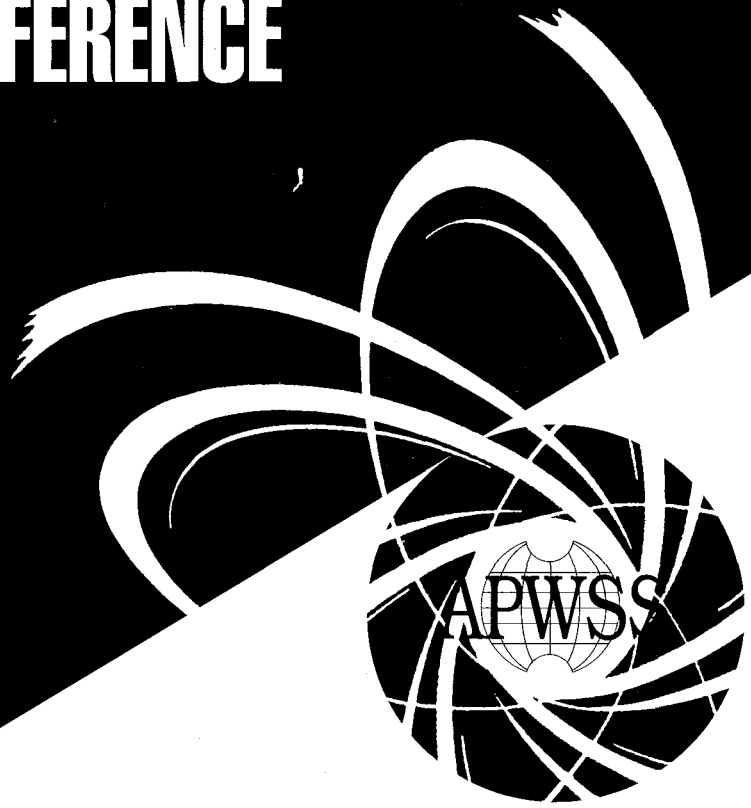
PROCEEDINGS I (B)

15TH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE



TSUKUBA
JAPAN
JULY 24-28
1995

Publishment of this Proceedings was partially supported by a grant-in-aid of the Tukuba EXPO'85 Memorial Foundation

PROCEEDINGS I (B)

15TH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE

TSUKUBA, JAPAN
JULY 24-28, 1995

THE ORGANIZING COMMITTEE OF
THE 15TH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE
1995

This volume of the Proceedings contains papers received by May 31 1995 from authors who have registered by that date.

Papers received on or after June 1, 1995 and those from authors whose registrations are received on or after that date will be published in the second volume of the Proceedings.

The order in which papers appear in this volume is similar to but not the same as their order of presentation in the program.

Printed by East Japan Printing Co., Ltd.
Tsukuba, Japan

ORGANIZATION

Organizer

The Organizing Committee of the 15th Asian-Pacific Weed Science Society (15th APWSS) Conference

Coorganizers

The Weed Science Society of Japan (WSSJ)
The Japan Association for Advancement of Phyto-Regulators (JAPR)

Supporters

Japan International Research Center for Agricultural Sciences, Ministry of Agriculture, Forestry and Fisheries (JIRCAS)
Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC)

Organizing Committee of the 15th APWSS Conference

Chairperson :	Ishizuka, K			
Vice-Chairpersons :	Hamaguchi, H.	Harada, J.	Ozawa, K.	Shibayama, H.
Secretary General :	Hyakutake, H.			
Secretaries :	Kobayashi, K.	Matsumoto, H.	Noritake, K.	Usui, K
APWSS Secretary :	Yamasue, Y.			
Treasurers : (Chairperson : Noguchi, K.)				
	Sato, E.	Takeshita, T.	Ueji, M.	
Planning : (Chairperson : Harada, J.)				
	Aizawa, H.	Asanuma, S.	Fukuki, K.	Hamada, K.
	Hyakutake, H.	Ichihashi, M.	Ichizen, N.	Kobayashi, K.
	Matsumoto, H.	Mizutani, M.	Morita, H.	Namazu, Y.
	Nemoto, M.	Noguchi, K.	Noritake, K.	Oki, Y.
	Sago, R.	Sato, M.	Shibayama, H.	Sugiyama, H.
	Takabayashi, M.	Takeshita, T.	Takeuchi, Y.	Ueji, M.
	Usui, K.	Yamane, S.	Yamasue, Y.	Yogo, Y.
	Yukimoto, M.			
Program : (Chairperson : Morita, H)				
	Aizawa, H.	Itoh, K.	Kobayashi, K.	Kurobane, I.
	Matsumoto, T.	Nemoto, M.	Noguchi, K.	Oki, Y.
	Omokawa, H.	Sago, R.	Shimizu, N.	Yukimoto, M.
Proceedings : (Chairperson : Sugiyama, H.)				
	Itoh, K.	Ito, M.	Kobayashi, K.	Kojima, K.
	Morita, H.	Nemoto, M.	Sago, R.	Sato, M.
	Ueji, M.	Yogo, Y.		
Excursion : (Chairperson : Noritake, K.)				
	Asano, H.	Ikai, T.	Kamoi, M.	Monma, K.
	Namazu, Y.	Takeshita, T.	Yogo, Y.	
Venue and Exhibition : (Chairperson : Sago, R.)				
	Aizawa, H.	Ikeda, M.	Isokawa, T.	Jikihara, K.
	Matsumoto, H.	Noritake, K.	Uno, Y.	Yamane, S.
	Yasui, K.	Yogo, Y.		

Fund Raising Committee

Chairperson :	Ishizuka, K.			
Vice-Chairpersons :	Hamaguchi, H.	Harada, J.	Hyakutake, H.	
	Ozawa, K.	Sato, E.		
Advisers :	Chisaka, H.	Konnai, M.	Kusanagi, T.	
	Matsunaka, S.	Miyahara, M.	Yoshizawa, N.	
Secretariat :	Kobayashi, K.	Matsumoto, H.	Noritaka, K.	Usui, K.

CONTENTS

I (B)

Status of Herbicide Utilization

Herbicide Behaviors in Soil

Weed Management in Rice

Weed Management in Upland Crops

Poster

Weeds

I (A)

Evolution and genetic diversity of Malaysian weedy rice

Duncan A.Vaughan*[1], H.Watanabe[2], Abdullah Md. Zain[3], K.Okuno[1]

[1]National Institute of Agrobiological Resources(NIAR), [2]Japan International Research Center for Agricultural Science(JIRCAS), Tsukuba 315, Japan, [3]MARDI Rice Research Center, Seberang Perai, Malaysia

Abstract. Weedy rice (*O.sativa* L.) in Malaysia, locally called "padi angin", was first recognised as a problem in 1988. Subsequently this weed was reported from a wide area in Malaysia's most important rice growing areas. Yield losses can be very high in infested fields. Weedy rice was intensively sampled in a grid fashion from all areas where weedy rice has been reported. In addition, locally growing cultivars of rice and wild rice were sampled. After DNA extraction the PCR method was used to generate randomly amplified polymorphic DNA's(RAPDs). Weedy rice is genetically very similar to cultivated rice based on RAPD analysis and distinct from wild rice (*O.rufipogon* Griff.) growing in Malaysia. Although evidence of introgression from cultivated rice to wild rice was detected, based on our results, weedy rice most probably evolved as a result of indirect selection for shattering types during periods of volunteer seeding (i.e., grains dropped prior to harvest are the main seed source for the next crop). Where weedy rice has been observed for the longest time, our results suggest distinct weedy races are emerging. Weedy rices which mimic the most popular cultivar, MR84, have appeared making it more difficult to accurately detect and assess weedy rice infestation.

Keywords: RAPD's, PCR method, wild rice, *O. sativa*

Introduction

Asian rice farmers have rapidly adopted direct seeding of rice in areas where rice was previously transplanted. Direct seeding is economically advantageous for farmers who have other employment and require a rapid method of planting rice. Direct seeding is traditionally favored when the availability of water for rice is uncertain particularly early in the growing season.

The two major rice growing areas of West Malaysia are the MUDA river irrigation area, Kedah and Perlis States and Tanjung Karang irrigation area, Selangor State (Fig. 1a). Wahab and Suhaimi (1991) first reported that infestation of weedy rice, which is locally called "padi angin", appeared in rice fields around Tanjung Karang in 1988. Two years later weedy rice was found in the MUDA irrigation scheme and by 1993 some fields were obviously infested.

The objectives of our study were to determine the origin of weedy rice and its diversity in MUDA and Tanjung Karang using the polymerase chain method, which can readily detect polymorphism. Three experiments were performed:- Experiment 1 to determine the relationship between wild, weedy and cultivated rice; experiment 2 and 3 to determine the diversity of weedy rice variation in

MUDA and Tanjung Karang, respectively, as revealed by random amplified polymorphic DNA's (RAPD's).

Materials and Methods

Experiment 1. Seven samples of wild rice, *O. rufipogon*, from the MUDA area, 9 rice varieties which have been widely grown over the last decade in West Malaysia were provided by the MARDI rice genebank and 28 samples of weedy rice of which 24 were from a transect across a single infested monitoring field near Aloe Star were collected in early 1994(Fig. 1b).
Experiment 2. 49 samples of weedy rice were collected between July and August 1994 in a grid fashion across MUDA were. A maximum of 6 samples were taken within each subdistrict. Within sampled fields seeds from a single panicle on a weedy rice plant closest to a randomly chosen location in the field was sampled. Towards the north of MUDA weedy rice was only occasionally found (Fig.1b).
Experiment 3. Weedy rice was sampled as in MUDA in August, December, 1994, and January 1995. Two samples of MR84 the most widely cultivated rice in West Malaysia was included in this experiment. The location of samples collected across Tanjung Karang is shown (Fig 1c).
DNA extraction, amplification and visualization

For experiment 1 the base of young tillers was sampled approximatly 50 days after planting in the quarantine greenhouse of the NIAR, Tsukuba, Japan. For experiments 2 and 3, 10 to 15, 7 day old seedlings were used. DNA was extracted using the standard CTAB method for small scale extraction of DNA (Williams et al., 1993). Briefly, 0.5-1g of fresh leaf tissue, finely ground after immersion in liquid nitrogen, was placed in CTAB containing 0.3% β-mercapoethanol and immersed in a water bath at 60°C for 1hr. The solution was mixed with chloroform/isoamyalcohol (24:1) and centrifuged. The supernatent was mixed with 50μl of 10% CTAB solution and equal volume of precipitation buffer. After standing for 30 min, and centrifugation, the residue was dissolved in 500μl of high salt TE with about 1μl of RNase for 1 hour at 50°C. After adding an equal volume of isopropanol and centrifuging, the residue was washed in 70% ethanol and dissolved in 20 to 100μl of 0.1 TE buffer. The concentration of DNA was adjusted to 5ng/μl by comparing with known standards on starch gels. Amplification of DNA was performed on a Techne Thermal Cycler by mixing 2μl of 5ng/μl DNA with a pre-mix of 6μl sterile distilled water, 1μl of 10xbuffer (Cetus), 0.2μl 25mM $MgCl_2$, 0.2μl of 10mM mixture of dNTP's and 0.5μl of primer (oligonucleotide 10-mers), 0.2μl of Taq polymerase (1u/μl). The thermal cycler was programmed for 45 cycles of 1 min at 93°C, 2 min at 35°C and 3 min at 72°C. A further cycle at 72°C for 10 min was performed before cooling and storing at -5°C. The amplification products were visualized on 1% agarose gels containing ethidium bromide. Binary data (presence or absence) of bands which showed clear polymorphism were analysised using

Clustan software. Wards coefficient of similarity was measured.

Results and discussion

A dendrogram for each experiment is presented (Fig. 2a,b,c). For experiment 1, 23 polymorphic bands were scored for 13 primers. Analysis of the binary data is presented as a dendrogram (Fig. 2a). The results show a clear separation of wild rice from weedy and cultivated rice, except for one sample of wild rice which is in the cultivated/weedy cluster. This one sample was from a wild population which grows near Aloe Star. It is atypical of *O. rufipogon* in the region since it is not or only weakly sensitive to photoperiod and thus flowers when other populations of this species do not. It seems possible that this population represents progeny from a hybrid with cultivated rice which has retained most of its wild characteristics. The results of this experiment clearly suggest that weedy rice evolved from cultivated rice and is not progeny of a cross with wild rice. The banding pattern of weedy and cultivated rices were very similar and in one case the same. Many morphological traits of weedy rice are similar to cultivated rice (Watanabe et al.,1994) which also suggests cultivated rice is the source of weedy rice.

The wild rice species, *O. rufipogon*, which has the same genome as rice and can form hybrids naturally with cultivated rice, grows in several parts of West Malaysia but not in the Tanjung Karang area. Since weedy rice was first reported from Tanjung Karang this supports the results which indicate weedy rice evolved from cultivated rice. However in MUDA, *O. rufipogon* is common around rice fields, but based on our results *O. rufipogon* does not seem to have been a cause of weedy rice in MUDA.

Rainfall in Malaysia is insufficient in some years to fill reservoirs sufficiently to supply the water needs of all rice farmers. As a consequence the practice of "volunteer" seeding became widespread. This practice uses dropped seeds from one seasons rice planting to act as a source of seeds for the next crop. This method of seeding was practiced on nearly 40% of MUDA rice land during the off(dry) season in 1987 (Ho,1991). More than 10,000ha of land were volunteer seeded during the off season between 1984 and 1988. Such a practice would result in the selection of easy shattering rices and could lead to the selection of spontaneous shattering types, which is the main characteristic of weedy rice.

Germplasm from MUDA were analysised in experiment 2 and from this material 18 polymorphic bands were scored from amplification products using 12 primers. Analysis of the binary data is presented(Fig. 2b). All 49 samples, having 42 different banding combinations, used in the analysis were weedy rice samples. No clear groupings are observed from cluster analysis. In experiment 3, Tanjung Karang weedy rice and MR84 were analysised 24 polymorphic bands were scored for 12 primers. Analaysis of binary data is presented(Fig. 2c). The 40 weedy rice samples and 2 samples of MR84 resulted in 37 different banding patterns. Cluster analysis reveals two distinct groups of almost equal size. One group contains MR84. The other group consists of weedy rices only.

Results presented for weedy rice in Tanjung Karang (Fig.2c) suggest that two distinct groups of weedy rice have evolved. Further studies of these two groups is planned. Spontaneously shattering rice has been observed (by DAV) in Tanjung Karang which, apart from the spontaneous shattering trait, is very similar to MR84. Fields which have weeds which so closely mimics the crop that it cannot be obviously observed is likely to be very difficult to control.

The changes in weedy rice populations in Malaysia appears to be very dynamic and requires careful monitoring. The causes behind ecotypic differentiation, which appears to be happening in Tanjung Karang, needs to be addressed since this may be resulting in the evolution of more pernacious weedy rices. The genetic nature of shattering and changes involved in the development of spontaneously shattering weedy rice, from easily threshed rice, may provide clues to preventing spontaneous shattering ecotypes arising.

The emergence of weedy rice in Malaysia appears to be the result of specific characteristics of rice farming in Malaysia. However, weedy rice has also recently emerged as a serious problem in other countries, such as Korea (Suh et al., 1992). In other parts of the world weedy rices have been a persistent problem for a long time and a major cause of yield loss in rice. With the wide spread adoption of broadcasting rice in areas where rice was previously transplanted, weedy rice may emerge rapidly. This may occur in areas where neither wild rice grows nor weedy rice has previously been reported. Cultural practices to prevent weedy rice emerging, such as elimination of dropped rice seeds and use of a reliable seed sources for planting, will be necessary in all broadcast rice areas and particularly where water control and supply are problematic.

References

Ho, N.K. 1991. Comparative ecological studies of the weed flora in irrigated rice fields in the MUDA area. MADA monograph no.44. pp.97.

Williams,J.G.K., M.K.Hanafey, J.A.Rafalski and S.V.Tingey. 1993. Genetic analysis using randomly amplified polymorphic DNA markers. Methods in Enzymology 218:704-740

Suh, H.S., S.Z.Park, and M.H.Hue. 1992. Collection and evaluation of Korean red rice 1. Regional distribution and seed characteristics. Korean J. Crop. Sci. 37(5)425-430.

Wahab, A.H. and O. Suhaimi. 1991. Padi angin - characteristics, adverse effects and methods of its eradication (in Malay with English abstract). Teknol. Padi. 7:27-31

Watanabe, H., M. Azmi and I.Md.Zuki. 1994. Morphological and ecological variation of weedy rice in the MUDA area. Paper at the 1st quarterly meeting under the MADA/MARDI/JIRCAS Joint Research Programme. 26 July 1994.

Fig.1 (a) West Malaysia's main rice growing areas of MUDA and Tanjung Karang
(b) Sample(●) and monitoring field(★) location in MUDA
(c) Sample(●) location in Tanjung Karang

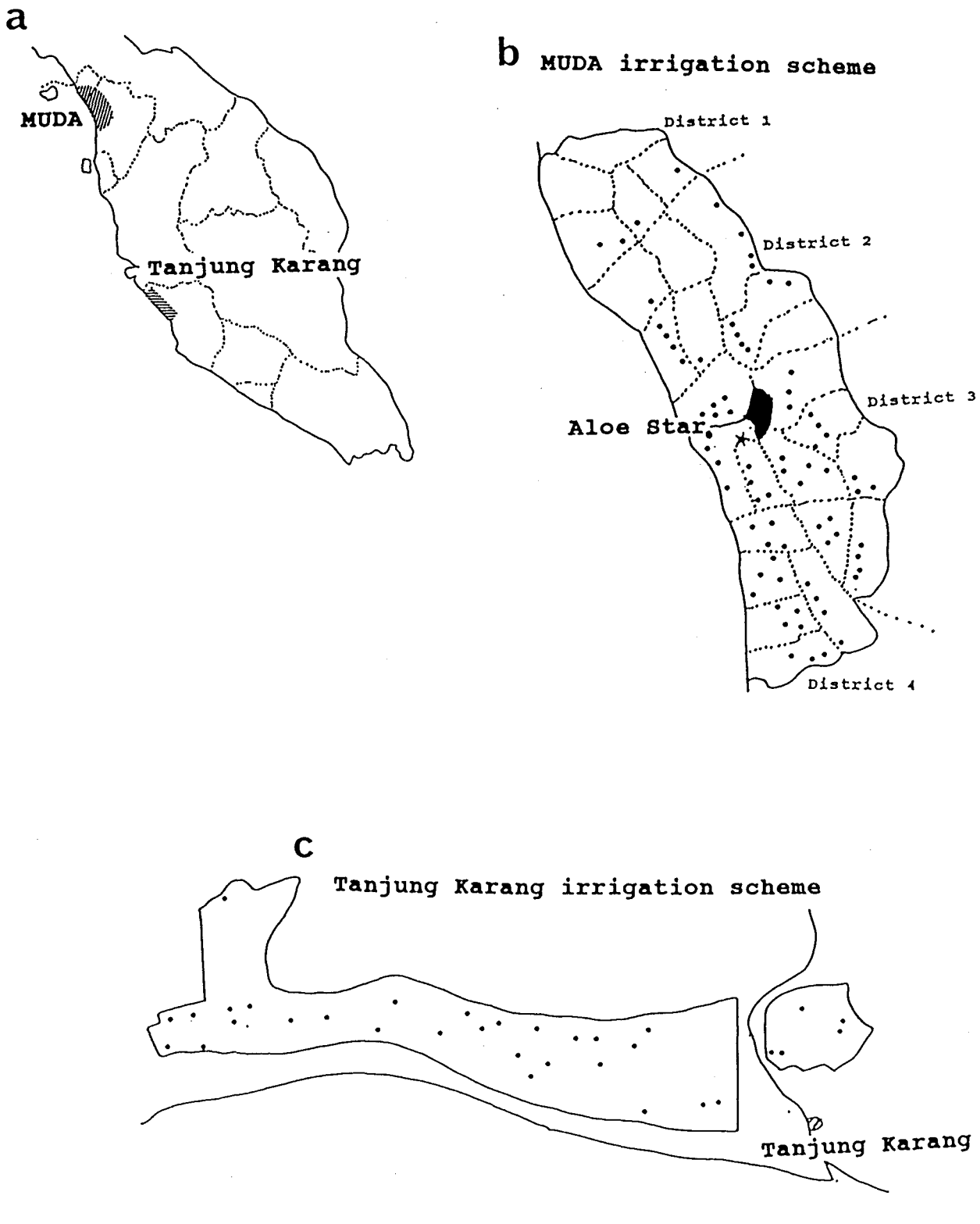
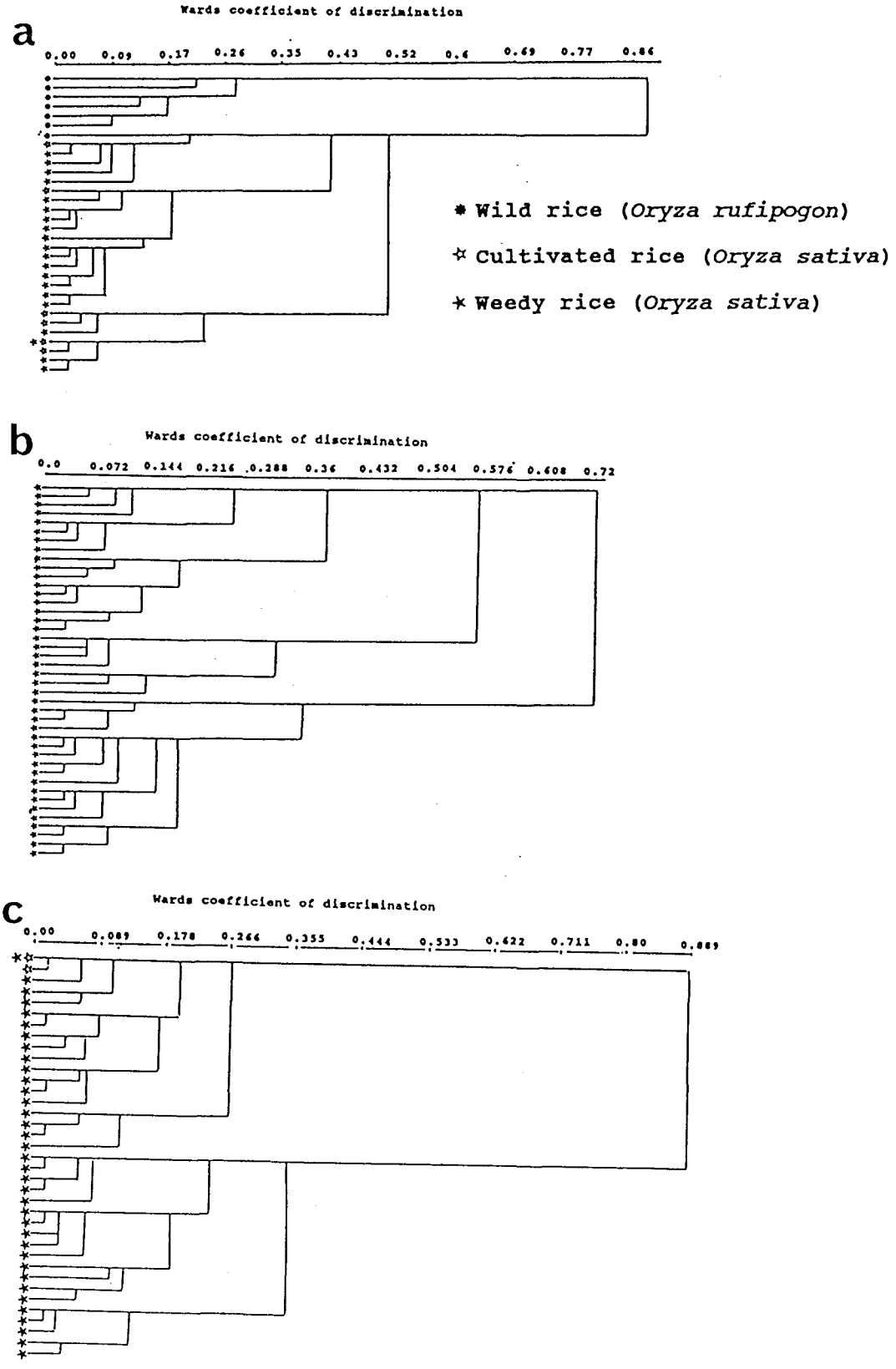


Fig. 2. Dendrograms showing cluster analysis of DNA banding patterns revealed by RAPD analysis of:
(a) wild (*), weedy (✶) and cultivated (☆) rice;
(b) weedy rice from MUDA
(c) weedy (✶) and cultivated (☆) rice from Tanjung Karang



Occurrence of Weedy Rice (Oryza sativa ssp spontanea) in Korea

Soon Chul Kim and Yun Jin Oh
National Yeongnam Agricultural Experiment Station
1085 Naidong, Milyang (627-130), Korea

Abstract. Weedy rice (a collective term for red rice and off-type rice) has rapidly increased in recent years and thus is widely distributedthroughout the nation. The major reasons for this phenomenon were postulated to be reduced tillage operation and poor water management as a result of reduced input to realize for low production cost. Even though the correct origin of weedy rice is not yet fully confirmed several possibilities are :

- shattered grains the previous year
- outcross between cultivated rices : between current year rice grains and shattered grains during harvest the previous year
- outcross between cultivated rice and red rice (dormant seeds)
- outcross between wild type rice and weedy rice (red rice)
- outcross between red rices
- dormant red rice or wild type rice itself

Numerous variations were harvested in farmers fields.
The typical conspicuous traits of weedy rice are heading date, culm length, pericarp color, awn lengh, awn color, grain shattering, grain shape(length/width ratio), spikelet fertility, etc. However, the variations are continuing and diverse intermediates are being harvested.

The occurrence of weedy rice is closely related with tillage operation, water management, cultivation method, and sowing date.

Key words. Rice, Weedy rice, Red rice, Distribution, Ecology

Introduction

Infestation of rice fields with weedy rice (including red rice) results in severe economic losses due to reduced quality and lower yields. It is, however, difficult to precisely describe the extent and severity of weedy rice(red rice) problem because of the confusion that exists in the literature as to the proper taxonomic classification of various weedy rices. Author use the term weedy rice to refer a collective term of red rice and hybrid progeny between red rice and cultivated rice or between cultivated species.
The weediness of weedy rice is attributed to its competitiveness with cultivated rice and to its ability to cross-polinate. In Korea, red rice is traditionally called as "Aeongmi" and collected 42 species at Chonnam province and 48 species at Kyonggi, Kyongbuk and Pyongnam provinces in 1941(Kim,1989). This red rice was no longer troublesome weed since 1970s. For maximum output, maximum input was practiced during this period in cultural practices such as heavy fertilization, good water management, good tillage operation, good weed control, etc. In recent, however, weedy rice(including red rice) has widely been infested and rapidly increased with adoption of labor-saving type cultivation technologies. The paper surveyed the current distribution status of weedy rice (red rice) and its taxonomic traits, agronomic traits and occurrence ecology.

Materials and Methods

Taxonomic Traits of Grains

Field observation was made from rice heading stage to maturity at the Yeongnam area (Kyongbuk and Kyongnam provinces) in 1994. Two hundred sixtytwo samples were collected from 22 City or Gun (county), 12 for Kyongbuk province and 10 for Kyongnam province, respectively. Sampling was done by visiting farm field when weedy rice(red rice) was conspicuously observed in a relatively large plain area. From the samples, grain shape, awn character and color, apiculus color, pericarp color, and endosperm type were observed.

Agronomic Traits

Agronomic traits of weedy rice(red rice)was measured in 1994 using samples collected from farm fields near Milyang area where Yeongnam Agricultural Experiment Station is located. For agronomic traits, heading date, culm length, panicle number and length, floret number, spikelet fertility, grain shape, lodging index with related characteristics, grain shattering and awn length were measured.

Occurrence Ecology

For study on occurrence ecology of weedy rice(red rice) the effect of cultivation methods,seeding date,tillage, double cropping and water management were evaluated from 1990 to 1994 at the experimental farm of the National Yeongnam Agricultural Experiment Station (NYAES) except water management which was done at the farmer's fields.
For cultivation methods,manual transplanting,mechanical transplanting, water seeding, wet seeding and dry seeding methods were included. Except when stated otherwise the cultural practices and sampling methods for all experiments followed the standard rice cultivation method(YCES, 1994 : RDA, 1983). This trial was conducted for four years since 1991. The fourth year provided thorough tillage(Autumn plow + 2 rotavations) and good water management(3~10cm water depth) while first 3 years received minimum tillage(one rotavation) and ordinary water management(0~5cm water depth). Direct seeding methods were sown on May 5~10 while transplanting methods were transplanted on June 5~10.
Rice seeds were also sown every one month or 10 day intervals from February 10 to June 20 in 1991 with dry drill seeding method for determining the effect of seeding date. To understand the effect of tillage operation three different tillage regimes were subjected : conventional tillage (Autumn plow + rotavation), reduced tillage(rotavation only) and zero tillage. This trial was carried out in 1991 with dry seeding method that was sown on May 10.
Barley, rye and itarian ryegrass were used as the related crops for rice based direct seeding technology. Rice was dry seeded on 10 June, 1990 after harvesting of related crops. For understanding the effect of water management, 94 farmer's fields were selected and categorized into 3 water regimes : good management(continuous flood), intermediate management(intermittend flood) and poor management(soil surface exposed, saturated). This was observed during rice growing season in 1994. Finally, postulated schematic diagram of the development of weedy rice suggested based on taxonomic and agronomic traits.

Results and Discussion

Taxonomic Traits of Grains

In farmer's fields most of weedy rices were came from nurserybed implying contaminated seeds were the important source of farm weedy rice. Only 14% of them was arised from shattered seeds in previous years (Table 1). The degree of contamination by weedy rice in weedy rice observed field was highly variable ranging from 0.5% to 27.0% for Kyongbuk province and from 1.0% to 35.2% for Kyongnam province, respectively (Table 1). It was difficult to estimate the degree of contamination and distribution pattern of all farmer's fields. This was due to the fact that field observation and sampling was done to only the field that had conspicuous rice plants which had greatly different from cultivated rice in heading date and plant height. Sometimes, hybrid swarms of many intergrades are existed in the same place and thus impossible to collect all the variations. In grain shape, both types of long grain and short grain, were collected even though long grain type was slightly higher than short grain type (Table 1).

Table 1. Origin, degree of contamination by weedy rice (red rice) and its taxonomic traits

Origin	Transplanted seedling 86%, Shattered seed 14%
Contamination range	Kyongbuk province 0.5~27.0%, Kyongnam province 1.0~35.2%
Grain shape (width/length)	Long grain 57% (> 2.0), Short grain 43% (< 2.0)
Awn length	> 3cm 11%, 1~3cm 13%, < 1cm 19%, 0cm 57%
Awn color	Purple 38%, Brown 19%, Yellow 43%
Apiculus color	Purple 32%, Brown 21%, Yellow 47%
Pericarp color	Red 62%, White 38%
Endosperm type	Waxy 20%, Non-waxy 80%

Suh et al(1990) also reported similar result. Awn length of weedy rice (red rice) was also greatly variable from longer than 3cm to 0cm. However, more than 50% of weedy rice(red rice) had no awn(Table 1). Among colored awn, purple awn was the majority having as high as ordinary yellow awn(Table 1). Brown awn was also observed. Apiculus color was also similar to the awn color : purple was the majority followed by brown(Table 1). Weedy rice(red rice) dominantly possessed red pericarp (62%) over non-colored pericarp (38%) (Table 1). Interestingly, 20% of weedy rice(red rice) was belonged to waxy grain endosperm type (Table 1).

Agronomic Traits

The most conspicuous agronomic traits for visiting field to collect weedy rice (red rice) was maturity with plant height. It is not difficult to find the situation that weedy rice (red rice) flowering later than cultivated rice. The difference between them was sometimes longer than one month. However, careful look can recognize that the difference was not discrete character. Due to introgression, collecting all the variables was not possible. Due to this fact, author arbitrary classified into three groups : early type(5 days later than cultivated rice), intermediate type(10 days late) and late type(30 days late). Some of the important agronomic traits of these 3 types were summarized in Table 2.

The plant height of three types of weedy rice (red rice) were not significantly different among them but significantly taller than cultivated rice. Late type of weedy rice (red rice) had the greatest number of floret number per panicle(255) even though the spikelet fertility was the least recording only 0.4%.

Table 2. Agronomic traits of weedy rice collected in the same field

Trait	Cultivated rice	Weedy rice		
		Early	Intermediate	Late
Heading date	Aug25d	Aug30c	Sep.5b	Sep.26a
Culm length(cm)	66b	94a	91a	89a
Spikelet fertility(%)	97.3a	56.1b	9.6c	0.4d
Panicle number/hill	11.8b	21.6a	20.6a	18.1a
Panicle length(cm)	15.6b	25.5a	27.5a	26.7a
Floret number/panicle	84c	206b	215b	255a
Grain length/width	2.11b	2.26ab	2.16ab	2.39a
Lodging index(3rd internode)	286a	141b	149b	162b
Culm diameter(mm)	3.47c	5.80b	6.29a	6.31a
Culm wall thickness(mm)	0.43c	0.90b	1.38a	1.09b
Grain shattering(%)	10.3c	47.8a	42.5a	25.6b
Germinability(%)	97a	86b	73c	42d
Awn length(cm)	0c	1.3b	4.5a	4.0a

Spikelet fertility was rapidly increased when the heading date became close to cultivated rice. Panicle number per hill and panicle length of weedy rice (red rice) were significantly greater and longer than cultivated rice. Late type had slightly long grain compared to cultivated rice based on grain length to width ratio. Great lodging tolerance of weedy rice (red rice) was exhibited due to advancement of related characters, culm diameter and culm wall thickness. However, no difference was found among weedy rice 3 types. Ease of shattering was another important charater of weedy rice (red rice). Low shattering value of late type compared to early and intermediate type might be due to low absolute spikelet fertility. Germinability was also greatly differed between cultivated rice and weedy rice (red rice) and among maturity types of weedy rices. Cultivated rice had the greatest germinability while late type of weedy rice had the least. The late type of weedy rice, however, had the longest awn length.

Occurrence Ecology

Transplanted rice field with thorough tillage and good water management hardly grow red rice while direct seeded rice field was easily infested by red rice (Table 3). Particularly, dry seeded rice field had drastically enhanced the population of weedy rice with year(Table 3). Water seeding method and wet seeding method hardly provide the opportunity to grow red rice. However, reduced tillage and insufficient water management caused the infestation of red rice at the transplanted, water seeded and wet seeded rices even though the degree of infestation and first appearance time were variable depending upon the cultivation method(Table 3). This result imply that heavily infested dry seeded rice field with red rice must be shifted either transplanting or water (or wet) seeding method with thorough tillage and good water management. For dry seeded rice, early seeding enhanced the growth of red rice (Table 3). Double cropped dry seeded rice with barley, rye or itarian ryegrass had significantly reduced the growth of red rice by 33%~66% (Table 3).

Based on field observation and agronomic traits of weedy rice (red rice) schematic diagram for developing diversed weedy rices postulated as follow. The first appearance of weedy rice in a given field might be originated by cross-pollination between cultivated rice and wild type rice or red rice which arised from dormant seeds in the soil. Wild type rice and red rice usually have strong dormancy and are able to survive in the soil for years (Wirjahardja et al.1983 : Baker and Sonnier, 1983 : Kim, 1989 : Cohn and Hughes, 1981). Thorough tillage operation and water management also strongly suppress the growth of weedy rice (red rice) (Baker and Sonnier, 1983 : LSUAC, 1987 : Seaman, 1983 : Smith, 1981 : TAES, 1988 : UA, 1988 : UC, 1983) and thus use as control method. Recent trends of reduced tillage and poor water management provided more opportunity to grow weedy rice (red rice). Even though the seeds of F1 hybrid between cultivated rice and wild type rice or red rice had very low spikelet fertility (less than 5%) as shown in Table 2. Few seeds develop into F2 generation in the following year. For the 2nd year, some of F2 seeds can also produce another F1 seeds by cross-pollination(backcross) with cultivated rice. Similarly, new F1 hybrid could be produced same reason as the first year. For the 3rd year, in the same manner, several types of F1, F2 and F3 generation will be existed together in the same field. In these evolutionary lines, the wild and domesticated types exist in the same field(sympatric) and produce hybrid swarms of many integrades particularly,in maturity and spikelet fertility. Therefore, the variables of these characters are continual not discrete.Even though the correct origin of weedy rice (red rice) was not yet fully confirmed several possibilities could be summarized as follow based on the research results discussed in this paper.

- shattered grains the previous year
- outcross between cultivated rices : between current year rice grains and shattered grains during harvest the previous year
- outcross between cultivated rice and red rice that was arised from the dormant seeds in the soil
- outcross between wild type rice and red rice (weedy rice)
- outcross between red rices
- dormant red rice or wild type rice itself

Table 3. Some occurrence ecology of weedy rice (red rice) as affected by cultural practices

Tillage	Plow+rotavation 100%, Rotavation 276%, No tillage 628%
Water management	Continuous flood 0.8%, Intermittend flood 29%, Soil surface exposed 100%
Cultivation method (no/1000㎡ at 3rd year)	Manual transplanting 0, Mechanical transplanting 13, Water seeded 14, Wet seeded 37, Dry seeded 3,960
Seeding date	Feb.10(100%), Mar.10(86%), Apr.10(81%), May 10(56%), May 20(31%), May 30(22%), Jun.10(14%), Jun.20(11%)
Double cropping	Rice single crop 100%, Rice-Barley 445%, Rice-Rye 67%, Rice-Itarian ryegrass 34%

References

1. Baker, J.B. and Sonnier, E.A. 1993. Red rice and its control P327 -333 in Weed control in rice. Int. Rice Res. Inst.(IRRI), LosBanos, Laguna, Philippines.
2. Cohn, M.A. and J.A. Hughes. 1981. Seed dormancy in red rice(Oryza sativa) I. Effect of temperature on dry-afterripening. Weed Sci. 29 : 402~404.
3. Kim, J.C. 1989. Studies on physio-ecological characteristics of red rice(Oryza sativa L.) and its competition with cultivated rice (Oryza sativa L.). Ph.D thesis, Chungbuk National University, Korea. 34P.
4. Louisiana State University Agricultural Center (LSUAC). 1987. Rice production handbook. Louisiana Univ. Agr. Center. 64P
5. Rural Development Administration (RAD). 1983. Handbook for agricultural research. RDA, Korea. 453P.
6. Seaman, D.E. 1983. Farmers weed control technology for water-seeded rice in north America. P167~177 in Weed control in rice. Int. Rice Res. Inst.(IRRI), LosBanos, Laguna, Philippines.
7. Smith, R.J.JR. 1981. Control of red rice (Oryza sativa) in water-seeded rice(O.sativa). Weed Sci. 29 : 663 - 666.
8. Suh, H.S., W.K. Hwa and M.H.Heu. 1990. Collection and character investigation of Korean red rice II. Geographical variation and grain character. J. Korean Crop Sic. 35 (suppl. 2) : 14 - 15.
9. Texas Agricultural Extension Service(TAES). 1988. Rice production guidelines. Taxas A&M Univ. Agri. Res. and Ext. Center. 61P.
10. University of Arkansas (UA). 1988. Rice production handbook. Cooperative Extension Service. Univ. Arkansas USDA and County Governments Cooperation. 61P.
11. University of California (UC). 1983. Integrated pest management for rice. Univ. California, Div. Agri. Sci. Pub. 95P.
12. Wirjahardja, S., Guhardja, E. and Wiroatmodjo, J. 1983. Wild rice and its control. P319~325 in Weed control in rice. Int. Rice Res. Inst. (IRRI), LosBanos Laguna, Philippines.
13. Yeongnam Crop Experiment Station. 1994. Rice cultivation method. P18~26 in Research plan for 1994. Yeongnam Crop Exp. Sta. RDA, Korea.

Occurrence of endogenous dsRNAs in wild rice and cultivated rice

Moriyama H.[1], Fukuhara T.[1], Hyakutake H.[2] and Nitta T.[1]

[1] Laboratory of Molecular Cell Biology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu, Tokyo, 183, Japan; [2] Plant Functions Laboratory, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan

Abstract A linear, 14 kb, double-stranded RNA (dsRNA) was found in both cultivated rice (*Oryza sativa L.*) and wild rice (*O. rufipogon*). Host plants with the dsRNA were apparently symptomless. The dsRNA was detectable in temperate (Japan) (8/8) and tropical (the Philippines) (6/6) cultivars of *japonica* rice. No cultivars of *indica* rice (0/6) harbored the dsRNA. Of wild rice, a 14 kb dsRNA was detected in *O. rufipogon*, W-1714, weedy type (Brazil) (1/17). Northern analyses showed that the dsRNAs of W-1714 and tropical *japonica* are not identical but homologous to that of temperate *japonica*. These dsRNAs of rice plants are likely to have evolved independently from a common ancestor.

Key words: Double-stranded RNA, *Oryza rufipogon*, *Oryza sativa* L., RNA plasmid, Vertical transmission

Introduction

Endogenous double-stranded RNAs (dsRNAs) are distributed extensively in the plant kingdom, from algae to higher plants (Ishihara et al. 1992; for reviews, see Dodds et al. 1984, 1988; Brown and Finnegan 1989; Milne and Natsuaki 1994). These dsRNAs appear to replicate independently of the cellular DNAs. Host plants with these plasmid-like dsRNAs are apparently asymptomatic. The dsRNAs have no obvious effect on the phenotype of their host plants. Although horizontal transmission of the dsRNAs from plant to plant has not been proved, dsRNAs are known to be transmitted to progeny cells during cell division, thus, their life cycles are plasmid-like rather than virus-like (for review, see Brown and Finnegan 1989).

A linear, high-molecular-weight dsRNA have been found in temperate *japonica* rice (*Oryza sativa* L.) (Fukuhara *et al.* 1993). It seems that it is not associated with distinct virus-like particles. The dsRNA occurs in every tissue and every developmental stage. It localizes in the cytoplasm, and is present at a constant concentration in most tissues (about 100 copies per cell). The dsRNA transmits vertically from generation to generation via seeds. The rice dsRNA {13,952 nucleotides (nt) in length} has one extremely long open reading frame (ORF) of 13,716 nt. The ORF appears to encode a large polyprotein which includes an RNA helicase-like domain and an RNA-dependent RNA polymerase-like domain (Moriyama *et al.* 1995). Here, we discuss the origin and evolution of these dsRNAs on the basis of results of hybridization and sequencing experiments.

