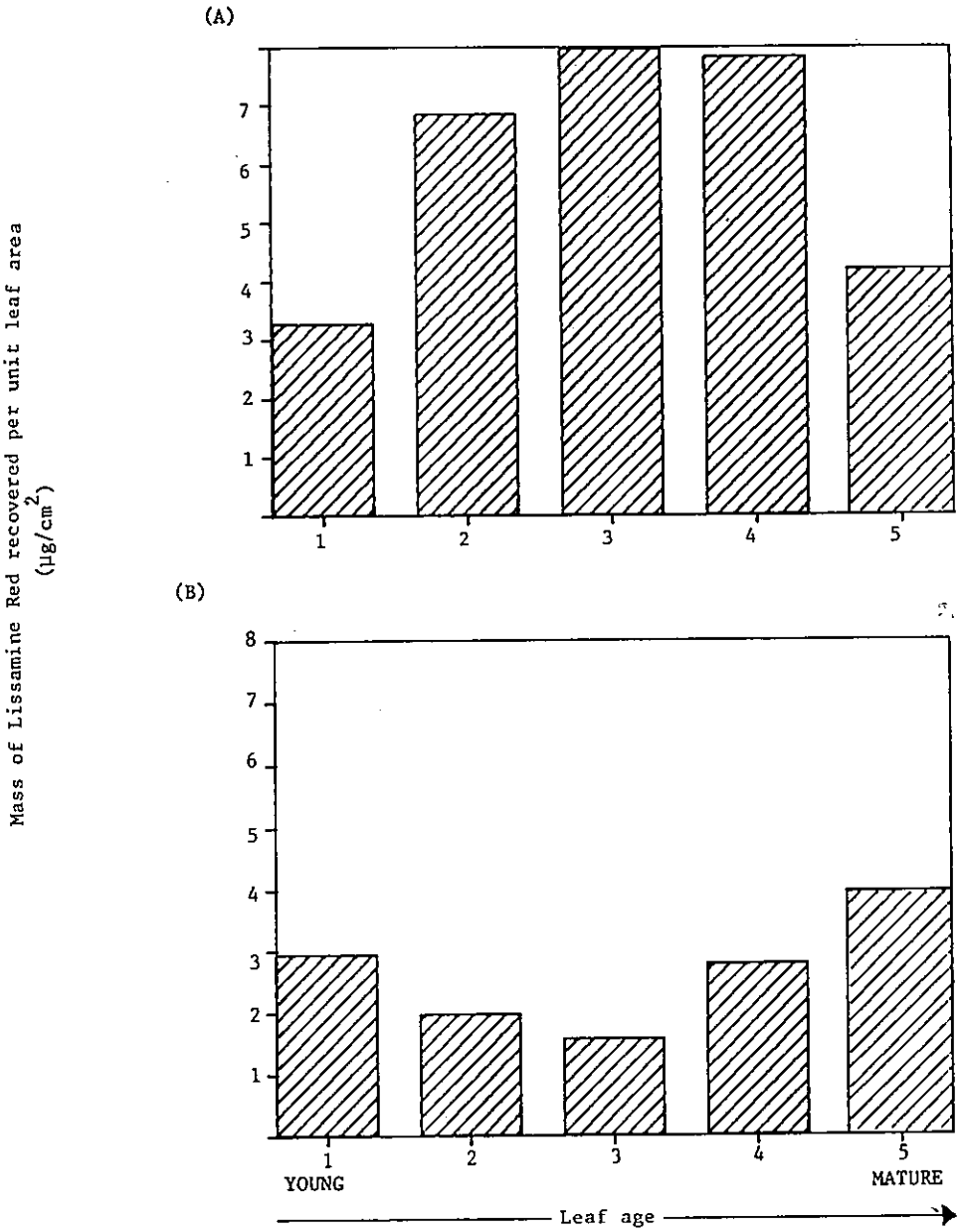
Table 2. Spray retention on *I. cylindrica* and *E. crus-galli* expressed as a % of that applied.

| Volume rate (l. $ha^{-1}$) | EO cotent of surfactant | Rate of suftactant (% w/v) | % Spray retained [1] | |
|---|---|---|---|---|
| | | | *I. cylindrica* | *E. crus-galli* |
| 200 | - | - | 47.2 | 19.0 |
| 20 | - | - | 52.0 | 21.0 |
| 200 | 2 | 0.25 | 59.8 | 27.4 |
| 200 | 7 | 0.10 | 70.0 | 26.2 |
| 200 | 7 | 0.25 | 53.8 | 24.5 |
| 200 | 7 | 0.50 | - | 29.2 |
| 200 | 7 | 1.00 | 59.8 | - |
| 20 | 7 | 0.10 | - | 77.2 |
| 20 | 7 | 0.25 | 64.0 | 67.6 |
| 20 | 7 | 2.50 | 70.0 | 56.2 |
| 200 | 20 | 0.25 | 64.6 | 26.2 |
| | | | (±4.20) | (±6.00) |

Values are means of 4 replicates (± 95% confidence intervals based on pooled standard deviation).

1 Spray solutions applied to *I. cylindrica* contained imazapyr at a dose equivalent to 0.35 kg ai/ha and Lissamine Red at a concentration of 2.0% w/v.
Spray solutions applied to *E. crus-galli* contained imazapyr at a dose equivalent to 0.175 kg ai/ha and Lissamine Red at a concentration of 0.5 % w/v.

Fig. 2. Distribution of spray deposits on (A) Imperata cylindrica and (B) Echinochloa crus-galli



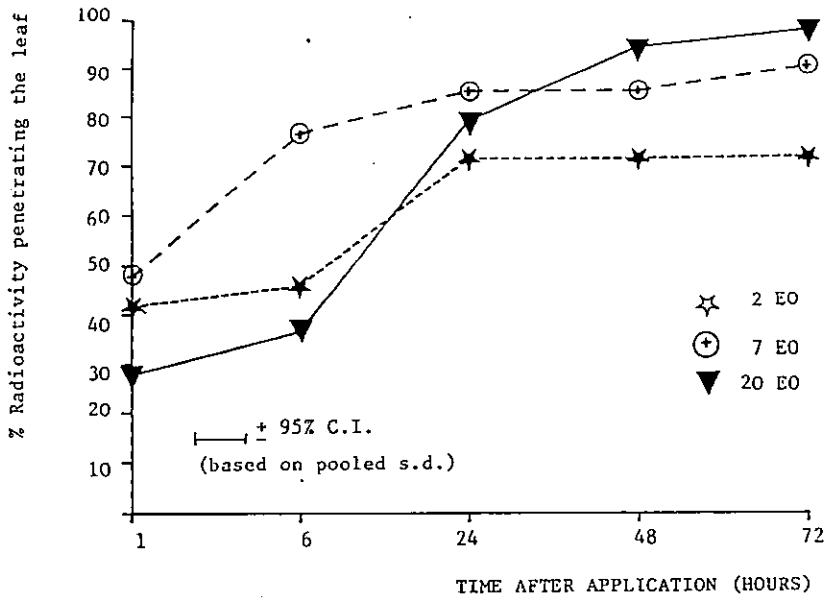



Fig. 3. Effect of surfactant ethylene oxide (EO) content on uptake of $^{14}$-C imazapyr by *Imperata cylindrica*.

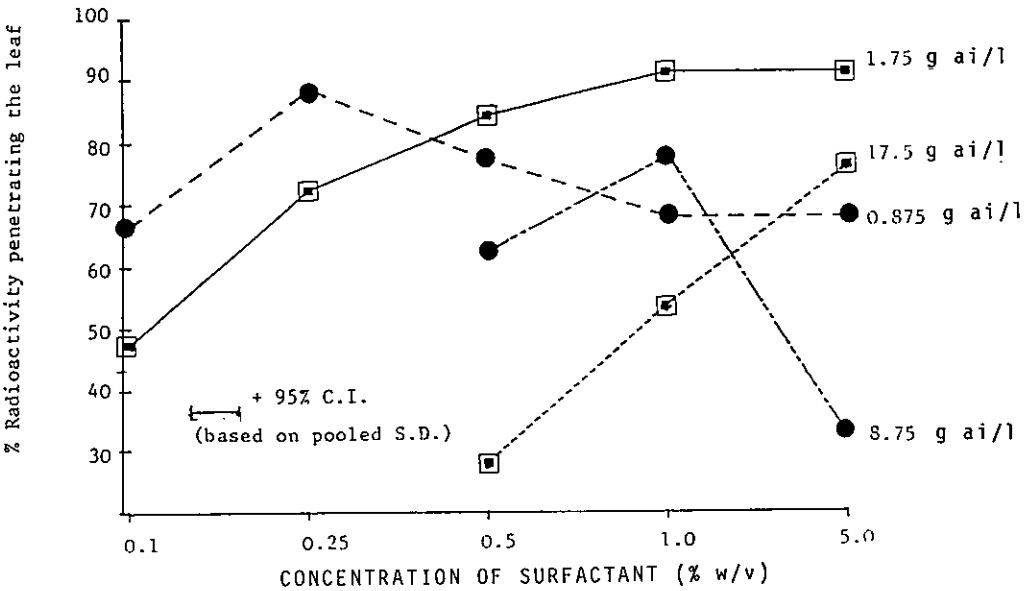



Fig. 4. Effect of surfactant concentration on uptake of $^{14}$-C imazapyr by ▣ *Imperata cylindrica* and ● *Echinochloa crus-galli* 6 hours after application.

**Flow rates** Flow rates through the spinning disc decreased with increasing surfactant concentration from 129 ml/min at 0.25 % w/v to 121 ml/min at 5·% w/v of A7. Of the surfactants tested the highest flow rate of 148 ml/min was recorded with 0.25 % w/v of A2.

**Droplet spectrum** Volume median diameters (VMD's) of 229-254 m and 190-254 m were measured for the hydraulic nozzle and the spinning disc, respectively. An increase in the EO number of the surfactant was found to decrease the VMD of the droplets produced, with 240 m and 192 m being recorded for 0.5 % w/v of A2 and A20, respectively. As the A7 concentration was increased from 0.25 % to 5 % w/v, a decrease in droplet diameter from 218 to 196 m was observed.

**Swath width** The spray distribution across and along the swath of the spinning disc was variable (Fig. 1). Differences in formulation did not affect spray dispersal, with a swath width of 1.0-1.2 m measured for all treatments.

**Retention and distribution on the plant** Total retention of herbicide on *I. cylindrica* was not significantly affected by the EO content or concentration of the surfactant, or by the method of spray application, but the absence of surfactant reduced retention. Decreased retention on *E. crus-galli* occurred as the rate of surfactant was raised at the lower volume rate. Greater retention resulted from spinning disc applications compared with those with the hydraulic system. In the latter, *I. cylindrica* retained a higher proportion of the applied spray than *E. crus-galli* (Table 2). The youngest leaf of *I. cylindrica* retained less spray than the older leaves, although the presence of A2 improved herbicide adhesion to this leaf. The least amount of spray solution was recovered from the base of the plant. Spray distribution of *E. crus-galli* was relatively uniform, with oldest, basal leaf collecting the greatest mass of spray deposit per unit area (Fig. 2). A decrease in the surfactant EO content from 20 to 2 at a concentration of 0.25 % w/v increased the area of an individual 1 l droplet deposit from 1.3 to 5 $mm^2$ on *I. cylindrica* and from 0.9 to 16.2 $mm^2$ on *E. crus-galli*. Increasing the concentration of A7 from 0.25 to 5 % w/v increased the deposit area after dry-down of a 1 l droplet on *I. cylindrica* from 2.0 to 18.1 $mm^2$.