Materials and Methods

Plant materials

Plants of temperate and tropical *japonica* rice (*Oryza sativa* L.) and wild rice were grown in a greenhouse at 28°C. Rice cultivars and species of wild rice are listed in Table 1. Seedlings of *Hordeum vulgare* L. (cv. Kashima) and *Secale cereale* L. (cv. Hatsuharu) were also grown in a greenhouse at 25°C.

Extraction and fractionation of dsRNA

Ten grams of seedlings or plant tissue were pulverized in a mortar after freezing in liquid nitrogen. The dsRNA was extracted by the SDS-phenol method and fractionated by column chromatography on CF-11 cellulose (Whatman, Maidstone, UK) as described by Morris and Dodds (1979). The dsRNA fraction was further purified by treatment with DNase I.

Cloning of cDNA

Complementary DNAs were synthesized from the dsRNA of *japonica* rice (cv. Nipponbare) by standard protocol (McCrae 1985) with the exception that a random hexanucleotide primer (Takara, Kyoto, Japan) was used instead of oligo-dT. Several oligonucleotide primers were synthesized on the basis of sequencing data obtained from the above cDNA clones, and then cDNA clones were obtained further by the method of Gubler and Hoffman (1983).

Northern hybridization

Purified dsRNAs were subjected to electrophoresis in a 0.8% agarose gel and then the dsRNAs were transferred to a nylon membrane (Zeta-probe; Bio-Rad). Northern hybridization was carried out according to the manufacture's protocol. Probes for hybridization were synthesized from the cDNA fragments of recombinant plasmids by use of a *Bca*BEST^{TM} Labeling Kit (Takara, Kyoto, Japan) and [α-^{32}P]dCTP (Amersham Co., Arlington Height, IL, USA).

DNA sequencing and homology searches

DNA sequencing was carried out by the dideoxynucleotide chain-terminating method using a 7-deaza Sequenase Ver. 2.0 DNA sequencing kit (United States Biochemical Corporation, Cleveland, OH, USA). Analyses of nucleotide and amino acid sequences and homology searches were performed with genetic information-processing programs (SDC-GENETYX; Software Development Co., Ltd., Tokyo, Japan).

Results

Occurrence of the dsRNAs in cultivated rice and wild rice

We examined whether the seedlings and tissues from many strains of cultivated rice (*O. sativa* L.) contained dsRNA or not (see Table 1, left column). The dsRNA of about 14 kb was detected in many strains of *japonica* rice by agarose gel electrophoresis. From a comparison of the amount of dsRNA with that of rice DNA [2.5×10^8 bp per haploid (Sorenson 1984)] in the total nucleic acids, the copy number of dsRNA in rice was roughly estimated to be 100 per cell. Thus dsRNA was detected not only in *japonica* rice from a temperate region (collected in Japan) (Fig. 1A) but also in tropical *japonica* rice (collected in the Philippines) (Fig. 2A). It was also detected in the Akamai strain (red-kerneled rice) (Fig. 1A, lane 5), which is considered to be the oldest cultivar among all extant strains of *japonica* rice. No dsRNA was found in cultivars of *indica* rice. We also examined eight species of wild rice and another species of African cultivated rice (*Oryza glaberrima*) (see Table 1, right column). Double-stranded RNAs were detected in only two strains of *O. rufipogon*, which is generally considered to be an ancestor of *O. sativa* (Second 1982). W-1714, which was collected in Brazil, contained 14kb dsRNA. W-0120, which was collected in India, contained 2.7 kb dsRNA.

Relationships of the dsRNAs among three groups of rice plants

All cloned cDNAs, which were derived from the dsRNA of *japonica* rice (cv. Nipponbare), hybridized to the dsRNA derived from all strains of temperate *japonica* rice we examined. For example, in the case of pRD39 (which contained a cDNA of about 1.6 kb), the intensities of all the hybridization signals were similar (Fig. 1B). This result indicated that the dsRNA in each strain had a very similar sequence.

We analyzed the extent of homology of the dsRNAs between tropical *japonica* rice, wild rice and temperate *japonica* rice by Northern hybridization. Fig. 2B shows the results obtained with pRD39 as the probe. The amount of dsRNA in each lane was the same in Figure 2A, but the intensity of the hybridization signals in lanes 2-7 (tropical *japonicas*) and lane 8 (W-1714) was lower than that of temperate *japonica* (lane 1). The signals of tropical *japonica* rice were stronger than that of W-1714. No hybridization signal was detected in 2.7 kb dsRNA from W-0120. The sequence of the dsRNAs of tropical and temperate *japonica* rice and W-1714 were not identical but homologous to each other.

Table 1. Occurrence of dsRNA in cultivated and wild rice

Cultivar of *O.sativa*			Presence of dsRNA	Species of wild rice		Presence of dsRNA
Temperate *japonica*	Nipponbare		+	*O. rufipogon*	W-1714	+
(Japan)	Aichiasahi		+		W-0120	+[a]
	Tsukinohikari		+		W-0106	-
	Musashikogane		+		W-0592	-
	Aikoku		+		W-0081	-
	Kusabue		+	*O. meridionalis*	W-1692	-
	Koshihikari		+	*O. australiensis*	W-0008	-
	Akihikari		+	*O. officinalis*	W-0002	-
Tropical *japonica*	Gendjah Gempel	BHB 721	+		W-0034	-
(Philippines)	Ketan Lumbok	BHB 738	+	*O. minuta*	W-0051	-
	Ketan lumbu	BHB 768	+	*O. punctata*	W-1577	-
	K. Rondo Marong	BHB 783	+		W-1564	-
	Pare bongor	BHB 740	+		W-0020	-
	Pring	BHB 773	+	*O. latifolia*	W-0019	-
javanica	Arborio		+	*O. grandiglumis*	W-1194	-
	Bluebonnet		-	*O. tisseranti*	W-1345	-
Indica	IR-26		-			
	IR-58		-	*O. glaberrima*[b]	W-0025	-
	LMW		-			
	Morak Sepilai		-			
	Qing-Er-ai		-			
	RD-10		-			

a: 2.7 kb. b: Another species of African cultivated rice.

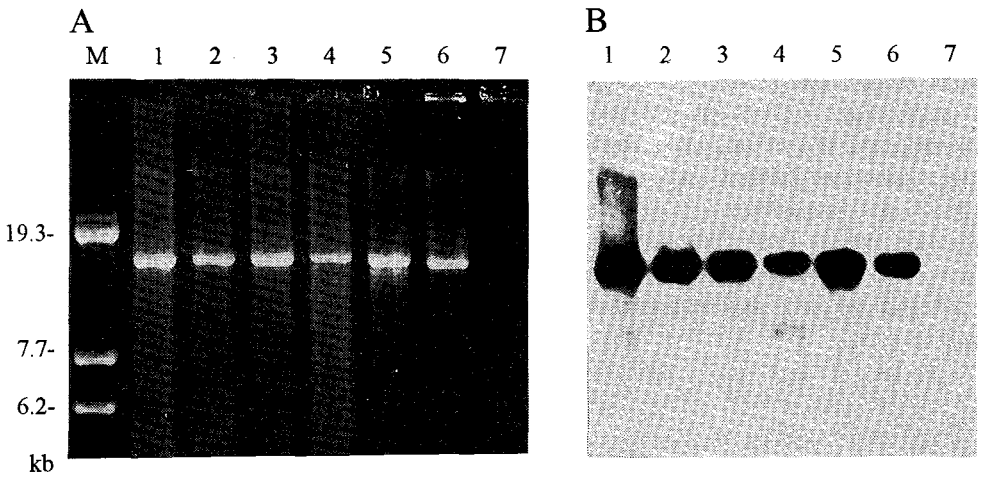


Fig. 1. A. Agarose gel electrophoresis of the dsRNA from several strains of cultivated rice (*O. sativa* L.). B. Northern hybridization analysis of the dsRNA using cDNA transcribed from the dsRNA of the *japonica* rice, cv. Nipponbare, (pRD39) as a probe. Lane M, size markers. Lanes 1-5, temperate *japonica*; lane 1, Nipponbare; lane 2, Aichiasahi; lane 3, Tukinohikari; lane 4, Musashikogane; lane 5 Akamai. Lane 6, *jawanica*, Arborio, Lane 7, *Indica*, IR-26.

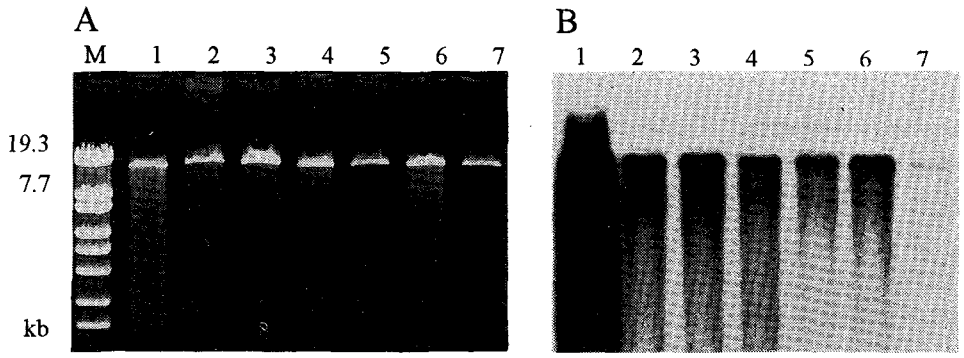


Fig. 2. A. Agarose gel electrophoresis of the dsRNA from several strains of tropical *japonica* rice and wild rice. B. Northern hybridization analysis of the dsRNAs using pRD39 as a probe. Lane M, size markers. Lane 1, temperate *japonica*, cv. Nipponbare (positive control). Lanes 2-6, tropical *japonica*; lane 2, Gendiah Gempel BHB 721; lane 3, Ketan Lumbok BHB 738; lane 4, Ketan lumbu BHB 768; lane 5, K. Rondo Marong BHB 738; lane 6, Pring BHB 773. Lane 7, *O. rufipogon*, W-1714. To show the partial hybridization with *O. rufipogon* dsRNA (lane 7), exposure time was prolonged.

Relationships between the dsRNA of rice and dsRNAs of the other gramineous plants

We also examined the extent of homology between the rice dsRNA and dsRNA of barley (*Hordeum vulgare* L.) or rye (*Secale cereale* L.). Some of the properties of the rice dsRNA reported here are very similar to those of dsRNA from barley (Zabalgogeazcoa and Gildow 1992; Zabalgogeazcoa *et al.* 1993). For example, the host plants with dsRNA are apparently symptomless, and dsRNA-free strains exist. The molecular weights and concentrations within host plants are similar. The largest among several dsRNAs from rye is similar in size to the dsRNA from rice, and rye plants that contain dsRNAs also possessed no symptoms. However, no hybridization signals were detected between rice and these two gramineous plants, showing that these dsRNAs are quite different to each other in sequence (Fig. 3).

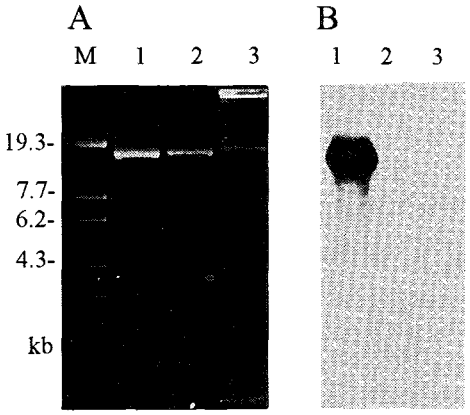


Fig. 3. A. Agarose gel electrophoresis of dsRNAs from other gramineous plants. B. Northern hybridization analysis of the dsRNAs using pRD39 as a probe. Lane 1, *Oryza sativa* (*japonica* rice, cv. Nipponbare). Lane 2, *Hordeum vulgare* (barley). Lane 3, *Secale cereale* (rye).

Discussion

Endogenous dsRNAs were detectable in many cultivars of *japonica* rice and two strains of *Oryza rufipogon* (W-0120 and W-1714). Except for the dsRNA of W-0120 (2.7 kb), these rice plants contained the dsRNAs of uniform size (14 kb) (Table 1 Figs. 1 and 2). Regardless of the strain, the observed level of the dsRNA relative to the total amount of the DNA was almost constant [0.3% (w/w); 100 copies/cell]. However, the sequences of the dsRNAswere not identical between three types of rice plants (temperate *japonica*, tropical *japonica* and *O. rufipogon*) (Fig. 2B). Northern analyses indicated that the rice dsRNAs were classified into at least three groups; one group was detected in temperate *japonica* rice collected in Japan, the second group was detected in tropical *japonica* rice collected in the Philippines, and the last group was detected in W-1714 collected in Brazil (Fig. 2B). This classification agrees with their geographical distributions. Since *O. sativa* is believed to have originated from *O. rufipogon* several thousand years ago (Second 1982), the dsRNAs of *japonica* rice and W-1714 are

likely to have evolved independently from a common ancestor. Then *japonica* rice plants might be divided into two groups geographically, and the dsRNAs of each rice plant have evolved independently. The differences indicate that the dsRNA of temperate *japonica* rice was not transmitted recently from tropical *japonica* rice or W-1714 or *vice versa* as a result of natural crossing.

Considerable base-substitution was found in the dsRNA of *japonica* rice, cv. Nipponbare. The entire sequence of 13, 952 nucleotides of the rice dsRNA was determined from a series of independent and overlapping cDNA clones (Moriyama *et al.* 1995). We sequenced more than 50 independent cDNA and RT-PCR (Reverse Transcriptase-PCR) clones to determine the entire nucleotide sequence of the rice dsRNA. Unexpectedly, many base-differences among independent cDNA clones were found within the entire sequence of the dsRNA. Six base-substitutions were found among four independent cDNA clones in a region of 500 nucleotides between nt 3,100 and 3,600. Five out of six substitutions lead to changes of amino acids in an open reading frame (ORF) (Fig. 4). The observed rate of base-substitution seems rather high, even if one takes into account possible errors during the synthesis of cDNA by reverse transcriptase. This suggests that the dsRNA is somewhat heterogeneous in cv. Nipponbare. Since the dsRNAs are not infectious and they are transmitted vertically from generation to generation via seeds, the dsRNAs might have been kept in autogamous rice plants. The base-substitutions in the dsRNA appear to have rapidly accumulated in each species of rice, and the sequence of *japonica* rice dsRNA has already diverged from that of wild-rice dsRNA. The high-molecular-weight dsRNAs in barley and rye did not hybridized to the cDNA derived from cv. Nipponbare (Fig. 3). These dsRNA in the three gramineous plants might be evolutionarily related to one another and many base-substitutions might have occurred within these dsRNAs. Because of a lack in proof-reading ability of the RNA-dependent RNA polymerase, the endogenous dsRNAs in the plant kingdom will evolved more rapidly than the host DNAs as seen in the other RNA replicons. These dsRNAs might have been evolved from a common ancestor. The origin of the high-molecular-weight dsRNA should be further investigated using many species of wild rice which are collected from all over the world.

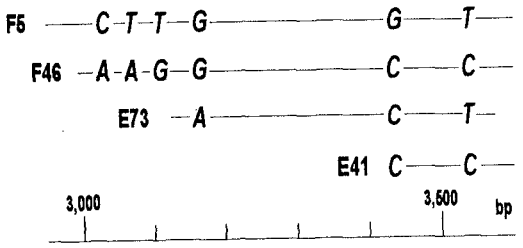


Fig. 4. An example of heterogeneity (base substitutions). Six base-substitution among four cDNA clones (F5, F46, E73 and E41) were found in a region of 500 nucleotides (from nucleotide position 3,100 to 3, 600).

Acknowledgment: We thank Dr. Gurdev S. Khush, International Rice Research Institute, Manila, The Philippines, for providing seeds of tropical *japonica* rice and for his useful suggestions. This research was supported in part by a Grants-in-Aid from Ministry of Education, Science and Culture of Japan (Nos. 0385080 and 04854071) to T.F.

References

Brown GG, Finnegan PM (1989) RNA plasmids. Int Rev Cytol 117, 1-56

Dodds JA, Morris TJ, Jordan RL (1984) Plant viral double-stranded RNA. Annu Rev Phytopathol. 22: 151-168

Dodds JA, Valverde RA, Mathews DM (1988) Detection and interpretation of dsRNA. In: Koltin Y, Leibowitz MJ (eds) Viruses of Fungi and Simple Eukaryotes, pp. 309-326. Marcel Dekker, New York

Fukuhara T, Moriyama H, Pak JK, Hyakutake H, Nitta T (1993) Enigmatic double-stranded RNA in Japonica rice. Plant Mol Biol 21: 1121-1130

Gubler U, Hoffman BJ (1983) A simple and very efficient method for generating cDNA libraries. Gene 25: 263-269

Ishihara J, Pak JK, Fukuhara T, Nitta T (1992) Association of particles that contain double-stranded RNAs with algal chloroplasts and mitochondria. Planta 187: 475-482

McCrae MA (1985) Double-stranded RNA viruses. In Mahy BWJ (ed.), Virology, a Practical Approach pp. 151-168. IRL Press, Oxford, Washington DC

Milne RG, Natsuaki T (1994) Cryptoviruses. Pathogens and host specificity in plant diseases. Vol. 3,

Moriyama H, Nitta T, Fukuhara T (1995) Double-stranded RNA in rice: a novel RNA replicon in plants. Mol Gen Genet (in press)
Morris TJ, Dodds JA (1979) Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69: 854-858
Rodriguez-Cousino N, Esteban LM, Esteban R (1991) Molecular cloning and characterization of W double-stranded RNA, a linear molecule present in *Saccharomyces cerevisiae*: identification of it single-stranded RNA form as 20s RNA. J Biol Chem 266: 12772-12778
Second G (1982) Origin of the genetic diversity of cultivated rice (Oryza spp.): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57: 25-57
Stuart KD, Weeks R, Guilbride L, Myler PJ (1992) Molecular organization of *Leishmania* RNA virus 1. Proc Natl Acad Sci USA 89: 8596-8600
Wang B, Li YN, Zhang XW, Hu L, Wang JZ (1990) Double-stranded RNA and male sterility in rice. Theor Appl Genet 79: 556-560
Zabalgogeazcoa IA, Gildow FE (1992) Double-stranded ribonucleic acids in `Barsoy' barley. Plant Sci 83: 187-194
Zabalgogeazcoa IA, Cox-Fostre DC, Gildow FE (1993) Pedigree analysis of the transmission of a double-stranded RNA in barley cultivars. Plant Sci 91 : 45-53

Anaerobic Seed Germinability and ADH Zymograms of *Echinochloa* Weeds from the Asian-Pacific Region

Y. Yamasue, Y. Minamiyama, S. Komatsu and T. Kusanagi
Weed Science Laboratory, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Abstract. Anaerobic seed germinability of rice paddy *Echinochloa* weeds, a key physiological character for their adaptation to flooded conditions, was studied with a reference to the zymograms of alcohol dehydrogenase (ADH), using strains from the Asian-Pacific region. Seeds of all twenty-two tested strains of *E. oryzicola* Vasing. (= *E. phyllopogon* Koss.) , an obligate weed in flooded rice, germinated well under nitrogen, and 20 of these had a specific ADH zymogram (A1A2A3). In *E. crus-galli* Beauv. var. *formosensis* Ohwi (= *E. glabrescens* Munro ex Hook. f.), a weed in flooded rice, all of eight tested strains had anaerobic germination, six of these were A1A2A3A4A5, one was A1A3A5, and one was A3A4A5. However, *E. colonum* Link. which is distributed in wetland rice and upland field crops, exhibited marked anaerobic seed germination in all of the seven strains used, and had the A3 zymogram pattern identical to that of *E. c.* var. *praticola* Ohwi, which is confined to be distributed in habitats with dry soil conditions such as paddy levees and open lands in Japan and requires oxygen for seed germination. Adaptive significance of anaerobic seed germinability and ADH zymogram will be discussed in *Echinochloa* weeds.

Key words. *Echinochloa* weeds, anaerobic germinability, ADH zymograms, physiological adaptation of weeds.

Introduction

Adaptive mechanisms of a weed at its habitat with human's disturbance are of our interest and we believe that an understanding of the mechanisms provides us with better weed management options in future than the present status of sole dependence on herbicides.

Echinochloa weeds of Japan have been well studied regarding various characters adaptive to their habitats [3, 8, 11, 12]. *E. oryzicola* Vasing. (=*E. phyllopogon* Koss.) and *E. crus-galli* var. *formosensis* Ohwi (= *E. glabrescens* Munro ex Hook. f.) (hereafter oryzicola and formosensis, respectively) are obligate weeds in flooded rice, and have mimicry in morphology to rice (*Oryza sativa* L.) as well as anaerobic seed (spikelet) germinability. On the other hand, *E. crus-galli* Beauv. var. *praticola* Ohwi (hereafter praticola) is a weed at upland habitats such as paddy levees, roadsides and open lands in Japan, and has larger tolerance to drought conditions, but no anaerobic germinability. Our previous studies showed that alcohol fermentation is the main pathway from which the paddy weeds yield energy for anaerobic seed germination [9,10], and that accessions of each species and variety of *Echinochloa* weeds collected in Kyoto Prefecture and its environs had specific zymograms of alcohol dehydrogenase (ADH): oryzicola; A1A2A3: formosensis, A1A2A3A4A5; and praticola, A3 [14].

The objectives of the present study are to extend our findings of differences in oxygen requirement for seed germination and ADH zymograms obtained from *Echinochloa* weeds in the region immediately surrounding Kyoto Prefecture to those originating from various countries in the Asian-Pacific region, and to determine these characters for *Echinochloa colonum* Link. (hereafter colonum), which is an important weed in wetland rice and upland field crop in the tropics[2], but which is not distributed in Japan.

Materials and Method

Plant materials.

Using seeds of various strains of oryzicola, formosensis, praticola and colonum from our *Echinochloa* weed collection, most of which were cytotaxonomically studied [8] and supplied by Dr. T. Yabuno, plants were grown at our experimental farm and the seeds inbred with application of paper bags were harvested in 1994. Seeds used for experiments were stored under room conditions for a year to release them from dormancy.

Anaerobic seed germinability.

Seeds of each strain were placed in 24-wells multidish (Nucleon, Denmark) at 25 seeds per well with 500 μl degassed distilled water and tested for anaerobic germinability at 20-30 °C (12/12 hrs) in the light under an N_2 atmosphere (99.99 % purity) in an anaerobic chamber. Anaerobic conditions inside were insured by flushing with N_2 gas for 10 min per hr. For comparison, aerobic germination was also determined with the multidishes placed outside of the anaerobic chamber. The experiment was conducted with three replications.

ADH zymograms.

Caryopses were obtained by removing floral lemma from spikelets and incubated at 20-30 °C in the light for about 12 hrs, and their ADH zymograms were determined as previously described by Yamasue *et al.*[14].

Results and Discussion

Twenty-two strains of oryzicola from ten countries used for the experiments. Aerobic germination of all these strains was 58 to 100 %, sufficiently higher to consider them well released from dormancy, and to evaluate anaerobic germinability (Table 1). Percent germination under nitrogen was not essentially different from that under aerobic conditions, and all strains were defined to have anaerobic seed germinability. Isozymatic bands of ADH detected with a seed were designated A1, A2, A3, A4 and A5, with the band specified by A1 showing the most rapid migration rate to anode, and with the band specified by A5 the slowest (Figure 1). Twenty of 21 oryzicola strains tested had an identical ADH zymogram having three isozymatic bands of A1, A2 and A3 (Table 1).

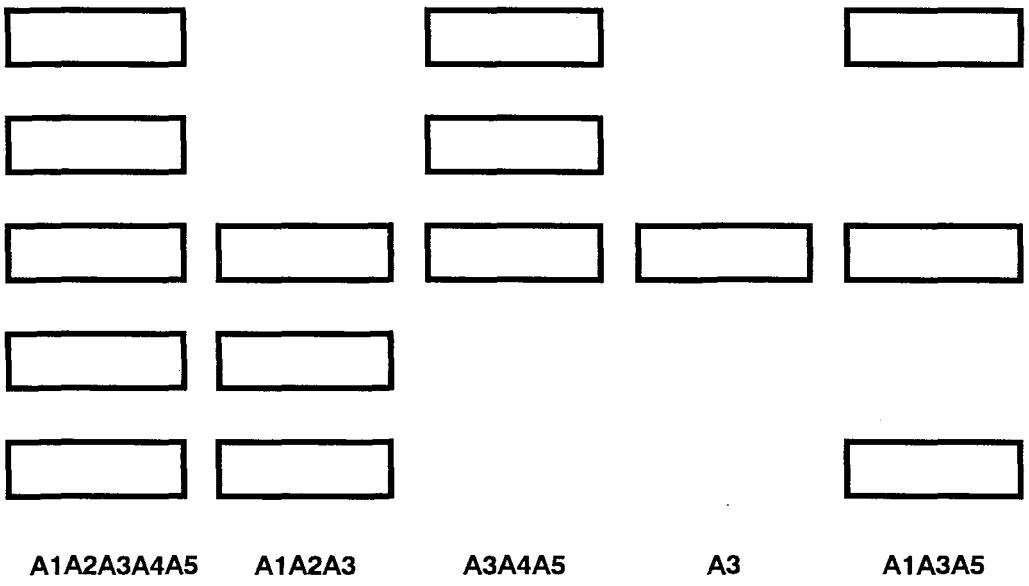


Figure 1. ADH zymograms observed in seeds of *Echinochloa* weeds. The bands were designated A1, A2, A3, A4 and A5, with the band specified by A1 showing the most rapid migration rate to anode, and the one specified by A5 the slowest.

Although formosensis strains studied were limited to eight from three countries, they all exhibited anaerobic seed germinability (Table 2). Seeds of six of these were A1A2A3A4A5 , one from Ishigaki of Japan, A3A4A5, and one from Okayama, A1A3A5. All of five praticola strains were from Japan and their seeds showed nearly perfect germination under aerobic conditions (Table 3). However, seeds of this upland weed showed little germination under the anaerobic conditions of nitrogen and thus were considered to have no anaerobic germinability. Moreover, A3 was the only isozymatic band in their ADH zymograms. Eight strains of colonum from four countries were evaluated for anaerobic seed germinability and ADH zymogram. Of these, seven strains showed about 90 % germination both under aerobic and anaerobic conditions, while one from Sri Lanka was 16% under anaerobic conditions (Table

4). However, all of eight strains had an identical ADH seed zymogram composed of a single A3 band , which was the same as that of praticola (Table 3).

Table 1. Anaerobic seed germinability and ADH zymograms of various strains of *Echinochloa oryzicola* Vasing., an obligate weed in flooded rice, from the Asian-Pacific region.

No.	Country	Site of collection	% Germination		ADH zymogram
			Air	N2	
1	Bangladesh	Dacca	100	77	A1A2A3
2	China	Jinghong	99	96	A1A2A3
3	China	Beijing	95	88	A1A2A3
4	China	Chanshan	-	-	A1A2A3
5	France	Camergue	67	40	A1A2A3
6	Indonesia	Bogor	100	85	A1A2A3
7	Korea	Fuyou	92	89	A1A2A3
8	Korea	Fuyou(73-75)	80	65	-
9	Korea	Daejeon	87	47	A1A2A3
10	Malaysia	Alor Setar	91	89	A1A2A3
11	Sri Lanka	Gampola	89	92	A1A2A3
12	Sri Lanka	Angunawala	73	79	A1A2A3
13	Taiwan	Tauyuen	85	80	A1A2A3
14	Taiwan	Taipei	84	80	A3A4A5
15	USA	Sacramento	81	64	A1A2A3
16	Japan	Asahikawa	91	99	A1A2A3
17	Japan	Kyoto	85	87	A1A2A3
18	Japan	Okayama	83	88	A1A2A3
19	Japan	Morioka	87	100	A1A2A3
20	Japan	Mito	60	80	A1A2A3
21	Japan	Kagoshima	96	81	A1A2A3
22	Japan	Matsuyama	58	73	A1A2A3

Table 2. Anaerobic seed germinability and ADH zymograms of various strains of *Echinochloa crus-galli* var. *formosensis* Ohwi, an obligate weed in flooded rice, from the Asian-Pacific region.

No.	Country	Site of collection	% Germination		ADH zymogram
			Air	N2	
1	Korea	Cheju	100	36	A1A2A3A4A5
2	Philippines	Cebu	100	96	A1A2A3A4A5
3	Philippines	IRRI	100	64	A1A2A3A4A5
4	Japan	Ishigaki	99	96	A3A4A5
5	Japan	kyoto	97	96	A1A2A3A4A5
6	Japan	Okayama	97	96	A1A3A5
7	Japan	Kagawa	100	96	A1A2A3A4A5
8	Japan	Amakusa	99	99	A1A2A3A4A5

We previously studied anaerobic seed germinability and ADH zymograms of *Echinochloa* weeds using about 200 accessions collected in the region immediately surrounding Kyoto Prefecture, Japan[14], and found that all strains of oryzicola and formosensis, obligate weeds in flooded rice, had anaerobic germinability, whereas most strains of praticola, an upland weed, required oxygen for seed germination and thereby were defined to have no anaerobic germinability. Alcohol fermentation catalyzed by ADH has been suggested to be the respiratory pathway on which oryzicola seeds depend at an early stage of seed germination[9, 10]. Thus, the physiological character of anaerobic germinability of oryzicola as well as its larger seed size appears to be adaptively significant since this rice paddy weed is confined to germinate under flooded conditions and the fermentation is a lower energy yielding respiratory pathway. Though a few exceptions were present, each species and variety of *Echinochloa* weeds had a specific ADH zymogram: oryzicola, A1A2A3; formosensis, A1A2A3A4A5; praticola; A3; and colonum; A3.

Table 3. Anaerobic seed germinability and ADH zymograms of various strains of *Echinochloa crus-galli* var. *praticola* Ohwi, an upland weed, from the Asian-Pacific region.

No.	Country	Site of collection	% Germination		ADH zymogram
			Air	N2	
1	Japan	Kanazawa	95	0	A3
2	Japan	Kyoto	93	11	A3
3	Japan	Okinawa	97	0	A3
4	Japan	Osaka	100	0	A3
5	Japan	Tokyo	88	8	A3

Table 3. Anaerobic seed germinability and ADH zymograms of various strains of *Echinochloa colonum* Link., a weed at wetland rice and upland crops, from the Asian-Pacific region.

No.	Country	Site of collection	% Germination		ADH zymogram
			Air	N2	
1	China	Guang zhou	100	84	A3
2	Myanmar	-	70	78	A3
3	Philippines	IRRI(81)	95	92	A3
4	Sri Lanka	-	100	98	A3
5	Sri Lanka	Sitiria	100	100	A3
6	Sri Lanka	Kandy(77-6)	100	92	A3
7	Sri Lanka	Kandy(77-3)	95	16	A3
8	Sri Lanka	Batticaloa	100	88	A3

In the present study, the differences in anaerobic seed germinability and ADH zymogram between species and varieties of *Echinochloa* weeds we previously observed in a limited region were found to be also applicable to strains from various countries in the Asian-Pacific region. Three of the four weeds studied here , oryzicola, formosensis and colonum, are those found in flooded or wetland rice, and all strains of them apparently had anaerobic germinability of seeds (Table 1, 2 and 4). While the other weed, praticola, is a weed at upland habitats such as paddy levees and open lands, and had little anaerobic germinability in all five strains presently used (Table 3) and the accessions used in our previous experiment[14]. Thus, we confirmed that this physiological character at seed germination is a key character for the paddy weeds to infest the habitats with oxygen deficient conditions. Although isozymes are usually considered to be neutral against selective pressures and used as a genetic maker of analyses of plant or animal populations, adaptive significance of ADH zymogram to seed germination and growth under flooding was suggested in maize (Zea may L.) [6, 7] and soft bromegrass (*Bromus mollis* L.)[1]. We have studied inheritance of anaerobic germinability and ADH zymogram using reciprocally crossed hybrids between formosensis and praticola, but no evidence was, however, found to directly relate anaerobic germinability to ADH zymograms[13]. Kennedy *et al.*[4, 5] also found no correlation of the number of ADH isozymes to flooding or anoxia tolerance in *Echinochloa* weeds in

North America. Present finding in the two *Echinochloa* weeds, praticola and colonum, with an identical zymogram (A3), but with distinct difference in anaerobic germinability (Table 3, and 4) may also support this although these weeds are suggested to phylogenically differ each other [8].

Acknowledgment: we sincerely appreciate Dr. T. Yabuno, a Professor Emeritus of The Osaka Prefectural University for his providing us with original seeds of *Echinochloa* weeds used in experiments.

References

1. Brown, A. D. H., D. R. Marshall and J. Munday. 1976. Adaptness of variants at alcohol dehydrogenase locus in *Bromus mollis* L. (soft bromegrass). Aust. J. Biol. Sci. 29: 389-396.
2. Holm, L. G., D. L. Plucknett, J. V. Pancho and J. P. Herberger. 1977. "The World Worst Weeds", The Univ. Press of Hawaii, Honolulu, pp. 41-46.
3. Kataoka, T. and S. Y. Kim. 1978. Oxygen requirement for seed germination of several weeds. Weed Res.(Japan) 23: 9-12.
4. Kennedy, R. A., S. C. H. Barrett, D. V. Zee and M. E. Rumpho. 1980. Germination and seedling growth under anaerobic conditions in *Echinochloa crus-galli* (barnyardgrass). Plant, Cell and Environ. 3: 343-248.
5. Kennedy, R. A., M. E. Rumpho and T. C. Fox 1986. Germination physiology of rice and rice weeds: Metabolic adaptations to anoxia. In "Plant Life in Aquatic and Amphibious Habitats" Crawford, R. M. M. (eds.), British Soc. Special Symposium, Blackwell, Oxford. pp.193-203.
6. Marshall, D. R., P. Broue and A. J. Pryor 1973. Adaptive significance of alcohol dehydrogenase isozymes in maize. Nature New Biology 244:16-18.
7. Schwartz, D. 1969. An example of gene fixation resulting from selective advantage in suboptimal conditions. Am. Nat. 103: 479-181.
8. Yabuno, T. 1966. Biosystematic study of the genus *Echinochloa*. Japan. J. Bot. 19:277-323.
9. Yamasue, Y., K. Ueki and H. Chisaka. 1987. Seed dormancy and germination of *Echinochloa oryzicola* Vasing. : An observation through respiration and several enzyme activities. Weed Res.(Japan) 32:188-197.
10. Yamasue, Y. and K. Ueki. 1987. Seed dormancy and germination of *Echinochloa oryzicola* Vasing. : Substansive evidences for alcohol fermentation at early germination. Weed Res.(Japan) 32:263-267.
11. Yamasue, Y. , Y. Asai, K.Ueki and T. Kusanagi. 1989. Anaerobic seed germination for habitat segregation of *Echinochloa* weeds. Japan. J. Breed. 39: 159-168.
12. Yamasue, Y., A. Nakamura, K. Ueki and T. Kusanagi. 1989. Drought resistance for habitat segregation of *Echinochloa* weeds. Japan. J. Breed. 39:337-343.
13. Yamasue, Y., M. Hamada and T. Kusanagi. 1989. Anaerobic seed germination of *Echinochloa* weeds: The inherent mode and relationship with isozymes of alcohol dehydrogenase. Mem. Coll. Agric., Kyoto Univ., No. 135: 43-52.
14. Yamasue, Y., T. Tanisaka and T. Kusanagi. 1990. Alcohol dehydrogenase zymogram, its inheritance and anaerobic germinability of seeds of *Echinochloa* weeds. Japan. J. Breed. 40: 53-61.

Thermal Control of Purple Nutsedge (*Cyperus rotundus*) Bud Break and Shoot Elongation

R. K. Nishimoto, J. E. Miles, and W. H. Sun

Department of Horticulture, University of Hawaii, Honolulu, Hawaii, 96822-2279 U.S.A.

Abstract. We conducted studies and reviewed our previous research to determine the effect of fluctuating temperature on bud sprouting of purple nutsedge (*Cyperus rotundus* L.) tubers. Bud break and subsequent elongation of the bud were strongly regulated by warm temperature. At relatively constant laboratory temperatures of 22 to 24 C, 84% of the tubers had bud break (at least one bud with a visible shoot > 1 mm and < 1 cm in length). However, only 10% of the tubers had at least one bud that elongated > 1 cm in length at 21 days, when both the percent of tubers with bud break and shoots elongated > 1 cm leveled off. With daily alternating temperatures of 22.5 / 27.5 C for 12 h each, 98% of the tubers had bud break and 85 % elongated. If the tubers were incubated for 2 wk at 20 C, about 30% of them remain unsprouted. Nearly all of those tubers could be induced to the bud break stage by a single 35 C shift ranging from 30 min to 12 h duration (Sun and Nishimoto, 1995). Once bud break occurs, a single shift of 35 C for 30 min caused only 56% of the tubers to produce shoots > 1 cm in length, while multiple warm temperature shifts caused nearly 100% of the tubers to produce shoots > 1 cm in length (Miles et al, 1994). These observations suggest that the sprouting of purple nutsedge tubers may involve two steps - the first is bud break, and the second is elongation of the emerged bud, and that both steps are regulated by warm temperature pulses.
Key words. Bud break, Dormancy, Tuber sprouting.

Introduction

Dormancy of weed propagules in the soil is one of the most difficult problems in weed management. Various degrees of dormancy cause irregular emergence of weeds and greatly contribute toward persistence of a species. While dormancy of purple nutsedge tubers occurs, the mechanism controlling dormancy is not understood. Apical dominance occurs within a tuber and in a chain of tubers (Muzik and Cruzado, 1953). Apical dominance in a tuber chain appears weak as it can be overcome by inversion of the chain (Muzik and Cruzado, 1953). Parker (1985) observed that the apical bud of a tuber in a growing chain remains dormant while the lateral buds sprout and produce rhizomes to extend the chain, and suggested that apical dominance is weak.

In contrast to using intact chains of tubers, single tubers have been used in studies to elucidate sprouting response to temperature. Soil temperature is perhaps the major factor affecting purple nutsedge tuber sprouting, provided moisture is adequate. The minimum temperature for purple nutsedge tuber sprouting was found to be 10 C, and the maximum temperature was 44 C (Tripathi 1967; Ueki 1969; Orcutt and Holt, 1990). Most studies used constant temperature regimes, but Tripathi (1967) observed that an alternating temperature regime caused more sprouting.

In this paper, we will review our recent work that shows fluctuating temperature to be more effective in stimulating tuber sprouting than constant temperature regimes (Miles et al, 1994), and that short duration warm pulses are most important in the sprouting stimulation process (Miles et al, 1994; Sun and Nishimoto, 1995). In addition, tuber sprouting appears to be a two stage process and evidence will be presented to show that warm temperature pulses appear to control the initial bud break process, as well as the subsequent shoot elongation.

Materials and Methods

Tubers were collected from the University of Hawaii's Waimanalo Research Farm; they were separated from the chains, and trimmed of roots and rhizomes in the laboratory. Tubers were sprouted on two layers of filter paper moistened with deionized water, in 15 cm Petri dishes. There were five replicates of 50 tubers each.

To quantify the difference between growth initiation or bud break and elongation, two experiments were conducted in which both the number of tubers with both bud break and elongation were counted. A tuber was considered to have initiated growth if at least one bud had produced a visible shoot at least 1 mm in length, and as elongated if at least one shoot was at least 1 cm in length. Temperatures in these experiments were alternating 22.5 / 27.5 C and a relatively constant ambient laboratory temperature of 22 to 24 C. The number of tubers which had bud break and the number elongated were counted daily. The experiment continued for 21 d, at which time both the number of tubers with bud break and with elongated shoots had leveled off; cumulative data at 21 d are presented.

Percent tuber sprouting was calculated based on the number of viable tubers in each Petri dish, as determined by the triphenyl tetrazolium chloride (TTC) test on unsprouted tubers. Tubers were split longitudinally and soaked in 0.1 % TTC at 30 C for 1 to 3 h, until a pink color response was observed in

known viable tubers. The TTC test was preceded by subjecting tubers to alternating 20 / 30 C for 1 to 2 wk, a temperature regime known to stimulate sprouting.

The experiment was repeated with tubers collected from the same location 5 d after tuber collection for the first experiment. Because of similar results, data from the two experiments were combined, and the percent of tubers which had initiated growth and the percent which had sprouted were compared.

Potential complication of warm temperatures in the field during the tuber collection process mandated that all of the subsequent experiments were conducted with tubers grown in the glasshouse, and tubers were collected and separated from purple nutsedge plants in a constant 20 C environment. The number of tubers with bud break while incubated at 20 C over a 15 to 18 d period are presented in this paper. Most subsequent experiments were conducted with tubers that did not sprout after at least 14 d at 20 C. The results from these experiments were presented at the 1994 and 1995 Weed Science Society of America Meetings, and will be discussed in this paper to describe how warm temperature regulates the bud break and elongation process.

Results and Discussion

Purple nutsedge sprouted much better under an alternating temperature regime (25 / 35 C) than a constant temperature regime (30 C) when both regimes had the same mean temperature (Miles et al, 1994). The alternating temperature regime with a mean temperature of 30 C caused 100 % sprouting; the 30 C constant temperature regime resulted in 77 % sprouting, and even the highest constant temperature regime of 35 C caused only 93 % sprouting. In addition, small temperature fluctuations from 2 C to 6 C of the alternating temperature cycle caused more tuber sprouting as the amount of temperature fluctuation increased (Miles et al, 1994). These results clearly demonstrate that purple nutsedge sprouted much better under fluctuating temperature regimes than a constant temperature regime set at the same mean temperature. In these initial experiments, a tuber was defined as sprouted if at least one bud on a tuber elongated more than 1.0 cm. It appeared that temperature controlled bud break and the subsequent elongation of the emerged bud, thus subsequent studies considered these stages.

At the 22.5 / 27.5 C alternating temperature regime, 98 % of the tubers had bud break, and 85 % of the tubers had shoots that elongated to at least 1 cm in length. However, at the temperature regime with near constant temperature (22 to 24 C), 84% of the tubers had bud break, but only 10% of the tubers had shoots that elongated to at least 1 cm in length (Table 1). There was an interaction between temperature and treatment effects, so comparisons were made separately for the different temperatures. At both temperature regimes the difference between the number of tubers with bud break and those that elongated was highly significant (Table 1). With both regimes, not all of the tubers that had bud break would elongate more than 1 cm (Table 2). Thus under certain conditions, tuber buds may initiate growth without continuing to grow. Moreover, there is a pronounced difference in this phenomenon for tubers exposed at these two different regimes, suggesting that the bud break and subsequent elongation process may involve two steps.

Table 1. Comparison of percent of tubers with buds initiating growth to more than 1 mm in length (bud break) and percent of tubers with shoots at least 1.0 cm in length (elongate) under two temperature regimes.

Regime[a]	Buds	Mean (%)	Standard. Error (%)	T Value	Probability > T
22 to 24	Bud break	84.1	2.1	23.2	<0.0001
	Elongate	10.1	2.4		
22.5 / 27.5	Bud break	98.3	0.8	4.8	0.0001
	Elongate	85.4	2.6		

[a]Minimum and maximum temperature in diurnal temperature alternation (degrees C)

When temperature is carefully controlled from the time that tubers are physically separated from the mother plants, only about 70 % of these tubers will have bud break when these tubers are incubated at 20 C for 2 wk (Figure 1). If those tubers without bud break (dormant tubers) were given only a single

35 C pulse ranging from 30 min to 12 h, more than 90% of the tubers had bud break (Sun and Nishimoto, 1995). We also showed that when tuber temperature reached 35 C in 3 min, and immediately returned to a 20 C environment, 62% of these tubers had bud break (Sun and Nishimoto, 1995). Bud break was only caused by warm temperature shifts; equivalent cold temperature shifts were ineffective in causing bud break (Sun and Nishimoto, 1995). Thus, it appears that a single short duration warm pulse can cause bud break to occur in dormant tubers.

Table 2. Comparison of percent of tubers with bud break but not continuing to elongate under two temperature regimes.

Regime[a]	Mean (%)	Standard Error (%)	T Value	Probability > T
22 to 24	87.5	1.9	30.2	0.0001
22.5 / 27.5	13.2	1.5		

[a]Minimum and maximum temperature in diurnal temperature alternation (degrees C).

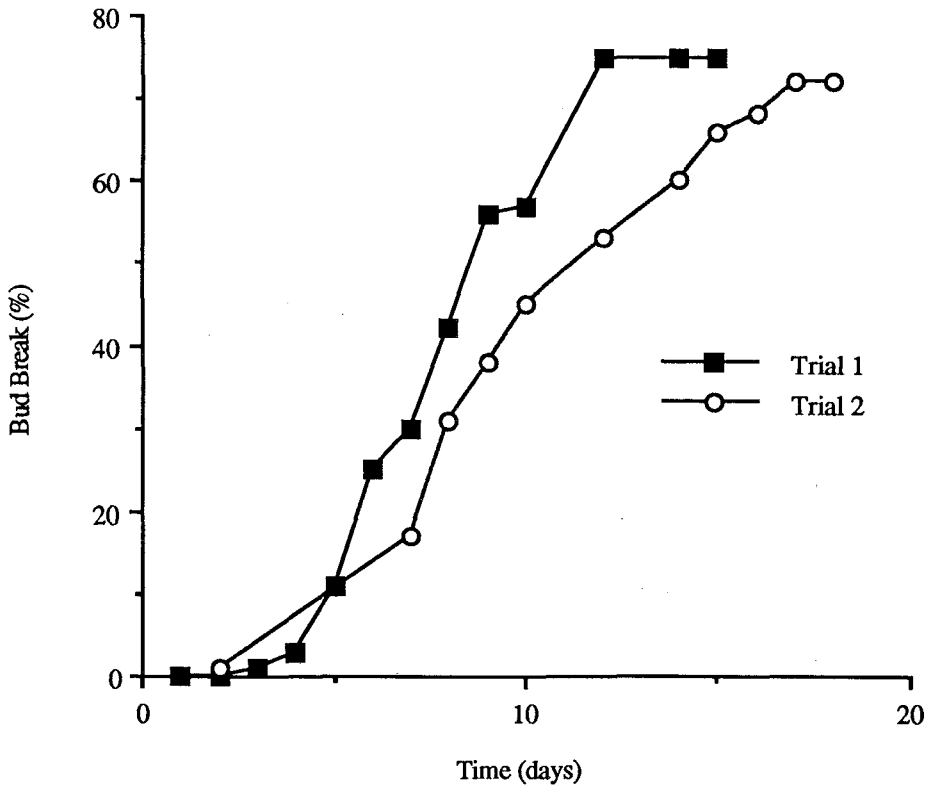


Figure 1. Time course development of tubers with bud break when incubated at 20 C.

In other experiments, we exposed tubers that had bud break, but without further elongation, to a single pulse of various temperature regimes for 30 min. Following a single pulse at 30 min for 35 C, 56 % of the tubers had shoots that elongated more than 1 cm (Miles et al, 1994). There was no shoot elongation if the tubers were maintained at 20 C. However, daily warm temperature shifts of 35 C from 0.5, 1, 6, 12 or 18 h caused nearly 100 % of the tubers to produce shoots that elongated more than 1 cm (Miles et al, 1994). Thus, once bud break occurs, a single warm pulse causes about one-half of the

tubers to produce elongated shoots, and multiple daily pulses causes nearly all of the tubers to produce elongated shoots.

Our research shows that temperature strongly controls bud break and the shoot elongation process of purple nutsedge tubers. It appears that a single warm pulse can cause bud break of dormant tubers, and that subsequent warm pulses are required for further elongation of the emerged bud.

The marked stimulation in bud break and shoot elongation of tubers in response to pulses of elevated temperatures may be key physiological responses to environmental signals related to emergence of tropical weed propagules. With a canopy over the soil surface or for positions deep in the soil profile, diurnal temperature alternations are relatively constant (Miles, 1991; Rubin and Benjamin, 1984). Diurnal temperature fluctuation would increase markedly and much greater sprouting would occur if a canopy is removed, or if tubers are located close to the soil surface. Thus, fluctuating temperature may be an important stimulatory signal for emergence of purple nutsedge.

References

Miles, J. E. 1991. Modeling the sprouting of *Cyperus rotundus* L. tubers in response to soil temperatures under soil solarization. PhD Dissertation, University of Hawaii, Honolulu. Pages 141-167.

Miles, J. E., R. K. Nishimoto, O. Kawabata, and W. H. Sun. 1994. Alternating temperature stimulates bud sprouting of purple nutsedge (*Cyperus rotundus* L.) tubers. Weed Science Society of America Abstracts. 34:72.

Muzik, T. J. and H. J. Cruzado. 1953. The effect of 2,4-D on sprout formation in *Cyperus rotundus*. American Journal of Botany 40:507-512.

Orcutt, D. R. and J. S. Holt. 1990. Temperature thresholds for bud sprouting in perennial weeds and seed germination in cotton. Weed Science Society of America Abstracts 30:47.

Parker, C. 1985. Growth patterns in *Cyperus rotundus*. Proceedings British Crop Protection Conference - Weeds Volume 1. pp. 387-394.

Rubin, B. and A. Benjamin. 1984. Solar heating of the soil: involvement of environmental factors in the weed control process. Weed Science. 32:138-142.

Sun, W. H. and R. K. Nishimoto. 1995. Trigger action in dormancy release of purple nutsedge (*Cyperus rotundus* L.) tubers by a single warm pulse. Weed Science Society of America Abstracts. 31:83.

Tripathi, R. S. 1967. Ecology of *Cyperus rotundus* L. II. Tuber sprouting in relation to temperature Proc. National Academy of Sciences, India (B) 37:409-412.

Ueki, K. 1969. Studies on the control of nutsedge (*Cyperus rotundus* L.): On the germination of a tuber. Proceedings 2nd Asian-Pacific Weed Control Interchange 2:355-369.

NUTRIENT CONTENTS IN SOME WEED SPECIES

P. Lekkong and P. Wanichanantakul
Department of Agriculture, Chatuchak, Bangkok 10900, Thailand

Abstract. Weed are severe, widespread in all fields and difficult to control. Control is possible, however, by making a weed useful, such as in a green manure. We sought to determine the macro and micro essential elements in 26 weed species: 12 grasses, 9 broad leaves and 5 sedges; there were upland, lowland and aquatic weeds. Plant samples were collected from fields at the flowering stage then dried and ground. Analysis of the nutrient contents in the shoots showed that N, P, K, Ca, Mg, S, Mn, Cu and Zn were low in the grassy weeds and high in both broad leaves and sedges. The N, P and K of the 12 grassy weeds varied from 0.80-1.7, 0.05-0.30 and 0.64-2.68%, respectively, *Rottboellia cochinchinensis, Imperata cylindrica,* and *Pennisetum setosum* had N contents below 1%, whereas most broad leaves and sedges had N, P and K of more than 1%, *Pistia stratiotes, Sphenoclea zeylanica, Jussiaea repens* and *Eupatorium odoratum* had N contents of 2.91, 2.96, 3.02 and 3.39%, respectively. Most sedges showed significantly higher K. The percentage of Ca, Mg and S contents of broad leaf weeds was comparable to the sedges. The amounts of Mn, Zn and S in broad leaves and sedges were similar, and were significantly greater than some grassy weed species.

Key words. Nutrient elements, weed species, green manure, sedges, broad leaves

Introduction

Weed problems are severe, especially in the tropical countries where wet and moist conditions favor weed growth. Crop competition with weed from the time they emerge, after planting the first job is weeding. Weed control can be done by chemical or mechanical control. However, control is possible, by making a weed useful, such as in a green manure or incorporate into the soil as a fertilizer and or source of livestock feed. In addition, study the nutrient content in weed specie should be helpful in estimating the amount of nutrient was removed or returned to soil. This work was conducted to determine the macro and micro essential elements in some weed species and legume cover crops as the comparison plants.

Materials and Methods

Sampling and sample preparation.

Twelve grasses: *Acrachne racemosa* Linn, *Dactyloctenium aegyptium* (L) P.B., *Digitaria adscendens* (H.B. K)., *Echinochloa crus-galli* (L.) Beauv., *Eleusine indica* (L.) Gaertn., *Imperata cylindrica* Raeuschel.,(L.) *Leptochlor chinensis* (L.) Nees., *Panicum repens* Linn., *Pennisetum polystachyon* (L.) Schult., *Pennisetum setosum* (Swz.) L.C.Rich., *Paspalum conjugatum* Berg., *Rottboellia cochinchinensis* L.F., nine broad leaves: *Stachytarpheta indica Vahl., Eichhornia crassipes* (Mart.) Solms., *Eupatorium odoratum* Linn., *Euphorbia geniculata* Ort., *Jussiaea linifolia* Vahl., *Jussiaea repens* Linn., *Sphenoclea zeylanica* Gaertn., *Pistia stratiotes* Linn., *Marsilea crenata* Presl., *five sedges: Cyperus difformis* Linn., *Cyperus rotundus* Linn., *Eleocharis dulcis* (Burm.f.) Henschel., *Fimbristylis miliacea* (L.) Vahl., *Fuirena ciliaris* (L.) Roxb., and three kinds of legume cover crops, *Centrosema pubescens , Calopogonium mucunoides* and *Pueraria phaseoloides.* The upland weeds and legume cover crops were collected from the Surat Thani Horticultural Research Centre and low land weeds collected from the Bangkhen Rice Station, in the late rainy season in 1994. Plant samples of weeds and cover crops were taken at the flowering stage and dried in the oven at 70° C for 48 hours. The samples were cut into small pieces and ground, and passed through a 40 mesh sieve and stored in the desiccator for the chemical analysis.

Total nitrogen determination

Digestion. Weigh 1 g of leaf samples into 500 kjeldahl digestion flasks, add the catalyst mixture of 10 g K_2SO_4 + 0.5 g $CuSO_4$ and 30 ml of conc. H_2SO_4. Place the Kjeldahl flasks on the heating mantle blocks

to digest under the fume hood, until the samples are clear, cool and transfer solution into 250 ml volumetric flasks with distilled water.
Distillation . Transfer 10 ml of the digested solution into a micro-kjeldahl distillation apparatus, then with quick delivery pipette, add 5 ml of 40% NaOH and rinse with a minimum of distilled water. Place the 125 ml Erlenmeyer flasks containing 20 ml of 0.3% boric acid and three drops of mixed indicator under the distillation apparatus, then distill the samples for 10 minutes.
Titration. Titrate the solution of boric acid and mixed indicator containing the "distilled off" ammonium with the standardized HCL.

Phosphorus, sulfur, potassium, calcium, magnesium, manganese, zinc and copper determination.
Wet digestion. Place 2 g of samples into 250 ml Erlenmeyer flasks. Add 20 ml of nitric acid and 7 ml perchloric acid and allow to predigest overnight under fume hood. Then moderately heat at least 2-3 hours, gradually increase the temperature until the mixtures become clear and remain about 5 ml solution in the flasks. Cool and fill the flasks with 50 ml deionized water. Filter the digested samples through an acid-washed filter paper (Whatman No-1) and make up to 100 ml with deionized water for a sample analysis.

Sample analysis : Phosphorus
Transfer 5 ml of aliquot into 50 ml volumetric flasks, prepare also a standard P and blank samples. Add 5 ml of the molybdate-vanadate solution and then make up to volume with distilled water, shake and allow to stand for 30 minutes. Measure absorbance at 420 mμ and compare with the absorbance of the phosphorus standard.

Sample analysis : Sulfur
Transfer 5-10 ml of the aliquot into 50 ml volumetric flasks, Add 10 ml of 2N ammonium acetate to bring the pH to 5, add 1 g of 30-60 mesh barium chloride then shake 1 minute before adding 2 ml gum acacia solution (0.25%) bring to the volume then mix and read absorbance at 410 mμ within 30 minutes and compare with the absorbance of the sulfur standard.

Sample analysis : Potassium, calcium and magnesium by atomic absorption spectrophotometer.
Pipette 5-10 ml of the aliquot into 50 ml volumetric flasks. Add 5 ml strontium chloride (5%) solution, make up to the volume with deionized water and prepare each of the K, Ca and Mg standards. Measure the nutrient elements by the atomic absorption spectrophotometer.

Sample analysis: Manganese, zinc and copper by the atomic absorption spectrophotometer.
Use aliquots from wet digestion to measure the amount of element contents with atomic absorption spectrophotometer and compare with the individual standard. If the concentration of each aliquot is too high, the dilution may be necessary.

The nutrient analysis methods were base on the methods of Chapman, 1961, Yoshida *etal.*, 1972 and Official method of analysis of association of official analytical chemist, 1970.

Results and Discussion

Analysis of nutrient contents in the shoots of 26 weed species and three legume cover crops showed that macro and micro nutrient elements in the grassy weeds were relatively low as compared to broad leaves and sedges. Analysis of grassy weeds found that *L. chinensis, A. racemosa, E. indica, D. adscendens and D. aegyptium* had N contents of 2.28, 1.70, 1.58, 1.38 and 1.26 % respectively, whereas *I. cylindrica, P. repens, P. conjugatum, P. setosum* and *R. cochinchinensis* had N content of 0.6-1.0 %. *L. chinensis* had the highest and *I. cylindrica* had the lowest N contents. *P. polystachyon* and *R. cochinchinensis* had P contents (>0.29 %) greater than other species (<0.20 %). Most of grassy weeds had K contents greater than 1.2% and a few species had K lower than 1%. Among the tested plants, *P. polystachyon* had the highest K (2.68 %) and *I. cylindrica* had the lowest K contents (0.65 %). Most grasses had Ca contents below 1%, Mg and S below 0.5 % except *E. indica,* had the highest Ca, Mg and S contents of 1.13, 0.35 and 0.54 % respectively. *I. cylindrica* and *P. setosum* had relatively low in Ca, Mg and S as compared with other species. The amount of Mn in grassy weeds had high variation, such as, *A. racemosa* and

R. cochinchinensis had Mn contents of 343 and 25 ppm respectively. Zn was found at the range of 20-40 ppm, except *R. cochinchinensis* and *E. indica* had high Zn contents of 76 and 57 ppm respectively, and had Cu between 5-10 ppm

Analysis of nutrient content in broad leaves showed that N and P were higher than grasses and sedges but the percentage of K was comparable to sedges. *E. oderatum, J. repens, S. zeylanica* and *P. stratiotes* had high N content of 3.39, 3.02, 2.96 and 2.91% respectively. Most sedges had N contents below 2%, except *C. difformis* had high N content (2.42%). *E. crassipes and P. stratiotes* showed high content of P (> 0.7%) whereas other broad leaves species had P at the range of 0.2-0.4 %. Most sedges had low P content (0.1-0.4%) but they were higher in K (> 2%). Broad leaves and sedges which had high K content were *E. crassipes* (3.34 %), *J. repens* (3.17%) and *C. difformis* (3.03%). The amount of Ca in broad leaves (0.5-2.5%) was significantly greater than sedges (< 0.5%) except *E. dulcis* (2.2%). The Mg and S in broad leaves and sedges were not significantly different. Sedge; *E. dulcis* and broad leaf; *P. stratiotes* showed significantly higher in Ca of 2.2 and 2.5 % respectively, Mg of 0.33 and 0.95% respectively and S of 0.63 and 0.62% respectively. The amount of micro nutrient in grasses, broad leaves and sedges were not significantly different, except *E. dulcis, P. stratiotes and E. crassipes* had significantly higher in Mn and Zn. Cu contents in three types of weeds were 5-10 ppm except, *E. odoratum* (17 ppm) were significantly higher than other weed species.

Analysis of legume cover crops found that *C. pubescens, C. mucunoides and P. phaseoloides* had significantly higher in N content (3.7-4.2%) as compared with weeds particularly grasses species, most had N less than 1.5% . The amount of P in cover crops was comparable to broad leaves, but was higher than grasses and sedges. K in cover crops tended to be lower than broad leaves and sedges. The amounts of Mg and S in cover crops were similar to broad leaves and sedge but were higher than grassy weeds. Most legume cover crops showed significantly higher in Zn (>59 ppm) and Cu (> 13ppm) but were lower in Mn as compared with weed species.

The results of analysis show that various weed species have quite high nutrient content, particularly broad-leaf weeds. This result indicates that weed has high ability in nutrient uptake so that weed completes crop for nutrient is important factor contributing to the stunted plants and reduce yields. It could be drawn that making weed useful is the efficient method of weed control.

References

Chapman, H.D., P.F. Pratt.1961. Method of analysis for soils, plants and waters. Univ. of Cal. Div. of Agr. Sci., 309 pp.

Official method of analysis of association of official analytical chemist. 1970. Eleventh edition, Benjamin Franklin Station, Washington, D.C., 1015 pp.

Yoshida, S., D.A. Forno, J.H. Cook and K.A. Gomez. 1972. Laboratory manual for physiological studies of rice (second edition). The International Rice Research Institute Los Banos Laguna, Philippines, 70 pp.

Table 1 Showing the macro nutrient contents in weeds and legume cover crops. (mean ± s.e.)

Weed species	% of macro nutrient elements					
	N	P	K	Ca	Mg	S
Grasses						
1 *Acrachne racemosa*	1.70±.10	0.14±.01	2.01±.16	0.81±.08	0.28±.04	0.35±.03
2 *Dactyloctenium aegyptium*	1.26±.41	0.08±.01	1.50±.20	0.58±.02	0.16±.01	0.18±.04
3 *Digitaria adscendens*	1.38±.11	0.17±.03	2.13±.23	0.34±.02	0.21±.01	0.16±.01
4 *Echinochloa crusgalli*	0.98±.04	0.18±.01	1.14±.06	0.35±.01	0.33±.02	0.22±.01
5 *Eleusine indica*	1.58±.01	0.23±.07	1.93±.06	1.13±.03	0.35±.01	0.54±.08
6 *Imperata cylindrica*	0.69±.02	0.06±.01	0.65±.10	0.25±.06	0.09±.00	0.06±.00
7 *Leptochloa chinensis*	2.28±.57	0.27±.07	1.87±.00	0.35±.03	0.27±.00	0.41±.09
8 *Panicum repens*	1.10±.06	0.08±.01	1.94±.09	0.40±.02	0.19±.00	0.25±.02
9 *Pennisetum polystachyon*	1.01±.04	0.29±.00	2.69±.06	0.43±.04	0.15±.00	0.17±.01
10 *Pennisetum setosum*	0.80±.03	0.05±.01	1.69±.10	0.11±.01	0.07±.01	0.08±.00
11 *Paspalum conjugatum*	1.01±.18	0.07±.02	1.26±.29	0.43±.02	0.24±.01	0.27±.04
12 *Rottboellia cochinchinensis*	0.87±.15	0.29±.08	0.91±.07	0.34±.05	0.19±.01	0.28±.01
Broad leaves						
13 *Stachytarpheta indica*	1.03±.04	0.08±.00	1.24±.01	1.00±.04	0.17±.01	0.13±.01
14 *Eichhornia crassipes*	2.30±.02	0.71±.02	3.34±.05	1.37±.02	0.54±.01	0.38±.01
15 *Eupatorium odoratum*	3.39±.16	0.23±.01	2.49±.09	1.49±.06	0.52±.01	0.22±.04
16 *Euphorbia geniculata*	1.72±.14	0.27±.05	1.81±.27	1.15±.11	0.25±.02	0.22±.03
17 *Jussiaea linifolia*	1.68±.12	0.39±.03	1.34±.19	1.18±.04	0.38±.01	0.16±.03
18 *Jussiaea repens*	3.02±.09	0.42±.01	3.17±.14	0.62±.04	0.43±.03	0.45±.02
19 *Sphenoclea zeylanica*	2.96±.10	0 .44±.01	2.06±.04	0.48±.02	0.30±.02	0.57±.07
20 *Pistia stratiotes*	2.91±.02	0.71±.01	2.85±.00	2.55±.04	0.95±.01	0.64±.07
21 *Marsilea crenata*	2.20±.20	0.39±.02	2.21±.20	0.55±.05	0.32±.04	0.44±.01
Sedges						
22 *Cyperus difformis*	2.42±.06	0.43±.01	3.03±.12	0.42±.02	0.34±.01	0.41±.06
23 *Cyperus rotundus*	1.84±.01	0.23±.00	2.38±.06	0.43±.01	0.19±.01	0.28±.03
24 *Eleocharis dulcis*	1.15±.04	0.10±.01	2.10±.09	2.20±.61	0.33±.03	0.63±.04
25 *Fimbristylis miliacea*	1.29±.04	0.20±.01	2.58±.11	0.43±.02	0.40±.05	0.43±.01
26 *Fuirena ciliaris*	1.82±.07	0.30±.00	2.55±.06	0.38±.01	0.32±.02	0.44±.03
Legume cover crops						
27 *Centrosema pubescens*	3.78±.15	0.31±.00	1.76±.04	1.53±.08	0.27±.01	0.45±.03
28 *Calopogonium mucunoides*	4.35±.30	0.33±.01	1.73±.09	1.46±.12	0.36±.01	0.30±.05
29 *Pueraria phaseoloides*	4.21±.14	0.35±.01	1.95±.02	1.47±.03	0.35±.02	0.38±.03

Table 2 Showing the micro-nutrient contents in weeds and legume cover crops. (mean ± s.e.)

Weed species	ppm of micro nutrient elements		
	Mn	Zn	Cu
Grasses			
1 *Acrachne racemosa*	343±72.1	33±4.30	7±0.001
2 *Dactyloctenium aegyptium*	316±24.8	39±1.10	5±0.000
3 *Digitaria adscendens*	53 ±14.2	30±1.80	7±0.001
4 *Echinochloa crusgalli*	178±21.5	34±8.90	7±0.001
5 *Eleusine indica*	89 ±8.70	57±2.30	10±0.000
6 *Imperata cylindrica*	34 ±6.50	11±1.40	5±0.000
7 *Leptochloa chinensis*	128±21.5	40±6.80	10±0.002
8 *Panicum repens*	133±21.2	19±1.20	5±0.000
9 *Paspalum conjugatum*	94±21.7	32±4.60	5±0.000
10 *Pennisetum polystachyon*	17±1.2	22±0.40	5±0.000
11 *Pennisetum setosum*	82±10.9	12±2.80	5±0.000
12 *Rottboellia cochinchinensis*	25 ±4.50	76±1.40	5±0.000
Broad leaves			
13 *Stachytarpheta indica*	101±8.00	30±1.00	10±0.000
14 *Eichhornia crassipes*	446±36.7	50±1.60	5±0.000
15 *Eupatorium odoratum*	68 ±7.10	35±1.20	17±0.001
16 *Euphorbia geniculata*	112±11.3	19±1.80	7±0.001
17 *Jussiaea linifolia*	372±13.6	48±2.20	10±0.000
18 *Jussiaea repens*	402±36.6	53±1.20	10±0.000
19 *Sphenoclea zeylanica*	222±10.8	35±2.40	5±0.000
20 *Pistia stratiotes*	2700±0.00	86±0.60	10±0.000
21 *Marsilea crenata*	467±0.00	47±5.50	8±0.001
Sedges			
22 *Cyperus difformis*	172±13.8	46±0.00	10±0.000
23 *Cyperus rotundus*	126±2.4	41±3.80	12±0.001
24 *Eleocharis dulsis*	1354±225.0	77±15.60	5±0.000
25 *Fimbristylis miliacea*	352±65.8	40±1.60	10±0.000
26 *Fuirena ciliaris*	218±8.8	50±2.80	7±0.001
Legume cover crops			
27 *Centrosema pubescens*	92±11.6	59±2.20	18±0.001
28 *Calopogonium mucunoides*	105±21.2	67±4.30	13±0.001
29 *Pueraria phaseoloides*	77±7.5	59±2.20	15±0.000

GROWTH HABIT OF AN AQUATIC WEED, APONOGETON UNDULATUS ROXB., AND ITS PHYSIOLOGICAL CHARACTERISTICS

T. Furuya, T. Mochizuki and Q. R. Islam*

Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan
* Canadian International Development Agency, Dhaka 1212, Bangladesh

Abstract The rootstock of the plant(Aponogeton undulatus Roxb.) is an important food source for the poor who live in the lowlands of Bangladesh which are often flooded. As a consequence of deep flooding, this plant has developed the ability to elongate and a tolerance to being submerged. In 1994, plants were potted and grown under 20 cm of water to study growth and submergence tolerance. At 3 rd leaf stage, the potted plants were submerged in water at a rate of 0, 2, 4, 6 and 8 cm per day. The plant have many submerged and floating leaves, reproductive organs(tubers) and flowering spikes. Tubers developed on the tip of the floating stem just under the water level. The plant elongation system involves either the petiole and the floating leaf or a separate floating stem, both of which rise with the water level; due to the limitation of the floating stem, elongation of petiole was more important. Plants grown in water rising at a rate of 8 cm / day were still not completely submerged after 16 days. At this time, the longest petiole length was about 140 cm and the plant height was about 1.6 m. From the results, we find that elongation of this plant depends on the depth and rising rate of water.

Introduction

Deep water or floating rice is the main crop grown in the Northeast Region of Bangladesh. The crop is often damaged by floods, as virtually all of this area is flooded to a depth of more than three meters annually. This flooding restricts crop production during the wet season.

Aponogeton undulatus Roxb. (Mammun 1989) grows in abundance throughout the deeply flooded land in Bangladesh. This plant is an aquatic perennial weed and its the rootstock is edible. The nutrient composition of rootstock consists of a large quantity of carbohydrates, proteins and some minerals. Therefore, the rootstock is an important food source for the poor who live in the lowlands. The demand for the rootstock increases when there is poor rice harvest. Thus, the rootstock provides some security during the times of food shortages in these low-lying areas.

The plant has developed the ability to elongate under deep flooding conditions. While little research has been made on the growth habit and its physiological characteristics of this plant, the present study examined its growth under water and its ability to elongate in increasing water depth.

Key Words : Floating leaf, Petiole elongation, Submergence tolerance, Rootstock, Aponogeton undulatus Roxb.

Materials and Methods

The following experiments were conducted in the summer of 1994 at Kyushu University in Fukuoka, Japan. Aponogeton undulatus Roxb. rootstock(tubers) collected in 1993 from lowland fields in Bangladesh were used. The tubers used were selected for size uniformity.

In this experiment, size a/5,000 pots were used. Each pot contained about 3.5 kg of air-dried paddy soil which had been fertilized with 2 g compound fertilizer(N-P-K:8-8-8%). One acquired germinated tuber was planted separately in either the beginning of April or at the end of August. Just after planting, the pot was submerged in a water tank at a depth of about 20 cm. When the plants had reached the third leaf stage, each pot containing one plant was gradually submerged at increasing depths by being suspended by string in a water tank and lowered at different rates; 0(control), 2, 4, 6 or 8 cm per day. This submergence tolerance experiment was terminated when the highest leaf was totally under the water surface. Average air-temperature during the experiment ranged from 20 to 30 °C and the amount of sunlight per day ranged from 12 hours 50 minutes to 14 hours 23 minutes.

Results

Fig.1 shows a diagram of an Aponogeton undulatus plant which was grown for 2 months after the tuber was planted and submerged under 20 cm of water. The plant produced submerged leaves (Fig.1-A),

floating leaves (Fig.1-B), flowering spike (Fig.1-C), tubers for reproduction(young plant, Fig.1-D) and the rootstock (Fig.1-E) which is the originally planted tuber that has grown larger. In the lot where tubers were planted under water at a depth of 20 cm in April, plants produced at first from two to five slender submerged leaves, followed by more than two spoon shaped floating leaves. Most of the floating leaves had petioles longer than 20 cm.

Seven weeks after plantation, the first floating stem was produced and a new tuber was formed on the tip of the floating stem just under the water surface. The new tuber was round shaped, and was covered with flimsy roots. The new tubers located on the tip of floating stems subsequently produced two or three leaves and developed into a young, miniature version of the parent plant complete with one or two elongated short floating stems and even secondary tubers. However a tertiary tuber was almost never formed. About ten weeks after being planted, the plant produced one flowering spike. The spike was flowered loosely.

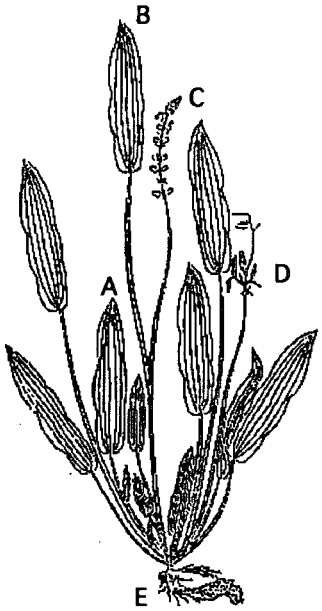


Fig. 1 Aponogeton undulatus Roxb. : A, Submerged leaf; B, Floating leaf; C, Flowering spike; D, Tuber(young plant); E, Rootstock; (water depth: 20 cm)

In nature the young plants are dispersed from floating stem presumably by wave action or other vibration. They then float on top of the water surface for a period of time and then established themselves as independent plants. Thus starting a new growing cycle. By the end of this study, the plant potted in April formed about 20 submerged leaves, 20 floating leaves, one flowering spike and 40 tubers with 17 floating stems.

Plant growth involves the elongation of the petiole, floating stem and leaf blade with the gradual rise of water. In this species, the floating leaf's petiole elongated faster than the submerged leaf's petiole, thus, petiole elongation is the most important because of the limitations of leaf elongation.

Table 1 shows the effect of different rates per day of rising water on each submerged potted plant in September 1994. The number of total living leaves was almost the same in all cases. However, the number of floating leaves on the water surface decreased with the depth of each plant. The plant submerged in water at 8 cm / day had only one floating leaf. In this case, the plant produced only one tuber and one long floating stem.

Table 1 Effect of different rates of rising water on each submerged potted plant in September, 1994

Rate of rising water	Days after Treatment	Submerged depth	Total number of living leaves	Number of floating leaves on the water surface
(cm / day)		(cm)		
0	30	20	11	11
2	30	80	12	5
4	30	140	11	3
6	19	134	9	2*
8	17	156	9	1*

* the number indicated was taken one day before the treatment day shown.

Furthermore, the plant submerged in water at 8 cm / day was not submerged completely 16 days after the beginning of the experiment. At this time, the longest petiole length was about 140 cm and the plant height was about 1.6 m. Plants grown in water rising at 6 cm / day were submerged completely after 19 days, but, plants grown in water rising at 2 cm / day and 4 cm / day were not submerged completely by the end of experiment (after 30 days).

Discussion

About one-third of total area in Bangladesh including 26,800 square kilometers of cultivated land is deeply flooded between May and October (MPO 1987). Several specialized characters that enable Aponogeton undulatus Roxb. to adapt to deep flooding and to have a submergence tolerance are researched in the study.

In many deep water areas, plants having poor elongation ability may be partially or completely damaged when submerged suddenly. Generally, plants are less tolerant to submergence at the early growth stages and the ability to elongate apparently increases with age. In this experiment, the potted plants submerged in water at the early growth(the third leaf stage) did not submerge completely during longer periods (Table 1). As the elongation speed of petiole of this plant was fast, this speed enables the plant to survive by providing better tolerance for being submerged. This capacity of the petiole to elongate at an early stage of growth is an important character for a plant what is submerged early in its growth.

As the plants grown in water rising at a rate of 8 cm / day did not submerge completely 16 days after the beginning of the experiment (Table 1), this plant has the inherent property to elongate with the rise of water. This plant may be useful in areas where the water level rises abruptly and where the water depth is more than 1.5 m. Thus, this plant is considered to have a high submergence tolerance. Submergence tolerance is related to the duration of submergence, water temperature, water turbidity, light intensity, water depth, and nitrogen content of soil. It seems that, like the deep water or floating rice(Mazaredo et al., 1981), the Aponogeton undulatus plant maintains a high level of carbohydrates, high photosynthesis and respiration rates, has green and active leaves, and nitrate reductase activity after submergence. These are matters of considerable interest.

The dominant nutritional problem for the rice-eating population of Bangladesh is an in adequate food supply, which includes issues like the supply of rice, the price of rice and low consumer purchasing power. Rice provides 69% of the calories and 51% of the protein to the people (Salam et al., 1991).

When considering the nutritional value of the rootstock, the Aponogeton undulatus plant can play an important role in enhancing the food supplies in Bangladesh. The rootstock is sold in the market and the price is a little below that of rice. The rootstock's principal component is starch (soluble carbohydrate) and about 8.5 % is protein, which is higher than rice. The rootstock can also be an important source of calcium, potassium, iron and zinc. One hundred grams of rootstock has been reported to contain 33.7 mg of phytic acid (inositol hexaphosphoric acid), which impedes mineral absorption by forming insoluble compounds with the minerals (Jesmin 1994). The rootstock of

Aponogeton undulatus Roxb. exceeds this protein-to-calorie concentration.

Therefore, increased utilization of the rootstock will directly enhance protein supplies. Moreover, the ideal combination of protein quality can be achieved if rice-based diets are supplemented with the protein from the rootstock. Thus, the rootstock of Aponogeton undulatus can become the dominant calorie and protein source in the diets of many low- income people. Propagation of the plant in this flooded area would be especially beneficial to low-income people and to those living in low-lying areas where market foods are limited.

In addition, the vegetative reproduction capacity of this plant highlighting the development of young plants(new tubers), is the most important morphological feature of this plant. The rootstock yield appears to depend on the number of young plants(new tubers) developed by each plant. Studies on developing a greater number of young plants(new tubers) will contribute to increasing the plant density and result in a greater rootstock yield.

Further study of Aponogeton undulatus will be most important in elucidating factors controlling plant growth, especially tuber formation, for weed control or food production.

References

Jesmin, T., 1994. A Study on the Nutritional Value of Aponogeton undulatus. M. Sc. thesis. Institute of Nutrition and Food Science, Dhaka University, Bangladesh, 83 pp.

Mazaredo, A. M. and B.S. Vergara. 1981. Physiological Differences in Rice Varieties Tolerant of and Susceptible to Complete Submergence. Proceeding of the international deep water rice workshop. Laguna, Philippines, 327-341 pp.

Mammun, A.A., 1989. Agro-ecological studies of weed in Bangladesh. Japan International Cooperation Agency, Bangladesh, 142 pp.

Master Plan Organization, 1987. Technical Report No. 14: Agricultural Production System. Ministry of Irrigation, Water Development and Flood Control, UNDP and world Bank, Dhaka, Bangladesh, 57 pp.

Salam, A., K. Kamal, S. Hossain, T. Islam, A.R. Islam, D.K. Bhadra, H. Rashid & T.U. Ahmed (eds), 1991. Report on the household expenditure survey 1988-89. Bangladesh Bureau of Satistics, Dhaka, Bangladesh, 201 pp.

Rapid Regeneration of Vulpia (*Vulpia bromoides)* in Pastures

P.M. Dowling[1*], B. Verbeek[2], D. Lemerle[2] and A.R. Leys[3]

[1]NSW Agriculture, Agricultural Research & Veterinary Centre, Forest Rd, Orange NSW 2800, Australia
[2]NSW Agriculture, Agricultural Research Institute, PMB, Wagga Wagga, NSW 2650, Australia
[3]National Parks & Wildlife Service, PO Box 1967 Hurstville, NSW 2220 Australia

Abstract. Vulpia spp. are undesirable annual grasses of temperate pastures in Australia and are responsible for reduced livestock productivity through interference with more desirable forage species and production of lower quality feed. Pastures consisting of pure subterranean clover (*Trifolium subterraneum)* and a mixture of subterranean clover and annual ryegrass (*Lolium rigidum)*, both infested with vulpia (*Vulpia bromoides)*, were treated with simazine in 1992 and compared with an unsprayed control over a two year period. Regeneration of vulpia was measured until 1994. After one year, the pure stand of clover treated with simazine had a vulpia density of 138 vulpia plants/m^2 which produced 551,195 seeds/m^2, compared with 3390 plants/m^2 producing 609,400 vulpia seeds/m^2 on the unsprayed control. In contrast, the pasture mixture of subterranean clover and annual ryegrass treated with simazine produced only 5,860 vulpia seeds/m^2 which was slightly fewer than that on the unsprayed control treatment of subterranean clover annual ryegrass (9,400 seeds/m^2). However, one year later in 1994, any differences due to simazine application in terms of plant density and seed production had largely disappeared, and only the inclusion of the ryegrass in the pasture mixture allowed for a sustainable reduction in the vulpia composition of the sward.

Key words: vulpia, competition, annual ryegrass, seed production, regeneration

Introduction

Vulpia is a small, fine-leaved annual grass which occurs as a naturalised volunteer in most Australian temperate pastures (8). It is most abundant in improved pastures in all regions of southern and central New South Wales, and is a major problem on the northern tablelands and the south coast of New South Wales, and in much of Victoria, South Australia, and Western Australia. There are at least five species belonging to the genus vulpia, but *V.bromoides* (L.) S.F.Gray and *V.myuros* (L.) C.C. Gmel. are the two most common species and usually grow in mixed populations over most of its geographical range (7).

Vulpia competes with more highly regarded species (*e.g.* introduced perennial grasses and annual ryegrass (*Lolium rigidum* Gaud.)), and produces lower quality forage. As such, it is widely recognised as an undesirable component of temperate pastures in Australia and is responsible for substantial reductions in livestock productivity. Estimates of the total cost of vulpia to Australian woolgrowers are in excess of $22 million annually (9). Woolgrowing and cropping are integrated enterprises on many farms, and in these situations vulpia dominant pastures cause additional losses to crop yields due to reduced nitrogen fixation by clover, carryover of root diseases such as take-all, and by direct competitive and allelopathic effects on both crops and sown pasture species.

Control of vulpia in pastures has revolved around the application of herbicides, either 'winter-cleaning' with simazine in May-July (5) or 'spraytopping' with glyphosate or paraquat in September-November (2), both of which can significantly reduce vulpia plant numbers over the short-term. Reduction of vulpia plant numbers over the longer-term however, has proved difficult, and is possibly due to carry-over of dormant seed and high seed production of vulpia. Clearly, additional management inputs which apply continuous pressure to vulpia during the growing season should complement the initial control provided by a herbicide, and this was confirmed in an earlier phase of the current experiment (6). The rapid rate at which pastures can be reinfested with vulpia and the influence of annual ryegrass presence on speed of regeneration is reported here.

Materials and Methods

The experiment was conducted on a medium textured red-brown earth at the Agricultural Research Institute, Wagga Wagga, New South Wales, Australia (Lat. 35° 3' S; Long. 147° 21' E; Alt. 219 m; Annual

average rainfall (AAR) 555 mm), and constituted the second phase of an experiment commenced in June 1990 (6).

Treatments

The basic structure of the experiment remained the same as in the first phase *i.e.* four pasture densities/composition (three densities of subterranean clover - S (*Trifolium subterraneum* L.); and a mixture of annual ryegrass (*Lolium rigidum* Gaud.) plus subterranean clover - C) x two fertility levels (low and high soil phosphorus) x two vulpia densities (low and high). In this phase, the initial vulpia densities were nil and high, resulting from application of simazine (0.63 kg a.i./ha - Flowable Gesatop 50% a.i.) to half the plots (-H, +H); and the density/composition treatment number was the same but the densities of the subterranean clover were similar. The effect of fertility level is not discussed here. Layout was a split-plot design (two fertility levels as the main plots, with the four pastures x two vulpia densities fully randomised within each fertility level) replicated four times.

Plant measurements

Plant densities were determined by counting all plants in 20 cores (4.5 cm diam.) taken at random from each plot in winter 1992, 1993 and 1994. Panicle densities were determined by counting all panicles in 10 quadrats (7.5 x 7.5 cm) harvested at maturity each year. Seed production was estimated from 50 panicles randomly selected from those harvested for panicle measurements. All spikelets on each of the 50 selected panicles were counted and the average number of spikelets calculated for each plot. A sub-sample of 10 panicles were randomly selected for estimating seeds per spikelet. Seeds were counted in three randomly selected spikelets on each of the 10 panicles, and the average number of seeds per spikelet calculated for each plot. Seed production/m^2 for each plot was then calculated by multiplying panicles/m^2 x spikelets/panicle x seeds/spikelet.