**Uptake of imazapyr** In *I. cylindrica*, the EO content of the surfactant added affected the uptake of imazapyr (Fig. 3). The same pattern was found for *E. crus-galli* except that initial uptake was greatest in the presence of A2. At both high and low imazapyr concentrations, uptake into *I. cylindrica* increased with a progressive increase in surfactant rate. In *E. crus-galli*, a surfactant concentration of 0.25 % w/v brought about the highest rate of uptake of a 0.875 g/l solution of imazapyr 6 hours after treatment. At a concentration of 8.75 g ai/l, imazapyr uptake into this species was greatest when 1.0 % w/v surfactant was present in the solution. Lower rates of herbicide uptake over the first 6 hours after application were found in both species at the higher compared with the lower concentrations of imazapyr (Fig. 4). No significant difference was found in uptake when imazapyr was applied as drops of 200 m or 400 m to *I. cylindrica*, with 80 % of the radioactivity from $^{14}C$ imazapyr penetrating the leaf 3 hours after application.

**Translocation** Redistribution of radioactivity from the site of application in the presence of the linear alcohol surfactants increased in the order A2 A20 A7. At low herbicide concentrations, raising the surfactant concentration from 0.1 to 0.25 % w/v improved translocation of radiolabel but further increase to 1.0 % w/v had no observable effect. At high herbicide concentration, redistribution of radiolabel was greatest at a surfactant concentration

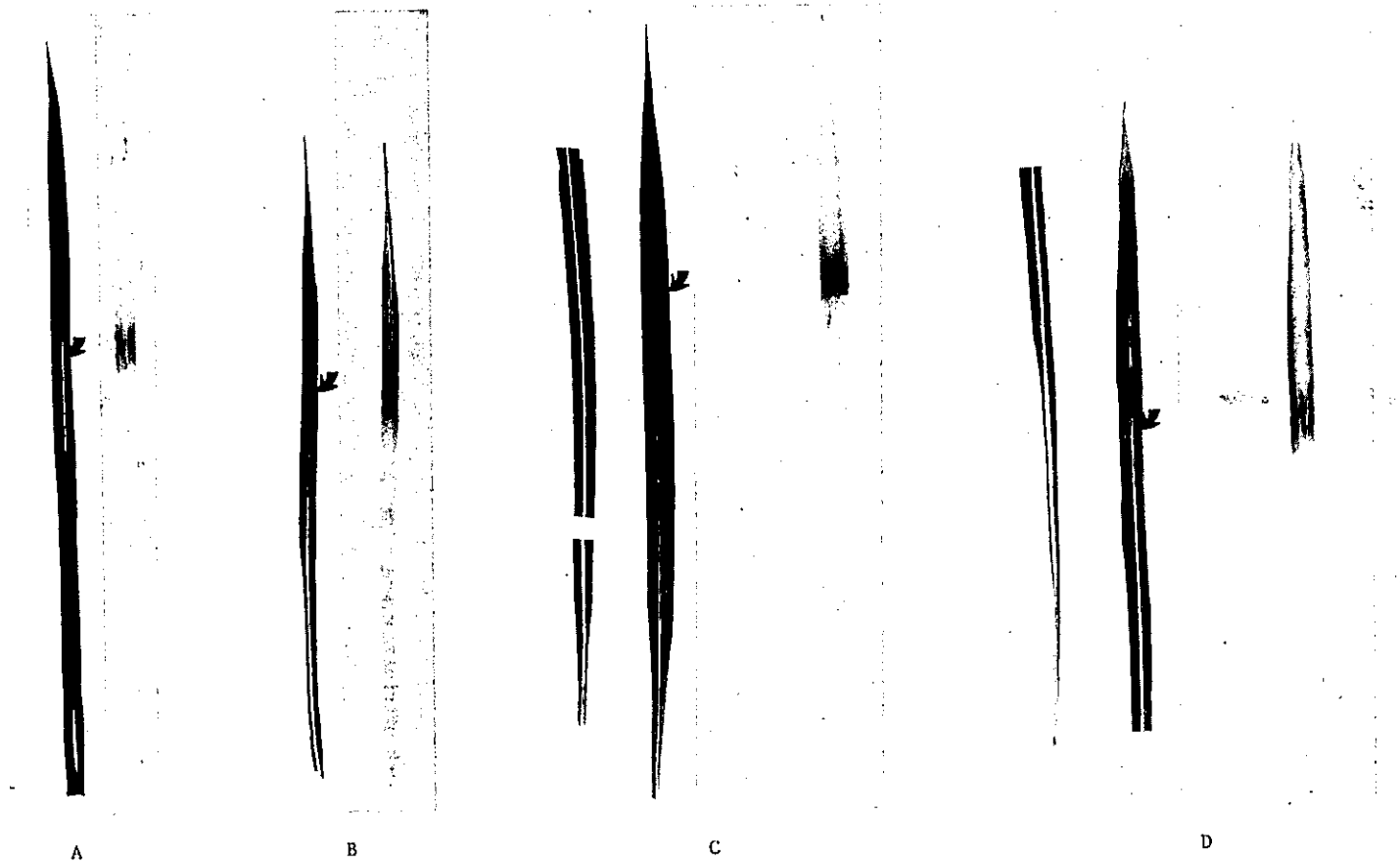



Fig. 5.1. Autoradiographs showing the effect of the surfactant ethylene oxide (EO) content and concentration on translocation of $^{14}$-C imazapyr (1.75 g/l) in Imperata cylindrica : (A) 0.25% w/v A2; (B) 0.25% w/v A7; (C) 0.25% w/v A20; (D) 5.0% w/v A7.

▼ Position of application of three 1 µl droplets

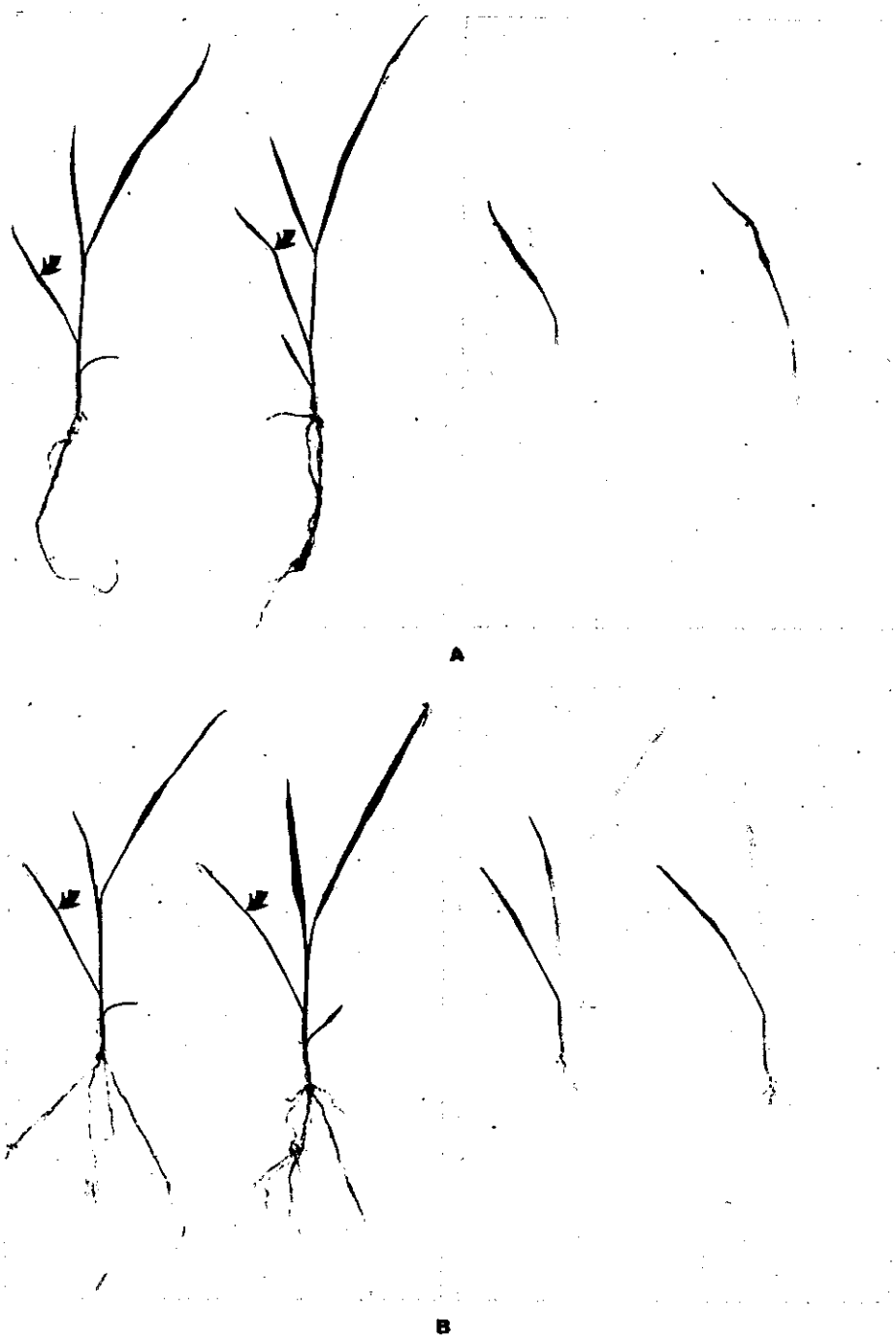
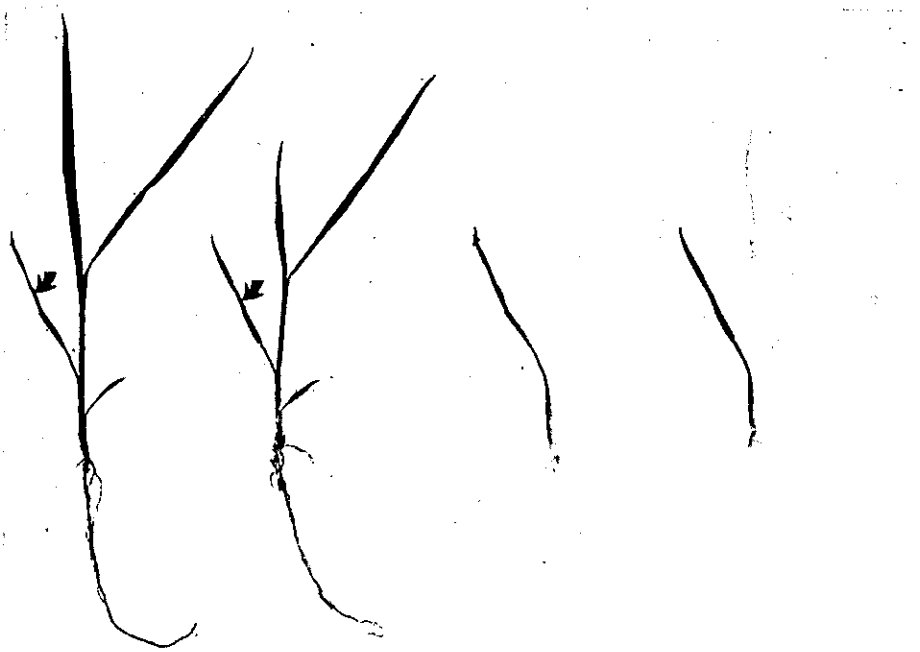



Fig. 5.2. Autoradiographs showing the effect of surfactant ethylene oxide (EO) content on translocation of $^{14}$-C imazapyr (0.875 g/l) in Echinochloa crus-galli : (A) 0.25% w/v A2; (B) 0.25% w/v A20.