Experimental design and analysis

For this phase of the experiment, the three subterranean clover densities were similar and were combined *i.e.* four pasture types were reduced to two. Data were analysed by the REML (Residual Maximum Likelihood) procedure (3), while the Wald test was used to test for significance between means.

Results and Discussion

In 1992, one month after application of simazine to the +H treatments, the population of vulpia was effectively reduced to zero from a mean of 2,990 plants/m^2 on the -H treatments (Fig. 1). There were fewer ($P<0.01$) vulpia plants present on the C-H treatments (1,600 plants/m^2) compared with the S-H treatments (4,390 plants/m^2). Seed production was negligible on the +H treatments, being reduced from a mean of 181,470 seeds/m^2 to 1,050 seeds/m^2. Presence of annual ryegrass in the sward (C-H) reduced seed production ($P<0.01$) from 296,780 seeds/m^2 to 66,160 seeds/m^2.

In 1993, vulpia plant numbers on the +H treatments were still lower ($P<0.01$) than the -H treatments. Respective numbers were 1,850/m^2 and 79/m^2. Presence of annual ryegrass (C) had no significant effect on plant numbers, but reduced seed production from a mean of 580,300 seeds/m^2 to 7634 seeds/m^2. The effect of simazine on seed production was not significant, indicating that in the time between plants and seeds being counted (July to November - four months), there was compensatory seed production on the +H plots.

In 1994, application of simazine (+H) had no significant effect on either vulpia plant numbers or seed production, though values for both parameters were now marginally higher where simazine had been applied. Presence of annual ryegrass in the sward (C) again reduced vulpia plant ($P<0.01$) and seed numbers ($P<0.05$). Values for both seed production and plant numbers were lower than in the previous years because of the dry seasonal conditions in 1994.

Vulpia plants/m^2 declined with time where simazine was not applied and where ryegrass was originally sown. The percentage decrease in plants/m^2 between 1991-92 and 1992-93 for the S-H treatment was 23% and 82%, while the decrease for the C-H treatment was 81% and 99%. For the C+H treatment, the respective decreases were 66%, 55%. For both +/- herbicide treatments, presence of ryegrass resulted in

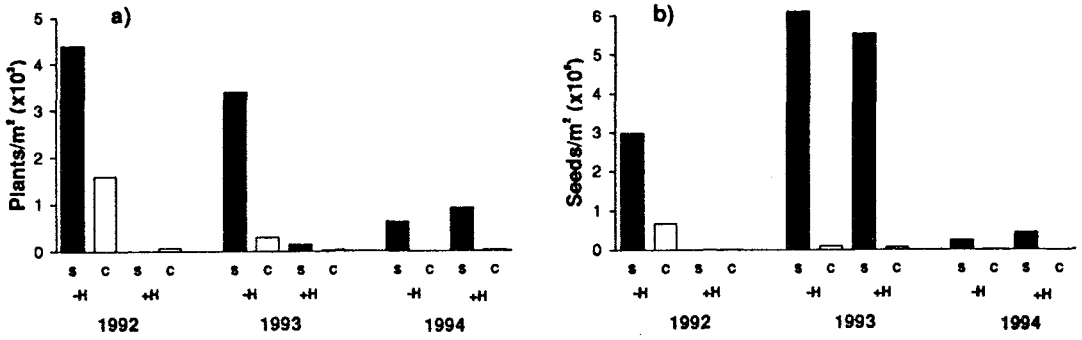


Figure 1. Effect of simazine application and pasture type on a) regeneration (plants/m^2) and b) seed production (seeds/m^2) of vulpia. Pasture type: S, pure subterranean clover; C, combination of subterranean clover and annual ryegrass. Simazine application: +H, simazine applied in 1992; -H, no herbicide applied in 1992.

greater decreases in vulpia plants/m^2 compared with where ryegrass was absent. Alternatively, where simazine was applied and ryegrass was absent, vulpia plants/m^2 increased dramatically but from a lower base. Seed production/plant remained low (<300 seeds/plant) on all treatments in all years except where simazine had been applied and ryegrass was absent in 1993 (4,000 seeds/plant), and in the absence of simazine where ryegrass was present in 1994 (527 seeds/plant). An estimate of the minimum seed production per plant required to at least maintain the vulpia population in the following season (equilibrium value) is 770 seeds/plant (2). Seed production of 4,000 seeds/plant is five times the estimated equilibrium value and the plant population would be expected to increase in the following year (Fig. 1). Therefore, vulpia populations should decline on the remaining treatments. All treatments followed this expected trend.

Vulpia is an extremely difficult species to control in pastures, largely because of its high seed production and resistance of its reproductive structures to removal by grazing. The short-term nature of chemical control demonstrated in this experiment shows that within two years of the herbicide being applied, vulpia plant populations can equal or exceed untreated populations. This rapid increase is attributed to the ability of vulpia, in the absence of competition, to produce high seed numbers from a low plant population. In addition, there is the likelihood of carryover of a small proportion of the seed produced in any one year beyond the following year, helping to ensure continuity of the species presence in pastures. The size of the dormant fraction of *V. myuros/bromoides* has not been clearly defined but estimates of 1-7% are common (4; G. Code; P. Dowling, unpublished data), and a dependence on year (1) and grazing pattern (4) is apparent. Longer term control to augment the lower vulpia plant numbers achieved from application of simazine clearly requires the inclusion of an additional grass, such as ryegrass, to compete with the residual vulpia plants. This is necessary if the rapid reinfestation of vulpia in a herbicide treated pasture sward is to be prevented, and vulpia populations are to be retained at low levels.

Acknowledgment: We gratefully acknowledge Ms H. Nicol for data analysis; Mr D. I. Pickering for producing the figure, and the International Wool Secretariat for funding the research.

References

1. Arnold, G.W. and G.W. Anderson (1987). The influence of nitrogen level, rainfall, seed pools, and pasture biomass on the botanical composition of annual pastures. *Australian Journal of Agricultural Research* **38**: 339-54.
2. Dowling, P.M. (1988). Effect of pre-season management and in-crop herbicides on weed seedling numbers and triticale grain yield. *Australian Journal of Agricultural Research* **39**: 783-92.
3. Engel, B. (1990). The analysis of unbalanced linear models with variance components. *Statistica Neerlandica* **44**: 195-219.
4. Jones, C.E., R.D.B. Whalley, J.V. Lovett and S. McIntyre (1992). Seed bank dynamics of *Vulpia*

spp. in pastures. *Proceedings of the 6th Australian Agronomy Conference*, Armidale NSW, p. 532.

5. Leys, A.R., B. Plater and W.J. Lill (1991). Response of vulpia (*Vulpia bromoides* (L.) S.F. Gray) and *V. myuros* (L.) C.C. Gmelin) and subterranean clover to rate and time of application of simazine. *Australian Journal of Experimental Agriculture* **31**: 785-91.
6. Leys, A.R., P.M. Dowling and B. Plater (1993). The effect of pasture density and composition on vulpia. *Proceedings of the 10th Australian and 14th Asian Pacific Weed Conference*, Brisbane Qld. pp. 190-2.
7. McIntyre, S. and R.D.B. Whalley (1990). Co-occurrence of *Vulpia* species on the northern tablelands of New South Wales. *Australian Journal of Botany* **38**: 445-50.
8. Rossiter, R. C. (1966). Ecology of the Mediterranean annual-type pasture. *Advances in Agronomy* **18**: 1-56.
9. Sloane, Cook and King (1988). The economic impact of pasture weeds, pests and diseases on the Australian wool industry. p. 22. (Australian Wool Corporation).

Strategic Use of Grazing Sheep For Long-Term Management of Vulpia (*Vulpia* spp.) in Pastures.

P.M. Dowling* and D. I. Pickering

NSW Agriculture, Agricultural Research & Veterinary Centre, Forest Road, Orange NSW 2800, Australia

Abstract. Vulpia is an undesirable annual grass of temperate pastures in Australia and is responsible for reduced livestock productivity through interference with more desirable forage species and production of lower quality feed. Severity of infestation in any one year is largely dependent on seed production in the previous year (*e.g.* a 25% infestation can produce approx. 500,000 seeds/m^2). Low cost herbicides can reduce vulpia presence in pastures but their effect is short-term. The aim of this project is to evaluate the effectiveness of grazing a vulpia infested perennial grass pasture with sheep, at key phases of vulpia development, so as to remove the apex of each elongating tiller, thus reducing seed production and reinfestation of the pasture in the following autumn. For this approach to be effective at practical grazing pressures however, the feed quality of the vulpia needs to remain sufficiently high so that sheep will readily graze the vulpia forage. In addition, the base of the tiller apex needs to be elevated sufficiently above ground level that grazing removes the tiller apex, resulting in death of that tiller and reduced seed production from any later developing tillers. This approach offers potential for the long-term management of vulpia in pastures without recourse to use of herbicides.

Key words: vulpia, annual grass management, strategic grazing, phenological development, growing-point elevation

Introduction

Vulpia (mostly *V.bromoides* ((L.) S.F. Gray) and *V.myuros* ((L.) C.C.Gmel.)) is a naturalised annual grass of Australian temperate pastures (11). In New South Wales, it appears to have increased in abundance over the last 15 years and this is attributed largely to the drought of the early 1980's and reduced fertiliser application depleting other more productive pasture components, thereby reducing competition for vulpia; together with the extensive use of selective grass herbicides (eg. aryloxyphenoxypropionates, cyclohexanediones) to which vulpia is resistant (8). Vulpia is not a weed in the classical sense in that it does not result in death when ingested by livestock, nor does it deter grazing of neighbouring species. However, it competes with more highly regarded species and produces lower quality forage. As such, it is widely recognised as an undesirable component of temperate pastures in Australia and is responsible for substantial reductions in livestock productivity.

Management of vulpia in pastures has centred around the application of non-selective herbicides (paraquat (0.1 kg a.i./ha - Gramoxone 20% a.i.; glyphosate (0.16 kg a.i./ha - Roundup 36% a.i.,) at head emergence in spring, and more recently, simazine (0.63 kg a.i./ha - Flowable Gesatop 50% a.i.) applied over the winter period. These procedures are referred to as spraytopping (4) and winter-cleaning (9). Spraytopping lowers populations of vulpia by reducing viability of developing seed, whereas winter-cleaning selectively removes vulpia from a mixed pasture. The aim with both approaches is to reduce seed-set since vulpia density in any given year is primarily dependent on seed production in the previous year. Chemical costs are low ($6/ha) but the treatments are relatively short-lived in effectiveness in the absence of additional management inputs. In pastures grazed by sheep, longer-term management of vulpia may be possible if the time(s) of grazing coincided with a critical phase of vulpia development such that its seed production potential was substantially reduced. Previous studies (5) have indicated that seedling regeneration of another annual grass commonly found in temperate pastures, barley grass (*Hordeum leporinum* L.), can be substantially reduced by resting from grazing in the previous winter followed by a heavy grazing with sheep in early spring. This paper outlines the experimental procedures adopted in determining whether lower populations of vulpia in pastures can be achieved by strategic use of grazing sheep.

Materials and Methods

Five experiments were commenced on an improved pasture at Gumble, 60 km north west of Orange in central New South Wales (Lat. 33° 06' S; Long. 148° 37' E). Soil is a red/yellow solodic derived from rhyolite. Surface texture is a fine sandy loam. Annual average rainfall (AAR) is 650-700 mm; elevation is 595 m. Rainfall received in 1994 was approx. 60% of AAR. The pasture is based on the perennial grass phalaris (*Phalaris aquatica* L.) - 60%, with vulpia (*V. bromoides, V. myuros*) as the next most dominant component (30%). Subterranean clover (*Trifolium subterraneum* L.) was present but was seldom greater than 10% of the pasture on a DM basis.

Treatments evaluated in the experiments were commercially accepted procedures commonly used to reduce the

incidence of vulpia in pastures (herbicide application - spraytopping, winter-cleaning; hay and silage making), together with grazing and fertiliser inputs (deferral of grazing over winter; litter presence over summer-autumn; grazing at different phenological stages in spring; fertiliser application - nitrogen, phosphorus). Not all treatments are discussed here.

The timing of the spring grazings were based on the phenological development of vulpia and approximated: 25%, 50% and 90% elongation (% of total number of tillers where internode elongation had clearly commenced); and 50% peeping (50% of total number of tillers where seedheads had commenced to emerge through the leaf sheath and were visible). This was in addition to the stages at which glyphosate and paraquat were applied in spraytopping treatments (75% and 85% peeping, respectively). Assessments were made at weekly intervals. For each stage, the grazing period was for seven days and the stocking pressure was 50 dry sheep equivalents (DSE)/ha. The general stocking pressure on the remainder of the paddock was 7.5 DSE/ha.

Median and maximum height of tiller apex bases above ground level, together with maximum plant height, was estimated from six pasture cores collected from the field at regular intervals during spring. Success of the treatments in reducing vulpia seed production will be assessed from soil cores taken in the following autumn.

Results and Discussion

For grazing management to have a role in reducing and maintaining the lower incidence of vulpia in pastures, its annual seed production needs to be reduced, since the presence of vulpia in a sward is largely dependent on seed produced in the previous year. Heavy grazing during the vegetative stage appears to **increase** rather than decrease the proportion of vulpia in the sward. This appears to be due to grazing encouraging greater tillering in the vulpia, in addition to selective grazing of the other more desirable species (*e.g.* subterranean clover) in the sward and consequently being more affected by the grazing than vulpia. Further, the chances of causing any comparative damage to the vulpia (and reducing seed-set) by grazing at this time is remote because of the prostrate nature of vulpia under grazing (14) and likely location of the tiller apices close to the ground.

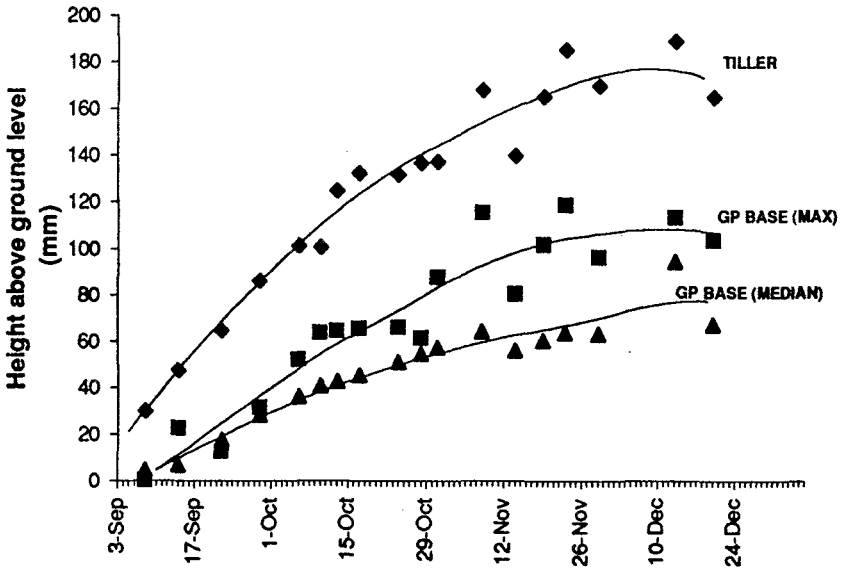


Figure 1. Maximum tiller height of vulpia above ground level in relation to maximum and median heights of the base of the tiller apex (TA) above ground level. Plant material was collected from the ungrazed control treatment.

Grazing after the reproductive stage has been initiated, and particularly during early spring, offers a better chance of reducing seed production, providing that the tiller apex is sufficiently elevated for removal by the grazing sheep, and if removed, death of that tiller. Depending on when the tiller apex is grazed in relation to the season, the vulpia plant may initiate a new tiller, but the potential seed production would

be markedly reduced because of later development of that tiller(s). The difficulty with this approach is that as pasture plants mature, structural carbohydrates increase in concentration resulting in a decline in digestibility and feeding value (13).

Maximum tiller heights on the dates the grazings were imposed (10%, 50%, 90% tiller elongation, 50% peeping) were 36, 63, 95, 110 mm, respectively. Median heights of the apex base above ground level were 5, 15, 32, 39 mm, for each of the four grazing times (Fig. 1). In vegetative pasture, sheep can graze very close to ground level, so with the possible exception of the 10% elongation stage, in at least 50% of the tillers, apices were sufficiently elevated to be prehended and removed by the grazing sheep. However, in denser and more productive pastures, the effective grazing height is suggested to be roughly half the maximum height of the feed on offer (3). In the current experiments, median heights in proportion to the maximum tiller height are: 14, 24, 34, 35% for the four grazing times respectively, and indicate that less than half the tillers would be grazed down sufficiently low to remove the apices at all four grazing times.

Ideally, the compromise between elevation of the tiller apex and feeding value in governing the success of tiller removal, would not result in the production of new tillers, resulting in the effective reduction of further seed production. The plant growth stage at which the probability of new tiller development is likely to occur after a grazing, may need to be considered and determined more precisely in relation to typical seasonal conditions.

For this approach to be of practical use to landholders in implementing grazing management to reduce seed production, easily recognisable stages of phenological development need to be determined and monitored. Figure 2 shows the progress with time of some measurable development stages during the reproductive phase of the vulpia population in the experimental plots in spring 1994. Ideally, development would be monitored using individual plants (e.g. 16), but in grazed pastures, vulpia plant populations are usually sufficiently high and many-tillered to cause difficulty in discerning single plants. For a faster and more practical estimate of development, progress is based on tillers rather than individual plants.

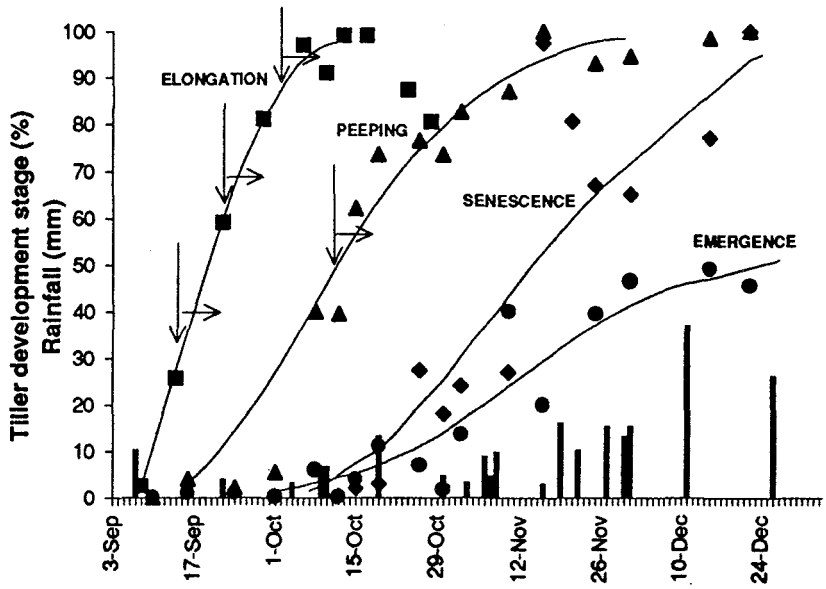


Figure 2. Progressive development stages of vulpia (tiller elongation, peeping of seedhead, plant senescence, seedhead emergence) during spring of 1994, and daily rainfall. Vertical arrows indicate time of grazing commencement; horizontal arrows indicate the grazing period. Rainfall (mm) is indicated by histograms.

For vulpia, the elongation and peeping stages provide a suitable measurement of progress in development over a three month period toward the latter stages of its growth cycle (Fig. 2). In addition, for most of

that period, the slope of the development curves increased sharply, providing more accurate assessment of rate of plant development. Alternatively, emergence (complete emergence of the seedhead through the sheath of the flag leaf) is not a good indicator of development, primarily because the seedhead of *V. myuros* seldom emerges fully from the leaf sheath as distinct from *V. bromoides* which fully emerges (15), and consequently emergence is more a reflection of the proportion of *V. myuros* in the vulpia population present rather than progress in development. Variation in the lines of best fit, particularly for senescence, appear to be influenced by the occurrence of rainfall at regular intervals during November and December when rainfall is not expected. The rainfall encouraged new tillers to grow, resulting in fluctuating rates of senescence.

The vulpia component of the sward maintained a high proportion of green material during the grazing periods, as indicated by the low vulpia senescence values (Fig. 2). Over that same period (10 September - 10 October), the proportion of leaf to stem declined from 78% (10% tiller elongation) to 43% (50% peeping). The likelihood of sheep grazing specific plant material depends on i) the rate at which it can be eaten, ii) its accessibility and iii) its relative acceptability (7). For vulpia to be preferentially eaten in a mixed pasture sward, there is probably little that can be done in practice to enhance ingestion rate. However, accessibility in a vegetative pasture can be modified in the field since a tiller is more likely to be defoliated if the grazed material is shorter and bulky (2). Spatial arrangement of the forage is clearly an important consideration (6, 3). Acceptability may also be manipulated in a vegetative pasture since sheep prefer to eat green rather than dry material, and leaf rather than stem (1), but the situation where the respective components of the pasture are at different stages of accessibility and acceptability is less clear. Significantly, it will be more difficult to enforce preferential or at least equal removal of plant material from the target species in a mixed sward when the target species has a lower ingestion rate and a lower level of acceptability than the other pasture components.

In these experiments, differential application rates of nitrogen and phosphorus (10), and low rates of glyphosate herbicide (12) would be expected to modify ingestion rate and to a lesser extent, acceptability. Accessibility in spring is being enhanced by resting the sward from grazing over the winter period. Under this grazing regime, there should be less tillering by the vulpia, tillers should be more erect and the tiller apex should be more elevated. Further, the chances of tiller removal can always be increased by raising the stocking rate (1). Stocking rates of 50 DSE/ha imposed in these experiments in 1994, a year of below average rainfall and pasture production, will need to be increased to at least 100 DSE/ha to cope with the greater pasture productivity expected in more normal years.

Effectiveness of the grazing treatments imposed in reducing seed production is presently being determined. Treatments may need to be modified in accordance with the results obtained and evaluated in relation to a drought year. If vulpia seed production can be significantly reduced by grazing, then there is the possibility that vulpia populations in a pasture can be continuously managed by strategic grazings rather than the current approach of using herbicides every 3-4 years for short-term control.

Acknowledgment: The technical input of Ms S. Cox and Ms K. Gilchrist during all phases of the experiments, discussions on technical aspects by members of the Pasture Development Group at Orange, and financial support provided by the International Wool Secretariat is gratefully acknowledged.

References

1. Arnold, G.W. (1981). Grazing behaviour. *In* 'Grazing Animals: World Animal Science'. (Ed F.H.W. Morley). pp. 79-104. (Elsevier: Amsterdam).
2. Black, J.L. and P.A. Kenney (1984). Factors affecting diet selection by sheep. II Height and density of pasture. *Australian Journal of Agricultural Research* **35**: 565-78.
3. Demment, M.W. and E.A. Laca (1994). Reductionism and synthesis in the grazing sciences: models and experiments. *Proceedings of the Australian Society of Animal Production* **20**: 6-16.
4. Dowling, P.M. (1988). Effect of pre-season management and in-crop herbicides on weed seedling numbers and triticale grain yield. *Australian Journal of Agricultural Research* **39**: 783-92.
5. Dowling, P.M., D.R. Kemp, D.L. Michalk, G.D. Millar and M. Goodacre (1993). Manipulating pasture composition -a matter of time, dollar$ and sense. *Proceedings of the 7th Australian*

Agronomy Conference, Adelaide SA. pp. 248-51.

6. Hodgson, J. (1982). Influence of sward characteristics on diet selection and herbage intake by the grazing animal. *In* 'Nutritional Limits to Animal Production from Pasture' (Ed. J.B. Hacker) pp. 153-66. (Commonwealth Agricultural Bureau: Farnham Royal, UK).
7. Kenney, P.A. and J.L. Black (1984). Factors affecting diet selection by sheep. I Potential intake rate and acceptability of feed. *Australian Journal of Agricultural Research* **35**: 551-63.
8. Leys, A.R. (1990). Control of annual grasses in pastures of southern Australia and implications for agriculture. *Proceedings of the 9th Australian Weeds Conference*, Adelaide SA. pp. 354-64.
9. Leys, A.R., B. Plater and W.J. Lill (1991). Response of vulpia (*Vulpia bromoides* (L.) S.F. Gray) and *V. myuros* (L.) C.C. Gmelin) and subterranean clover to rate and time of application of simazine. *Australian Journal of Experimental Agriculture* **31**: 785-91.
10. Ozanne, P.G. and K.F.M. Howes (1971). Preference of grazing sheep for pasture of high phosphate content. *Australian Journal of Agricultural Research* **22**: 941-50.
11. Rossiter, R.C. (1966). Ecology of the Mediterranean annual-type pasture. *Advances in Agronomy* **18**: 1-56.
12. Simpson, R.J., K.L. Gatford, C. Siever-Kelly, Leury, R.J. and H. Dove (1993). Improved feeding value in senescing grass pasture after application of glyphosate. *Proceedings of the XVII International Grassland Congress*. pp. 558-9.
13. Simpson, R.J. and Dove, H. (1994). Plant non-structural carbohydrates, diet selection and intake. *Proceedings of the Australian Society of Animal Production* **20**: 59-61.
14. Smith, R.C.G., E.F. Biddiscombe and W.R. Stern (1972). Evaluation of five mediterranean annual pasture species during early growth. *Australian Journal of Agricultural Research* **23**: 703-16.
15. Wheeler, D.J.B., S.W.L. Jacobs and B.E. Norton (1990). Grasses of New South Wales. (The University of New England Publishing Unit, Armidale).
16. Zadoks, J.C., T.T. Chang and C.F. Konzak (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**: 415-21.

LIFE CYCLE AND SEED LONGEVITY OF *ECHINOCHLOA CRUS-GALLI* COMPLEX IN DIRECT SEEDED RICE IN MALAYSIA

[1]M. AZMI, [2]M. MASHHOR, [3]K. ITOH AND [1]H. WATANABE
[1]MARDI Rice Research Centre, 13200 Kepala Batas,
Seberang Perai, Malaysia.
[2]Department of Biology, Universiti Sains Malaysia,
11800 Penang, Malaysia.
[3]Department of Lowland Farming, Tohoku National
Agricultural, Experiment Station, Omagari,
Akita 014-01, Japan.

Abstract:

Echinochloa crus-galli complex is rated as the most troublesome weed in direct seeded rice (DSR) fields in Malaysia. *Echinochloa crus-galli* complex consists of *E. crus-galli* var. *formosensis* and *E. crus-galli* var *crus-galli.* Variety *crus-galli* produces panicles at 45-55 days after emergence (DAE); 5-10 days earlier than var. *formensis.* Both varieties complete their life cycles within 85-95 DAE compared to 115 to 120 DAE of DSR. They produce an abundance seeds, as high as 48,000/plant. Studies on seed longevity indicated that of var. *formensensis* is longer in the soil than var. *crus-galli.* This was reflected by drastic reduction in germination percentages of var. *crus-galli* in the rice field beginning with 2.94% in the 1st season to 0.02% in the 6th season. Variety *formosensis* exhibited a similar trend in germination percentages from 7.33% (1st season to 0.08% (6 season). Survival test showed that after six seasons, none of the var. *crus-galli* seeds were viable while 7% of buried seeds of variety *formosensis* still survived.

Keywords: direct seeded rice, *Echinochloa crus-galli,* life-cycle, seed longevity, seed dormancy

INTRODUCTION

There are five species of *Echinochloa* found in rice granary areas in Peninsular Malaysia; namely *E. crus-galli* complex (6x, 2n=54), *E. oryzicola* (2n=36), *E. stagnina* (2n=54), *E. picta* (2n=126) and *E. colona* (2n=54). Most of them are annuals except *Echinochloa stagnina* and *E. picta* which are perennial species. *Echinochloa crus-galli* complex (barnyard grass) is the most dominant weed in direct seeded rice (1) and all of them are locally known as *rumput sambau.*

The most significant difference between the two varieties of *E. crus-galli* complex based on the morphological characteristics are short awn or awnless with open panicles and shiny spikelets (*E. crus-galli* var. *formosensis)* and long awn with closed or compact panicles (*E. crus-galli* var. *crus-galli*) (1). The morphology of *E. oryzicola* is relatively similar to the plant type of *E. crus-galli* var. *formosensis* (except structure of spikelet). In addition, the species could be differentiated by the shape of the lower empty glume and the size of lower emplty glume which is more than 1/3 the size of spikelet (2). On the other hand, lower empty glume of var. *formosensis* is less than 1/3 of its spikelet size.

Echinochloa crus-galli complex has more intraspecific morphological variations than most other species (2). The species are erect to decumbent and from 1 to 1.5 meters tall at maturity. Varieties of *E. crus-galli* which infest rice fields reproduce mainly by seeds. Both varieties produce abundant tillers and seeds.

The objectives of this study were to determine the life cycle of *E. crus-galli* complex and their seed longevity buried in the soils.

MATERIALS AND METHODS

a) Experiment 1: Study on life cycle

Seeds from both varieties *E. crus-galli* were sown in pots in four replicates. In this study, seeds of these weeds were first soaked in distilled water for 12 hours and incubated for another 18 hours until pregerminated. Five pregerminated seeds were sown in each pot. Only one plant/pot was allowed to grow until maturity. Growth measurements (i.e. plant height, number of tillers), days to heading, 1000-seed weight were recorded.

b) Experiment 2: Study on seed longevity of *E. crus-galli* complex

The experiment was carried out in MARDI Rice Research Centre, Bertam, Seberang Perai, from main season 1990 to off season 1992. Soils from an experimental rice field which were free from *Echinochloa crus-galli* seeds was collected and placed in stainless steel containers (50 x 50 x 50cm). The seeds of *E. crus-galli* var. *crus-galli* and var. *formosensis* were collected from two different rice fields in Muda area in December 1989. Ten thousand seeds of each variety were mixed thoroughly into the soil to a depth of 10 cm. Three treatments based on water regimes were tested i.e. 1) terrestrial condition, 2) rice field condition, 3) waterlogged/flooded condition. After puddling, pregerminated rice seeds (MR 84) were sown at a spacing of 25 cm x 25 cm in each container. Germinated *Echinochloa crus-galli* seeds were counted within 25 cm x 25 cm at the centre of containers every week. Normal fertilizer applications were used. This experiment was carried out for three years (6 seasons) until the main season 1992/93. When completed, three litres of soil were taken out from each container. The weed seeds in the soil were separated by washing with running water.

The seeds were put in petri dishes lined with filter paper. One hundred seeds were used for germination test. Germination test was conducted for one week under room temperature (30°C). Water was added to keep the filter paper moist during germination test. Number of germinated seeds were counted, and ungerminated seeds were used for viability test. The seeds were cut into half and put in 1% soluble of 2, 3, 5-triphenyltetrazolium chloride (TTC) under 30°C in the dark for 24 hours. The embryo of viable seed changed colour to red whereas the embryo of dead seed remained colourless.

RESULTS AND DISCUSSION

a) Life cyles of *E. crus-galli* complex

Both varieties of *E. crus-galli* slightly differ in time to maturity, height, tillering habit and size of stems, panicles and seeds. The first three leaves were produced three to four days after sowing. They started producing tillers at 14-16 days after emergence (DAE) (Figure 1). Their active tillering to maximum tillering stages were from 20 to 40 DAE. *Echinochloa crus-galli* var. *crus-galli* headed a few days earlier than *E. crus-galli* var. *formosensis* at 44-55 DAE and 55-65 DAE respectively. The former variety has the ability to produce more panicles than the latter counterpart from the side tillers. *Echinochloa crus-galli* var. *formosensis* could produce seeds up to 37331 seeds/plant and *E. crus-galli* var. *crus-galli* up to 47850 seeds/plant (Table 1). Azmi and Itoh (1) reported *E. crus-galli* produced less seeds in the fields due to impact of competition with direct seeded crop. Variety *formosensis* was found to produce seeds as high as 12,129 and 10,776 for var. *crus-galli*. One thousand seed weights of *E. crus-galli* var. *formosensis* were heavier than *E. crus-galli* var. *crus-galli* at 2.65 and 1.64 g respectively. The former variety has 2.99 mm long spikelet and the latter at 2.85 mm. Azmi and Itoh (1) reported that seed size and weight of *E. oryzicola* were bigger and heavier than *E. crus-galli* complex.

b) Seed longevity of *E. crus-galli* complex

Majority of *E. crus-galli* seeds of both varieties germinated during the first season (off season 1990) of trial irrespective of the treatments (Table 2). Both varieties of *E. crus-galli* seeds showed higher germination percentages in the first season under terrestrial condition at 25% for var. *crus-galli* and 12.6% for var. *formosensis*. The trend in reduction of germinated seeds for both varieties in the first season to the sixth season whereby var. *formosensis* seeds showed higher percentages of germinated seeds than var. *crus-galli* under all studied conditions. Therefore, var. *formosensis* has a better seed longevity than var. *crus-galli*. It should be noted that most seeds germinated during the first three weeks of sowing of rice. Viability test was carried out when the trial was completed indicated that var. *formosensis* seeds recorded higher no. of viable seeds in terrestrial condition (18.6%) followed by rice field condition (7.0%) and flooded condition (3.7%). None of the var. *crus-galli* seeds found viable after the study period. The experiment showed that var. *formosensis* has longer seed longevity than var. *crus-galli*. Therefore, *E. crus-galli* var. *formosensis* could be more threatening than var. *crus-galli* in direct seeded rice.

In conclusion, *E. crus-galli* complex seeds in the soil showed different states of dormancy and germinated at early crop growth stages and thus could be fairly difficult in weed control and management. This is probably due to the fact that the species belong to annual weed which has high seed production ability and longer seed dormancy periods. These factors can generally result in many seeds that can survive for several planting seasons. This phenomenon will increase the size of seed bank in the rice fields. Therefore, long term weed control strategy is required for an effective control on *E. crus-galli* complex.

Acknowledgements: The authors are grateful to Mr. Sobri Hassan for his technical help and Puan Mahani Maulud for typing the manuscript.

REFERENCES

1) Azmi, M. and Itoh, K. (1991). *Echinochloa crus-galli* complex. In Life cycles of rice field weeds and their management in Malaysia (Itoh ed.). pp. 17-27.

2) Yabuno, T. (1983). Biology of *Echinochloa* species. Proceedings of the Conference on Weed Control in Rice.

Table 1. Comparison in growth stages of two varieties of *E. crus-galli* complex

Days after sowing (DAS)	Growth stage	Variety	
		E. crus-galli var. *formosensis*	*E. crus-galli* var. *crus-galli*
0	Sowing of seeds	-	-
-	Germination	Germinate after 5 DAS	Germinate after 4 DAS
4 - 7	2-3 leaves	Long and narrow leaf	Broad leaf
14 - 16	Early tillering	Second tiller at 15-16 DAS	Second tiller at 14-15 DAS
17 - 18	Tillering	Third tiller	Third tiller
> 20	Active tillering	Compact tillering	Open tillering
44 - 65	Heading	First panicle at 48 DAS and 50% heading at 55-64 DAS	First panicle at 44 DAS and 50% heading at 46-55 DAS
66 - 95	Maturation	90-95 DAS	85-90 DAS
1000 seed weight		2.65 g	1.64 g
Seed size :length :width		 2.99 mm 1.70 mm	 2.85 mm 1.44 mm
Other characteristics		Usually erect and seldom producing panicles from side tillers	Able to produce panicles from side tillersbeside from normal tillers

DAS = Days after sowing

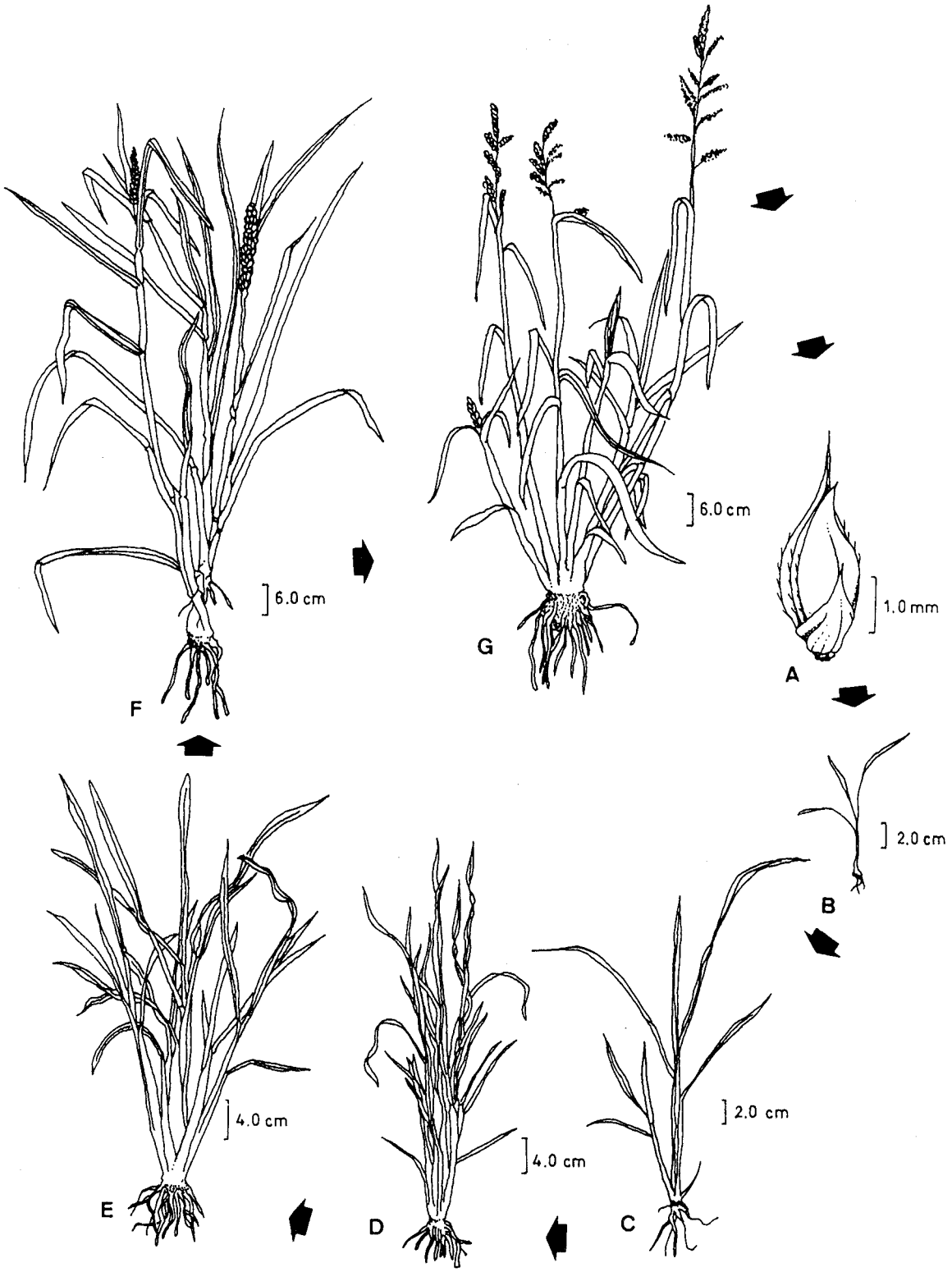


Figure 1. Life cycle of *Echinochloa crus-galli* var. *formensensis* (A = seed, B = 3 leaf-stage (4-7 days after sowing, DAS), C = First tiller stage (14 - 16 DAS), D = Active tillering stage (20-25 DAS), E = Maximum tillering/booting stage (35-40 DAS), F = Heading stage (48-50 DAS); G = Flowering/maturation stage (50-95 DAS)

Table 2. Seed germination and viability test of *E. crus-galli* complex after buried in the soil for a certain period

Season	*E. crus-galli* var. *crus-galli*			*E. crus-galli* var. *formensensis*		
	Terrestrial condition	Rice field condition	Flooded condition	Terrestrial condition	Rice field condition	Flooded condition
	%					
Off season 1990	25.00 (9999)	2.94 (1174)	6.72 (2688)	12.55 (5018)	7.33 (2933)	7.43 (2970)
Main season 1990/91	0.60 (240)	6.44 (2576)	4.77 (1909)	1.94 (774)	3.29 (1317)	2.79 (1115)
Off season 1991	1.76 (704)	1.36 (543)	0.56 (224)	2.12 (847)	1.83 (730)	1.71 (683)
Main season 1991/92	0.05 (21)	0.06 (25)	0.05 (21)	0.55 (219)	0.78 (310)	0.52 (209)
Off season 1992	0.02 (8)	0.05 (20)	0.01 (4)	0.27 (109)	0.31 (123)	0.02 (7)
Main season 1992/93	0.01 (5)	0.02 (7)	0.003 (1)	0.16 (62)	0.08 (32)	0.003 (1)
Total	27.44 (10976)	10.86 (4345)	12.12 (4847)	17.58 (7033)	13.58 (5432)	12.48 (4990)
Germination*	0	0	0	15.6	3.7	3.3
Viable seed**	0	0	0	3.0	3.3	0.7
Viable seed	27.44	10.86	12.12	36.18	20.58	16.48
Death seed	72.56	89.14	87.88	63.82	79.42	83.52

* Germination test after 6 seasons
** Tetrazolium test after 6 seasons

Figure in parenthesis shows no. of seeds germinated/m^2

SEED GERMINATION, SEEDLING ESTABLISHMENT AND GROWTH PATTERNS OF WRINKLEGRASS (*ISCHAEMUM RUGOSUM* SALISB.), A NOXIOUS WEED OF RICE FIELDS IN MALAYSIA

L.A.N. Nabi and B.B. Baki
Botany Dept., University of Malaya, 59100 Kuala Lumpur, Malaysia

Abstract. Studies were conducted in 1993 - 1994 in an insect-proof house and laboratory to evaluate seed germination, seedling establishment and growth patterns of wrinklegrass (*Ischaemum rugosum* Salisb.) under different regimes of light, temperature, moisture, depths of inundation and experimental media. Seeds of *I. rugosum* exhibited induced and enforced dormancy. Seed germination was light-dependent (positively photoblastic) while the rate of germination was temperature-dependent. In the light, the seeds exhibited quasi-simultaneous germination pattern with most seeds germinated within six day after sowing. The optimum temperature for germination ranges between 25 and 35°C. Nitric acid at a concentration of 0.01N inflicted damage and cause death to the emerged seedlings. *Ischaemum rugosum* seeds failed to germinate when they were sown in soil at 0.5 cm depth and inundated at 2 - 14 cm. Seeds sown in moist soils without inundation had a germination percentage of about 15%. Seeds floated freely on water surface had a germination percentage of about 98%. Seedling establishment and survival were inversely proportional to the depths of inundation. Being a C_4 plant. *I. rugosum* is well adapted to hot, arid and high light intensity. Under favourable environmental conditions, *I. rugosum* has a highly branched habit and growth form. The genet can reach 2m in height. Each genet produced *ca* 22 culms bearing 106 rachis. Floral initiation began *ca* 12 weeks after sowing. It has a highly prolonged reproductive phase producing > 6000 ripened seeds/genet within 12 weeks after the emergence of the first spike.

Keywords: *Ischaemum rugosum*, #ISCRU, germination, seedling growth, growth patterns, germination media, photoblastic.

INTRODUCTION

Wrinklegrass (*Ischaemum rugosum* Salisb.) is a member of Poaceae. It is a noxious and vigorous perennial weed. Ranked as the 44th world's worst weeds by Holm *et al.* (1977), wrinklegrass is reported as a serious weed in 26 countries. The species is a true indigene of the Asian, American and African tropics. Wrinklegrass inflicted an estimated annual loss of 46 million tons of rice worldwide (Moody, 1991). Wrinklegrass is well adapted to a variety of habitats, *viz.* wet, inundated and non-irrigated rice fields up to 2400 m in altitude (Baki, 1992). In Malaysia, wrinklegrass is considered a serious weed in major rice fields in the states of Selangor, Perak, Penang and Kedah.

In spite of the rapid spread of wrinklegras in rice and sugarcane fields, there is a paucity of information pertaining to the ecology and control of the weed (Sudhakara, 1986; Chandrasena, 1988, 1989; Azmi, 1989; Colon, 1989; Lubigan, 1989; Bhatia, 1989; Singh, 1990; Taria, 1991).

An understanding of seed germination, seedling establishment and growth patterns are necessary prequisites in arresting the growth potential and further infestations of new sites by the weed aligned to efforts in developing the appropriate control measures. It is with the objective of evaluating the effects of some environmental factors, *viz.* different regimes of light, temperature and moisture, depths of inundation and experimental media on the germination and growth patterns of *I. rugosum* that this paper attempts to address.

MATERIALS AND METHODS

General. Seeds of wrinklegrass (*Ischaemum rugosum* Salisb.) were collected from rice fields in Penang, Malaysia in 1993. The seeds were then dried in the oven at 35°C for one week and stored in a refrigerator at 5°C until they were used for experimentation. Four experiments were conducted on the germination characteristics, seedling survivorship and general growth patterns of wrinklegrass.

Composite list of weeds (1989), WSSA, Champaign, Illinois, USA.

Seed germination. Germination experiments were conducted in the laboratories of the Botany Department, University of Malaya, Kuala Lumpur in 1993 - 1994. In evaluating germination, the standard procedures for seed testing recommended by the International Seed Testing Association (1966, 1985) and the methods used for overcoming the dormancy of IR64 rice by Nugrahu *et al.* (1991) were adopted. Ten treatment regimes inclusive of the control were used, *viz;* T_1 (untreated seeds/H_2O), T_2 (untreated seeds soaked in H_2O for 24 hrs/H_2O), T_3 (untreated seeds soaked in H_2O for 24 hrs/0.2N KNO_3). Treatments T_4 and T_5 were similar to T_3 but 0.5% concentration of H_2O_2 and 0.001N HNO_3 media were used, respectively while seeds were ovendried and then soaked in water. Enough oven-dried seeds were soaked in H_2O for 24 hrs and placed in petri-dishes with moistened filter papers. Distilled water, 0.02N KNO_3, 0.5% concentration of H_2O_2 and 0.001N HNO_3 and were used as experimental media for T_7, T_8, T_9 and T_{10} respectively. The pH of the chemical media used, i.e. H_2O, KNO_3, H_2O_2 and HNO_3 were 7.0, 2.5, 2.8 and 2.5, respectively. All the treatments were subjected to six temperature regimes, *viz.* 15, 20, 25, 30, 35 and 40°C. Each treatment has four replications. Ten seeds were placed in a 9-cm diameter plastic petri dish previously lined with Whatman filter papers. The filter papers were moistened with 6 ml of water or chemical solutions where appropriate. For each treatment and each temperature regime, one set of petri dishes was maintained in a growth chamber and was exposed to fluorescent light with an intensity of 630 E $m^{-2}s^{1}$. Another set of petri-dishes was wrapped in a double layer of aluminium foil and was maintained in the same growth chamber to evaluate germination under dark conditions. Germination was recorded every three days and the percent germination was determined twelve days after seeds were sown. Seeds were considered to have germinated normally when the radical has emerged for 0.5 mm or more.

The following experiments were conducted in an insect-proof glasshouse. The daily mean temperature of the glasshouse was *ca* 27°C while RH was *ca* 85% and the mean daily sunshine was 6.5 hours. Necessary precautions were taken against pests and diseases.

Seed germination in soil inundated at different levels. Twenty-five seeds were sown at a depth of 0.5 cm in each of 24 pots (30 cm in diameter x 30 cm in height). The pots were previously half-filled with Java series soils. The pots were assorted randomly into 8 sets, each set represented one treatment with three replications. Each set was randomly assigned to one of the 8 levels of inundation used in the experiment (0 (control), 2, 4, 6, 8, 10, 12 and 14 cm). The water level in each pot was maintained at constant depth with augmentation of water where appropriate. The number of emerged seedlings was recorded every two days for a period of 30 days.

Germination and seedling survivorship of seeds floated freely in water. Each of 21 graduate cylinder (60 cm height x 6 cm diameter) were filled with Java soils to 15 cm depth. They were then assorted into 7 sets each representing one treatment with 3 replications. Each treatment was randomly assigned to one of the seven water depths (2, 4, 6, 8, 10, 12 and 14 cm). Water was added to each treatment in graduate cylinders to the required level. Twenty-five seeds were dropped on the water surface in each graduate cylinder. Water was added to maintain required water level. The number of germinated seeds was counted every two days for 12 days. The experiment was terminated after one month and the survived plants of each treatment were counted. The lengths of the shoots were measured.

Life cycle. Ten seeds were sown in each of 20 plastic pots (25 cm diameter x 20 cm height) previously filled with moist silt loam padi soils of the Java series. The physio-chemical properties of the soils were: pH (4.4); 0.M (1.98%); organic carbon (1.15); total N (0.12%); C/N (9%); Na (0.08 meq/100 g) and the available P (128 ppm) (Aris, J., pers. comms.). One week after sowing, the seedlings were thinned leaving one seedling/pot. Two weeks after the seeds were sown, 10 uniform plants were selected for the study. The pots were arranged in a completely randomized design and the positions of the pots were changed every week to minimise edge effects. The plants were watered once daily from above by using a fine spray. The plants were supplemented monthly with urea, muriate of potash and triphosphates at a rate of 100 : 30 : 20 respectively. The growth parameters and their recording schedules were: plant height, number of plant nodes and internodes at maturity, number of

culms/plant/week, number of rachis/plant/week, spike length/plant, number of spikes/plants collected simultaneously upon ripening and the time of spike emergence when appropriate.

The experiment commenced on 29 March 1993 and was terminated on 29 September 1994 upon the completion of the life cycle. The plants were harvested and dismembered into shoots and roots and dried in the oven at 60°C for three days. Seeds were collected simultaneously as they ripened and were dried in the oven at 40°C for one week.

Analysis of variance, regressions analysis, and comparisons of treatment means by using Tukey's test at 1% and 5% levels of significance were made whenever applicable to the data. The polynomial equation, $y = e_0 + e_1x + e_2 x^2$ was used for fitting the germination data (where y represents the treatments T_1 - T_{10}, x represents temperature and e_0, e_1 and e_2 are the coefficients).

RESULTS AND DISCUSSIONS

Germination tests. Wrinklegrass seeds of all treatments under all temperature regimes failed to germinate when incubated under continuous darkness. The exception was for treatment T_5 where 10% of the seeds germinated at 30°C while T_8 registering the same rate of germination at 35°C.

In light, however, the seeds exhibited quasi-simultaneous germination pattern. The seeds of all treatments (Figure 1) failed to germinate at 15°C. At the other temperature extreme of 40°C, 65% and 85% seed germinated for T_3 and T_8 treatments, respectively. Seeds of T_5 and T_{10} failed to germinate at 40°C. At the other temperature regimes of 20, 25, 30 and 35°C, treatments T_5 and T_{10}, had high seed germination percentages recorded. Nitric acid at a concentration of 0.00N inflicted damage and caused death to the emerged seedlings. Treatments T_1 and T_6 showed the low germination percentages at all temperature regimes except at 25°C where 95% and 98% germination were recorded respectively.

The optimum temperatures for germination were different between treatments. The optimum temperatue for germination for T_1 and T_6 was 20°C; in others these were between 25°C and 35°C.

When the means of treatments T_1 and T_6, T_2 and T_7, T_3 and T_8, T_4 and T_9 and T_5 and T_{10} were tested, the null hypothesis of drying of seeds in the oven has no effect on germination was accepted. Soaking of seeds for 24 hours, however, was found to have a significant effect on germination as the means of treatments T_1 and T_2 were compared.

The dependence of wrinklegrass seeds on the light, as a prerequisite for germination, classify this species as positively photoblastic (Berrie, 1987) exhibiting induced dormancy (*sensu* Harper, 1977). The failure of wrinklegrass seeds to germinate at low temperatures is an important attribute which may help to explain its restricted spread within the tropical regions of Asia, Africa and America. The ability of the seeds to germinate in media with low pH explains the ability of this species to survive in acidic soils. Using chemicals as germination media appeared not only to enhance germination, but also widened the optimum temperature range.

Treatments T_1, T_3 and T_8 appeared to be the most favourable ones in enhancing seed germination provided that light is not a limiting factor.

Seed germination in soils inundated at different levels. Seeds of wrinklegrass failed to germinate in all the treatments except for those sown in moist soil or in soils inundated with 2 cm of water, where germination percentages were *ca* 15% and 1% respectively. Seed burial, arguably, inhibit seed germination due to inaccessibility of light. Buried weed seeds at any depth induces enforced dormancy (Hill, 1977). Seeds buried in soil inundated with water at any depth became totally dormant. Seeds buried at a depth of 0.5 cm in moist soil experienced enforced dormancy with *ca* 85% failing to germinate, while those soils covered with 2 cm of water or more had all their seeds remaining dormant.

Seed germination and seedling survivorship floated freely in water The results indicated that the average germination percentage of wrinklegrass seeds floated freely on water surface was 98%. Seedling survivorship was found to be inversely proportional to water depth in the graduate cylinders (Fig. 2). Mean seedling survivorship at the respective depths of 2 to 14 cm were 100, 100, 80, 60, 40, 33 and 8%. On the contrary, seedling mortality was positively proportional to the depths of inundation. The seedling height of the survivors was also inversely proportional to the water depth

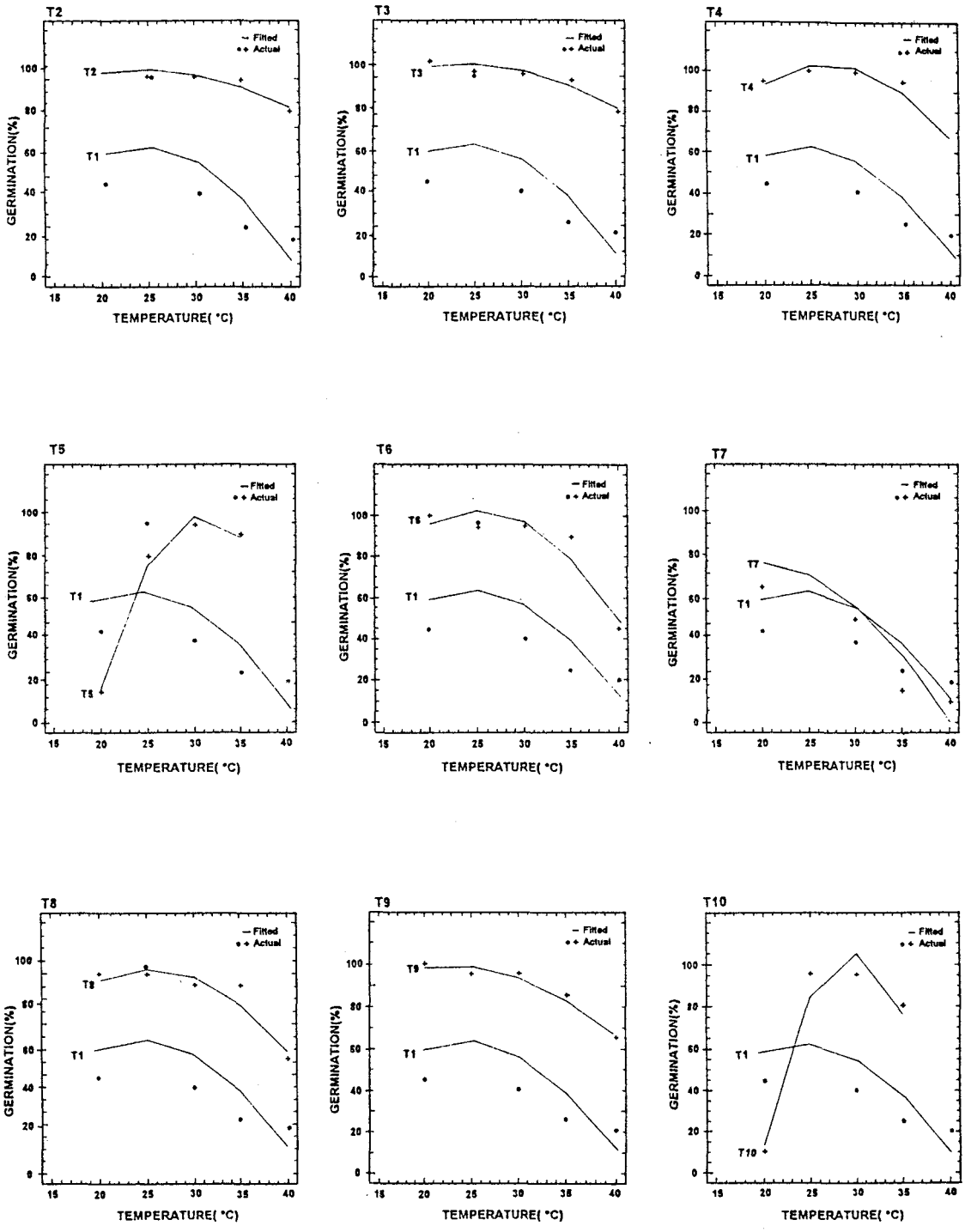


Fig. 1 : Fitted and observed effects of different temperature regimes and experimental media (T_2, T_3 ..... T_{10}) on germination percentage of wrinklegrass *vis-a-vis* the control (T_1) (refer to the text for detailed notations of the treatments).

(Fig. 3). At the water depth of 2 cm, the seedlings registered *ca* 60 cm in height *vis a vis* their counterparts at 14 cm water depth and this was only *ca* 16 cm in height.

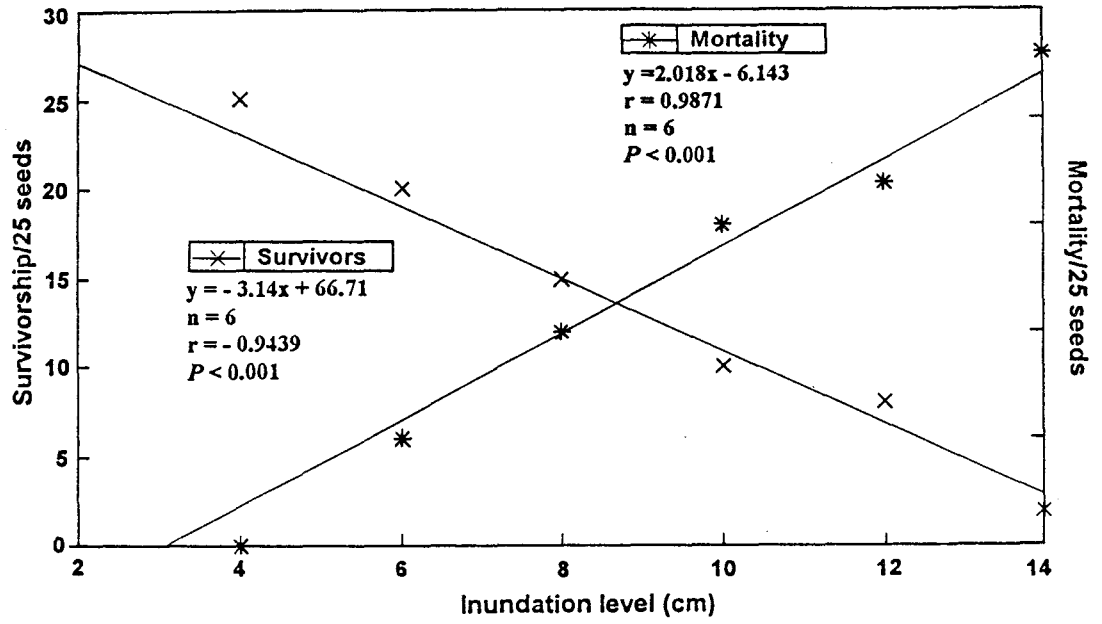


Figure 2 : Seedling survivorship and mortality in relation to water inundation level in soil surface in the graduate cylinders

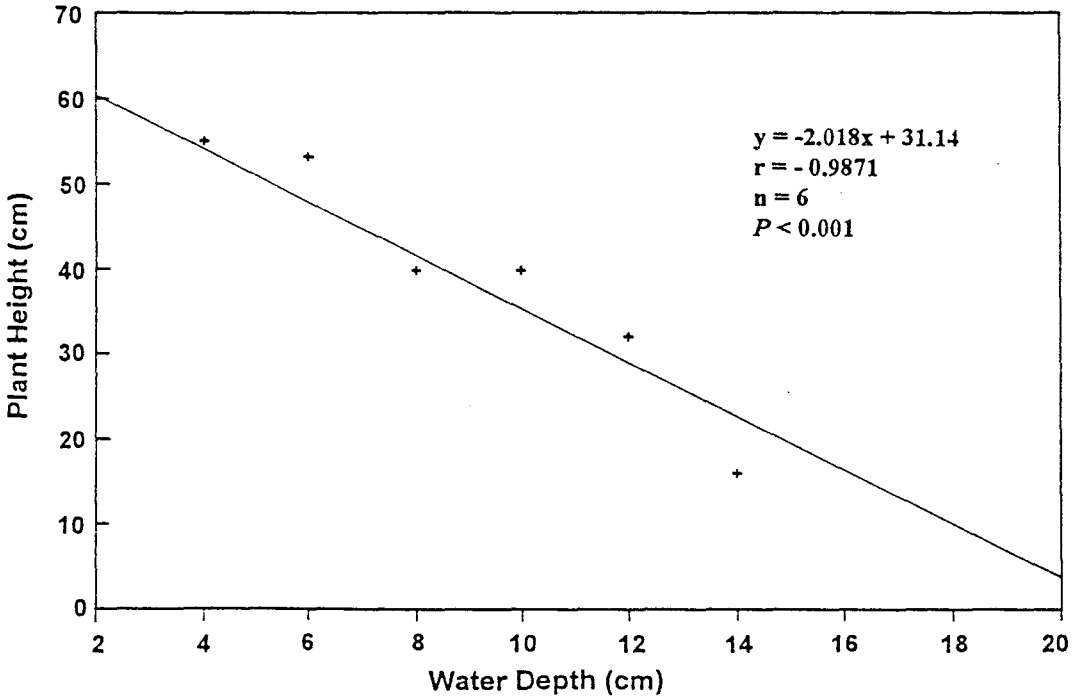


Figure 3 : The relationship between plant height and water depth over soil surface

Wrinklegrass is a C_4 plant which is well adapted to hot, arid, and high light intensity climates. Under favourable conditions it has a highly branched growing habit and growth form. Wrinklegrass also produces a large number of seeds ripened over a period of 10 weeks. Except for light, seeds of wrinklegrass have no special requirements for germination. These characteristics make wrinklegrass an ideal weed (cf Baker, 1965). Seed could be dessiminated by water from one rice field to another. While floating on water surface, it can establish itself and exert a severe biological constraints to rice production in all flooded rice fields in Malaysia and elsewhere in the tropics.

Life cycle A schematic diagram of the life cycle of wrinklegrass is represented in Figure 4. The major growth characteristics of wrinklegrass recorded are listed in Table 1. The results, however, showed significant intra-specific differences between the growth parameters of the ten genets used in the study. The average height of wrinklegrass was *ca* 161 cm and showed strong linear correlation

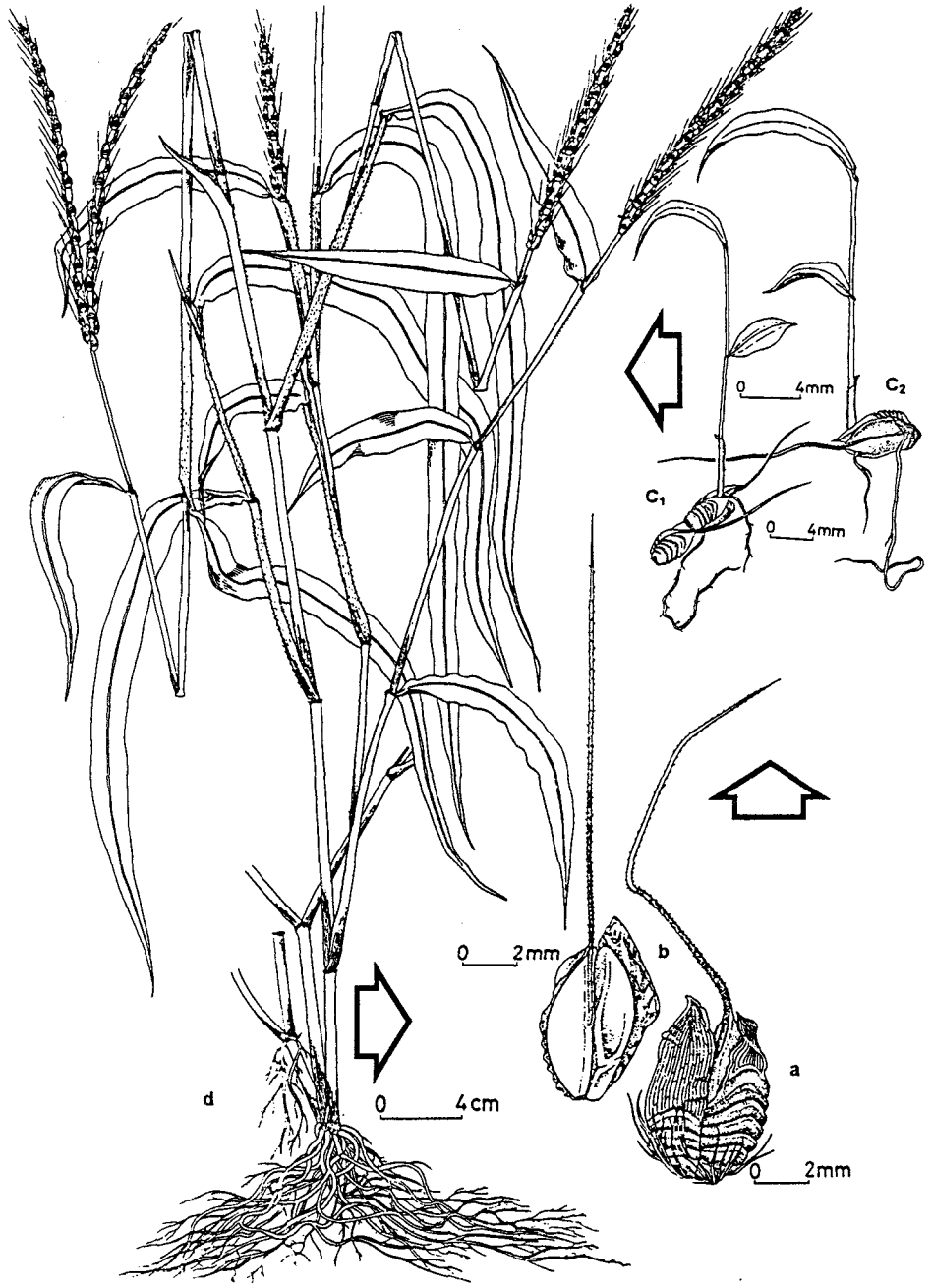


Figure 4 : Schematic representation of the life cycle of *Ischaemum rugosum* Salisb. a, b, dorsal and ventral views of the seed respectively; C_1, C_2, 2 week-old seedlings; d, mature 12- weeks old plant.

Table 1: Selected growth characteristics of *Ischaemum rugosum* Salisb.

No.	Parameter	Value
1	Plant height	161 cm (range 141-182 cm)
2	Number of nodes/plant	8
3	Length of internodes	6, 13, 16, 19, 19, 22
4	Number of culms/plant	22
5	Number of rachis/plant	106
6	Spike length	13 cm (range 3-16 cm)
7	Approximate time of spike emergence	12 weeks
8	Approximate time of spike ripening	14 weeks
9	Number of seeds/spike	57 (range 12-70 cm)
10	Spikes dry weight/plant	24.5 g
11	Seed net weight/plant	24.1 g
12	Shoot dry weight/plant	48.0 g
13	Root dry weight/plant	5.0 g
14	Total dry weight/plant	77.5 g
15	Weight of 1000 seeds	4.0 g
16	Number of seeds/plant	6116
17	Reproductive effort (*Re*)	0.32
18	Vegetative effort (*Ve*)	0.68

of height increase with time (Figure 5). The increase in plant height of each genet ceased after emergence of the first spike. The genets were highly branched with *ca* 22 culms/genet. The main branch of each culm comprised of *ca* 8 nodes. The average length of the internode was *ca* 15 cm. The average shoot dry weight was 48 g/genet. The number of rachis/genet was 106 each bearing one or more spikes. The mean time for floral initiation was 12 weeks. Spikes ripened two weeks after

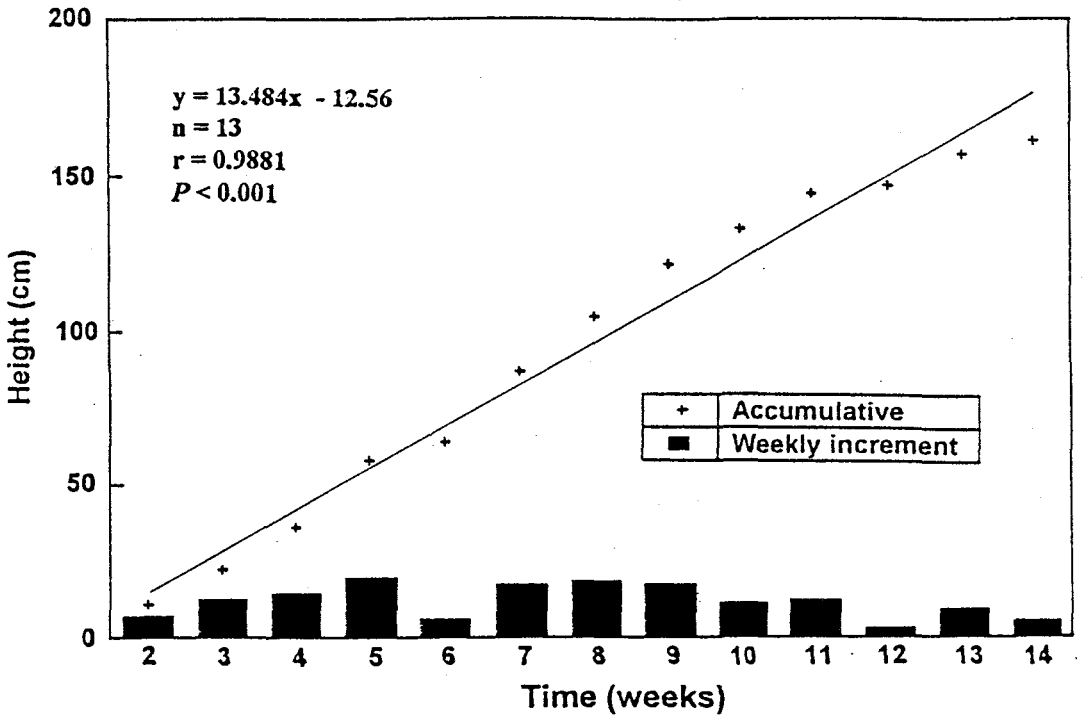


FIGURE 5 : Average height of ten *Ischaemum rugosum* Salisb. plants from seedling stage to maturity

emergence. Spike length was 13 cm and each spike had *ca* 57 seeds. The net seed weight/genet was 24 g, while the mean number of seeds/genet was > 6000. Seed production continued for 10 weeks after the ripening of the first spike. The vegetative and reproductive efforts of each genet were 0.68 and 0.32, respectively.

The results indicated that wrinklegrass has a relatively rapid growth habit and allocated approximately one third of its net primary productivity for seed production. It also has a highly prolonged reproductive phase extended up to 10 weeks after the emergence of the first spike. This arguably accounted for about one half of the plant longevity.

Acknowledgement. The authors wish to express their appreciation to Mohamed Nor and to Ibrahim Abogussa for their assistance in data analysis. Thanks are also expressed to Anuar Pandak and Zainal Mustafa for the technical help and Tan Lip Hong for typing the manuscript.

REFERENCES

Anon. 1966. Proceedings of the International Seed Testing Association. 1966. International Rules for Seed Testing. pp 1-152.

Azmi, M. 1988. Weed competition in rice production. *Proceedings of the National Seminar and Workshop on Rice Field Weed Management.* pp 141-152.

Baker, H.G. 1965. Characteristics and modes of origins of weeds. In: H.G. Baker and G.L. Stebbins (eds). *The Genetics of Colonizing Species*, pp 147-72. Academic Press, New York.

Baki, B.B. 1992. In: *Plant Resources of South-East Asia No. 4. Forages.* (L't Manetje and R.M. Jones, eds.). Pydoc. Scientific Publishers, Wareningen. pp 146-148.

Bhatia, R.K., Sandhu, K.S. 1984. Relative toxicity of herbicides to germinating paddy weed seeds. *Indian Journal of Weed Science 21*: 1-2, 83-87.

Berrie, A.M.M. 1987. Germination and dormancy. *Advanced Plant Physiology.* English Language Book Society/Longman. pp 440-468.

Chandrasena, J.P.N.R. 1988. Floristic composition and abundance of rice field weeds in four low country wet zone districts of Sri Lanka. *Tropical Pest Management 34*: 3, 378-287.

Chandrasena, J.P.N.R. 1989. Weed problems of rice in Sri Lanka. *Proceeding of the 4th EWRS Symposium on Weed Problems in Mediterranean Climates, Vol.* 2: 293-300.

Chandrasena, J.P.N.R. 1989. A survey of rice field weeds in Ratnapura and Kurunegaia districts of Sri Lanka. *Journal of the National Science Council of Sri Lanka 17*: 187-211.

Colon, C. and Antigua, G. 1989. Main weed of flooded rice in Cuba. *Cienciang y Tecnica en la Agricultura. Arroz. 12*: 137-142.

Harper, J.L. 1977. *Population biology of plants.* Academic Press, London, England.

Hill, T.A. 1977. *The biology of weeds.* The Institute of Biology's Studies in Biology No. 79. Edward Arnold Publishers Ltd., London. pp 1-29.

Holm, L.G., Plucknett, D.L., Pancho, J.V. & Herberger, J.P. 1977. *The World's Worst Weeds. Distribution and Biology.* The University Press of Hawaii, U.S.A. p 295-298. International Seed Testing Association. 1985. International rules for seed testing. *Seed Science and Technology 13*: 2, 299-355.

International Seed Testing Association. 1966. The germination test. *Proceeding of International Seed Test Association 31*: 1, 49-93.

Lubigan, R.T., Moody, K. 1989. Effect of herbicide on Ischaemum rugosum. *Internation. Rice Research Newsletter 14*: 2, 38-39.

Moody, K. 1991. Weed management in rice. In: *Handbook of Pest Management in Agriculture* (D. Pimentel, ed.), pp 301-328. CRC Press, Boca Raton, Florida, USA.

Nugraha, U.S. and Soejadi. 1991. Predrying and soaking of IR64 rice seeds as and effective method for overcoming dormancy. *Seed Science and Technology 19*: 307-313.

Singh, I., Kolar, J.S. 1990. Control of *I. rugosum* Salisb. (Wrinklegrass) in transplanted rice. *Indian Journal of Weed Science* 22: 3-4, 46-50.

Sudhakara, K., Nair, R.R. 1986. Weed control in rice under semi-arid system. *Agricultural Journal of Kerala 24*: 2, 211-215.

Effect of Seeding Depth and Soil Moisture Regime on Emergence of *Ischaemum rugosum* and *Echinochloa glabrescens*

A.N. Rao and K. Moody*
International Rice Research Institute
P.O. Box 933, Manila, Philippines

Abstract. *Ischaemum rugosum* Salisb. and *Echinochloa glabrescens* Munro ex Hook. f. are major weeds of wet-seeded rice in the Philippines. The objectives of this study were to understand the adaptations of *I. rugosum* and *E. glabrescens* to the irrigated rice ecosystem and to quantify the effect of seeding depth and soil moisture regime on their emergence and establishment. The greatest number of *I. rugosum* and *E. glabrescens* seedlings emerged and established on saturated soil and when the soil was kept saturated for 7 days after seeding followed by flooding to a depth of 2.5 cm. *I. rugosum* and *E. glabrescens* germinated even when seeding was done on the water surface. Under these conditions, establishment was greater at a water depth of 2.5 cm and when flooding was alternated with saturated soil condition. The germination of both weed species was reduced with increase in seeding depth and continuous flooding. Probably, due to seed buoyancy, the ability to germinate on the water surface and establish from shallower water depths or during intermittent periods of irrigation and saturation, *I. rugosum* has become a dominant weed in irrigated wet-seeded rice in addition to the rainfed rice ecosystem.

Key words: *Ischaemum rugosum*, *Echinochloa glabrescens*, seeding depth, soil moisture, water depth, seedling establishment, weed seedling emergence

Introduction

Knowledge on germination behavior of seeds, propagules and other aspects of weed biology help to gain insight into the mechanisms of specialization and distribution of the species. Such studies will offer an opportunity to locate vulnerable points in the life histories of weeds when they can be successfully attacked for their control (Misra, 1969). *I. rugosum* and *E. glabrescens* are the major weed problems in direct-seeded rice ecosystems in the Philippines, Malaysia and Vietnam (Rao and Moody, 1994).

Flooding is an important factor in delaying seedling emergence and minimizing weed populations in rice fields (Moody and De Datta, 1982). Smith and Fox (1973) reported that few or no seedlings of *Echinochloa crus-galli* (L.) P. Beauv. emerged when the soil was flooded. Seeding depth and flooding reduced germination, survival and growth of *E. glabrescens* (Diop and Moody, 1984).

The objectives of this study were to understand the adaptations of *I. rugosum* and *E. glabrescens* to the irrigated rice ecosystem and to quantify the effect of seeding depth and soil moisture regimes on their emergence and establishment.

Materials and Methods

Separate experiments were conducted for each species. Small pots containing 1.7 kg lowland soil, which had 41% clay, 38% silt, 21% sand, pH of 6.9, 0.128% N, 38 mg/kg available P, 1.17 meq/100 g exchangeable K, 17.8 meq/100 g exchangeable Ca and 12.0 meq/100 g exchangeable Mg, were used. A split plot design with seeding methods assigned to the main plots and water regimes to the subplots was used and there were three replications. Seeding methods were: a) on the soil surface or on the water surface if water was present, b) on the soil surface, c) at 2 cm soil depth and d) 4 cm deep. Fifty weed seeds were sown in each pot. The water regimes were: a) saturated soil, b) continuously flooded with 2.5 cm water c) continuously flooded with 5 cm water, d) saturated soil for 7 days followed by flooding with 2.5 cm water, e) continuously flooded with 10 cm water, f) alternating regime of 2.5 cm water - saturated soil - 2.5 cm water, and g) alternating regime of 5 cm water - saturated soil - 5 cm water.

The number of seedlings that emerged and became established were recorded at 10 and 20 days after seeding (DAS), respectively. Seedling height and shoot dry weight (g/pot) were determined at 20 DAS. Data were analyzed using the methods described by Gomez and Gomez (1984).

Results and Discussion

Maximum emergence, seedling establishment, seedling height and dry weight of *I. rugosum* and *E. glabrescens* were observed when seeded on the saturated soil surface (Figs. 1 and 2). Saturated soil conditions for 7 DAS and later flooding with 2.5 cm water was equally conducive for the establishment and growth of both species except when *I. rugosum* was seeded 4 cm deep and *E. glabrescens* was seeded on the soil surface. Drost *et al.* (1982) observed greatest emergence of *E. glabrescens* when the soil moisture content was between 70 and 100%. During a survey made in Central Luzon and Iloilo, Philippines (Rao and Moody, unpublished), many farmers reported that they drain the fields during the first 3 to 10 DAS for better rice establishment. This creates an environment conducive for the emergence and establishment of both *I. rugosum* and *E. glabrescens*.

Emergence and establishment of seedlings of *E. glabrescens* and *I. rugosum* were observed even when seeds were placed on the water surface. Establishment was greater when the water depth was 2.5 cm and when flooding was alternated with saturated soil conditions. Seeds germinated (Figs. 1A, C) and seedling growth occurred when seeds were sown on the surface of 10-cm deep water (Fig. 2) but the seedlings did not become established (Figs. 1B, D) because they failed to anchor in the soil.

I. rugosum is a major weed of rainfed (Estorninos *et al.*, 1982) and irrigated rice (Rao and Moody, unpublished) in Central Luzon. Due to the buoyant nature of the seed and ability to germinate on the water surface and establish from shallow water depths or during intermittent periods of flooding, *I. rugosum* has become a dominant weed in irrigated wet-seeded rice in this area.

More *E. glabrescens* seedlings emerged and established when seeded 2 and 4 cm deep and flooded than *I. rugosum* seedlings. *Echinochloa oryzicola* (Vasing.) Vasing germinates under anoxic conditions which contributes to its ability to grow and compete in flooded rice fields (VanderZee and Kennedy, 1981). It appears that *E. glabrescens* also possesses the same feature.

Emergence, establishment, seedling height and weight of both species (Figs. 1 and 2) was reduced with increase in seeding depth and continuous flooding. Singh *et al.* (1991) observed that *I. rugosum* seeds emerged from up to 5 cm soil depth but continuous submergence checked its germination. Diop and Moody (1984) reported that deeper seeding and flooding reduced seedling emergence of *E. glabrescens*. The results of this study emphasize the importance of early flooding for the suppression of *E. glabrescens* and *I. rugosum* in wet-seeded rice.

References

Diop, A.M. and K. Moody. 1984. Effect of seeding depth and flooding regime on germination and growth of *Echinochloa glabrescens*. Philipp. J. Weed Sci. 11:65-69.

Drost, D.C., K. Moody and J.D. Doll. 1982. Effect of length of dry period following establishment of wet-seeded rice (*Oryza sativa*) on weed emergence and weed community composition. Abstract No. 217 *in* Abstracts of papers presented at the Annual Meeting of the Weed Science Society of America, 7-12 February 1982, Boston, Massachusetts, USA.

Estorninos, L.E. Jr., D.C. Navarez and K. Moody. 1982. Farmers concepts about weeds and weed control practices in rainfed areas of the Philippines. Pages 507-518 *in* International Rice Research Institute. Report of a workshop on cropping systems research in Asia. Los Baños, Laguna, Philippines.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. Wiley, New York, USA.

Misra, R. 1969. Ecological studies of noxious weeds, common in India and America, which are becoming increasing problem in the upper Gangetic plains. USAID Project, FG-IN-231-A7-CR-106. Department of Botany, Banaras Hindu University, Varanasi-5, India.

Moody, K. and S.K. De Datta. 1982. Integration of weed control practices for rice in tropical Asia. Pages 37-47 *in* M. Soerjani, D.E. Barnes, and T.O. Robson, eds., Weed control in small farms. Asian-Pacific Weed Science Society and BIOTROP Special Publication No 15, Bogor, Indonesia.

Rao, A.N. and K. Moody. 1994. Ecology and management of weeds in farmers direct-seeded rice (*Oryza sativa* L.) fields. International Rice Research Institute, Los Baños, Philippines. 81 p.

Singh, T., J.S. Kolar and R.K. Bhatia. 1991. Emergence behavior of *Ischaemum rugosum* (Wrinkle grass) as affected by variable temperature, submergence period and depth of seed placement in soil. Indian J. Weed Sci. 23:56-60.

Smith, R.J., Jr. and W.T. Fox. 1973. Soil water and growth of rice and weeds. Weed Sci. 21:61-63.

VanderZee, D. and R.A. Kennedy. 1981. Germination and seedling growth in *Echinochloa crus-galli* var. *oryzicola* under anoxic conditions: structural aspects. Am. J. Bot. 68:1269-1277.