Position of application of three 1 µl droplets



A

B

Fig. 5.3. Autoradiographs showing the effect of surfactant concentration on translocation of $^{14}$-C imazapyr (0.875 g/l) in Echinochloa crus-galli : (A) 0.25% w/v A7; (B) 5.0% w/v A7.

Position of application of three 1 µl droplets

of between 0.5 and 1.0 % w/v. Less translocation occurred at the higher herbicide concentrations and with a surfactant rate of 5 % w/v in both species (Fig. 5).

## DISCUSSION

Controlled droplet application of imazapyr to *I. cylindrica* theoretically offers considerable biological and economic advantages over conventional hydraulic equipment. However, this work supports reports from the field that the performance of spinning discs can be both unreliable and inferior in comparison with that of conventional spray machinery. Due to the confounding effects of herbicide concentration, spray volume and droplet spectrum, clear interpretation of data from the published spray trials is impossible. By considering the different application variables separately at each stage of the spraying operation, some key factors affecting biological activity have been identified in the present work:

**Herbicide concentration** Under the conditions of our trials, an increase in imazapyr concentration was associated with a decrease in biological performance. Spray production, herbicide retention and droplet spread on the leaf were not affected by the concentration of imazapyr. Although initial uptake was reduced by high concentrations of a.i., this effect was not significant 6 hours after application. Doubling the diameter of individual droplets did not influence uptake, despite the potential for variation in the concentration gradient across the cuticle due to differences in droplet dry-down time and the quantity of a.i. per unit area. The process most greatly affected by imazapyr concentration was redistribution of the a.i. from the treated area after penetration of the leaf cuticle had occurred. High herbicide concentrations are likely to damage the underlying plant membranes thus inhibiting transport.

**Surfactant properties** Altering the EO number of the linear alcohol surfactants added to the formulations caused variations in imazpyr activity. Of the surfactants tested, A7 improved herbicide performance to a greater extent than A2 or A20 in both test species. The differences in flow rate found with A2, A7 and A20 were considered of minimal importance to the overall activity of imazapyr due to the low repeatability of the results. No significant difference was found in the quantity of spray solution recovered from the leaf surfaces of *I. cylindrica* when the EO content of the surfactant was altered, showing that the droplet size differences observed were not sufficiently great to affect retention. This result also indicates that the variations in biological performance of imazapyr in the presence of the three surfactants could not be attributed to differential herbicide adhesion. Uptake data revealed differences in imazapyr absorption when the EO content of the surfactant was altered, but these did not always correlate with the biological effect of the corresponding treatments in the two species. This suggests that the biological activity of imazapyr is not solely determined by foliar penetration. Redistribution of radiolabelled herbicide was greatest with A7 and A20, but was relatively poor with A2. There is no evidence that A2 gave sufficiently rapid initial uptake of imazapyr to cause herbicide damage to the underlying tissues, which would restrict translocation. This is supported by the fact that A7 showed both high initial uptake and good translocation in *I. cylindrica*. Another explanation could be that A2 itself penetrated the leaf and inhibited subsequent translocation.

**Surfactant concentration** Increasing the surfactant concentration to 0.25 % w/v improved the biological performance of imazapyr but any further increase led to reduced activity in both very

low and medium volume application. Herbicide retention on *I. cylindrica* was not significantly affected by the surfactant rate, whereas an increase in surfactant rate reduced retention on *E. crus-galli* at the very low volume rate. These findings cannot be explained by the small decrease in flow rate or the trend to a decreased VMD of droplets that occurred as surfactant concentratoin was raised. The improvement in imazapyr uptake with increasing rates of surfactant may be due to the solubilisation of the herbicide by the surfactant micelle, thereby increasing the effective concentration gradient across the cuticle. All the concentrations used were supramicellar.

**Spray application method** Our results indicate that, in general, the biological activity of each formulation correlated most closely with the uptake and translocation of radiolabel within the plant. On this basis, some of the formulations tested should have caused greater damage to the target weed than was actually achieved. This implies that the method of spray production was also important in determining the biological performance of imazapyr. Variability in spray deposition by the spinning disc was found under laboratory conditions. It is probable that this led to inadequate herbicide coverage of the plants, reducing the potential activity of the formulations.

In conclusion, it is suggested that a linear alcohol surfactant containing an average of 7 EO residues at a concentration of 0.5-1.0 % w/v, in combination with the minimum possible lethal dose of imazapyr, would have potential for excellent control of *I. cylindrica* in very low volume applications. High surfactant concentrations of 5 % w/v, typical of commercial very low volume formulations, should not be employed as they cause a restriction of herbicide translocation within the plant tissue. Increasing the volume rate from 20 to 50 l/ha may improve spray coverage of the plants and hence the consistency of control.

## ACKNOWLEDGEMENTS

This research was carried out as part of an MSc degree and we are grateful to the U. K. Science and Engineering Research Council for the award of a post-graduate studentship to JKT and to the U. K. Overseas Development Administration for additional financial assistance. Thanks are also extended to American Cyanamid Company for supplying 14-C and unlabelled imazapyr and for generously providing funding for JKT to present this paper; to ICI for supplying the surfactants; to Ken Williams for the photography; and to all those at Silwood Park who contributed to this work.

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# COMPARATIVE STUDY OF MAMMALIAN GENOTOXICTIES IN TWO HERBICIDES

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## ABSTRACT

Genotoxicities of alachlor and butachlor to Chinese hamster ovary (CHO) cells are reported. Alachlor shows higher cytotoxicity, induces a more significant cell cycle delay than butachlor, and is positive for both sister chromatid exchange (SCE) and chromosome aberration (CA) inductions. Clastogenic effect of alachlor and cytotoxicity of butachlor are increased by the metabolic activation. Genotoxicities for two butachlor-containing herbicide mixtures are also included. Butachlor when mixed with chlomethoxynil induces higher SCE and chromosome aberration than a formulation with pyrazoxyfen.

## INTRODUCTION

Alachlor and butachlor are two herbicides with similar structure differing only in having a methoxy or butoxy group, respectively, on the *N*-methyl moiety (Fig. 1). According to EPA'S report, alachlor is a potential human carcinogen inducing lung tumor in mice and stomach, thyroid and nasal turbinate tumors in rats (1). However, similar attention has not been paid to butachlor as well. Investigations on the genotoxicity of butachlor have been limited to bacteria (9, 11) and green alga (13). This is probably because butachlor is applied mainly to rice field which are common in certain Asian and South American areas and is not marketed in the United States. A large segment of our environment is exposed to butachlor due to the fact that, for past five years, this herbicide is the most extensively used pesticides in Taiwan. Studies of deleterious effect of this herbicide to humans as well as to other living things is consequently an important issue. Long-term studies using animals is impractical here in this country. We therefore report results of some comparative studies on *in vitro* genotoxicities of this two herbicides in Chinese hamster ovary (CHO) cells trying to estimate the potential mutagenicities of butachlor to mammalian cells.

## MATERIALS AND METHODS

**Cell** The methodology for culturing of CHO cells followed those described previously (5, 6, 12). Briefly, cells were supplied by Drs. T. C. Lee and K. Y. Jan of the same Institute, recloned and cryostored in liquid nitrogen. Routinely, cells were thawed right before each experiment and grown at 37°C in a humidified atmosphere of 5% $CO_2$ in air, in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, 0.22% sodium bicarbonate and

antibiotics including penicillin (100 units/ml) and streptomycin (100 ug/ml). All the materials for cell culture were purchased from Gibco, Grand Island Biological Co., Grand Island, NY. In order to keep karyotypical stability, cells used for experiment were limited to the 1st and 2nd passages after thawing.

**Herbicides**

Alachlor : 2-chloro-2',6'-diethyl-*N*-(methoxymethyl) acetanilide, 95% technical grade.

Butachlor : 2-chloro-2' ,6'-diethyl-*N*-(butoxymethyl) acetanilide, 90% technical grade.

Chlomethoxynil : 2,4-Dichloro-3'-methoxy-4'-nitrodiphenylether, 90% technical grade.

Pyrazoxyfen : 2-[4-(2,4-dichlorobenzenzoyl)-1,3-dimethyl pyrazol-5-yl oxy]acetophenone, 97.5% technical grade.

All technical grades were supplied by Taiwan Agricultural Chemicals and Toxic substances Research Institute. Herbicides were dissolved in dimethyl sulfoxide (DMSO, E. Merck) prior to their additions to cell culture.

**Cytotoxicity assay** Cells of 3 x $10^5$ were incubated with herbicide of different concentrations for 24 hours and then trypsinized by trypsine/EDTA (0.05%/0.02%, Sigma). From each treatment group 200 cells were taken and replated in a 60-mm petri dish in triplicate. After incubation for 1 week, colonies were fixed with absolute methanol (E. Merck) and stained in 5% Giemsa solution (E. Merck). Colonies formed were counted under an Olympus dissecting microscope and % survivals were calculated by dividing colony numbers in each treatment by those in control treatment. At least 3 replicates were performed for each treatment.

**Inductions of SCEs, cell cycle delay and chromosomal aberration** Three x $10^5$ cells were seeded in a 60-mm petri dish and allowed to grow overnight (less than 24 hours). Old media were then replaced by using fresh media containing BrdUrd (10 uM, Sigma) and tested herbicide or control chemical of DMSO. DMSO in control group was 1% and in treated groups were kept at levels of less than 1%. Incubations were continued at dark for another 24 hours. Two hours prior to the end of incubation, colcemid (0.2 ug/ml, Sigma) was added to the culture. Metaphase cells were harvested by shake-off and air-dried techniques (5). Sister-chromatid differential (SCD) stain of chromosomes was performed by a modified fluorescence plus Giemsa technique (4). Totally 30 secondary metaphase (M2) cells were randomly sampled from blindly-coded slides from each treatment and SCEs scored by using an Olympus Vanox-S photomicroscope. At the same time, 100 cells were sampled for each treatment to calculate the ratio of 1st metaphase (M1) and M2 cells. Cell cycle delay estimated by replication index calculated from the ration of M2/(M1+M2) followed those described by Schneider at al. (10). Methods for chromosomal aberration induction were basically the same as those for SCEs except that there was no BrdUrd added in the culture and the post-treatment incubation period was 18 instead of 24 hours. Examination of chromosomal aberration followed those of Galloway et al. (3) and Margolin et al. (8). For those studies with metabolic activation, mammalian hepatic extracts (S9 mixture) were added according to the methods described by Lin et al. (6) and Wang et al. (12). Methods for statistical analysis of both SCEs and chromosomal aberration followed those described previously by Margolin et al. (7), Dean and Danfold (2), Galloway et al. (3) and Margolin et al. (8).

## RESULTS AND DISCUSSION

Fig. 1. Chemical structures of alachlor and butachlor --- similarities and difference.