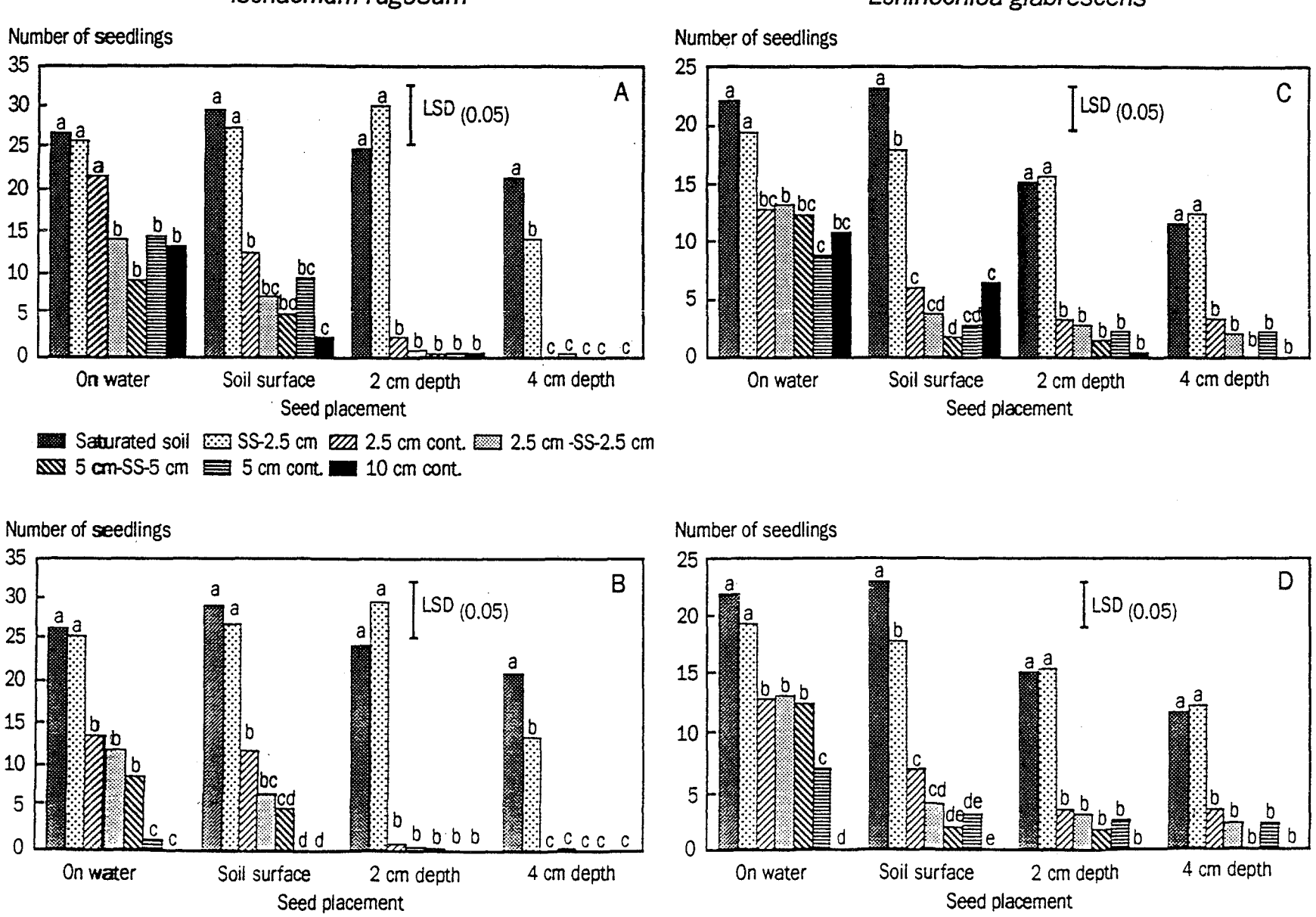


Fig. 1. Effect of seeding depth and soil moisture regime on emergence (A and C) and establishment (B and D).

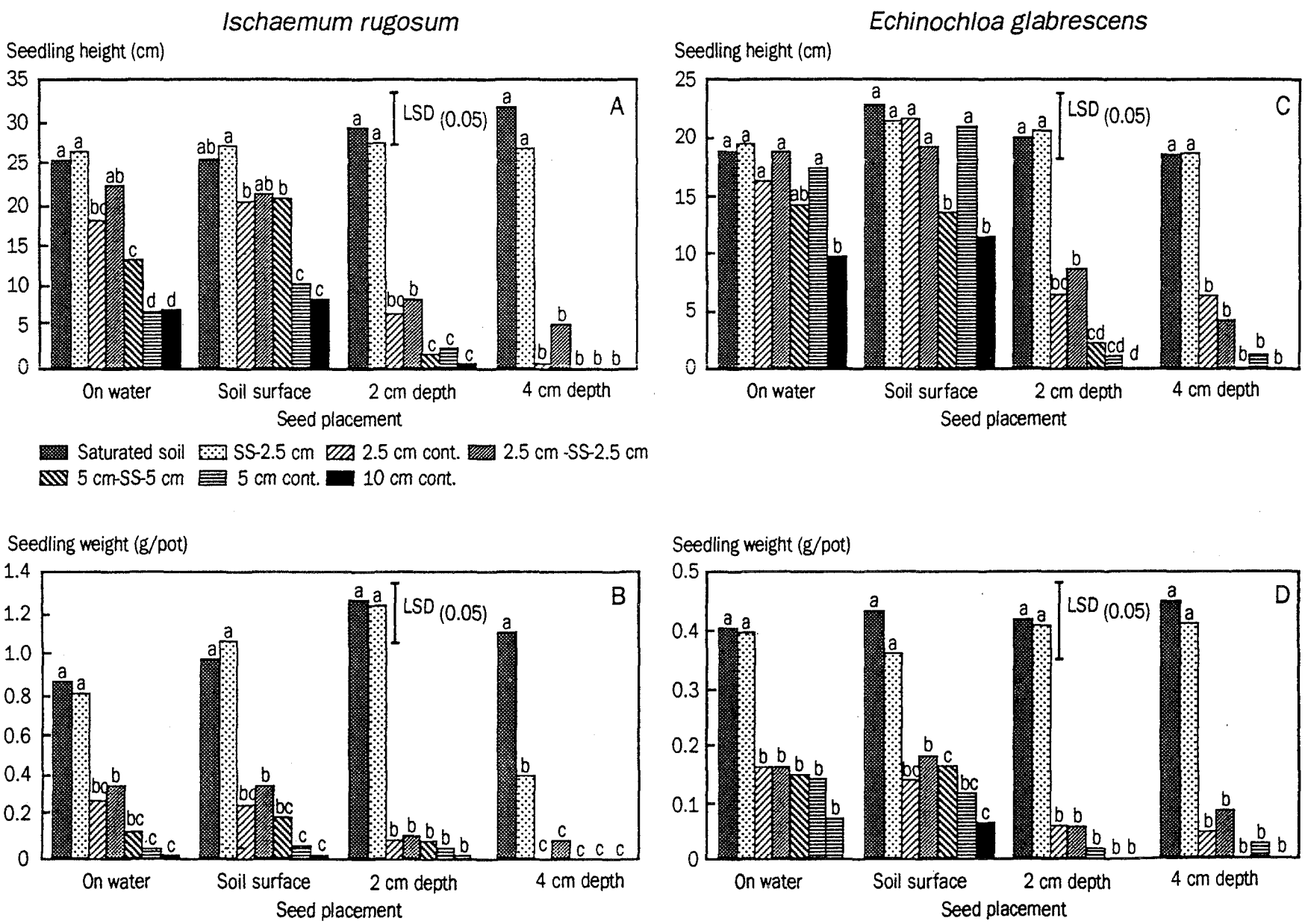


Fig. 2. Effect of seeding depth and soil moisture regime on seedling height (A and C) and seedling weight (B and D).

The Response of Wild Oats to Climate Change: Future Application to a Generic Model for Annual Weeds

Chris C. O'Donnell & S.W.Adkins*
Department of Agriculture, The University of Queensland, Brisbane, Australia

Abstract. A changing climate could have negative consequences for Australia's agricultural sector if there is an increase in the abundance and geographical distribution of annual weeds. The Co-operative Research Centre for Tropical Pest Management is presently developing a generic pest model that will enable Australia's agricultural and environmental managers to respond to changes in the incidence and abundance of pests as a consequence of the enhanced greenhouse effect. Weeds and plant pathogens are included in this definition of pests. This study is the first of a series of experiments designed to examine the sensitivity of *Avena fatua* L. to the interactive effects of temperature and CO_2 fertilisation and provide validation data for the annual weeds component of the model. Wild oat has been chosen for the case-study because of its economic importance in winter cereal growing regions and the extensive literature published on its biology. Seeds from six near-isogenic lines of wild oat were germinated and grown in controlled environment chambers under either ambient CO_2 (357 ppmv) or elevated CO_2 (480 ppmv). Three soil moisture treatments were also imposed. Preliminary results indicate that developmental times, as measured by time to anthesis and maturity, were reduced and that seed numbers, plant dry weights, and heights were increased for the plants grown under elevated CO_2. There was no significant difference in dormancy between either CO_2 treatment. These results indicate that the CO_2 fertilisation will have an important effect on the life-cycle processes and population dynamics of wild oat. By applying the data to the generic pest model it will be possible to define the impacts of annual weeds under climate change.

Key words: annual weeds, climate change, wild oat, generic model, carbon dioxide

Introduction

The "Enhanced Greenhouse Effect" (EGE) refers to the radiative forcing of the Earth's climate through the anthropogenic loading of the atmosphere with radiatively active gases. Chemical analyses of Antarctic ice-cores have revealed that the pre-industrial atmospheric concentration of carbon dioxide (CO_2) was 280 parts-per-million by volume; 77 ppmv less than current levels (Neftel *et al.*, 1985, Pearman *et al.*, 1986). Direct monitoring of atmospheric CO_2 concentrations at Mauna Loa, Hawaii, since 1958 has indicated that the annual rate of increase has risen from 0.6 ppmv in 1958 to 1.8 ppmv at present (Keeling *et al.*, 1989). Combustion of fossil fuels and deforestation are the prime causes of these increases. Climate modelling studies indicate that an equilibrium doubling of CO_2 is likely to cause global mean surface temperatures to increase by 1.5° to 4.5°C (Houghton *et al.*, 1990). These studies also indicate that there will be changes in precipitation patterns as well as changes in the frequency and intensity of extreme rainfall events (Houghton *et al.*, 1990). A changing climate will have important consequences for Australia'a agricultural sector which already experiences high year-to-year fluctuations in yields as a consequence of variable weather conditions. It is probable that there will be changes in the incidence and abundance of pests, diseases and weeds as a consequence of changing climatic means. Because weeds have a broad genotype it is possible that in a changed climate they will achieve greater competitive fitness than the crops with which they compete. The balance between existing competitive interactions may also be affected by differential physiological responses to the CO_2 "fertilisation effect" which has been reported to enhance plant growth by as much as 30 to 40% for C_3 species at doubled current CO_2 concentrations (Kimball 1983; Cure & Acock 1986; Idso *et al.*, 1987; Gifford 1988). The mechanism for this growth enhancement appears to be stimulation of photosynthesis. Present CO_2 levels are sub-optimal for C_3 photosynthesis because the key photosynthetic enzyme, ribulose bisphosphate carboxylase/oxygenase (RuBP), has an affinity for oxygen as well as carbon dioxide. This results in losses to photorespiration at current ambient CO_2:O_2 levels. As the CO_2 concentration of the atmosphere increases, C_3 plants achieve higher rates of carbon fixation because more carbon is available to enter the photo-reductive carbon cycle. Plants that use the C_4 photosynthetic pathway have a CO_2 concentrating mechanism located in the mesophyll cells. The CO_2 is then pumped to the bundle sheath cells maintaining a high CO_2:O_2 ratio and favouring the carboxylation of RuBP thereby preventing photorespiration. Increasing the atmospheric CO_2 concentration does not confer as large an advantage on C_4 species because the internal CO_2 concentration is already near saturation (Wong, 1979).

The Co-Operative Research Centre for Tropical Pest Management, Brisbane, Australia, has undertaken a generic approach that will strengthen Australia's capacity to define and respond to the impacts of pests, diseases and

weeds under EGE conditions. The model GENSECT, which is presently under development, is a generic population simulation model that will allow users to construct simulation models of a species phenology and abundance based on its life-cycle stages and the processes that affect each of these.
For the annual weeds component of the model, wild oat has been chosen for the initial sensitivity study because of its key pest status in Australia's winter cereal growing regions and the vast literature published on its biology and ecology. The aims of this study were to examine the response of wild oat to CO_2 fertilisation at an initial temperature thus providing a base data set to which future experiments could investigate the interactive effects of higher temperatures and CO_2 fertilisation..

Materials and Methods

Caryopses from six Australian near-isogenic lines of wild oat spanning 12°of latitude (Table 1) were placed in petri dishes on two No.1 Whatman filter papers. Each caryopsis was pierced and 5 mL of 10 *u*M gibberelic acid (GA_3) was added to promote germination (Adkins *et al.*, 1986). When germinated, seedlings were transplanted into 20 cm diameter plastic pots containing University of California potting mix and then placed in two identical controlled environment growth chambers (Conviron model PGW36, FSE Scientific Ltd, Brisbane, Australia; internal dimensions 240x150x200 cm). Each chamber held 36 pots. Light intensity was adjusted to 506 *u*mol m^{-2} s^{-1} at mean canopy height and temperature was set at 20±1 °C day / 16±1 °C night with a 12 hour square-wave photoperiod and relative humidity was 70-90%. One cabinet was enriched with FOG grade CO_2 (Air Liquide, Brisbane, Australia) to 480 ppmv. Supply of CO_2 was monitored and controlled using an ADC 2000 CO monitor (ANRI Instruments and Controls P/L, Victoria, Australia) in conjunction with a solenoid valve. When the CO_2 concentration fell below the set level the monitor opened the solenoid valve allowing CO_2 to effuse into the chamber until the concentration rose again to the set level at which point the solenoid valve closed. On day 14 seedlines were thinned to three plants per pot. After day 20 the soil moisture deficit treatments were applied. Using de-ionised water the potting mix in each pot was watered daily by weight to either field capacity, -0.1MPa , or -1.0 MPa. This provided two replicate pots per soil moisture treatment per line (2x3x6). Every 10 days Aquasol (Hortico Industries Australia P/L) soluble liquid fertiliser at the rate of 0.8 gL^{-1} was applied as part of the normal watering regime.

Water-use was measured on youngest fully-expanded leaves with a LI-1600 steady state porometer (Li-Cor, Lincoln, Nebraska) and as each pot was weighed daily, volume of water added was also recorded. Time to anthesis and maturity was recorded. Primary and secondary seeds were harvested from each plant as they matured, air dried for *ca.* 3 days in the laboratory at 22±2 °C and then placed in a deep freezer until required for experimentation (*ca.* 60 days). At maturity plant height was measured from the soil surface to the top of the tallest panicle. Plants were then separated into root, shoot and leaf fractions and placed in a drying oven at 80°C for 72 hours to obtain dry weights. Germination trials were conducted on a sample of 30 primary caryopses from each treatment. Caryopses were placed in 9cm-diameter petri dishes on two No.1 Whatman filter papers to which 5ml of de-ionised water was added. The petri dishes were then placed in air-tight plastic containers and incubated in dark incubators set at 20±1 °C. Germination, defined as protrusion of the coleorhiza through testa and pericarp, was recorded every 48 hours. Filter papers were replaced at regular intervals to reduce fungal contamination.

Line	Abbreviation	Latitude
Springsure	Sp	24° 08' S
Toowoomba	Tb	27° 32' S
Narrabri	Nb	30° 19' S
Wellington	Wt	32° 35' S
Wickepin	Wn	32° 47' S
Rutherglen	Rg	36° 05' S

Table 1. Australian near-isogenic lines of wild oat used in this study.

Results & Discussion

Phenology.

The vegetative, reproductive and maturation phases were initiated earlier in the elevated CO_2 plants than those grown under ambient CO_2 with elevated CO_2 plants attaining physiological maturity approximately 7 days earlier than the control plants (Fig. 1a). Water stress did not significantly reduce the time to maturity in any line for either CO_2 treatment. Under a changed climate maturation times could be expected to be faster than those indicated here because of the effects of increases in mean temperatures. Maturation times for wild oat are shortened as temperature increases (Adkins *et al.*, 1987). Results from crop simulation models for wheat, also a member of the grass family that shares the same photosynthetic pathway as wild oat, indicate that whilst CO_2 fertilisation alone increases above-ground biomass, the interactive effects of increases in temperaure of as little as 3 °C can reduce yield by 24% (Gifford 1988) or up to 60% (Wang *et al.*, 1992) through faster maturation times.

In modelling expected distribution and abundance of wild oat under climate change, the maturation rates of crops with which wild oat competes, has to be considered. If maturation times for wild oat were significantly slower than those of the crop then the abundance of wild oat could be expected to decline because seed would be removed with the harvested crop and not deposited to the soil-seed bank.

Seed Characters.

Seed production averaged across all lines and soil moisture treatments was increased by 27% for the elevated CO_2 plants compared to ambient CO_2 plants (Fig. 1b). Although -0.1 MPa and -1.0 MPa water stress reduced seed production across both CO_2 treatments, elevated CO_2 plants suffered a relatively smaller reduction (32% & 28%) compared to normal CO_2 plants that had a 50% & 58% reduction (Fig. 1c). Caryopsis dry weights were reduced in four out of the six elevated CO_2 lines (Fig. 1d) probably due to faster maturation times and subsequent shorter grain filling times. Total seed biomass was 22% higher for the elevated CO_2 treatment as a result of greater seed numbers per plant but seed weight as a ratio of total plant weight was higher for the ambient CO_2 plants (0.38) than for the elevated CO_2 plants (0.33). Initial germination trials have not shown any significant differences in the level of dormancy between CO_2 treatments. As the seed is the basic unit of the population, greater seed numbers, assuming similar crop maturation rates, would indicate potential for increases in wild oat populations.

Morphology.

There was no significant difference between CO_2 or soil moisture treatments in the number of fertile or vegetative tillers produced (data not shown). Plant dry-weights were 45% higher for the elevated CO_2 plants (Fig 1e) with water stress reducing the dry weights in both CO_2 treatments by the same relative amounts. The increase in dry weights of the elevated CO_2 plants could be attributed to higher rates of photosynthesis and not just more phoyosynthesising surface as a result of greater leaf area development. Although destructive harvests were not made over the course of the experiment, leaf dry weights at maturity and leaf weight ratios can be used as an indication of the leaf area development of the plants and also as a comparison between the amount of photosynthesising and respiring material present in the plant. A necessary assumption here is that leaf senescence was similar across both treatments. Althoug elevated CO_2 plants had 39% more leaf dry weight than ambient CO_2 plants, the leaf-weight-ratio of elevated CO_2 plants was 0.26 compared to 0.31 for ambient CO_2 plants, suggesting that a factor other than greater leaf area or photosynthesising surface was responsible for the increase in dry weights of the elevated CO_2 plants. It has been reported that stimulation of photosynthesis or higher net assimilation rates occur for plants grown under high CO_2 (Gifford 1988, Conroy & Hocking 1993). It is suggested that this factor is operating here.

One of the most striking differences between CO_2 treatments was in the amount of dry matter allocated to root production. Root weight ratio for elevated CO_2 plants was 0.22 compared to 0.11 for ambient CO_2 plants. This is best understood by considering water use (below). Elevated CO_2 plants also attained greater heights (Fig. 1f).

Water Use.

Although elevated CO_2 plants had lower transpiration rates per unit leaf area as a consequence of reduced stomatal conductances, daily water use was higher across all three water treatments (Figs 2 a,b,c). This effect is a consequence of greater leaf area development and has been documented previously (Gifford 1988) and supported by Leuning *et al.*, (1993) using a mechanistic simulation model. From approximately 70 days-after-sowing (DAS) the water-use trend was reversed with high CO_2 plants reducing their water demand as they

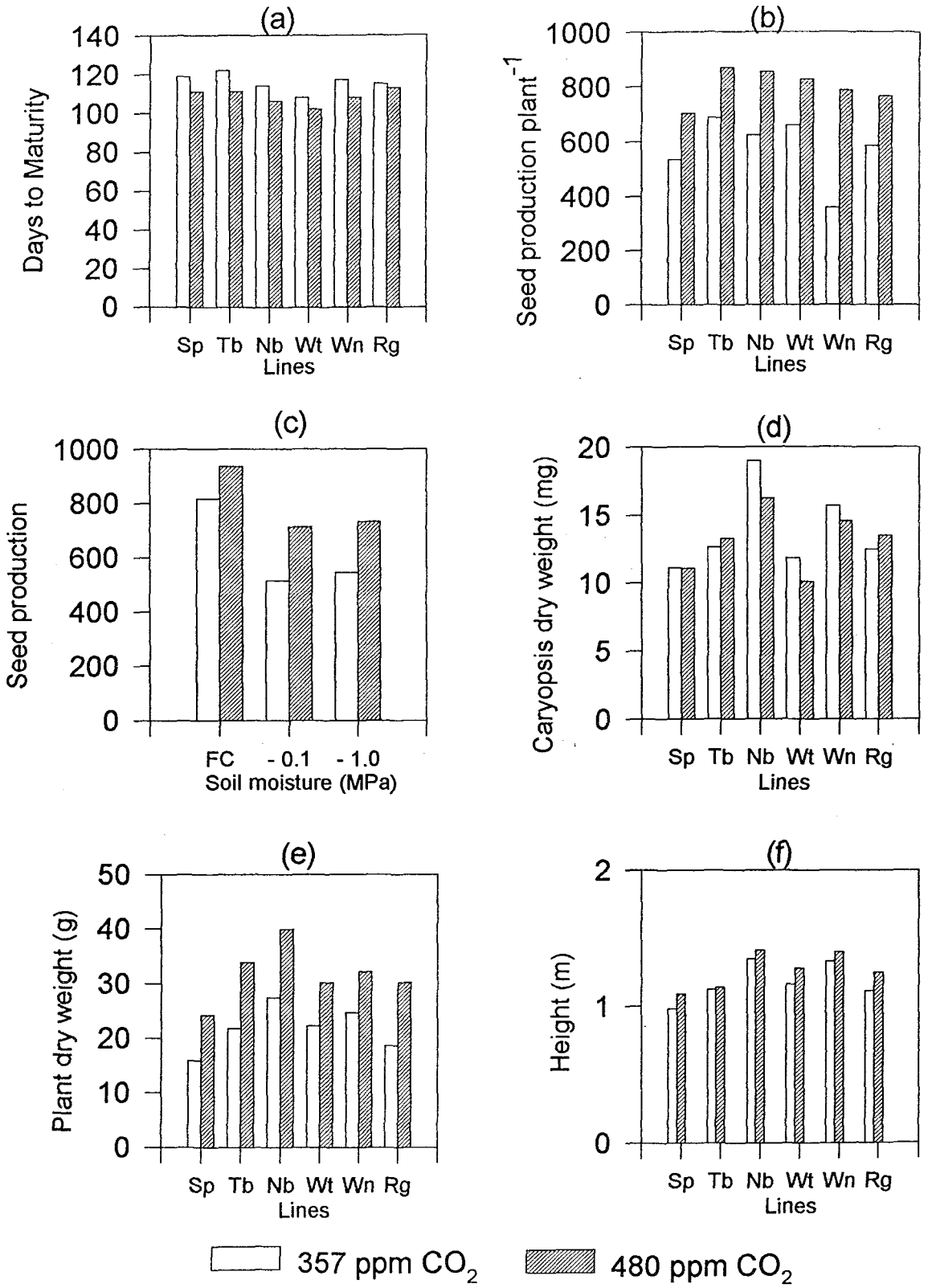


Figure 1: Effect of CO_2 fertilisation on (a) Days to maturity (b) Seed production (c) Seed production per water treatment (d) Caryopsis dry weight (e) Plant dry weight and (f) Height ; for six Australian lines of wild oat.

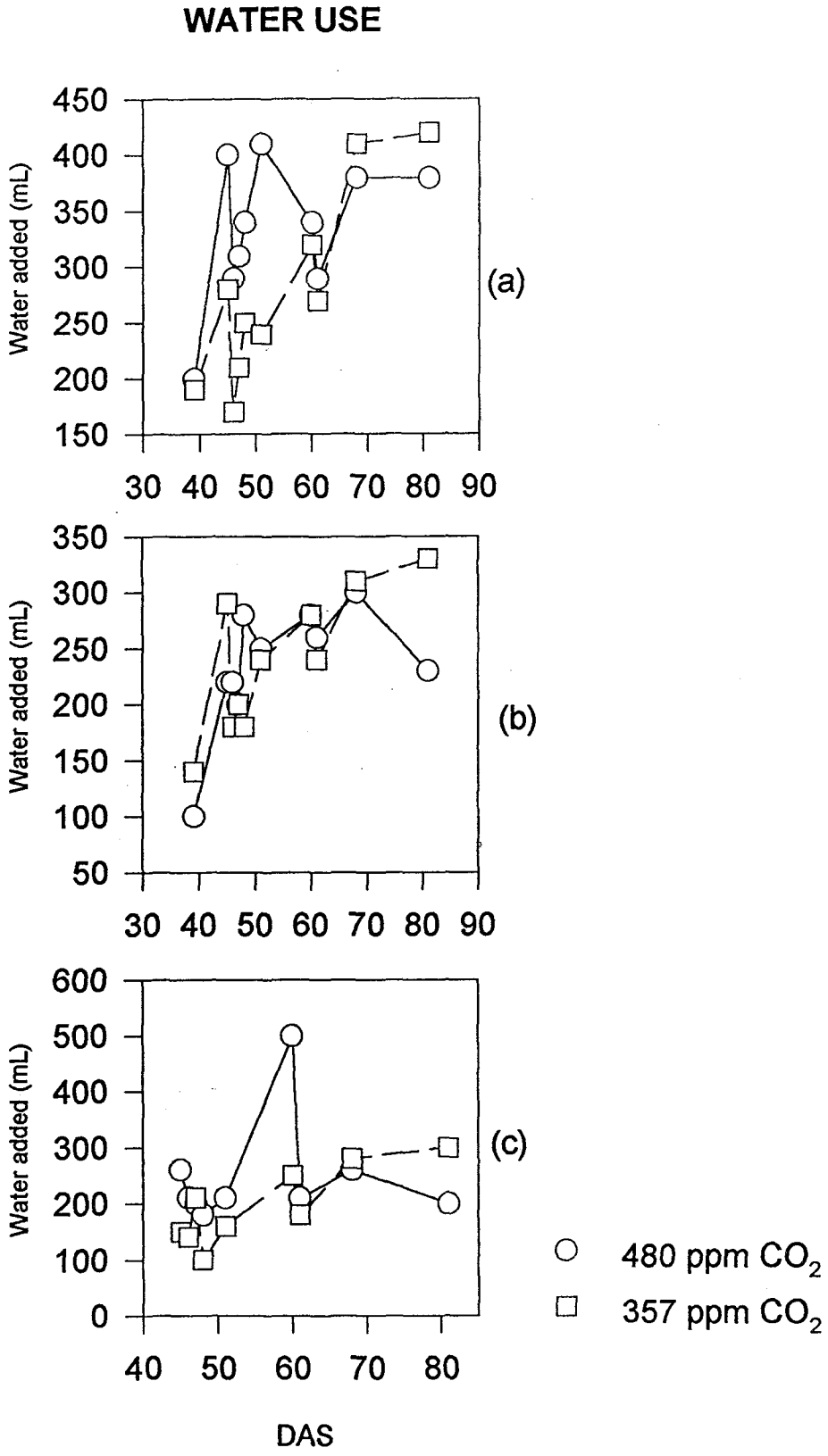


Figure 2: Daily water use for (a) field capacity plants
(b) -0.1 MPa soil moisture stress (c) -1.0 MPa soil moisture stress

approached maturity. As CO_2 fertilisation causes wild oat will to have a higher water requirement, a condition that will be magnified under warmer temperatures and subsequent higher evaporative demand, water availability could become a major limiting factor controlling the distribution and abundance of wild oat in a changed climate.

Acknowledgement: This project was funded under the Dedicated Greenhouse Research Grants Scheme and accordingly the authors acknowledge the support and financial assistance of the National Greenhouse Advisory Committee and the Department of Environment, Sport & Territories, Commonwealth of Australia.

References

1. Adkins, S.W., M. Loewen, S.J. Symons. (1986). Variation within pure lines of wild oat (*Avena fatua)* in relation to degree of primary dormancy. Weed Science 34:859-864.
2. Adkins, S.W., M. Loewen, S.J. Symons. (1987). Variation within pure lines of wild oats *(Avena fatua)* in relation to temperature of development. Weed Science **35**:169-172.
3. Conroy, J., P. Hocking. (1993) Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. Physiologia Plantarum **89**: 570-576.
4. Cure, J.D. and B. Acock. (1986). Crop responses to carbon dioxide doubling: A literature survey. Agricultural and Forest Meteorology **38**:127-145.
5. Gifford, R. (1988) Direct effects of higher carbon dioxide concentrations on vegetation pp506-519 in *Greenhouse: Planning for climate change.* G.I. Pearman Ed. CSIRO, Melbourne.
6. Houghton, J.T., G.J. Jenkins, J.J. Ephraums Eds. (1990). Climate change - The IPCC scientific assessment. 365pp. Cambridge University Press. Cambridge.
7. Idso, S.B., B.A. Kimball, M.G. Anderson, J.R. Mauney. (1987) Effects of atmospheric CO_2 enrichment on plant growth: the interactive role of air temperature. Agriculture, Ecosystems and Environment. **20**: 1-10.
8. Keeling, C.D., R.B. Bacastow, A.F. Carter, S.C. Piper, T.P. Whorf, M. Heimann, W.G. Mook, H. Roeloffzen. (1989). A trhee-dimensional model of atmospheric CO_2 transport based on observed winds: 1. Analysis of observational data. Geophysical Monograph 55, D.H. Peterson *ed.* pp 305-363. American Geophysical Union, Washington.
9. Kimball B.A. (1983). Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agronomy Journal. **75**: 779-788.
10. Leuning, R., Y.P.Wang, D. de Pury, O.T. Denmead, F.X. Dunin, A.G.Condon, S. Nonhebel, J. Goudriaan. (1993). Growth and water use of wheat under present and future levels of CO_2. Journal of Agricultural Meteorology (Japan) **45**(5): 807-810.
11. Neftel, A., E. Moor, H. Oeschger, B. Stauffer. (1985). Evidence from polar ice cores for the increase in atmospheric CO_2 in the past two centuries. Nature **315**: 45-47.
13. Pearman, G.I., D. Etheridge, F. de Silva, P.J. Fraser. (1986). Evidence of changing concentrations of atmospheric CO_2, N_2O and CH_4 from air bubbles in Antarctic ice. Nature **320**: 248-250.
14. Wang, Y.P., Jr Handoko, G.M. Rimmington. (1992). Sensitivity of wheat growth to increased air temperature for different scenarios of ambient CO_2 concentration and rainfall in Victoria, Australia - a simulation study. Climate Research **2**: 131-149.
15. Wong, S.C. (1979) Elevated atmospheric partial pressure of CO_2 and plant growth. Oecologia (Berl.) **44:** 68-74.

Ecological Distribution and Habitat Segregation in Two Closely Related Ruderal Plantains, *Plantago asiatica* L. and *P. major* L.

K. Matsuo
National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki 305, Japan

Abstract. *Plantago asiatica* and *P. major* are very similar in microhabitat preference(both inhabiting parking sites, gardens, lawns and roadsides), but they exhibit rather conspicuous habitat segregation. With exception of small overlapping zones in urban areas, *P. asiatica* is predominant in rural areas, while *P. major* monopolizes urban areas. A preliminary study on the environmental factors indicated that *P. major* tends to become predominant in sparse and short plant communities that develop on bare ground, while *P. asiatica* is a member of more closed communities.

Key Words: Habitat preference, Coexistence, Introduced species, *Plantago*

Introduction

In and around urban areas, size and composition of vegetation are influenced by the kind and intensity of artificial management practices. Groups of species occur in disturbed habitats in cities such as roadsides, parking lots, gardens and open spots. We called these species "ruderal species". The habitats of ruderal species are frequently disturbed by trampling, mowing and so on, thus native species are frequently replaced by introduced species with similar life history traits. In Sapporo and its vicinity, the population of *P. major* (introduced species) are expanding as rapidly as and, in some sites, even faster than native plantain, *P. asiatica* (Ito, 1984).

In this preliminary study, I report on the ecological distribution and habitat segregation of *Plantago asiatica* L. and *P. major* L. in relation to environmental factors and their habitat preferences.

Materials and Methods

Plantago major (greater plantain; broad-leaved plantain), a weed of European origin, has been spread all over the world and now is well known as a cosmopolitan species, even in Asia which is dominated by another native ruderal species, *P. asiatica* (asiatic plantain). *P. major* is expanding its distribution especially in dairy regions and urban areas(Sager & Harper, 1975). In Japan, too, the occurrence of this species has been confirmed in Sapporo, Hokkaido (Fujiwara, 1957), Yokohama and Fujisawa, Kanagawa Prefecture (Asai after Osada, 1976) and Tokyo (Osada, 1976). Nevertheless, this species has been considered minor compared with the native ruderal plantain species , *P. asiatica*, which is expanding its distribution range even into subalpine zones ca. 2500m in altitude after extensive human disturbance (e.g. road construction) in central Honshu (Kawano & Matsuo, 1983).

Table 1. Key to closely related plantain species, *P. asiatica* and *P. major*.

	P. asiatica	*P. major*
Number of seeds per capsule	4 - 6	8 - 12
Shape of seed	elliptic in shape	irregular in shape (round, elliptic or triangular)
Size of seed	1.8 - 2.0mm in long	1.0 - 1.9mm in long
Features of seed coat	irregularly net-like ornamentation, but no stripe on the surface	elevated striate ornamentation

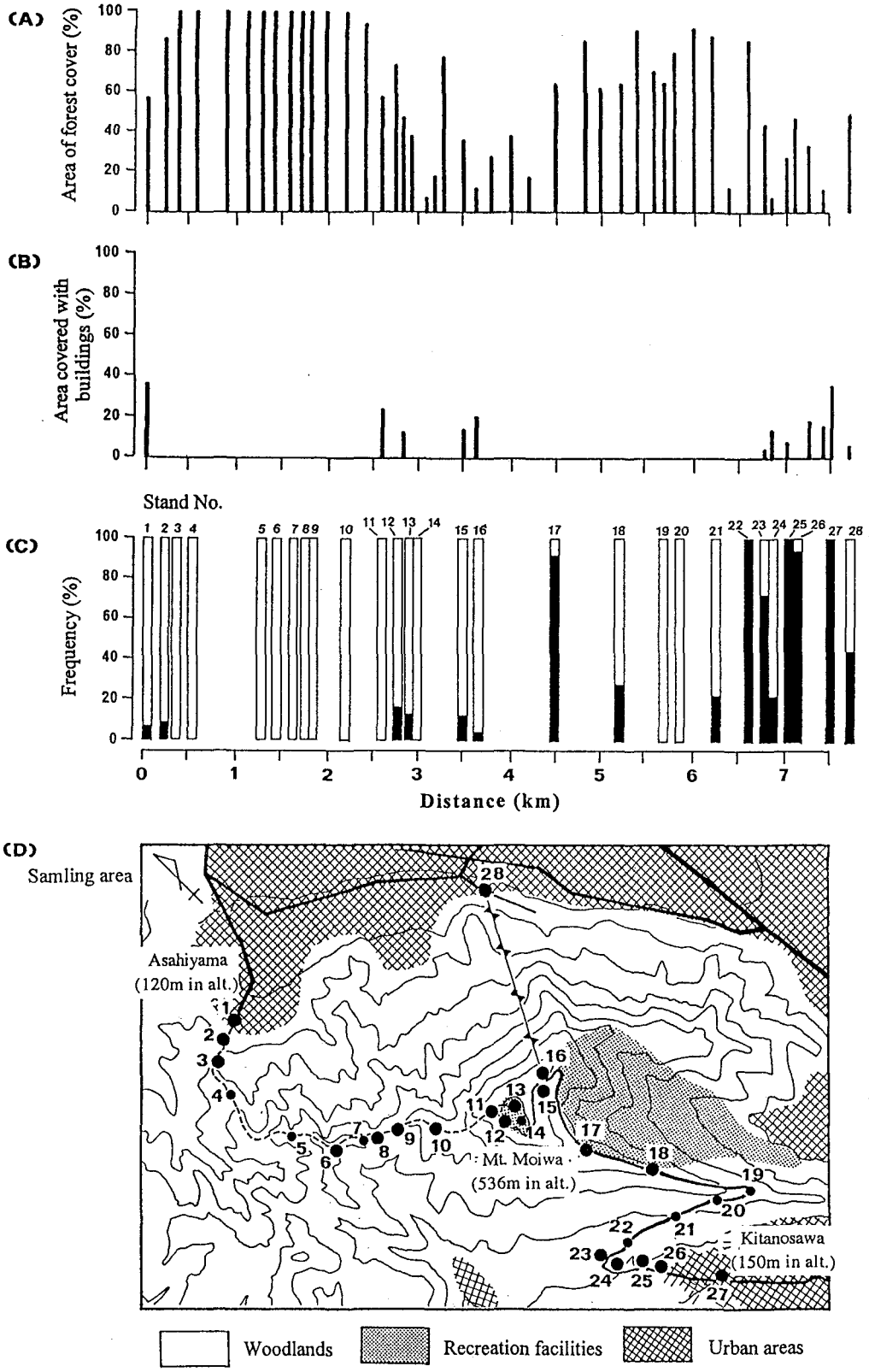


Fig. 1. Percentage of forest cover per 160m × 160m (ca. 2.6ha) around the sampling stand and its neighborhood, (A) ; in an area covered with buildings, (B). Occurrence frequency of *P. asiatica* (open bars) and *P. major* (filled bars) along a road passing through Mt. Moiwa (C). In Fig. 2-D, figures show the stand numbers. Thick lines, thin lines and dashed lines indicate more than 5.5m road width, 2.5-5.5m road width and less than 2.5m road width, respectively. And a ropeway links near the top of Mt. Moiwa (Stands 16) and the foot of the moutain (Stand 28).

P. asiatica and *P. major* can identified by the morphological differences in their seeds (Table 1; after Matsuo, 1989), therefore surveys were conducted in the seed-maturing season (late August to early November). In Sapporo and its vicinity, 143 sites were surveyed. About 30 *Plantago* individuals were collected at each site and the morphological features of seeds in each individual were observed by binocular.

In addition, the same sampling method was used for the survey at 28 sites situated along ca.7.5km long road through Mt. Moiwa, along which the degree of human impact gradually increases from natural woodlands to urban communities, forming a gradient. As a preliminary study on the elements controlling the separate distribution of the two species, the following factors were measured at 103 1mx1m quadrats randomly chosen in Sapporo and its vicinity: plant coverage and vegetation height, i.e., the mean height of dominant plant species; relative light intensity; and ground hardness (penetration resistance).

Results

Of 143 sites examined, 62 were dominated by *P. major*, 26 by *P. asiatica* and 55 shared by these two plantain species. The urban areas and farmlands were predominantly covered by *P. major*, while the open spaces of woodlands were monopolized by *P. asiatica*, both species inhabiting roadsides , parking sites, gardens, turf, and other open spots. The stands shared by these two species were mainly on the border between woodlands and housing lots. This patter of distribution was also observed along the 7.5km long road (Fig.1), *P. major* predominantly growing in more developed areas and *P. asiatica* predominating in woodland areas. Such recreation facilities as ski grounds and some amusement parks have been constructed in woodlands areas of Mt. Moiwa (Stands 12 to 18).

The environmental conditions at 103 sites are shown in Fig.2. *P. major* is evidently a species of sparser and shorter plant communities developed on harder ground than *P. asiatica*, which prefers relatively closed communities. The stands shared by those two species showed intermediated values of vegetation height, relative light intensity and ground hardness. And the coverage was the highest in the coexisting stands.

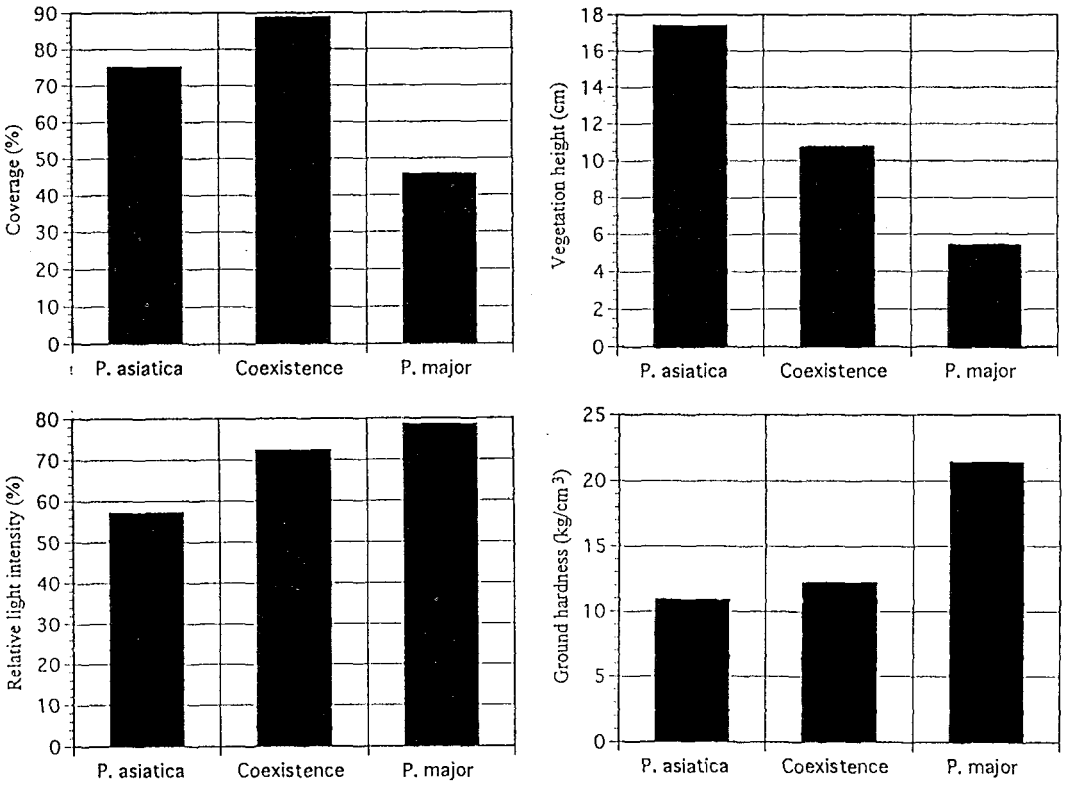


Fig. 2. Environmental conditions at 1m × 1m stands inhabited by *P. asiatica*, both species (coexistence) and *P. major*.

Discussion

The segregated distribution of *P. major* from its related native species *P. rugelii* was observed in Canada (Hawthorn, 1974). Hawthorn and Cavers (1978, 1982) revealed that more resources are allocated to reproductive organs such as scapes, capsules and seeds in *P. major*, but to the root system in *P. rugelii*, suggesting that *P. major* is more competitive in urban areas, because seed recruitment rate is critical for population recovery when habitats are frequently disturbed by human activities. In *P. major* of England, form with erect leaves is more competitive in wild grasslands than another form with prostrate leaves that usually dominates garden and lawns (Warwick, 1980). However, *P. major* and *P. asiatica* do not show sharply segregated distribution when they coexist within a small microsite. This fact suggest that segregated distribution of these two species may not be due to direct competition through shading but be due to more complicated mechanisms, probably including the seed recruitment rate and tolerance to various other environmental factors.

In addition to *Plantago* species here reported, somewhat similar cases of habitat segregation between closely related plant species have been known in *Taraxacum* (*T. officinale* and native species such as *T. japonicum*, *T. platycarpum* and *T. hondoense*: Naito ,1975; Hotta, 1978; Sawada et al., 1982; Ogawa & Mototani, 1985) , *Artemisia* (*A. rubripes* and *A. montana*: Nakayama, 1985) and *Cardamine* (*C. flexuosa* and *C. hirsuta*: Kudoh et al., 1992) in Japan. In particular, *T. officinale* has rapidly been expanding its range all over the Japan, taking over all possible habitats (Hotta, 1978). Sawada et al. (1982) demonstrated that *T. officinale* extends its leaves more quickly than related native species, suggesting that *T. officinale* is more competitive because it shades the natives. However, according to Ogawa and Mototani (1985), such direct competition is unlikely, and *T. officinale* outcompetes when they coexist with related natives. The mechanisms by which the introduced plant species frequently can dominate the related natives could be clarified by more detailed comprehensive studies on the physiological and ecological aspects of these species.

References

Hawthorn, W.R. 1974. The biology of Canadian weeds. Ⅳ. *Plantago major* and *P. rugelii*. Can. J. Pl. Sci. **54**: 383-396.

Hawthorn, W.R. and Cavers, W.R. 1978. Resource allocation in young plants of two perennial of Plantago.Can.J. Bot. **56**: 2533-2537.

Hawthorn, W.R. and Cavers, W.R. 1982. Dry weight and resource allocation patterns among individuals in populations of *Plantago major* and *P. rugelii*. Can. J. Bot. **60**: 2424-2439.

Fujiwara, I. 1957. First report of Plantago major L. distribution in Hokkaido. Kagaku, **27**: 40-41. Iwanamishoten, Tokyo (in Japanese).

Hotta, M. 1978. On the distribution of indigenous dandelions (Taraxacum) in the Kansai district. Nankiseibutsu **20**:1-6 (in Japanese).

Ito. K. 1984. New localities of Hokkaido plants (2). Jour. Jap. Bot. **59**: 189-190 (in Japanese).

Kawano, S. and Matsuo, K. 1982. Studies on the life history of the genus Plantago. Ⅰ. Reproductive energy allocation and propagule output in wild population of a ruderal species, Plantago asiatica L., extending over a broad altitude gradient. J. Coll. Lib. Arts. Toyama Univ. **16**: 85-112.

Kudoh, H., Ishiguri, Y. and Kawano, S. 1992. *Cardamine hirsuta* L. a new ruderal species introduced into Japan. J. Phytogeogr. & Taxon. **40**: 85-89.

Matsuo. K. 1989. Biosystematic studies on the genus *Plantago* . Ⅰ. Variations in *Plantago japonica* and its related species, with special reference to its identity. Acta. Phytotax. Geobot. **40**: 37-60 (in Japanese with English summary).

Naito, T. 1975. Notes on the ecesis of *Taraxacum* plants. Biol. Sci. Tokyo. **27**: 195-202 (in Japanese).

Nakayama, S. 1985. Reproductive characteristics under competitive conditions in some Artemisia species. Shuseibutsugakukenkyu. **9**: 85-108 (in Japanese).

Ogawa, K. and Mototani, I. 1985. Invasion of the introduced dandelion and survival of the native ones in the Tokyo metropolitan area of Japan. Japan J. Ecol. **35**: 443-452.

Osada, T. 1976. Colored illustrations of naturalized plants of Japan. 425pp. Hoikusha, Osaka. (in Japanese).

Sager, G.R. and Harper, J.L. 1964. Biological flora of the British Isles. Plantago major L. J. Ecol. **54**: 189-205.

Sawada, S. , Takahashi, S. and Kasaishi, Y. 1982. Population dynamics and production processes of indigenous and naturalized dandelions subjected to artificial disturbance by mowing. Jap. J. Ecol. **32**: 143-152.

Warwick, S. I. 1980. The genecology of lawn weeds. V. The adaptive significance of different growth habit in lawn and roadside populations of Plantago major L. New Phytol. **85**: 289-300.

Effect of Rice Cultural Practices on Emergence of *Echinochloa crus-galli* and *Monochoria vaginalis*

J. S. Kim[1] and J. C. Chun[2]
[1]Department of Life Resource Science, Woosuk University, Wanju 565-800, Korea
[2]Department of Agricultural Chemistry, Jeonbug National University, Jeonju 560-756, Korea

Abstract. Seed occurrence patterns of *Echinochloa crus-galli* (L.) P. Beauv. and *Monochoria vaginalis* (Burm. f.) Presl. were investigated in irrigated and rainfed rice cultures. There was not much difference in seed population of *E. crus-galli* between irrigated and rainfed fields, but *M. vaginalis* occurred in about 1.6-fold greater number in rainfed fields. In seed distribution patterns under the two conditions, about 50% and 30% of *E. crus-galli* and *M. vaginalis*, respectively, were found in the uppermost 5 cm layer. Under both conditions *M. vaginalis* was distributed to a depth of 25 cm, but distribution of *E. crus-galli* was only 15 cm deep. Early rice transplanting brought about greater emergence of the two weeds than optimal or late transplanting. Emergence of the weeds was significantly greater in single cropping system of rice than in double cropping systems of rice followed by barley and/or strawberry.

Key words: Seed population, Rice cultural practice, *Echinochloa crus-galli, Monochoria vaginalis*

INTRODUCTION

Rice cultural practice in Korea has been changed from hand-transplanting in 1970s to machine-transplanting by the late 1980s and in turn to direct-seeding since the early 1990s. Moreover, double-cropping system becomes more prevalent. This trend results from strong demand for establishing labor-saving rice cultural practice and utilizing effectively the limited land. Consequently weed occurrence in rice field has varied with the cultural practice accepted. Intensive use of annual herbicides has given rise to heavy infestation of perennial weeds in machine-transplanting rice field[1)]. The weed problem, however, gradually returns again to annual weeds in direct-seeded rice[4)].

The present study, therefore, was undertaken to determine difference in seed population of *E. crus-galli* and *M. vaginalis* between irrigated and rainfed paddy soils and to investigate emergence pattern of the weeds due to different transplanting dates and cropping systems.

MATERIALS AND METHODS

Seed Population.

Seed reserve and vertical distribution of *E. crus-galli* and *M. vaginalis* were determined in two different paddy conditions. Soil types of irrigated and rainfed paddy fields were clay and sandy clay loam, respectively, while such other soil characteristics as pH and organic matter content were not quite different between the two soils. Water condition of the two fields has kept without change for more than 10 years. In each condition 5 locations were randomly selected to determine seed population, and ten soil cores, 15-cm diameter and 15-cm deep, were taken after harvest in 1993. The cores were bulked and the soil was thoroughly mixed. Sub samples of 100 g were taken from each bulked sample. There were two sub samples for each field. To investigate the vertical distribution of seeds, ten soil cores, 8-cm diameter and 30-cm deep, were taken and sectioned every 5-cm. The same horizon from each sample were bulked to give five replicates of the six horizons.

The sub samples were air-dried and thoroughly pulverized. Weed seeds were separated by placing each sub sample in 60-mesh brass sieve and washing it under a running stream of tap water to remove all silt, clay and fine sand. The remaining material was washed into 1-L beaker filled with water. After settling the floating matter was decanted into a 6- by 8-cm fine mesh nylon bag. This step was repeated until no material floated. The samples were dried and seeds were counted with the aid of a binocular microscope.

Seeds were first incubated for 30 days in a laboratory germinator operated at 12-h day/12-h night temperature regimes of 30/25°C and the number of germinated seed was recorded. Viability of the remaining seeds was then determined using 2,3,5-triphenyltetrazoliumchloride (TTC) according to the method of Eagly and Chandler[2)]. Total numbers of viable seed consisted of the sum of the germinated seeds with the non-germinated seed stained pink to red with TTC.

Emergence Pattern.

Effects of transplanting dates and cropping systems on emergence pattern of *E. crus-galli* and *M. vaginalis* were investigated in the above irrigated rice field. Rice transplanting dates employed were May 12, May 26, and June 9 as early, optimal, and late transplanting timing, respectively. Cropping systems used were rice only, rice followed by barley, and rice followed by strawberry. The cultural practices were conducted in 1992 and 1993. Four fields were selected and a quadrate area of 0.5- by 0.5-m was established in four locations from each field. During the rice growing period standing water was maintained at a depth of 5-cm. Emerging weeds at the marked quadrate were counted at 2-week intervals. No herbicide was applied and frequent hand weeding outside the marked quadrates was done during the experimental period.

RESULTS AND DISCUSSION

Seed Bank

Seed reserves of *E. crus-galli* and *M. vaginalis* varied with water management condition in rice field. There was not much difference in seed population of *E. crus-galli* between irrigated and rainfed fields, but *M. vaginalis* occurred in about 1.6-fold greater number in rainfed field than in irrigated field (Fig. 1). In both conditions *M. vaginalis* was about 4- to 5-fold greater in number of seeds than *E. crus-galli.*

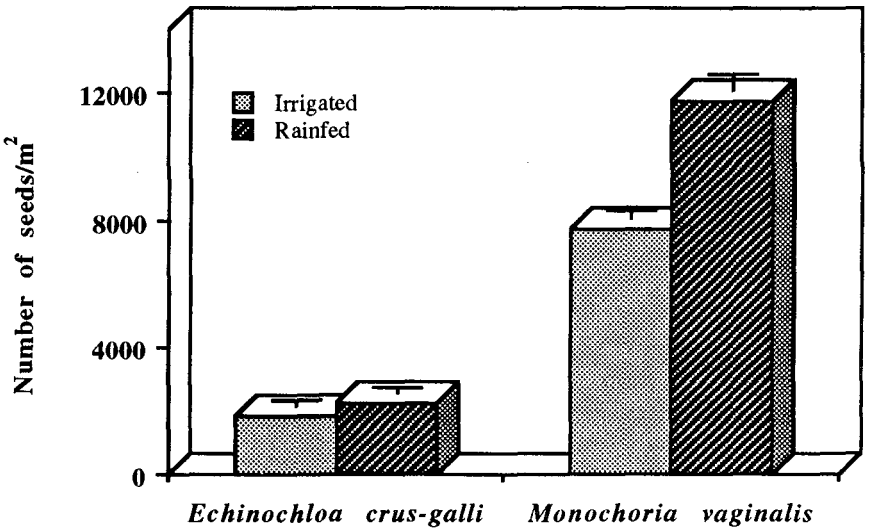


Fig. 1. Weed seed population as affected by water condition in rice field.

Vertical distribution of the two weeds in soil was similar between irrigated and rainfed fields, but the distribution pattern varied with the weed species involved (Fig. 2). About 50% and 30% seeds of *E. crus-galli* and *M. vaginalis*, respectively, were found in the uppermost 5-cm layer. On the other hand, seed occurrence of *E. crus-galli* was restricted at the depth of 15-cm, while *M. vaginalis* was found at 25-cm deep. Most of *E. crus-galli* seeds were present in plowing layer. This finding suggests that *E. crus-galli* emerges within a short period of cropping season, while occurrence of *M. vaginalis* may be erratic during a relatively longer period.

Effect of Transplanting Date

Transplanting date of rice affected emergence pattern of *E. crus-galli* and *M. vaginalis* (Fig. 3). *E. crus-galli* emerged for 4 weeks after transplanting (WAT) was about 69% of the total emergence when rice was transplanted at 2 weeks earlier than the optimal or conventional transplanting date. As the transplanting date was delayed, however, the number of occurrence for 4 WAT increased to about 86% and 83% at the optimal and late transplanting, respectively. In *M. vaginalis* more than 85% emergence of the total number was obtained for 10, 8, and 8 WAT at early, optimal, and late transplanting date,

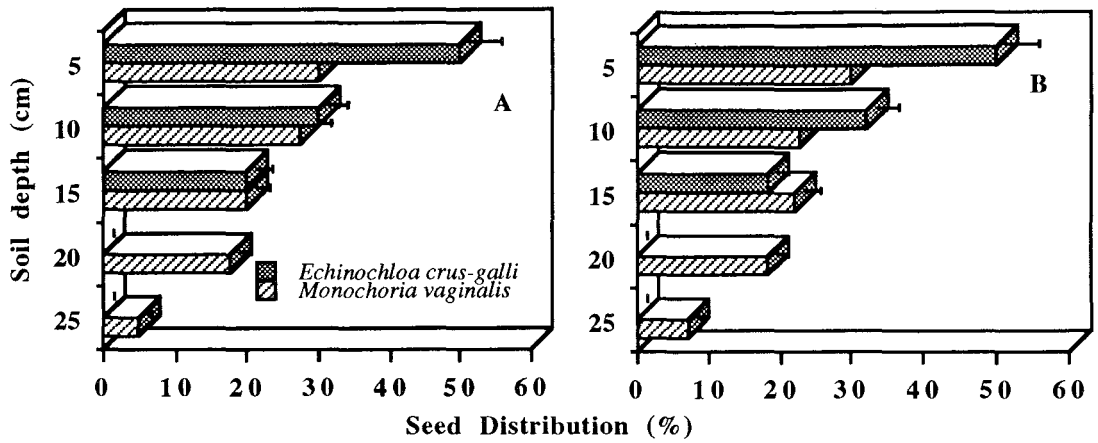


Fig. 2. Distribution of weed seeds at different soil depths in irrigated (A) and rainfed (B) fields.

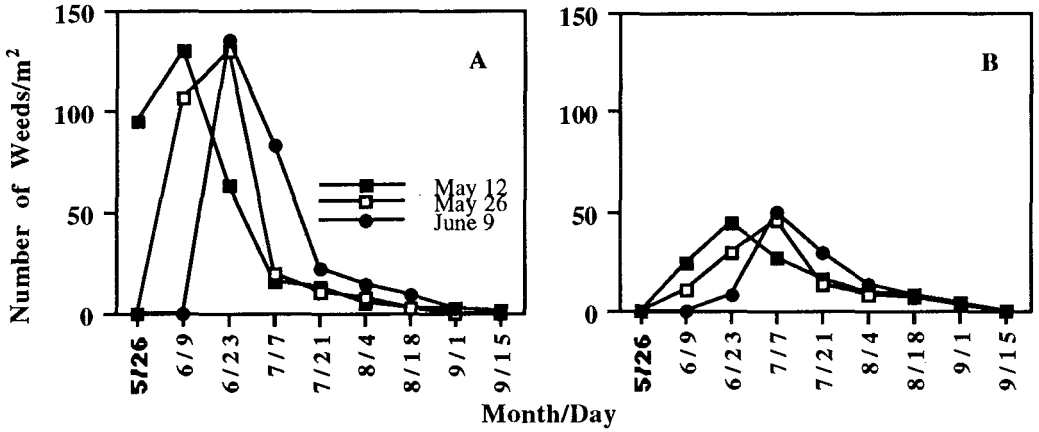


Fig. 3. Emergence pattern of *Echinochloa crus-galli* (A) and *Monochoria vaginalis* (B) as affected by rice transplanting date.

respectively. This indicated that occurrence period of *M. vaginalis* after transplanting was longer than *E. crus-galli*.

As the transplanting date was delayed, total number of emergence throughout the cropping season decreased in both the weeds. *E. crus-galli* emerged at the optimal and late transplanting dates decreased by about 16% and 20%, respectively, as compared with at early transplanting date. A similar trend was also found in *M. vaginalis*, but decrease in emergence due to the delayed transplanting was about 11% and 14%, respectively. Increase in emergence at early transplanting was attributed to increase in the overall cropping period.

Effect of Cropping System

In the experimental field dominant weed species were mostly annual weeds. In 1992 community dominance was 0.72 and the value did not greatly changed in the following year (Table 1). For the two years importance value ranged from 40 to 41% on *E. crus-galli* and from 33 to 36% on *M. vaginalis*. The minor weeds such as *Eleocharis acicularis* showed less than 8% of importance value. Dominant occurrence of annual weeds in recent is considered to result from intensive use of sulfonylurea herbicides which are effective to perennial sedges, but ineffective to grasses.

Emergence of *E. crus-galli* and *M. vaginalis* varied with cropping system employed. Both the weeds emerged significantly greater number in rice monoculture than in double cropping systems (Fig. 4). However, emergence of *E. crus-galli* was about 2.4-fold greater than *M. vaginalis*, regardless of the

Table 1. Community dominance and importance value of major weeds in paddy rice field studied.

Year	Community dominance	Major weed	Importance value (%)
1992	0.72	*Echinochloa crus-galli*	41
		Monochoria vaginalis	33
		Eleocharis acicularis	8
		Aneilema japonica	4
		Ludwigia prostrata	4
		Cyperus difformis	3
		Leersia japonica	2
1993	0.67	*Echinochloa crus-galli*	40
		Monochoria vaginalis	36
		Eleocharis acicularis	7
		Aneilema japonica	5
		Ludwigia prostrata	5
		Sagittaria pygmaea	3
		Rotala indica	3

cropping patterns employed. Decrease in emergence of the weeds in double cropping systems was due to increased land disturbance by crop rotation. A similar trend was reported by Guh et al.[3)]

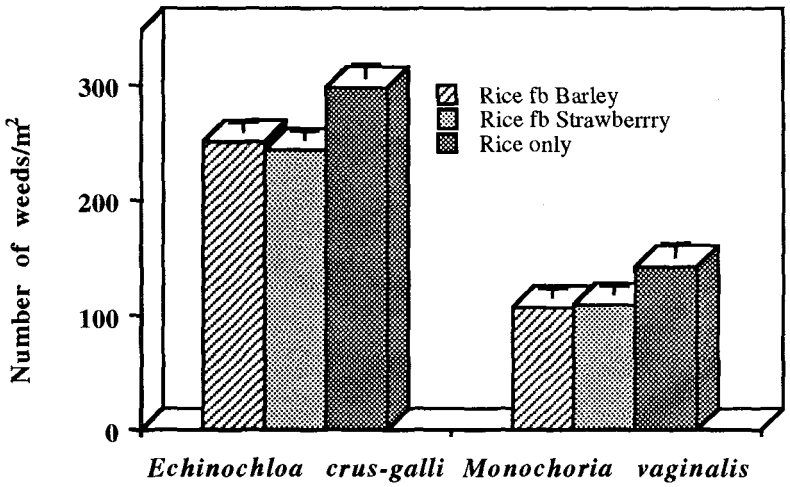


Fig. 4. Weed emergence in paddy field as affected by cropping system.

ACKNOWLEDGMENT: The research grant given by Korea Science and Engineering Foundation (893-1504-017-2) is gratefully acknowledged.

REFERENCES

1. Choi, C. D., S. C. Kim, and D. Y. Hwang. 1989. Effect of repeated use of same herbicide on weed growth in lowland rice. Kor. J. Weed Sci. 9:39-45.
2. Egley, G. H. and J. M. Chandler. 1978. Germination and viability of weed seeds after 2.5 years in a 50-year buried seed study. Weed Sci. 26:230-239.
3. Guh, J. O., S. L. Kwon, and S. M. Heu. 1983. Differential weed competition of two rice cultivars under various cropping patterns. Kor. J. of Weed Sci. 3:57-68.
4. Ryang, H. S. and J. S. Kim. 1992. The status, problems and countermeasure of direct rice seeding in Honam province. - On weed control. Kor. J. of Weed Sci. 12:271-291.

Species Coexistence through Shoot Morphological Performances

H. Ikeda, T. Ohkuro and M. Nemoto
National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki 305, Japan

Abstract. Shoot morphological response to intra- and interspecific interactions between orchard grass (*Dactylis glomerata* L.) and bird's-eye (*Veronica persica* Poir.) was examined in greenhouse experiments. We measured the shoot growth and morphology of one target plant for each species, surrounded by six conspecific (monoculture) or heterospecific (mixed culture) neighbours planted at different distances from the target plant. There were no significant effects of either species of neighbour or distance between plants on the above-ground dry mass of target plants, in both species. However, the mean stem length of *D. glomerata* targets was significantly greater in monoculture than in mixed culture, resulting in taller plants when they were set up with conspecific neighbours. *V. persica* targets showed a significant change in the coefficient of variation for the angles between terminal internodes and the soil surface, indicating the fact that this angle varied more in mixed culture than in monoculture. These findings suggest that the plastic responses of plant form to the identity of neighbouring species allow plants to achieve comparable biomass, playing an important role in coexistence between these species.

Key words. coexistence, morphological plasticity, plant form, shoot growth, species identity.

Introduction

Plant form can vary according to the growing environments (e.g. Waller 1986). Many workers have reported morphological responses to environmental stress (e.g. Salzman & Parker 1985), disturbance such as herbivory (e.g. Crawley 1983) and locally rich patches of resources (e.g. Hutchings & Slade 1988). There are also several studies that explore adaptive interpretations of the plant form (e.g. Givnish 1982).

Species interactions are affected by the spatial distribution of individual plants and their plant form. As Tremmel and Bazzaz (1993) pointed out, neighbour canopies show species-specific architecture. However, little is known about plant morphological responses to spacing and identity of neighbouring species. Thus we carried out experimental studies to compare the shoot growth patterns and morphology of target plants, surrounded by conspecific and heterospecific neighbours planted at different distances from the target plants. In this paper, we focus on two questions: (1) How plants respond morphologically to the spacing and identity of neighbouring species; and (2) What an ecological significance of their morphological performances is in response to conspecific and heterospecific neighbours.

Materials and Methods

We used orchard grass (*Dactylis glomerata* L.) and bird's-eye (*Veronica persica* Poir.); these species often co-occur in young meadows in Japan (Nemoto 1982). *D. glomerata* is a perennial tussocky pasture grass, and *V. persica* is a winter annual, herbaceous weed in arable land, gardens and disturbed sites such as roadsides. The latter species usually has a prostrate growth form (Boutin & Harper 1991). Both species are very common in the temperate region of the world.

Seeds of each species were germinated in a greenhouse in late December 1990, in a plastic container (47 cm long, 32 cm wide, 6 cm deep) filled with granulated loam. Seedlings of comparable size (5 cm long for *D. glomerata* and 1 cm long for *V. persica*) were transplanted to fresh plastic containers (47 cm x 32 cm x 6 cm deep) filled with an artificial soil composed of vermiculite and granulated loam (1:1 by volume) on 23 January 1991. The initial artificial soil in each container contained about 1.4 gN, 1.7

gP, 1.4 gK and 0.4 gMg. Each container was set up with six neighbouring plants of a single species surrounding a central target plant. The neighbours were planted at a distance of either 5 cm or 10 cm from the target plant. The plants were set up in both monoculture and two-species mixed culture with three replicates of spacing between plants. All plants were kept well watered every two days but not fertilized.

On 20-26 April 1991, we measured the three-dimensional position of nodes (for *V. persica*) and stems (for *D. glomerata*) for the target plants. Since some plants of *V. persica* fruited at that time, we harvested the aboveground parts of the target plants, separated them into leaves, stems and inflorescence, and weighed them after drying at 85°C for more than 48 h. Then we calculated node (tiller for *D. glomerata*) number, mean internode (stem for *D. glomerata*) length, coefficient of variation (C.V.) for the internode lengths, the mean angle (radian) between terminal internodes (> 5 cm long) and the soil surface, and the C.V. for the angles of terminal internodes (> 5 cm long) for the target plants.

Analysis of variance (ANOVA) tests were performed on the raw and log or square-root transformed parameters as necessary to obtain the homogeneity of variances using Bartlett's tests. When the homogeneity of variances was still not obtained in these transformed parameters, we used the rank transformation for ANOVA (Thompson 1991).

Results

There were no significant effects of either species of neighbour, or distance between plants, on the total aboveground dry mass of target plants for *D. glomerata* and *V. persica*. Most target plants of *V. persica* flowered, but only one tiller of a *D. glomerata* target plant flowered (it was in mixed culture, with neighbours 5 cm distant) in this experimental period. For both target species, there were also no significant effects of either species of neighbour, or distance, on the dry mass of leaves, stems and inflorescences.

However, the mean number of tillers of *D. glomerata* was significantly lower when neighbours were 5 cm away, than when they were 10 cm distant (Fig. 1; $P<0.05$), but it did not matter which species of neighbour was present (Fig. 1). There were no significant effects of species of neighbour, or distance between plants, on the mean number of nodes of *V. persica* (Fig. 1).

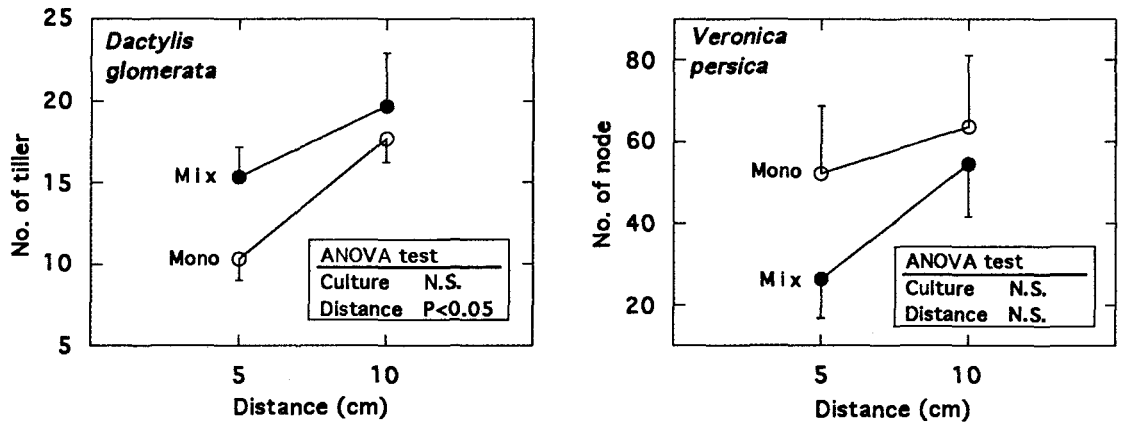


Fig. 1 Effects of culture and distance between plants on mean tiller and node numbers (plant^{-1}) for target plants of *Dactylis glomerata* and *Veronica persica*, respectively. Error bars indicate 1 SE ($n = 3$).

Stems of *D. glomerata* were significantly longer in monoculture than in mixed culture (Fig. 2; $P<0.05$) and there was also a significant interaction between species of neighbour and distance (Fig. 2, $P<0.05$). There were no significant effects of species of neighbour, or distance, on the mean internode length of *V. persica* (Fig. 2). In addition, for both species, there were no significant effects of species of neighbour or distance on the coefficient of variation (C.V.) of internode (stem for *D. glomerata*) lengths within a plant. However, the C.V. of *V. persica* was significantly greater than that of *D. glomerata* ($P<0.001$).

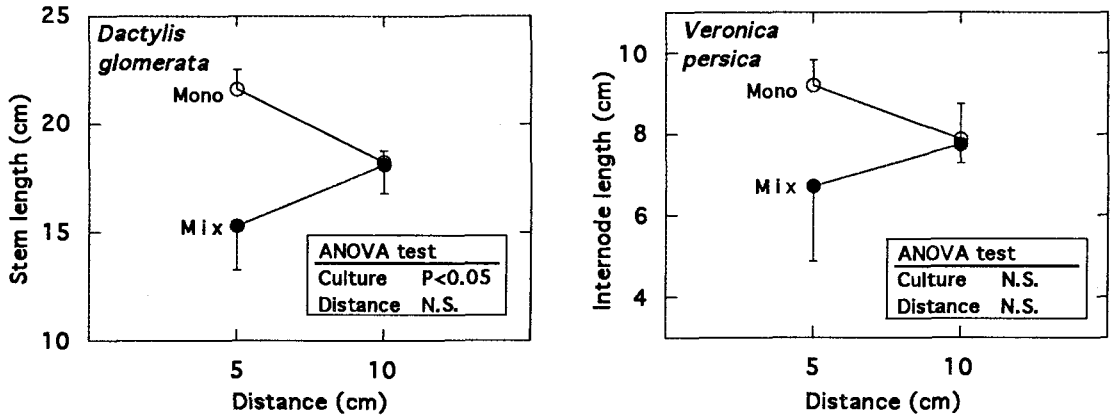


Fig. 2 Effects of culture and distance between plants on mean stem and internode lengths (cm) for target plants of *Dactylis glomerata* and *Veronica persica*, respectively. Error bars indicate 1 SE ($n = 3$).

The mean angle between stems and the soil surface was significantly lower for *D. glomerata* when neighbours were 5 cm away, than when 10 cm distant (Fig. 3; $P<0.01$), but there were no significant effects of species of neighbour (Fig. 3). In *V. persica*, there were no significant effects of species of neighbour or distance on the mean angle between terminal internodes (> 5 cm long) and the soil surface (Fig. 3). However, this species showed a significant change in C.V. for angles between terminal internodes (> 5 cm long) and the soil surface, with significantly greater variation evident in mixed culture than in monoculture (Fig. 4; $P<0.05$). This effect was not seen in *D. glomerata* (Fig. 4).

Discussion

No significant differences in the shoot biomass of target plants for *D. glomerata* and *V. persica* according to either species of neighbours or their distance from the targets indicate that the plants were not sufficiently crowded to show diminished growth. However, the closer neighbours of either species significantly decreased the number of tillers of *D. glomerata* targets (Fig. 1). This confirms previous studies with the different plant spacing in regular square arrangement of 30.5 and 61.0 cm apart for three years experiments (Lambert 1963), and of 3.3, 5.0 and 7.7 cm apart for 63 - 84 days experiments (Terai & Kanda 1978), but few studies have examined the effects of spacing on tiller production when plants are set up at an equal density. Our result suggests that the decrease in tiller number was simply caused by the reduction in space available for target shoots to extend.

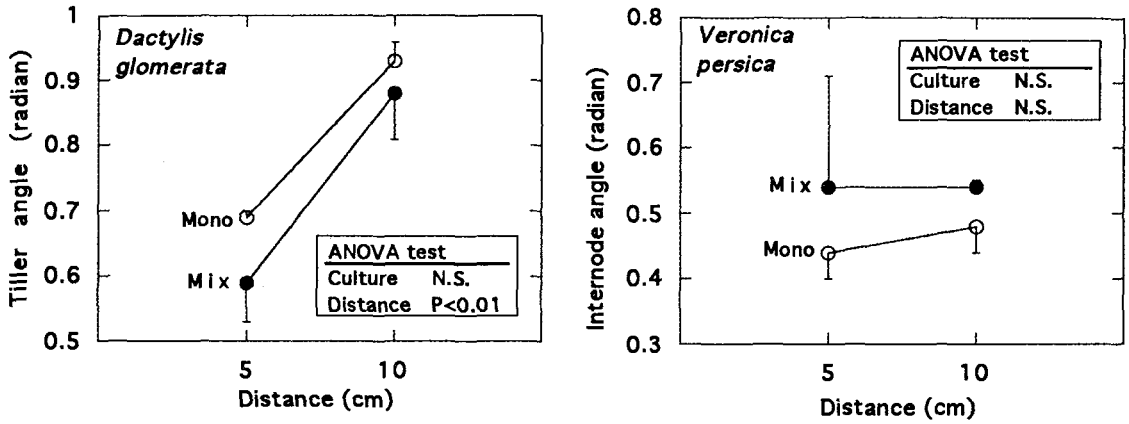


Fig. 3 Effects of culture and distance between plants on mean angle (radian) between tillers of target plants for *Dactylis glomerata* (or terminal internodes, >5 cm, for *Veronica persica*) and the soil surface. Error bars indicate 1 SE (n = 3).

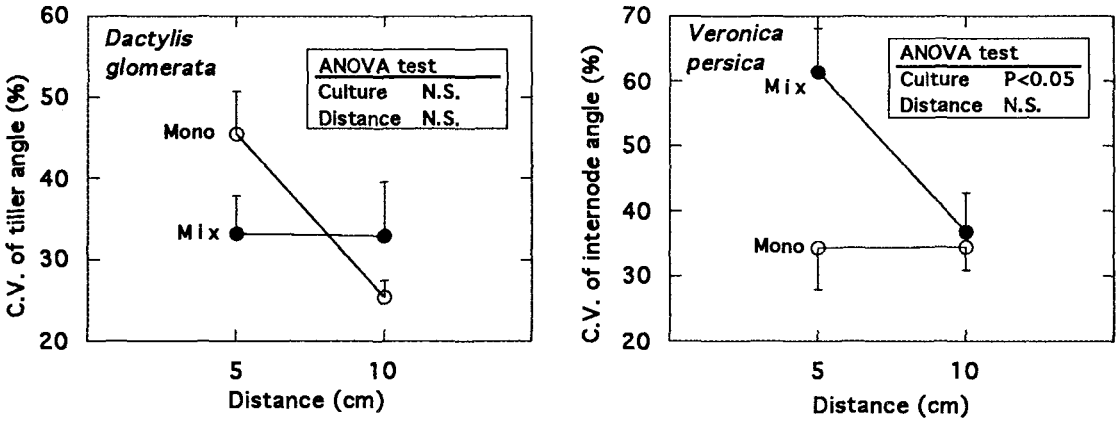


Fig. 4 Effects of culture and distance between plants on mean coefficient of variation (%) of angles between tillers of target plants for *Dactylis glomerata* (or terminal internodes, >5 cm, for *Veronica persica*) and the soil surface. Error bars indicate 1 SE (n = 3).

When neighbours of both species were closer, there was a decrease in the angle between tillers of *D. glomerata* targets and the soil surface (Fig. 3). Since the many shoots of neighbouring plants were observed on the tillers of target plants, one interpretation of the reduced tiller angle may be that the neighbouring shoots leant on the target tillers and weighted them. Few studies have investigated the angle between tillers and the soil surface, but a lower tiller angle (and therefore reduced plant height) may indicate inferior competitive ability (e.g. Mitchley & Grubb 1986, Gaudet & Keddy 1988). Thus the change in tiller angle may have ecological significance for competitive interactions.

Contrary to our results, many previous studies have described the species-specific effects of neighbours on the biomass of target plants (Goldberg & Fleetwood 1987, Gurevitch *et al.* 1990,

Tremmel & Bazzaz 1993). We found, however, some interesting morphological responses. *D. glomerata* had longer stems when set up with conspecific neighbours than with heterospecific ones, resulting in a taller plant (Fig. 2). Conspecific neighbours are usually stronger competitors than heterospecific ones because of greater overlap in resource requirements, but the taller plants are favoured in competition for light (Givnish 1982) and so could avoid the growth suppression. On the other hand, there were no similar effects on internode length in *V. persica*, which has a prostrate growth form. In this latter species, longer internodes do not result in a taller plant, but a more extensive one. Similarly, Lovett Doust (1987) revealed that stolon internode lengths of *Ranunclus repens* L. were not significantly affected by light (full or 66% relative intensity) and nutrient (twice or one-fifth full strength Long Ashton Nutrient Solution, applied every two days) treatments.

Higher variation in the terminal shoot angle of target *V. persica* plants set up with *D. glomerata* (Fig. 4) also indicate the influence of the identity of heterospecific neighbours on the shoot morphology. Similarly, the quality of transmitted radiation resulting from contrasting canopies of pasture grasses affected the growth and morphology of *Trifolium repens* L. (Thompson & Harper 1988), and *Portulaca oleracea* L. seedlings avoided growing towards their neighbours by sensing the spectral composition (red/far-red ratio) of light (Novoplansky *et al.* 1990). Although we have no data on either the light quality or photon flux density in each neighbour canopy, it may be reasonable to infer that the higher canopies of the tussocky grass (as opposed to the prostrate herb) may result in more patchy light conditions. Thus the shoot behaviour of *V. persica* may reflect the patchy shading caused by neighbouring canopies of *D. glomerata*.

It is generally suggested that taller tussocky plants will be favoured in conspecific competition leading to an increase in competitive performance. This means that the conspecific individuals can coexist through symmetric competition. In contrast, more variable directions of prostrate shoot growth allowing avoidance of competition will be favoured under heterospecific canopies. These flexible morphological behaviours may allow target plants to achieve similar shoot biomass irrespective of neighbour species identity. It seems that morphological plasticity to avoid shoot competition will bring about neutralism between neighbouring individuals; further studies of the nature of plasticity of plant form will help us to understand mechanisms of competitive interactions and coexistence in plant communities.

In conclusion, our findings suggest that the shoots of *D. glomerata* and *V. persica* respond in a morphologically different way to conspecific and heterospecific neighbours, and their plasticity of plant form may play an important role in coexistence between these species.

Acknowledgement: We thank Y. Yamagata, National Institute for Environmental Studies, for his valuable advice, and the members of Research Institute of the Japan Association for Advancement of Phyto-regulators, for giving us the seeds of *V. persica*. This study was supported by the Science and Technology Agency of Japan.

References

Boutin, C. & J. L. Harper, 1991. A comparative study of the population dynamics of five species of *Veronica* in natural habitats. *Journal of Ecology*, 79: 199-221.

Crawley, M. J., 1983. *Herbivory. The dynamics of animal-plant interactions*. Blackwell Scientific, Oxford, London, 437 p.

Gaudet, C. L. & P. A. Keddy, 1988. A comparative approach to predicting competitive ability from plant traits. *Nature*, 334: 242-243.

Givnish, T. J., 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist*, 120: 353-381.

Goldberg, D. E. & L. Fleetwood, 1987. Competitive effect and response in four annual plants. *Journal of Ecology*, 75: 1131-1143.

Gurevitch, J., P. Wilson, J. L. Stone, P. Teese & R. J. Stoutenburgh, 1990. Competition among old-field perennials at different levels of soil fertility and available space. *Journal of Ecology*, 78: 727-744.

Hutchings, M. J. & A. J. Slade, 1988. Morphological plasticity, foraging and integration in clonal perennial herbs. Pages 83-109 *in* A. J. Davy, M. J. Hutchings & A. R. Watkinson (eds.). *Plant population ecology*. Blackwell Scientific, Oxford, UK.

Lambert, D. A., 1963. The influence of density and nitrogen in seed production stands of S 37 cocksfoot (*Dactylis glomerata* L.). *Journal of Agricultural Science*, 61: 361-373.

Lovett Doust, L., 1987. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). III. Responses to light and nutrient supply. *Journal of Ecology*, 75: 555-568.

Mitchley, J. & P. J. Grubb, 1986. Control of relative abundance of perennials in chalk grassland in southern England: I. Constancy of rank order and results of pot- and field-experiments on the role of interference. *Journal of Ecology*, 74: 1139-1166.

Nemoto, M., 1982. Dynamics of weed community in meadow in its early stage of establishment. *Bulletin of Institute for Agricultural Research, Tohoku University*, 33: 81-91.

Novoplansky, A., D. Cohen & T. Sachs, 1990. How portulaca seedlings avoid their neighbours. *Oecologia* (Berlin), 82: 490-493.

Salzman, A. G. & M. A. Parker, 1985. Neighbours ameliorate local salinity stress for a rhizomatous plant in a heterogeneous environment. *Oecologia* (Berlin), 65: 273-277.

Terai, K. & M. Kanda, 1978. Mortal response and growth of individual plants in a population of orchard grass (*Dactylis glomerata* L.). II. Aggression of weedy species and tiller mortality of orchard grass. *Journal of Japanese Society of Grassland Science*, 4: 319-324.

Thompson, G.L. 1991. A note on the rank transform for interactions. *Biometrica*, 78: 697-701.

Thompson, L. & J. L. Harper, 1988. The effects of grasses on the quality of transmitted radiation and its influence on the growth of white clover *Trifolium repens*. *Oecologia* (Berlin), 75: 343-347.

Tremmel, D. C. & F. A. Bazzaz, 1993. How neighbour canopy architecture affects target plant performance. *Ecology*, 74: 2114-2124.

Waller, D. M., 1986. The dynamics of growth and form. Pages 291-320. *in* M. J. Crawley (ed.). *Plant ecology*. Blackwell Scientific, Oxford, UK.

PATH ANALYSIS OF TWO SYMPATRIC GRAMINOIDS (*ECHINOCHLOA CRUS-GALLI* SSP. *CRUS-GALLI* (L.) BEAUV. AND *ISCHAEMUM RUGOSUM* SALISB.) IN COMPETITION WITH RICE (*ORYZA SATIVA* L. VAR. MR84)

B. B. Baki, [a*], S. Suhaimi,[a] and J. Abdul Munir[b]
[a]Botany Department, University of Malaya 59100 Kuala Lumpur, Malaysia and
[b]Statistical Unit, MARDI, GPO Box 12301, 50774 Kuala Lumpur.