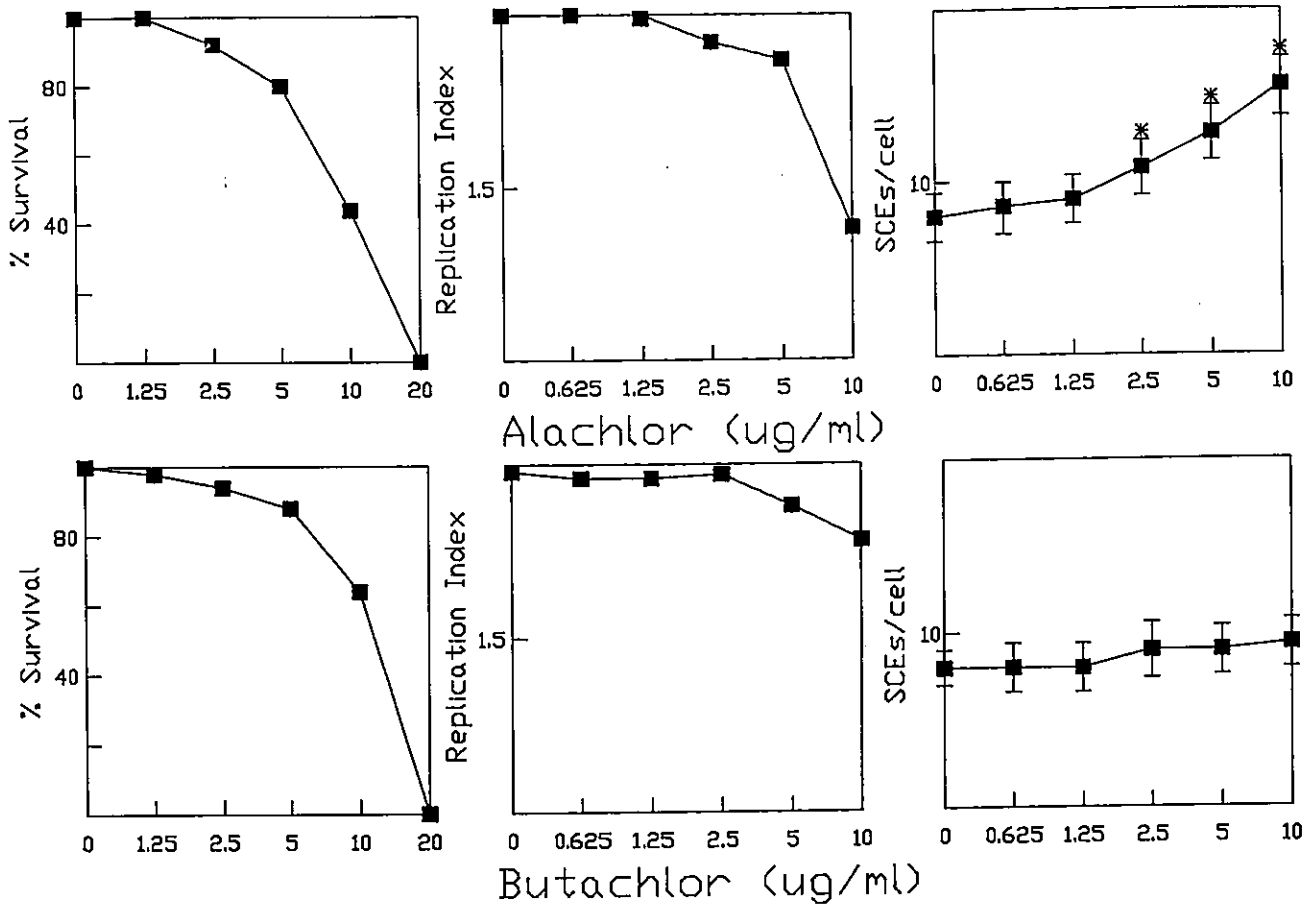


Fig. 2. Cytotoxicities, replication index and sister chromatid exchanges induced by alachlor and butachlor in Chinese hamster ovary cells. '*' indicates that SCEs induced are significantly difference from control ( according to Galloway et al., 1985 )

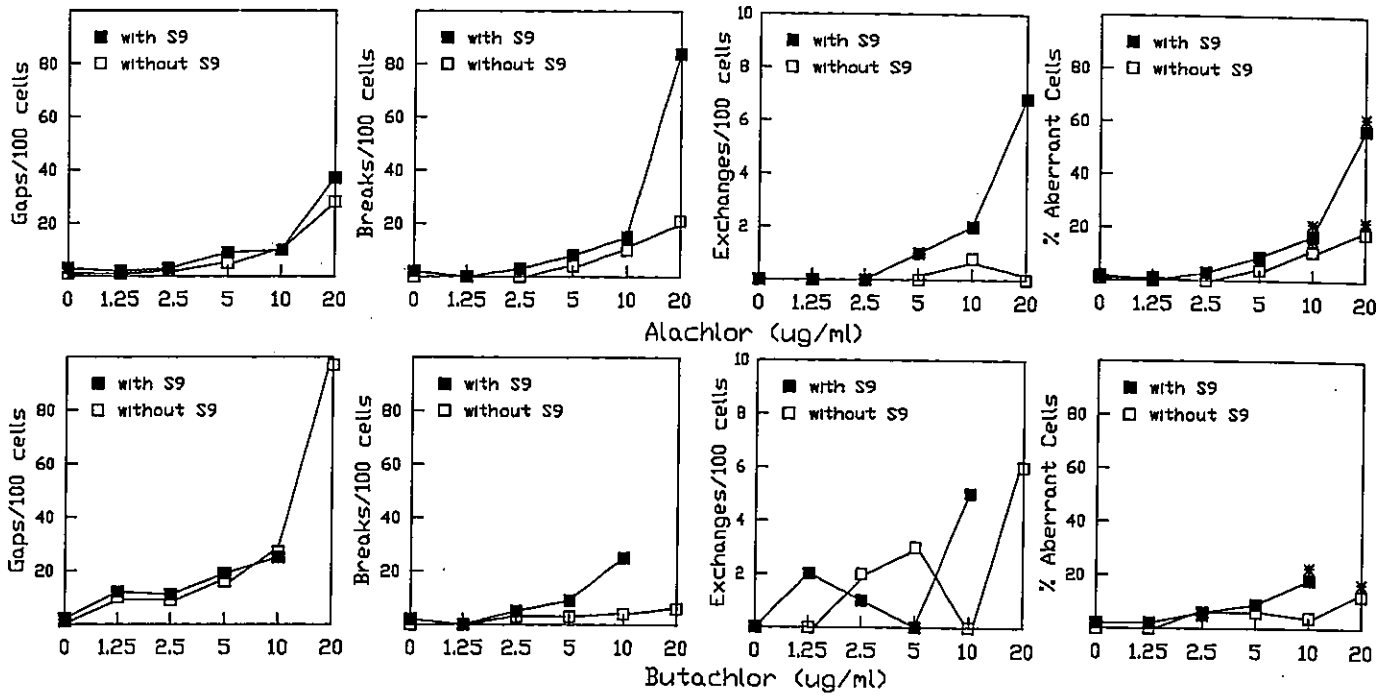


Fig. 3. Chromosome aberration induced by alachlor and butachlor in Chinese hamster ovary cells. '*' indicates that the induction is significantly different from control ( $p<0.01$, according to Margolin et al., 1983 )

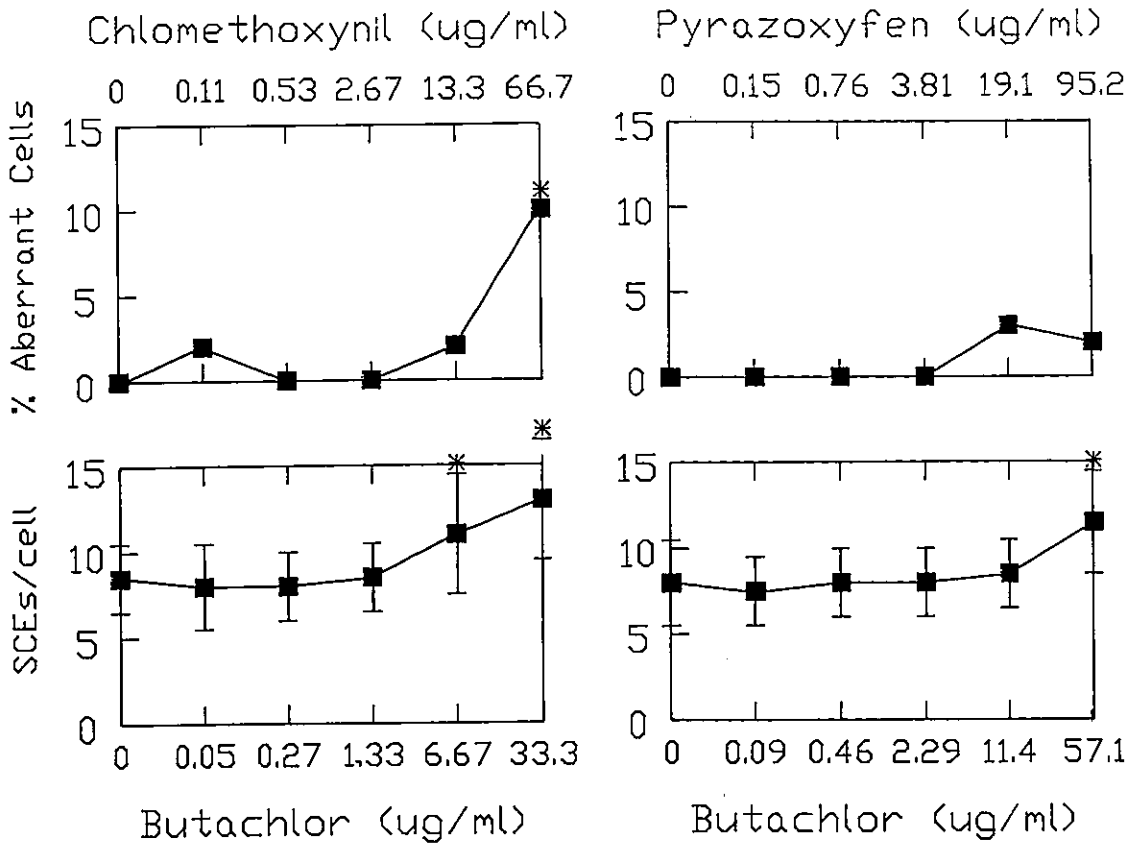


**Fig. 4. Sister chromatid exchange and chromosome aberration induced by two butachlor-containing pesticide mixtures. '*' indicates same meaning as in previous figures.**

Alachlor and butachlor shows differently in their genotoxicities. Alachlor is more toxic to CHO cells than butachlor (Fig. 2). At 5 and 10 ug/ml, alachlor kills 20 and 55% cells, respectively. In those treatments with butachlor, CHO cells retain 86 and 62% survivals respectively. At 20 ug/ml, the survivalities in treatments with both herbicides are less than 1%.

Alachlor also is more significant in inducing cell cycle delay in CHO cells (Fig. 2). Difference between two herbicides is especially apparent in the highest dose scored (10 ug/ml). Butachlor allows about 86% cells proceeded to the second cell cycle (replication index, 1.86). At the same dose level, alachlor withholds about 60% cells staying at the 1st cell cycle (replication index, 1.4).

In SCE inductions, alachlor shows its genotoxicity significantly higher than control at three doses including 2.5, 5 and 10 ug/ml (Fig. 2). Butachlor, on the other hand, does not induce any significant SCEs within dose ranges we have assayed. Inductions by these two herbicides, however, are both dose dependent with trend probabilities both less than 0.005. According to Galloway et al. (3), alachlor is positive and butachlor is questionable weak positive in SCE inductions.

Induction of chromosome aberration was performed with and without metabolic activation (Fig. 3). In both conditions, alachlor again shows stronger clastogenic effect than butachlor. In the presence of S9 mixture, alachlor significantly increases its potential for chromosome aberration in CHO cells. Types of chromosome aberration which significantly increased after the metabolic activation are chromatid exchanges and breaks. According to the results of % aberrant cells induced, alachlor is a positive clastogen to CHO cells with two doses induced significant aberrant cells ($p<0.01$, according to Margolin et al. (7) and significant dose response of the induction (trend probability, $< 0.001$, according to Galloway et al. (3) and Margolin et al. (8). With only one dose significantly induces chromosome aberration and dose dependent in induction, butachlor is a questionable positive clastogen to CHO cells.

Herbicide combining butachlor and chlomethoxynil (1:2) shows positive in SCE while questionable positive in chromosome aberration inductions (Fig. 4). SCEs induced by these two herbicides individually fails to confirm any of them as a positive agents for the induction (data not shown in this report). Combination of butachlor and pyrazoxyfen (3:5) also is not positive either in SCE or chromosome aberration induction.

Lack of a thorough genotoxicity data for butachlor is a big problem for safety regulation of this herbicide in Taiwan. According to our results, butachlor is not as toxic to mammalian cells *in vitro* as alachlor. Whether the genotoxicity differences showed in our report are due to the small variation in structure between these two herbicides is unknown. Possibility that different impurities in them could play the key role is not excluded by the author. More investigations using analytical grades of the herbicides are suggested to elucidate the facts.

## ACKNOWLEDGEMENT

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# THE MECHANISM OF ALLELOPATHIC ACTION OF *LYCORIS RADIATA* TO OTHER WEEDS

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## ABSTRACT

The mechanism of allelopathic action which inhibit the formation of germinating radicle tissues was, (1) Initial cells of germinating radicles were found to be hypertrophy by the allelopathic component. (2) Allelopathic effects was observed that the hypertrophy of initial cell caused inhibition of cell division to be root cap and therefore the number of root cap cells was decreased with the passage of time. (3) Since initial cell division was inhibited by the allelopathic component, cell line numbers of epidermis, cortex, and central cylinder were decreased. (4) Because of the inhibition of procambium differentiation, formation of some organs such as vessel were interfered. (5) Consequently, the physiological balance of nutrient and water was disturbed and the atrophy of root system appeared. (6) It was found that not only *Lycoris radiata* but also *Pueraria lobata, Setaria viridis*, and *Rumex crispus* have the same kind of allelopathic effect with *Lycoris radiata*. As a result of the study of interaction between *Lycoris radiata* which grow naturally on levees and both sides of country lanes and other weeds, fundamental material for biological control of harmful weeds was gained. It is suggested that this material and some results of the mechanism of allelopathic action gained are very important for the biological control of weeds by using allelopathy, the development of new natural herbicides and the introduction of allelopathy into crop plants in the future.

## INTRODUCTION

For the past thirty years, many researches on allelopathy have been rapidly persued; through these energetic reserches, various kinds of chemical compounds which cause allelopathic effects have been precisely identified. The role and the function allelopathy has in natural ecology or ecosystem affected by humans have been also studied in the process.

In many reports, however, to express the crop production control caused by important weeds, which means allelopathic effects in amount or the correlation between allelopathic effects and competition, the changes in weight and size which were caused by it were mostly used.

In my research the mechanism of allelopathic action to plants was studied at the points based on cell, tissue, and nucleus. A component included in *Lycoris radiata* was used for experiment; the components of *Pueraria lobata, Setaria viridis*, and *Rumex crispus* were also attempted in experiment to find out the same effects as *Lycoris radiata*. These facts seem to give some fundamental knowledge for development of new natural herbicides and further successful use of allelopathy into weed control in the future.

**1. Allelopathic action and its mechanism on *Solidago altissima***

*Solidago altissima* is observed to be completely controlled by *Lycoris radiata*; so none of them is found in areas where *Lycoris radiata* naturally grow. Of all organs of the plant, germinating radicles are most susceptible to the influences of the inhibitory component of *Lycoris radiata*. Especially apical meristem of them is significantly interfered its growth by the component; as a result, the cells are observed to be hypertrophied. I reported more details about this phenomenon to Weed Science Society of Japan.

In this report, cell movement of germinating radicles affected by the component, the tissue form, and the mechanism of allelopathic action on germinating radicles are to be discussed.

**2. The mechanism of allelopathic action of *Lycoris radiata* on *Rumex crispus***

As *Lycoris radiata* have a strong power to control *Rumex crispus*, their propagation by seedlings is seldom observed. In this part, allelopathic action and its effects on tissue formation of *Rumex crispus* when they are affected by *Lycoris radiata* are to be stated based on the cell movement, especially initial cells.

## MATERIALS AND METHODS

Undiluted solution of the inhibitory component obtained from *Lycoris radiata* was adjusted to 1000ppm and two to eight-fold dilutions were respectively prepared for experiment. Subsequently bio-test was carried out with *Solidago altissima* and *Rumex crispus* by putting germinated radicles of about one millimeter long into various dilutions to study the effect of the inhibitory component. Twenty-four, 48, and 72 hours after the experiment started, the radicles were fixed with modification of Navashin's fluid; after paraffin sections which were made with them were dyed with hematoxylin and Eosine, permanent preparations were made to be examined.

## RESULTS AND DISCUSSION

***Solidago altissima***

**Allelopathic action on root cap cells and its length** From the experiment, root caps of germinating radicles of *Solidago altissima* were found to be inhibited in length as Table 1 shows. The allelopathic effect on root cap-forming cells is designated in Table 2. These two tables show that the number of cells distinctly decrease, root cap gets shorter as time passed. considered from this fact, it is quite possible that a group of initial cells can be bare without the cover of root cap when root cap cells continue to decrease.

Root cap cells secrete a mucous substance, which makes it easy to get into the soil; at the same time, it helps root elongation physically. Accordingly it is physiologically essential for sound development of plants. Without root cap, therefore, cell supply is continuously prevented being caused less production of cells.

**The action on initial cells of germinating radicles** Table 3 shows the movement of initial cells affected by the inhibitory action of *Lycoris radiata* in comparison with the ones in controls. According to this table, it is clear that an initial cell affected by the action gets longer and wider though the one in the control gets longer while it gets narrower in width. this difference between them is considered to be one extraordinary effect caused by the allelopathic action; it also seems to result in hypertrophy of a cell. As it is obvious from

Table 1. The effect of inhibitory ingredient of *Lycoris radiata* to the length of root cap in germinative radicle of *Solidago altissima*.

| Hours | Section | | | | |
|---|---|---|---|---|---|
| | Control[1] | Formulated concentrate | 2 | 4 | 8 |
| 24 | 100 | 58 | 62 | 69 | 82 |
| 48 | 100 | 20 | 26 | 38 | 62 |
| 72 | 100 | 13 | 24 | 46 | 91 |

1 Relative ratio to control as 100%.

Table 2. The effect of inhibitory ingredient of *Lycoris radiata* to cell in root cap of *Solidago altissima*.

| Number of cells | Hours | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Control | 54 | 46 | 49 |
| Formulated concentrate of ingredient A | 30 | 26 | 11 |

Table 3. The effect of inhibitory ingredient of *Lycoris radiata* to the initial cell in germinative radicle of *Solidago altissima*.

| Hours | Sections | Items | | |
|---|---|---|---|---|
| | | Width | Length | Area |
| 24 | Control | 100 | 100 | 100 |
| | Formulated concentrate | 132 | 113 | 144 |
| 48 | Control | 100 | 100 | 100 |
| | Formulated concentrate | 166 | 141 | 231 |
| 72 | Control | 100 | 100 | 100 |
| | Formulated concentrate | 215 | 130 | 270 |

1 Relative ratio to control as 100%.

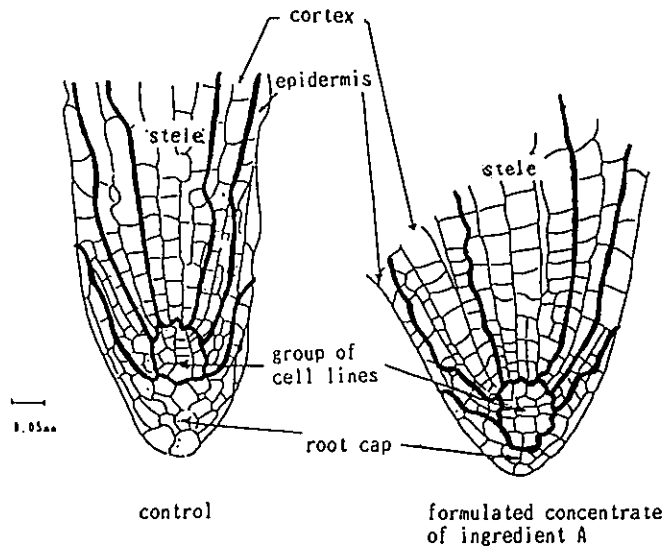


**Fig. 1. The effect of inhibitory ingredient A of *Lycoris radiata* to germinative radicle of *Solidago altissima*. (24 hours after)**

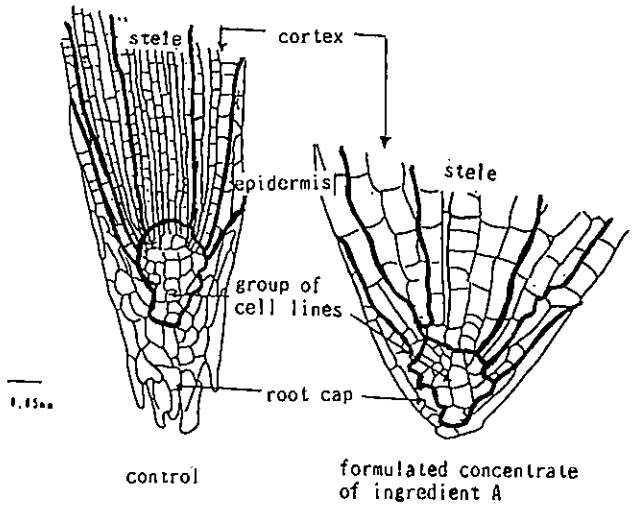


**Fig. 2. The effect of inhibitory ingredient A of *Lycoris radiata* to germinative radicle of *Solidago altissima*. (48 hours after)**

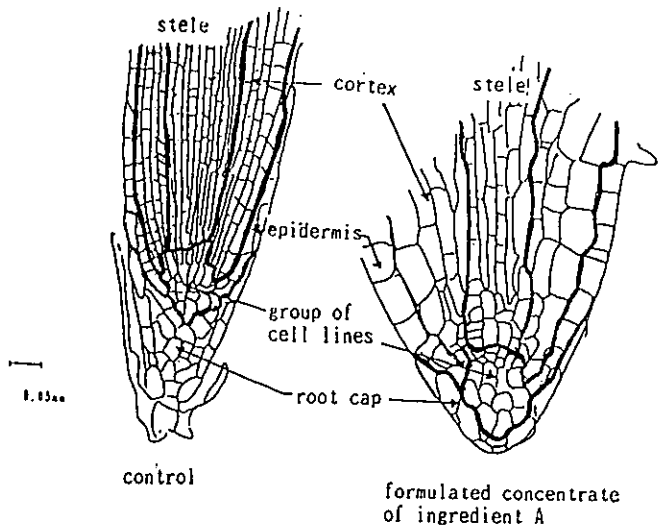


**Fig. 3. The effect of inhibitory ingredient A of *Lycoris radiata* to germinative radicle of *Solidago altissima*. (72 hours after)**

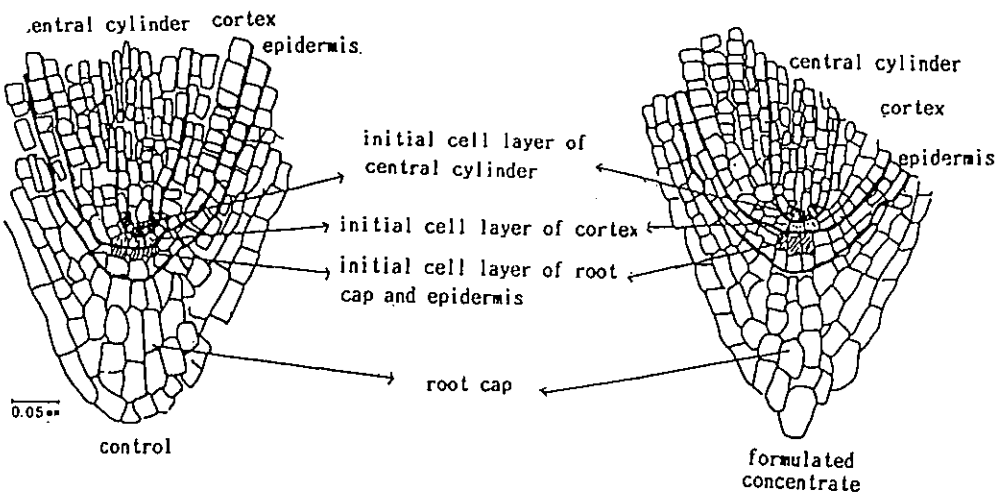


**Fig. 4. Allelopathic action of inhibitory component of *Lycoris radiata* on germinating radicles of *Rumex cripus*. (after 24 hours)**

the Table, the areas of cells in undiluted sections treated for 48 and 72 hours are more than twice as the ones of controls.

**The Allelopathic action on the number of cell lines of germinating radicles.** Figs. 1, 2, and 3 show the vertical sections of root tips which were treated for 24, 48, and 72 hours respectively; each value obtained is shown in Table 4. According to these Figures and Table, it is clear that the numbers of cell lines of central cylinder and cortex increased in the control, which meant internal development of germinating radicles. However, it was observed that the cells in line which were affected by the component were inhibited compared with the control although they were somehow increased.

Central cylinder consists of fibrovascular bundle and parenchyma around it. Fibrovascular bundle is formed in the following process: first procambium start to differentiate near apical meristem, from which phloem start its differentiation into the mature one close to the end of lateral meristem. At the end of it xylem appears, which is an important tissue concerned with the procambium of hypocotyl. As it can be considered that the increase in central cylinder means the differentiation of procambium, it seems to have an important effect on transportation of nutrient and water in the plant. Therefore it seems reasonable that the tissues of which formation is inhibited should cause the insufficient transportation of nutrient and water of the plant. In the experiment, the plants, *Solidago altissima*, were observed to form vessel about 96 hours after germination; on the other hand, no vessel formation was observed in the individuals under inhibitory action in an undiluted solution to eight-fold dilution sections.

**Allelopathic action and cell nucleus affected** Table 5 shows allelopathic effect on cell nucleus size of apical meristem of germinating radicles of *Solidago altissima*. Cell nucleus generally get smaller as the plant grows; it was observed in the control, too. On the contrary, in the experimental section, this tendency was not so distinct as in the control; especially in the 72 hr treatment section, the size of cell nucleus was observed more than twice as the one in the control as shown in Table 5.

The morphologically distinct differences observed on cell nucleus of apical meristem caused by the inhibitory component of *Lycoris radiata* seem to affect the proper function of apical meristem cells.

**Morphological changes of germinating radicles affected by the inhibitory component** As shown in Fig. 1, it was observed that roots had become distorted in the experimental section of 24 hr treatment; in addition, it is shown in the Figure that root cap is thinner than control and therefore initial cells are closer to the end of the root.

In the control with 48 hr treatment, it was observed that the numbers of cell lines of central cylinder and cortex were increased, and root's internal structure got much more complicated compared with the one in the 24 hr treatment section; root cap cells were also properly increased. In the experimental section, however, these characteristics in Fig. 2 were distinct: root's external appearance was distorted, the numbers of cell lines of central cylinder and cortex did not increase so much as the ones observed in the control; root cap cells were decreased, so a group of initial cells got closer to the outside.

As time passed, germinating radicles with 72 hr treatment as control showed not so remarkable changes but full and harmonious development. On the contrary, the form of the tissues observed in the experimental section (72 hr treatment) was obviously different from the one in the control; weaken root caps were also observed as shown in Fig. 3.

Table 4. The effect of inhibitory ingredient of *Lycoris radiata* to the number of cell lines in germinative radicle of *Solidago altissima*[1].

| Tissues | Sections | Hours | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| Stele | Control | 4 | 10 | 10 |
| | Formulated concentrate[2] | 3 | 4 | 6 |
| Cortex | Control | 4 | 8 | 10 |
| | Formulated concentrate | 5 | 5 | 6 |
| Epidermis | Control | 2 | 2 | 2 |
| | Formulated concentrate | 2 | 2 | 2 |

1 The number of cell lines being five cells distance from each initial cell.

2 The number of cell lines in right and left about cortex and epidermis. ingredient A is formulated concentrate.

Table 5. The effect of formulated concentrate of inhibitory ingredient of *Lycoris radiata* to nucleus of cell in meristem part of germinative radicle of *Solidago altissima*.

| Sections | Hours | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Control[1] | 100 | 100 | 100 |
| Formulated concentrate | 113 | 192 | 233 |

1 Relative ratio of diameter of nucleus to control as 100%.

Table 6. Allelopathic action of inhibitory component of *Lycoris radiata* on root cap length of germinating radicles of *Rumex crispus*.

| Hours | Control[1] | Formulated concentrate | Sections | | |
|---|---|---|---|---|---|
| | | | 2 | 4 | 8 |
| 24 | 100 | 76.5 | 38.2 | 70.6 | 75.0 |
| 48 | 100 | 73.1 | 84.6 | 92.3 | 93.0 |
| 72 | 100 | 74.1 | 86.2 | 92.3 | 93.0 |

1 Relative ratio to control as 100%.

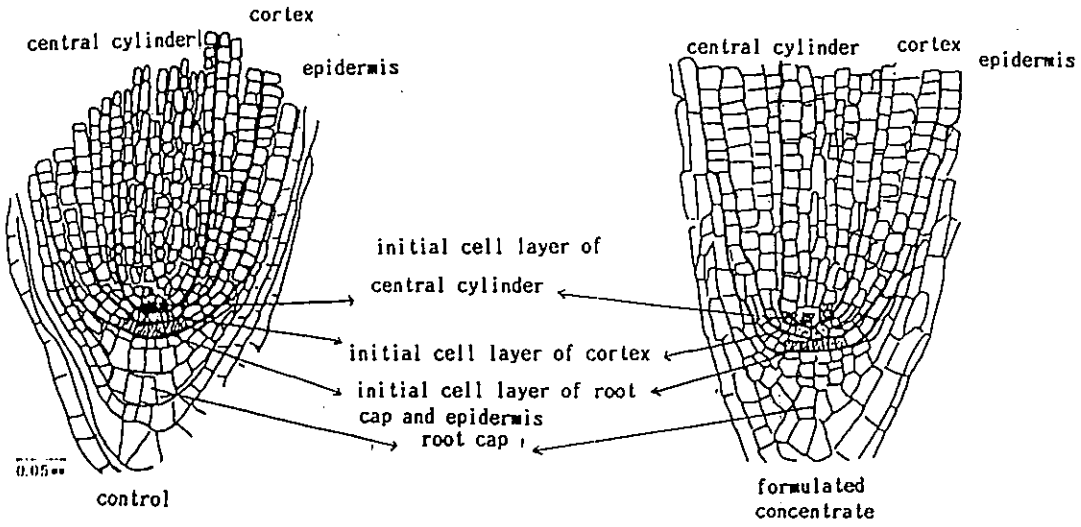


**Fig. 5. Allelopathic action of inhibitory component of *Lycoris radiata* on germinating radicles of *Rumex cripus*. (after 48 hours)**

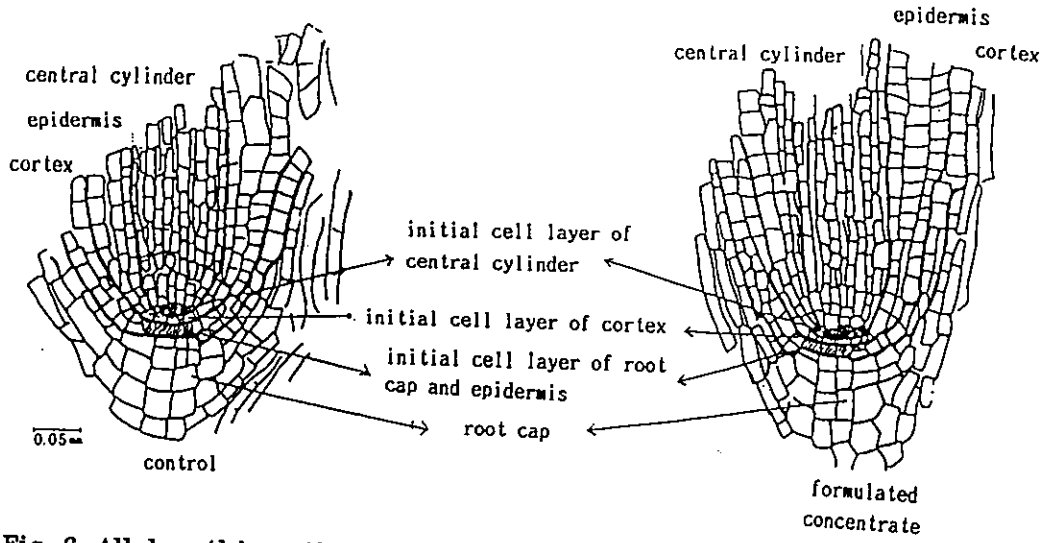


**Fig. 6. Allelopathic action of inhibitory component of *Lycoris radiata* on germinating radicles of *Rumex crispus*. (after 72 hours)**

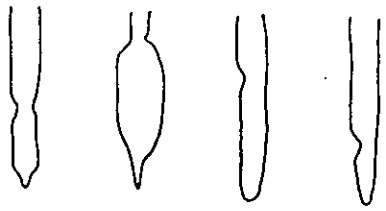

**Fig. 8. Narrow parts of root cap caused by the inhibitory component.**

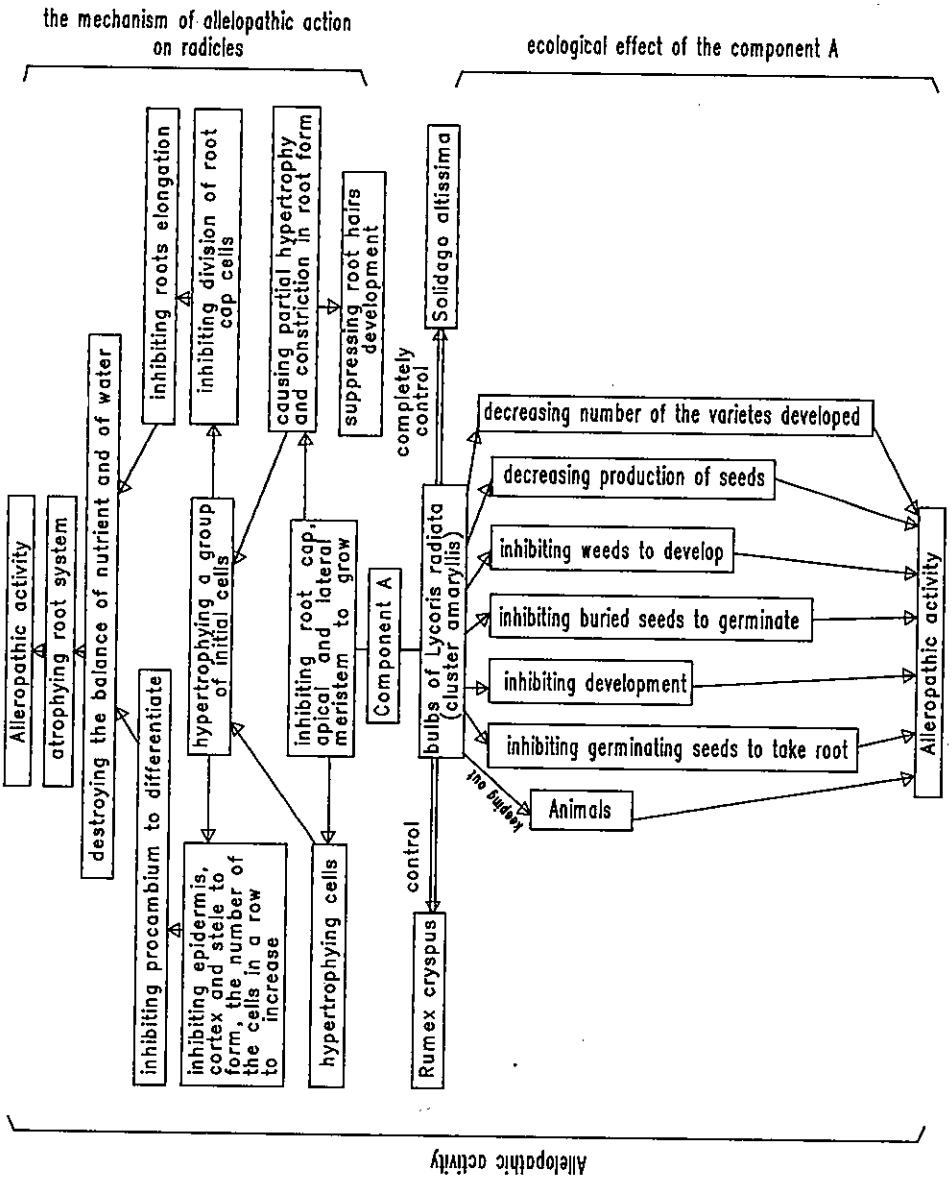


**Fig. 7. The systematic mechanism of allelopathic action of Lycoris radiata.**

*Rumex crispus*

**Inhibitory action of root cap cells and their lengths** It was found that *Rumex crispus* was inhibited in root cap length of germinating radicles as shown in Table 6. Table 7 designates the changes in the number of root cap cells. According to these results, the root cap length of *Rumex crispus* which was treated with undiluted solution was only 70 percent of the one in the control; the number of root cap cells was also decreased by the inhibitory action of the component in *Lycoris radiata*.

**The action on initial cell layer of germinating radicles of *Rumex crispus*** Of all the initial cells of *Rumex crispus*, three groups of cell layers, initials developing into root cap, cortex, and initials into cortex and central cylinder which could be easily distinguished are shown in Figs. 4, 5, and 6.

Obviously different development compared with the control was observed by the allelopathic action: cell areas of root cap, epidermis, and central cylinder with 72 hour-treatment were found to be hypertrophy. The cortex area decreased than the control though cells with 24 and 48 hour-treatment were found to be hypertrophy.

These results seem to indicate that the initial cell which is affected at the very beginning as hypertrophy or atrophy can have an important effect on cell division into each tissue.

**The action on the number of cell lines of germinating radicles** Three parts of initial cells in layers repeat division, each part developing to be mature tissue, such as central cylinder, cortex, root cap, and epidermis forming cell lines.

Table 9 shows the inhibitory action on the formation of central cylinder at this point. Though few effects were noticed on cortex and epidermis, irregular arrangement of cells were sometimes observed in the experimental section.

**The allelopathic action on nucleus of initials of germinating radicles** The size of resting nucleus affected by the inhibitory component (1000ppm) is shown in Table 10, which designates that the nucleus diameter was generally shorter or longer. Especially the nucleus diameter of a cell of central cylinder was 120 percent hypertrophied.

Although Takahashi (4) has reported that the hypertrophy of cell nucleus as a result of allelopathic action results in cell elongation, no relation between the size of cell nucleus and initial cell-enlargement was observed.

The results of this experiment seem to suggest that the hypertrophy of nucleus of initial cells caused by the allelopathic activity exert a real influence on tissue formation as a result of cell division.

**Allelopathic mechanism caused by the component included in *Lycoris radiata*** *Lycoris radiata* which completely control *Solidago altissima* and *Rumex crispus* relatively have been found to cause the decrease of root cap cells and the inhibition on formation of new root cap cells. The allelopathic component induces hypertrophies in the form of initial cells; this change results in the decrease of the number of cell lines which are to be central cylinder and cortex as a result of inhibition in normal cell activity. Consequently the tissue differentiation into procambium, the formation of organs such as vessel was prevented; these phenomena were considered to cause even the inhibition of proper function of transportation of nutrient and water and so the tissue differentiation. It was also found that central cylinder of apical meristem cell got bigger compared with the one in the control and the physiologically normal activity of cells was prevented.

Table 7. Allelopathic action of inhibitory component of *Lycoris radiata* on cell line number of root cap of *Rumex crispus*.

| Hours | Control[1] | Formulated concentrate | Sections 2 | 4 | 8 |
|---|---|---|---|---|---|
| 24 | 100 | 84.2 | 92.1 | 81.5 | 94.7 |
| 48 | 100 | 72.9 | 62.5 | 81.2 | 83.3 |
| 72 | 100 | 88.0 | 78.5 | 83.3 | 95.2 |

1 Relative ratio to control as 100%.

Table 8. Allelopathic action of inhibitory component of *Lycoris radiata* on initial cells of germinating radicles of *Rumex crispus*[1].

| Hours | Initial cell | Items Length | Width | Area |
|---|---|---|---|---|
| 24 | Rootcap epidermis | 588.0 | 104.6 | 182.5 |
| | Cortex | 136.0 | 95.8 | 131.0 |
| | Central cylinder | 70.0 | 78.0 | 58.2 |
| 48 | Rootcap epidermis | 96.1 | 80.1 | 76.1 |
| | Cortex | 104.2 | 99.1 | 116.5 |
| | Central cylinder | 130.0 | 122.0 | 163.0 |
| 72 | Rootcap epidermis | 129.8 | 77.3 | 113.9 |
| | Cortex | 102.2 | 91.7 | 91.8 |
| | Central cylinder | 137.0 | 108.3 | 146.5 |

1 Relative ratio to control as 100%.

Table 9. Allelopathic action of inhibitory component of *Lycoris radiata* on cell lines of germinating radicles of *Rumex crispus*[1].

| Tissue | Sections | Hours | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| Central cylinder | Control | 8 | 10 | 11 |
| | Formulated concentrate | 6 | 17 | 19 |
| Cortex | Control | 7 | 6 | 6 |
| | Formulated concentrate | 6 | 6 | 6 |
| Epidermis | Control | 2 | 4 | 3 |
| | Formulated concentrate | 3 | 2 | 3 |

1 Relative ratio to control as 100%.

Table 10. Allelopathic action of inhibitory component on diameter of initial cell nucleus of germinating radicles of *0Rumex crispus*[1].

| Initial cell | Hours | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Central cylinder nucleus | 86.0 | 121.7 | 123.2 |
| Cortex nucleus | 92.1 | 117.6 | 76.3 |
| Epidermis nucleus | 107.1 | 85.1 | 94.3 |

1 Relative ratio to control as 100%.

Allelopathic action and its mechanism on the two weeds are shown in Fig. 7, on the basis of the results stated above and the report written by Takahashi (2-5). It suggests several things as follows:

By the mechanism of allelopathic action, *Solidago altissima* is being completely controlled for its seedling-production while *Rumex crispus* is being relatively controlled. Other weeds were found to be inhibited for their development in the native habitat of *Lycoris radiata*, which subsequently cause the decrease of the number of developed weeds and growth inhibition, yielded less seeds. It was also found that bulbs of *Lycoris radiata* had an effect to keep away mice and moles.

*Lycoris radiata* have some allelopathic effects on several weeds by allelopathy included in them, in which root cap, apical and lateral meristems are inhibited and so their growth; hypertrophies of each cell in the plant consequently cause extraordinary form of radicles, producing narrow parts and partial hypertrophy of radicle form as shown in Fig. 8. Described in details, it was also found that the hypertrophies of the initial cells by this component resulted in the growth inhibition of epidermis, cortex, central cylinder, and consequently decreasing the cell line number. Cells were inhibited for their differentiation into root cap causes inhibition of root elongation and, differentiation into procambium. The physiological balance of nutrient and water is thus disturbed which leads atrophy of roots. This process was considered to be the mechanism of allelopathy on plants. *Pueraria lobata*, *Setaria viridis*, and *Rumex crispus* were also found to have the same kind of effects on some plants.

This mechanism of allelopathy on some plants seems to suggest us some possibility for development of new natural herbicides and introduction of allelopathy into crop plants in the future.

## ACKNOWLEDGEMENT

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# ANTHOCYANIN OF *TRAPA* SPP.

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**ABSTRACT**

*Trapa* spp. might be a good source of anthocyanin because the underside of the leaves as well as the stems, bulb and horn of the mature plant is red. Our analytical result showed that these parts of the plant are indeed rich in anthocyanin which is most probably a glycoside of cyanidin. By eluting with acidic 60% ethanol, the anthocyanin of *Trapa* was isolated as a single band in Sephade G-25 column. Confirmation of cyanidin as the aglycone was done as follows : Cellulose thin layer chromatography with Forestal solvent showed a red cyanidin spot with Rf = 0.5. The red spot turned blue upon exposure to ammonia vapor. The acidic butanolic solution of cyanidin had Smax = 546nm and its absorption spectrum gave a bathochromic shift of +16nm after the addition of AICI3.

## INTRODUCTION

Anthocyanins are ubiquitous plant pigments contributing to the red coloring of different plant parts. They contribute to the color of strawberries, cranberries and even of wines. Some derivatives of anthocyanins have medicinal and insecticidal properties.

Anthocyanins, pigments ubiquitously present in the cellular sap of plants, are being found to have many important effects and uses. The anthocyanin cyanidin-3-2-glucoside has been reported to be a major factor in the resistance of cotton to the tobacco budworm. Artificial food colorants are presently gaining unpopularity because of their potential health hazard. Alternatively, development of natural pigments as food colorants is being favored and anthocyanins have been suggested as natural sources of red pigments. Cyanidin is a derivative of catechin, which is a drug sold under the trade names "Catergen" and "Cianidanol" for the treatment of various liver diseases.

*Trapa* spp. is a floating water plant found in many parts of Thailand. Since the underside of the leaves as well as the stems and bulbs of the mature plant have a red hue, they might be a good source of anthocyanin. These parts of the plant are discarded when the plants are gathered for their edible horns.

There are species of the family Trapaceae in Thailand---*Trapa bispinosa* Roxb., *T. natans*. (*T. quadrespinosa* Roxb.) and *T. bicornis* Asb. The external structure of these species is quite similar. They are floating plants in fresh water. At first, the root system is fibrous, but later becomes adventitious at the same node with submerged leaves. The stem is elongate with

distinct node and internode, and extends to the water level. The leaves are dimorphic--- those that float are rhomboidal and arranged in apical rosette while those submerged are filliform. The flower is axillary, white and small. It has calyx and corolla of four compartments but ovary of two carpels. The fruit develops under water in the form of a drupe. The drupe of *T. bispinosa* has two pointed horns, that of *T. bicornis* has two roundish horns and that of *T. natans* has four pointed horns. The two cotyledons are unequal and the seed is exalbuminous. The germination period is 10-14 days, from seedling to the first anthesis is 101-104 days and from anthesis to the ripened fruit is 38-40 days.

This study concerns the extraction of anthocyanin from *Trapa* spp. which may be a good source of the substance to be used as food colorant or for drug or insecticidal purposes.

## MATERIALS AND METHODS

The samples of *T. bispinosa* plants used in the experiment were rinsed in distilled water and blotted dry with tissue paper. The leaves, stems and bulbs were separated and analyzed individually for moisture and anthocyanidin. Moisture was determined by drying to constant weight in a 60°C oven. Determination of anthocyanidin was done according to the method of Bate-Smith (1). Anthocyanin was isolated from the whole plant by Sephadex G-25 chromatography according to the method of Somers (2).

Cyanidin, the acid degradation product of anthocyanin, was identified by silica gel thin layer chromatography according to the method of Strumeyer and Malin (3).

## RESULTS AND DISCUSSION

The anthocyanidin content of *T. bispinosa* is shown in Table 1. The leaves have the highest content and the stem the lowest.

Fig. 1 shows the elution pattern of anthocyanin from Sephadex G-25 column using acidic 60% ethanol as eluent. The chromatogram in Fig. 2 shows the separation of anthocyanin as a distinct band.

Gyanidin is the acid decomposition product of anthocyanin. Its properties given in Table 2 were also exhibited by the cyanidin sample in this experiment.

Fig. 3 shows the upper part of *T. bispinosa* plant. Fig. 4 shows the underside of the plant.

The extracted anthocyanin pigment was tried as food coloring in gelatin molds and icing as shown in Fig. 5 and Fig. 6, respectively.

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Table 1. Average moisture and anthocyanidin contets of *T. bispinosa*.

| | Moisture % | Anthocyanidin mg% (wet basis) |
|---|---|---|
| Leaves | 95.6 | 260 |
| Stem | 92.4 | 194 |
| Bulb | 91.8 | 206 |

Table 2. Properties of cyanidin.

| | |
|---|---|
| Color of TLC spot | red |
| Effect of ammonia vapor | red blue |
| $R_f$ in Forestal solvent | 0.5 |
| max in acidic ethanol | 546 nm |
| Bathochromic shift | 16 nm |

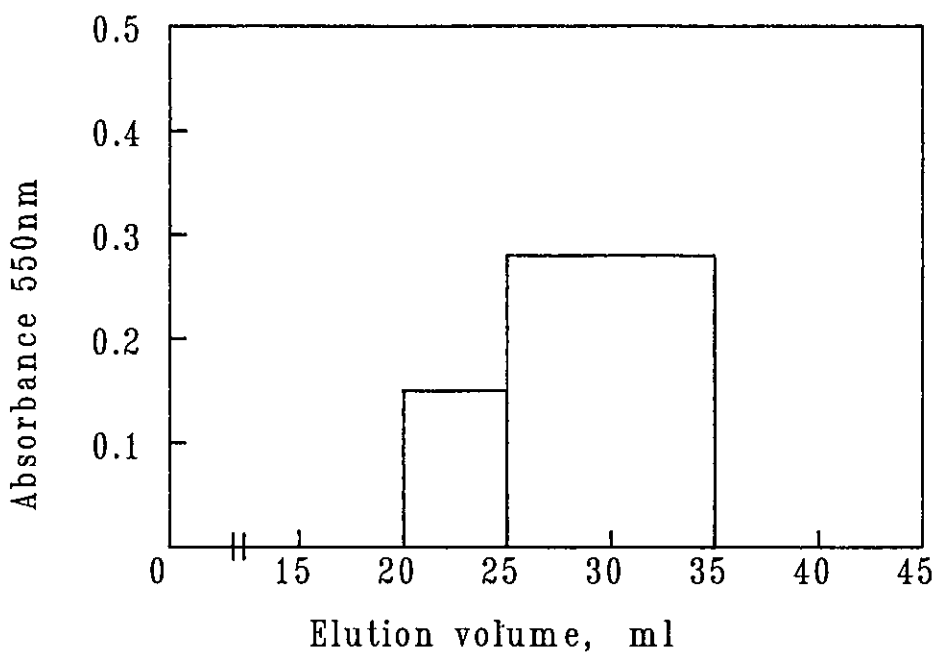
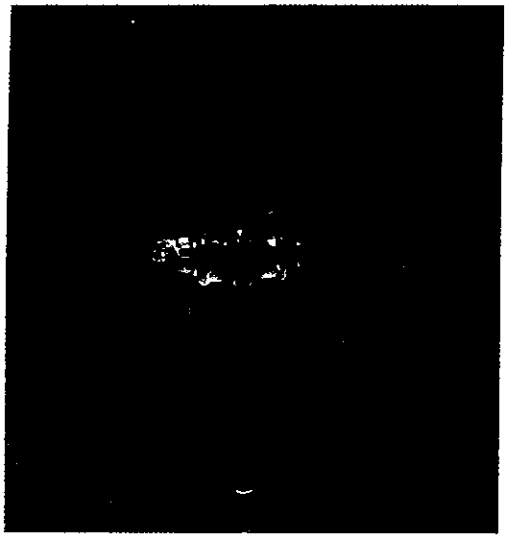



Fig. 1. Elution of anthocyanin from Sephadex G-25 with acidic 60% ethanol.



Sephadex G-25

Eluent: acidic 60% ETOH.

Fig. 2. Anthocyanin chromatogram.

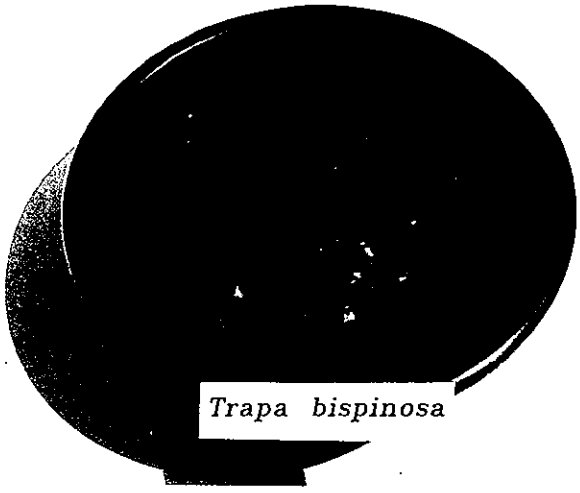



Fig. 3. Top view of *T. bispinosa* plant.

*Trapa bispinosa*

Fig. 4. Underside of *T. bispinosa* plant.

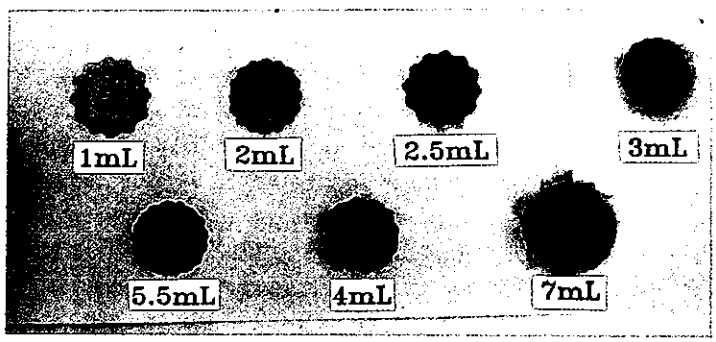



Fig. 5. Anthocyanin as food colorant in gelatin molds.

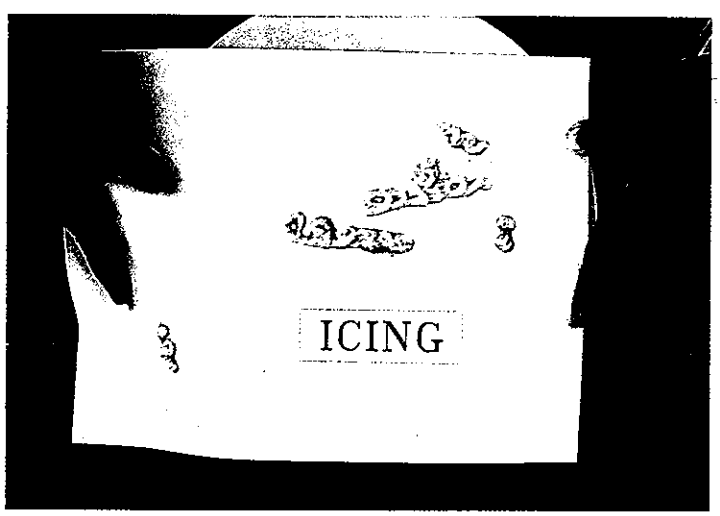



Fig. 6. Anthocyanin as food colorant in icing.

# WEED SCIENTISTS AND SEAWIC-PARTNERS IN WEED RESEARCH

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## ABSTRACT

Information pertaining to weeds and related subjects is a basic component of weed research and agricultural development. A scientist may spend hours, days, and even months in looking for information to solve his particular problems or meet his particular needs. The Southeast Asian Weed Information Center (SEAWIC) at BIOTROP hopes to meet the need for a single nearby source of packaged selected information in the region, and help identify gaps in information so that new research can be done to fill them. SEAWIC, a specialized information center on weed science, provides information services on the identification, distribution, biology, ecology, management, utilization and control of weeds significant to the region. It also covers information on weed interference, herbicides, and related subjects. The center's database of documents consists of published articles, books, and unpublished research/project/travel reports, dissertations, theses and other studies. These are indexed and abstracted (when no abstract is available). Retrieval is done with computers, using the title, author, subject, and keyword fields or combination of these. The center also has an herbarium of Southeast Asian weeds. Data concerning these specimens (data bank) are inputted and stored in a computer. At present, SEAWIC is developing a computerized system of identifying Southeast Asian weeds. SEAWIC publishes a quarterly newsletter (WEEDwatcher), illustrated weed leaflets, and annotated bibliographies. It provides literature searches and selective dissemination of information(SDI), question-and answer; and identification services. Scientists can ask for photocopies of articles and computer-generated bibliographies and can avail of SEAWIC's other services. On the other hand, SEAWIC solicits direct contributions (articles, books, and studies) from scientists, who are both producers and users of information.

## GENERAL INFORMATION

The Southeast Asian Weed Information Center (SEAWIC) was established in January 1986 by SEAMEO-BIOTROP to act as a clearing house of information on Southeast Asian weeds and related subjects.

SEAWIC's information network involves weed scientists, herbaria, and libraries/documentation centers in Indonesis, Malaysia, the Philippines, Singapore, and Thailand.

Many regional and local journals do not find their way to international abstract journals. Not to mention the countless unpublished research reports, project reports, theses, and dissertations hidden in various institutions in Southeast Asia. SEAWIC's emphasis is on these materials.

SEAWIC collects, processes, stores, and disseminates weed information from both published and unpublished materials, using microcomputers. Such records in the database can be searched and retrieved in a very short period of time by subject, keyword, author, title and year or combinations of these.

SEAWIC also collects, processes and stores weed herbarium specimens from the five above-mentioned countries. Information on these weeds (description, taxonomy, habitat, distribution, control, etc.) are being completed and will be stored in microcomputers for easy retrieval. A computerized system of identification is also being developed.

SEAWIC offers literature searches and selective dissemination of information (SDI), question-and-answer, weed identification and document delivery services.

SEAWIC also publishes a newsletter, WEEDwatcher, which is a means of keeping weed scientists and others up to date on developments in weed science in the region. The newsletter also acts as a medium of communication among weed scientists and is the main link between SEAWIC and its clientele.

Weed scientists are both producers and users of information in SEAWIC'a database.

SEAWIC aims to lessen the users' load, so that they need not waste much time looking for the information they want.

SEAWIC solicits direct contributions and suggestions from users. By knowing the research results of weed scientists, SEAWIC can help identify the gaps in weed information so that new research can be done to fill them. The production of information by the scientist and the handling of information by the information/subject specialist go hand in hand helps the R and D process to function efficiently.