Abstract. A series of pot experiments was conducted in an insect-proof house at the Institute of Advanced Studies, University of Malaya, Kuala Lumpur in 1993-1995 to evaluate the differential competitive ability of two sympatric graminoids (*Echinochloa crus-galli* ssp. *crus-galli* (L.) Beauv. and *Ischaemum rugosum* Salisb.) with rice (*Oryza sativa* L. var. MR84) and the causal relationships between grain yield components using path analysis. The analysis quantified direct effect of the graminoids and MR84 rice densities on the yield components (grain weight, number of filled and unfilled grains, number of grains/ panicle, number of panicles/ plant (=genet), number of reproductive and non-reproductive tillers) of weedy graminoids and MR84. The path analysis model illustrated the direct and indirect effects of yields components on fecundity and grain yield/plant. The direct effects of MR84 and weedy graminoid densities on the number of panicles/plant and number of seeds/plant were always positive. Conversely, the effects of density on the number of tillers/plant, grain weight/plant, percent filled panicles/plant varied from positive to negative reflecting the unequal influence of density dependent factors on such parameters. The analysis indicated that the number of tillers/plant and the number of panicle/plant were the key yield components determining the response of fecundity and grain yield to crop-weed competitions. *Echinochloa crus-galli* and *Ischaemum rugosum* in competition with rice var MR84 showed significant differences in path coefficients for each yield component recorded, reflecting the differential competitive ability of the two weedy graminoids against the rice crop.

Keywords: *Oryza sativa* L. var MR84, *Echinochloa crusgalli* (L.) Beauv., *Ischaemum rugosum* Salisb., correlation coefficient, partitioning, direct effects, indirect effects, interference, path coefficients, yield components, regression analysis, weed competition, weed density.

INTRODUCTION

A number of empirical models and methods have been developed to study rice yield responses to weed and crop density (Yoshida and Parao, 1976; Wells and Faw, 1978; Cousens, 1985; Cousens *et al.*, 1978; Spitters *et al.*, 1989; Yamada, 1961; Counce, 1987; Jones & Snyder, 1987; Counce *et al.*, 1989; Kropff *et al.*, 1992; Weaver *et al.*, 1992). While additional studies have established that crop density can be a critical factor influencing the rice yield losses to weeds (Dunand, 1988; Pantone and Baker, 1991a,b), weed density alone, may not be a reliable predictor of crop yield due to variability in size and spatio-temporal pattern of emergence. This is especially so when weeds emerge in discrete flushes (Kropff *et al.*, 1992). To alleviate these limitations (Kropff & Spitters, 1991; Spitters, 1983, 1984 and 1989) among others, recently developed dynamic and mechanistic simulation models of crop- weed competition which relate crop yield losses to duration and critical time of weed competition (Kropff *et al.*, 1992; Weaver *et al.*, 1992).

The impact of weed and crop density in the yield components of rice are central to an understanding of the way weed competition influences yield potential of rice (Smith, 1968, 1974; Zimdahl, 1980; Kwon *et al.*, 1991; Pantone *et al.*, 1992; Azmi, 1994). There was a strong evidence on the negative impact on rice grain weight (biomass/grain) with seeding rate (Jones and Snyder, 1987). Azmi & Othman (1987) recorded reduced rice grain weight due to shading during flowering and ripening. Studies by Reddy (1976), among others, failed to show any detrimental effect on grain weight due to increased rice density. It was the studies by Kim (1986), Counse *et al.*, (1989), Hill *et al*, (1990), Nyarko & De Datta (1991) and Pantone *et al.*,(1992), among others, which established the negative impact and correlations between increased density and seeding rates on the yield components each as percentage

of filled grains; the number of grains/panicle, the number of florets/panicle and the number of panicles/plant.

There is strong tendency among researchers in their interpretation of grain yield data to focus only on separate correlations between yield and each yield component (Abdullah, M.Z. pers. comms.). Path analysis as being used only by breeders and geneticists on yield grain data facilitates analysis of a hypothetical network of causal relationship between components and grain yield. In the analysis of cereals, the consequence of observed correlations between yield components and assigns a relative importance to yield component-yield relationships which can be direct against indirect. Further, additive effect (which tend to reinforce each other) *vis-a-vis* substractive ones (which tend to annihilate each other) may be identified. In addition, path analysis generate standardized partial regression coefficients; these are referred as path coefficients (Li, 1975; Afifi and Clark, 1984; Loehlin, 1987). These coefficients have the advantage of being independent of the original unit of measurements in which comparisons of the relative importance of correlations associated with hypothesized causal relationships between different yield components and grain yield may be made.

Path analysis, originally developed by Wright (1954) has been used intensively in agronomy, horticulture, ecology and weed science (eg. Dewey and Lu, 1959; Duartye and Adams, 1972; Hamcoch, 1985; Jordan, 1985; van Brugger and Arneson, 1986; Farris and Lechowicz, 1990). Pantone *et al.*, (1989, 1992) developed path analysis models to study the crop-weed competition on yield components of wheat and rice either in monoculture or in mixtures. With the exception of grain weight, weed competition had a significant negative effect on the yield components of wheat. In rice, the path analysis indicated that the number of panicle/plant and florets/panicle were the most important yield components determining the responses of fecundity and grain yield to competition.

A path analysis model of rice in monocluture or in competition either with barnyardgrass (*Echinochloa crus-galli* ssp. *crus-galli* (L.) P. Beauv.) or wrinklegrass (*Ischaemum rugosum* Salisb.) in presented in Figure 1.

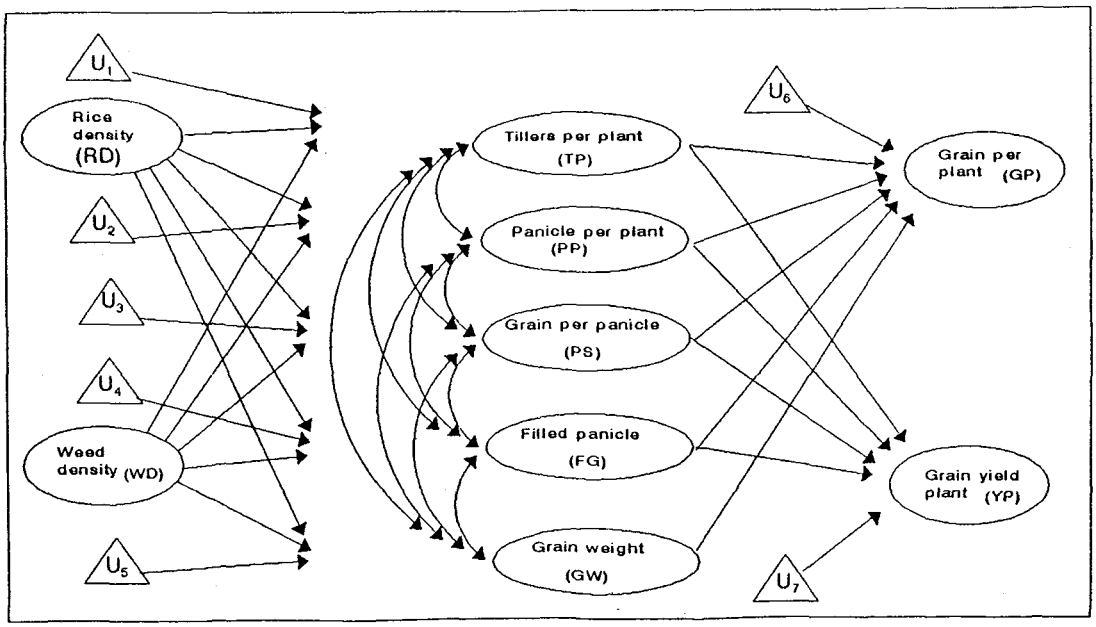


Figure 1: Path analysis diagram for the relationship between planting densities (RD & WD) of rice var MR84 or weeds (*Echinochloa crus-galli* ssp. *crus-galli* (L.) Beauv. and *Ischaemum rugosum* Salisb.) and yield components (TP, PP, PS, FG and GW) and effect of yield components on grain and fecundity (GP & YP). U_1, U_2, U_3, U_4, U_5, U_6 & U_7 are undetermined residuals.

Both weed species were chosen for the study due to their economic importance as dominant species and ability to inflict heavy losses to rice yields. The analysis helps to facilitate the partitioning of correlations between the yield components (number of tiller/plant (=genet), panicles/plant, panicle size, filled grains, unfilled grains, grain weight) and yield (grain yield/plant and grain/plant) into direct and indirect effects. For example, there are five path, which is denoted by the path coefficient from panicles/plant to grain yield/plant. The indirect effect through grain size which is equal to the product of simple correlation between panicles/plant and panicle size and the path coefficient from panicle size to grain yield/plant. The indirect effect *via* grain weight, which is equal to the product of simple correlations between panicles/plant and grain weight and/or between number of tiller/plant and panicle/plant and/or grain weight and path coefficients from grain weight to grain yield/plant. The sum of direct and indirect effect represents the correlation between panicles/plant and grain yield/plant.

Arguably, if there is no correlation between independent variables, it follows that path coefficient in equivalent to the correlation between the predictor (independent) and criterion (dependent) variable. This appears to be the case for the path coefficients along the path from rice var MR84 density and wrinklegrass or barnyardgrass densities to panicle/plant, panicle/plant, panicle size, filled grains and grain weight.

This paper reports results of a series of study with two-fold objectives, viz: (i) to asses the effect of crop-weed competition on yield components of rice var MR84 and wrinklegrass or barnyardgrass, and (ii) to determine the relationships between yield and yield components.

MATERIALS AND METHODS

Pot experiments were conducted in an insect-proof house at the Institute of Advanced Studies, University of Malaya (IASUM), Kuala Lumpur in 1993-1995. The IASUM area has a mean monthly rainfall of *ca.* 200mm, daily mean temperature of 27° C, RH of *ca.* 85% and mean daily sunshine of 6.5 hours.

Pre-germinated seeds of wrinklegrass and barnyardgrass collected from Seberang Prai granary in 1994 and a commercial rice variety MR84 from MARDI Research Station, Seberang Prai were sown in pots previously filled with moist silt loam padi soils of the Java series. The physico-chemical characteristics of the soils were as follows: pH (4.4); O.M (1.98%); organic carbon (1.15%); total N (1.93%); C:N (11.5); Na (1.40meq/100g); K (0.5 meq/100g); Mg (3.50meq/100g); P (65.5ppm); CEC (18.28) (Aris Junus, pers. comms).

Each pot was accorded with different density regime of wrinklegrass, barnyardgrass and rice either in monoculture or in mixtures. The density regime of rice plants in monocultures were 1, 3, 6, 9 and 12 plants/pot denoted as C_1, C_2, C_3, C_4 and C_5 respectively, while for barnyardgrass or wrinklegrass these were 12, 9, 6, 3 and 1 plant(s)/pot with the following respective notations as C_9, C_{10}, C_{11}, C_{12} and C_{13}. The density regime of the rice-barnyardgrass or rice-wrinklegrass mixtures was 12 plants/pot with in the following notations and proportions, viz: C_6 (3 rice plants + 9 weed plants); C_7 (6 rice plants + 6 weed plants); C_8 (9 rice plants + 3 weed plants). The details of sowing positions in relation to densities and proportional representations of rice, wrinklegrass and barnyardgrass plants per pot is shown in Figure 2. Each density regime has three replications and the pots were arranged in complete randomised design (CRD) (Gomez & Gomez, 1985).

The seedlings were watered twice daily from above using a five rose and were kept inundated 2 to 3 cm until the onset of booting stage. At booting stage, excess water was drained; the soils nevertheless were kept moist.

Compound fertilizers consisting of urea, muriate of potash and tripos were applied at the rate of 100:30:20, respectively, as top dressings in two equal split doses i.e. at 30 and 60 days after transplanting (DAT).

Adequate plant protection measures were taken against pests, diseases and weeds (other than those accorded in the experiments).

Several growth parameters were recorded at appropriate time intervals. These were total number of tillers/plant, number of productive tillers/plant, number of non-productive tillers/plant (at 10 days interval from transplanting until harvest), number of panicles/plant, panicle weights, number of filled grain/panicle, number of unfilled grain/panicle, number of grains/plant, weight of grains/plant, weight of filled grains/panicle and weight of unfilled grains/panicle (at harvest).

Path analysis was used to study factors effecting plant yield by facilitating the partitioning of

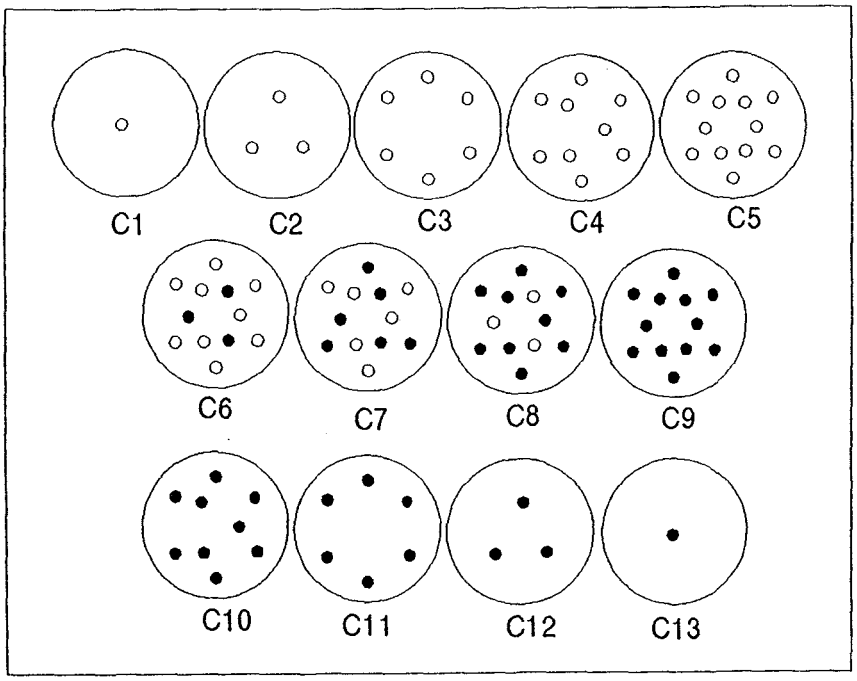


Figure 2: Spatial arrangement of rice and weeds in a replacement series experiment ○ =rice; ● =weed

correlation between plants densities and yield component, viz: MARDI rice var MR84 density (plant/m^2) (*RD*); wrinklegrass planting density (plant/m^2) (*WD*); barnyardgrass planting density (plant/m^2) (*WD*); number of tiller/plant (MR84) (*TP*), panicle/plant (*PP*), panicle size (grain/panicle) (*PS*), percent filled grains (filled grain/panicle) (*FG*), grain weight (g/grain) (*GW*), grain yield/plant (*YP*) (g) and grain/plant (*GP*) into direct and indirect effect. The direct effect between plants densities and grain yield will explain the effect of plants densities to the yield component [tiller/plant (*TP*), panicle/plant (*PP*), Grain/panicle (*PS*), filled panicle (*FG*) and grain weight (*GW*)] (Figure 1). The indirect effect between yield component shows the interactions between one yield component to the other and the direct effect between yield components and grain yield denote the value of the importance of yield components to the grains yields.

Multiple linear regressions adapted from Droper and Smith (1981) and SAS Institute (1985) were used to calculate the path coefficients, simple correlation coefficients and the undetermined residuals (U_s) of the regression $\sqrt{(1-R^2)}$ (Williams *et al.*, 1990) based on the hypothetical models of Figure 1.

RESULTS AND DISCUSSION

Direct effect of plant densities on yield components

The direct effect of rice *(RD)* and barnyardgrass *(WD)* densities on the number of tillers/plant *(TP)* and number of panicle/plant (=genet) *(PP)* in weed and crop plants were always negative. Conversely, the effect of *RD* and *WD* on number of grain/panicle *(PS)*, weight of filled grain/panicle *(FG)* and grain weight *(GW)* were not uniformly negative (Figure 3). It follows that the negative effects of *RD* and *WD* on *TP* and *PP* would mean that increased *RD* or *WD* would reduce *TP* and *PP*. The high value of path coefficient of direct effect of *RD* and *WD* on *TP* of rice indicated that rice density *(RD)* and *E. crus-galli* density *(WD)* were important attributes in the reduction of tiller/plant *(TP)* of rice (Figure 3).

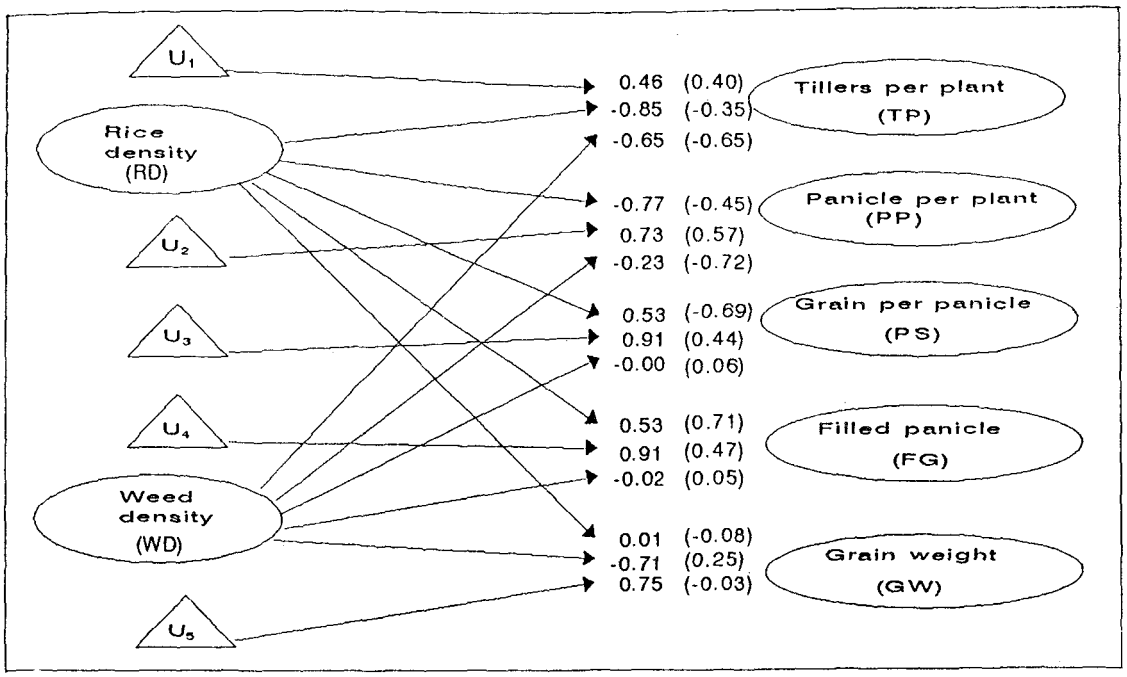


Figure 3: Path analysis diagram for the relationship between planting densities of rice var MR84 (RD) or barnyardgrass (WD) on yield components (TP, PP, PS, FG and GW) (Path coefficient values in parentheses are for barnyardgrass and outside parenthesis are for rice var MR84) (U_1, U_2, U_3, U_4 & U_5 are undetermined residuals)

The path coefficient value for direct effect of *RD* on *PP* was higher in rice *vis-a-vis E. crus-galli.* It was apparent that rice or *E. crus-galli* when grown at high densities had lower number of panicles/plant *(PP).* Increasing the density of rice in the rice-weed mixtures apparently may lead to decreasing number of grains/panicle *(PS)* of *E. crus-galli* but can increase the number of grains/panicle *(PS)* of rice.

The path coefficients for direct effect of *RD* on filled panicle *(FG)* of rice and *E. crus-galli* were positive (>0.50) indicating that the increase in *RD* will bring parallel increase in *FG* of rice and *E. crus-galli.* Conversely, *WD* did not bring about any meaningful effect *FG* of rice and *E. crus-galli.* The path coefficient of direct effect on *RD* and *WD* were either positive or negative on the grain weight *(GW)* of rice and *E. crus-galli.* Weed density *(WD)* appeared to have significant negative effect on *GW* of rice with the path coefficient value > 0.50. Likewise in *E.crus-galli,* the direct effect of *WD* on *GW* was positive with the path coefficient value *ca.* 0.25. The respective path coefficient values of direct effect of *RD* on *GW* of rice and *E. crus-galli* were 0.01 and -0.08, indicating minimal effect.

The direct effect of rice and *I. rugosum* densities on *TP, PP, PS and FG* of rice and *I. rugosum* were always negative (Figure 4). The path coefficient value of direct effect *RD* on number of panicle/plant of rice was higher *vis-a-vis* the values of other components signifying that *PP* of rice was highly affected by rice density. For *I. rugosum* yield components, *FG* showed fairly high negative value of path coefficient (-0.72) reflecting strong negative influence of *RD* on *FG* of *I. rugosum.*

The path coefficient for direct effect of *I. rugosum* density *(WD)* on rice yield components was remarkably high (-0.83) on *GW* followed by *PS* (-0.73), *FG* (-0.72), *PP* (-0.52) and *TP*(-0.48). Conversely, for *I. rugosum* yield components, the order of influence in the path coefficient values was: *TP > PP > GW > PS.* Increased density of *I. rugosum* inflicted subtantial reduction in *TP* of the weed and *PS* of rice. the density of *I. rugosum (WD)* had a positive direct effect on *FG* of the weed. Its path coefficient value was *ca.* 0.25, indicating that increased density of *I. rugosum* may only lead to marginal increase in *FG.* Residual values (U_1, U_2, U_3, U_4 and U_5) for all yield components either for rice, *I.rugosum* and *E. crus-galli* were > 0.50 signifying that undetermined factors, *viz:* soil humidity, temperature and internal plant physiology may have strong effect on yield components in these plants.

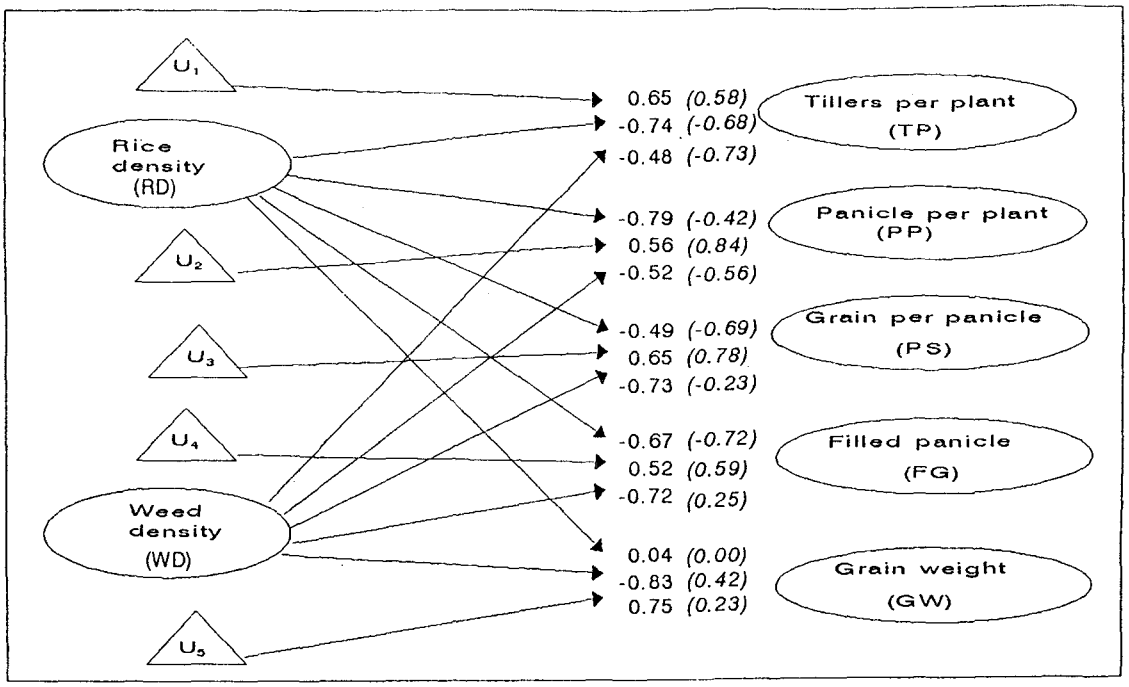


Figure 4 : Path analysis diagram for the relationship between planting densities and the yield components of rice var MR84 (RD) or wrinklegrass (WD) on yield components (TP, PP, PS, FG and GW) (Path coefficient values in parentheses are for wrinklegrass and outside parenthesis are for rice) (U_1, U_2, U_3, U_4 & U_5 are undetermined residuals)

Indirect effects among yield components

Path coefficient values of the indirect effect among yield components of rice and *E. crus-galli* were always positive or neutral (Figure 5). Measures such as *TP* of rice did not have any bearing on *PP* and *vice- versa but only had minor influence in the increase in PS, FG and GW of rice.* For *E. crus-galli, TP* apparently did not have any bearing on *PP* and had a minor effect on *FG.* Conversely, the *TP* of *E. crus-galli* have a strong bearing on *PS* and *GW.*

The *PP* for rice had a major effect on *PS, FG* and *GW* based on high path coefficient values (Figure 6). This indicated that increase in *PP* will lead to parallel increase in *PS, FG* and *GW.* For *E. crus-galli,* the number of *PP* only had a minor effect on weight of *FG* but have a fairly strong effect on *PS* and *GW.*

The *PS* for rice and *E. grus-galli* did not appear to affect the weight of *FG.* Conversely, the number of *PS* have a substantial bearing on *GW* (Figure 5). Increase in *GW* of rice and *E. crus-galli* will bring parallel increase on *FG.*

In direct effect between yield components of rice and *I. rugosum* were always positive. For example, the *TP* of rice and *I. rugosum* greatly affected *PP* and *PS* of rice and *I. rugosum* with respective path coefficient values ranging from 0.92 to 1.00 (Figure 7). It follows that increasing *TP* of rice or *I. rugosum* will bring about parallel increase in *PP* and *PS* of both rice and *I. rugosum.* The number of tillers/plant *(TP)* also affected *FG* and *GW,* but in relatively smaller degree *vis-a-vis PP* and *PS* either in rice or *I. rugosum.*

In rice, *PP* have a close relationship with *PS* and *FG* and *GW* where, increasing in quantum of one component will lead to correspondent increase in the other. Likewise *PS, FG* and *GW* highly affected each other (Figure 6). Conversely, in *I. rugosum, PP* only had moderate effect on *PS* and *GW.* Path coefficient values of indirect effect of *I. rugosum* between *PS, FG* and *GW* were closely correlated with one another and an increase in the value of one component can bring about parallel increase in other yield components.

Direct effect of yield components on weight of grain/plant (GP) and number of grain/plant (YP)

Despite differences in their path coefficient values, *TP* and yield components, *(PP PS and GW)* each had positive effect on *GP* of rice (Figure 7). The grain yield/plant *(YP)* of *E. crus-galli* was

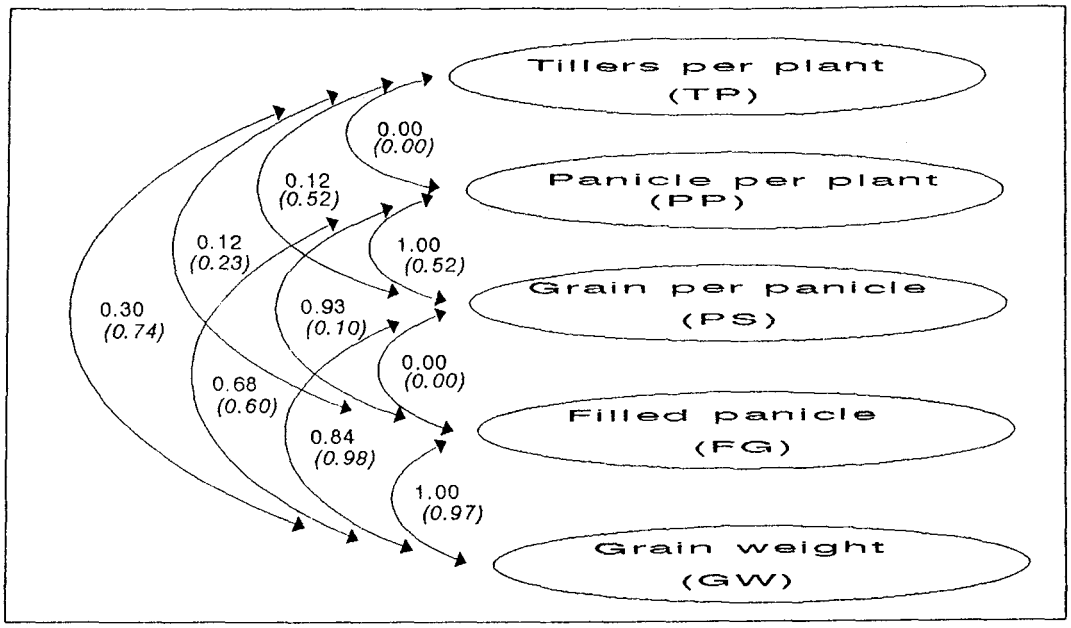


Figure 5 : Path analysis diagram of the indirect effect between yield components of rice var MR84 and barnyardgrass (TP, PP, PS, FG and GW) (Path coefficient values in parentheses are for barnyardgrass and outside parenthesis are for rice).

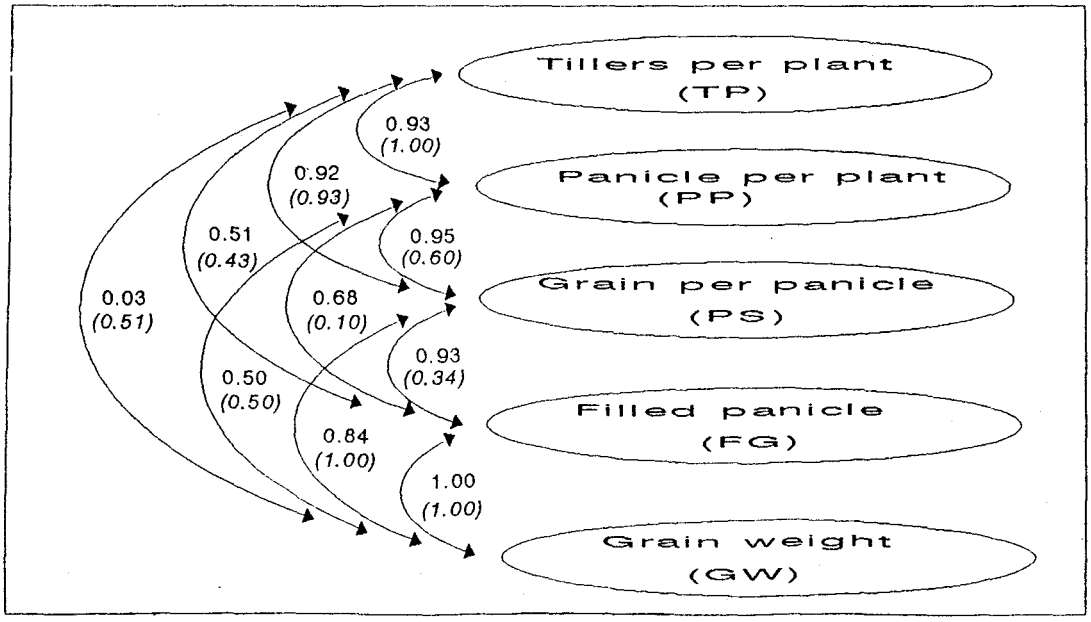


Figure 6: Path analysis diagram of the indirect effect between yield components of rice var MR84 or wrinklegrass (TP, PP, PS, FG and GW) (Path coefficient values in parentheses are for barnyardgrass and outside parentheses are for rice).

influenced by *TP, PS, FG* and *GW* with path coefficient values ranging from -0.4 to 0.46. The number of panicle *(PP)* had negative bearing on *YP* for *E. crus-galli.*

Direct effects of *TP* and *PP* on *YP* of rice were sporadically negative (Figure 7). For example, *TP* had a bigger effect on *GP,* registering path coefficient value of 0.67 *vis-a-vis GW* (0.51), *PP* (0.45) and *PS* (0.38) indicating differential influence of these yield components on *GP.* Similar trends of influence, though of varying intensities of *TP, PP, PS* and *GW* on *GP* of barnyardgrass were also observed.

All yield components either of rice or *I. rugosum* have positive effect on *GP* and *YP* except for direct effect of *FG* on *GP* and *TP* on *YP* of rice (Figure 8). Among rice yield components, *PP,* showed fairly high path coefficient value (0.93) on *GP* where increasing in *PP* will greatly increase *YP.* Likewise, the increase in *TP,* will increase *YP* and *GP.* For *I. rugosum* yield components, *TP* and *PP,* each have strong effect on *GP* and *YP* as shown by highly positive path coefficient values.

The residual values (U_6) on the weight of grains/plant *(GP)* for rice and *I. rugosum* was very low. Conversely, the residual value (U_7) on number of grains/plant, was moderately high either for rice or *I. rugosum.* It follows that the undetermined factors (U_6) did not effect the weight of grains/plant *(GP)* but highly affect number of grain/plant *(YP)* for both graminoids (U_7).

CONCLUSIONS

The density of rice, barnyardgrass and wrinklegrass plants either had positive or negative effect on their respective yield components (Figure 3 & 4). Similar findings have been recorded by Pantone *et al.* (1992). Rice density *(RD)* in the barnyardgrass-rice or wrinklegrass-rice mixtures had negative effect on *TP, PP* and *PS* of barnyardgrass and wrinklegrass. However, at low density, rice did not affect the production of the weedy graminoids reproductive components (Figure 3 & 4). On the contrary, wrinklegrass and barnyardgrass at high densities reduced the quantum of several yield components (Figure 3 & 4). Pantone *et al* (1992) indicated that the direct effect of yield components of Mars rice on its grain yield/plant *(YP)* was exceptionally consistent. Conversely, this study indicated that the direct effect of different yield components of rice on grain yield/plant *(YP)* was either positive or negative registering different path coefficient values both for the crop and the two weed species (Figure 3 & 4). From the study by Pantone *et al.* (1992), the relative importance of direct effect of grain per plant *(GP)* on grain yield plant *(YP)* was in the order of *PP > PS > FG* and *GW.* However, this study indicated that the hierarchial importance of direct effect of yield components on *GP* and *YP* in wrinklegrass were *TP > PP > GW > PS* (Figure 6), while in barnyardgrass this was *PP > PS > TP > FG* (Figure 5). The parallel relative importance of direct effect of yield components in rice on *GP* and *YP* was in the order of *FG > TP > GW > PP > PS.*

Acknowledgements: The authors wish to thank Anuar Pandak for technical assistance and Nor Azah Khalid for typing the manuscript.

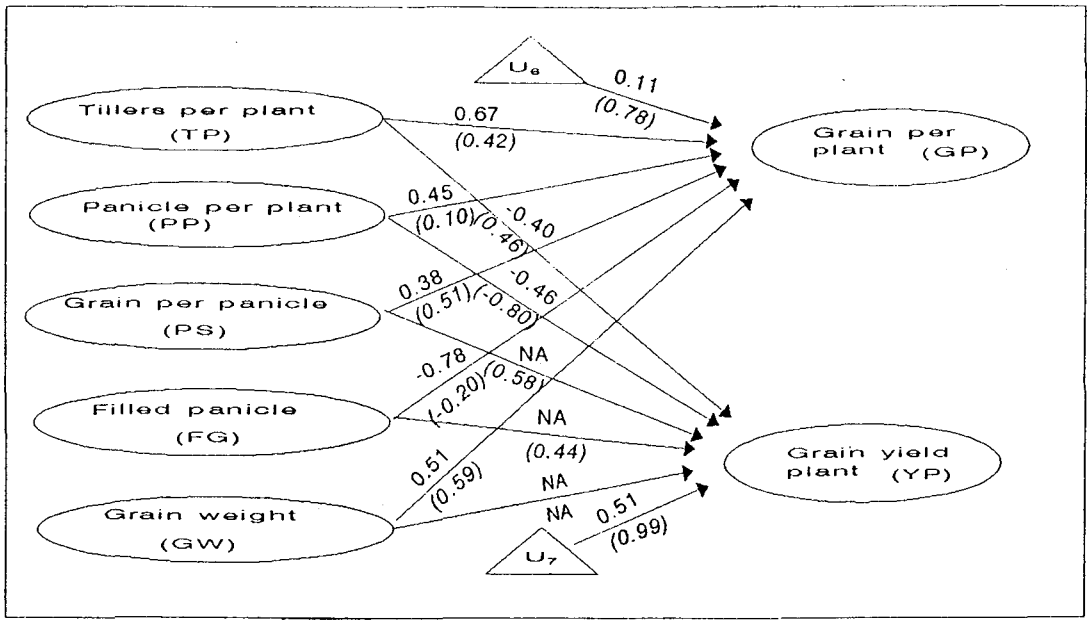


Figure 7 : Path analysis diagram for the direct effect of yield components of rice var MR84 and barnyardgrass (TP, PP, PS, FG and GW) on fecundity (GP & YP) (Path coefficient values in parentheses are for barnyardgrass and outside parenthesis are for rice) (U_6 and U_7 are undetermined residuals)

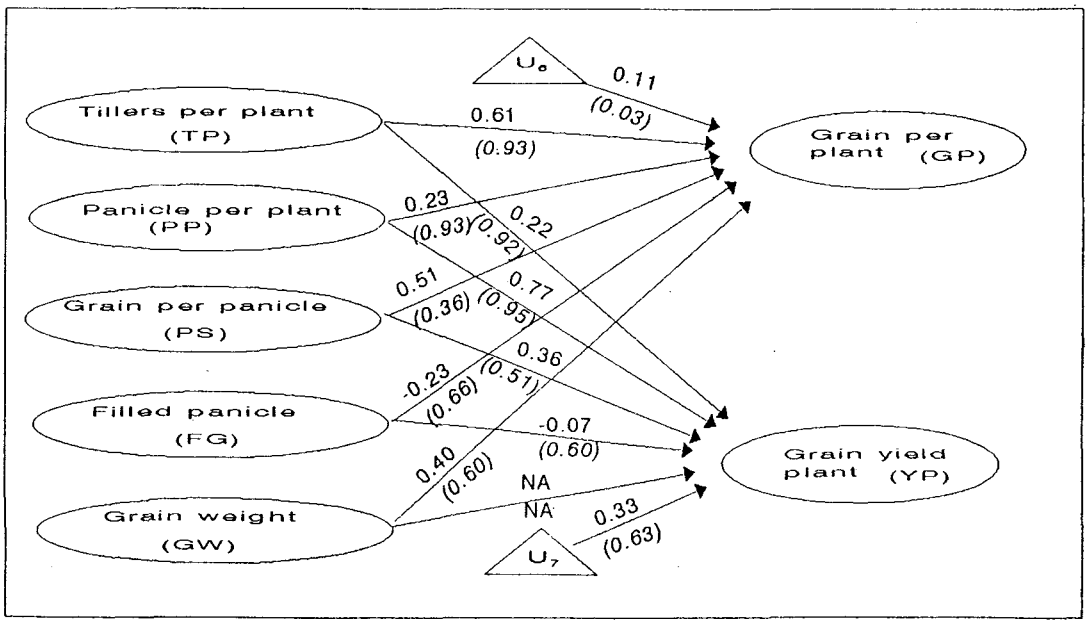


Figure 8 : Path analysis diagram for the direct effect of yield components of rice var MR84 and wrinklegrass (TP, PP, PS, FG and GW) (Path coefficient values in parentheses are for wrinklegrass and outside parenthesis are for rice) (U_6 and U_7 are undetermined residuals)

REFERENCES

Afifi, A. A. & Clark, V. 1984. *Path analysis: In Computer - Aided Multivariate Analysis. Lifetime Learning* Publication Belmont, *CA*.pp. 234-37

Akobundu, O. 1987. *Weed science in the Tropics Principles and Practices.* John Wiley & Sons, p 127-33

Azmi, M. 1994. Biologi dan kawalan *Echinochloa crus-galli* (L.) Beauv dalam tanaman padi tabur terus. *Ph.D. Thesis, Universiti Sains Malaysia*, Pulau Pinang, 185 p.

Azmi & Othman.1987. Saingan rumpai dengan beberapa varieti dalam tanaman padi tabur terus. *Teknologi Padi* **3:** 33-38.

Baki, B. B. 1988. The seed population dynamics of *Echinochloa crus-galli* (L.) Beauv. pp. 273-90 In: *Proceedings of the National Seminar and Workshop on Rice Field Weed Management (Lam, Y. M., A. W. Cheong & M. Azmi, eds.).*

Baki, B.B & Md. Khir, A. R. 1983. Weeds in major rice growing areas in Peninsular Malaysia: Detection and classification of groups of ecologically related species by multivariate analysis. pp. 4-27. *Proceedings of the First International Symposium of Weed Science in the Tropics* (Rosli, M. & Rajan, A. eds.): m.s. 4-27. Serdang.

Blackshaw, R. E., Anderson, G. W. & Dekker, J. 1987. Interference Of *Sinapis arnensis* L. And *Chenopodium album* L. in spring rapeseed (*Brassica rapus* L.). *Weed Research 27:* 207-13

Counce, P. A. 1987. Asymptotic and parabolic yield and linear nutrien content responces to rice population density. *Agronomic Journal 79:* 864-69

Counce, P. A., Moldenhauer, K. A. K. & Marx, D. B. 1989. Rice yield and plant yield variability responses to equidistant spacing. *Crop Science 29:* 175-79

Cousens, R. 1985. An empirical model relating crop yield to weed and crop density and a statistical comparison with other model. *Journal of Agricultural Science 105:*513-21

Cousens, R., Moss, S. R., Cussans, G. W. & Wilson, B. J. 1987. Modelling weed population in cereals. *Review of Weed Science 3:* 93-112

Dewey, D. R. & Lu, K. H. 1959. A correlation and path coefficient analysis of components of crested wheat grass production. *Agronomic Journal 51:* 515-18

De Wit, C. T. & van Den Bergh, J. P. 1965. Competiton between herbage plants. *Netherland, Journal Of Agricultural Science* **13**: 212-21

Duarte, R. A. & Adam, M. W. 1972. A path coefficient analysis of some yield component relationships in field beans (*Phaseolus Vulgaris). Crop Science 12:* 579-82

Dunand, R. T. 1988. Red Rice- Its impact on grain quality and its culture control. A Review Of Research In Loiusiana. 1960-1982. *Louisiana Agriculture Experimental Station Bulletin,* 792p

Draper, N. R. & Smith, H. 1981. *Applied Regression Analysis* 2nd Edition. John Wiley & Sons, New York, p 65-66

Farris, M. A. & Lechowicz, M. J. 1990. Functional interation among trait that determine reproductive success on a native annual plant. *Ecology 71:* 548-57

Gomez, K. A. & Gomez, A. A. 1984. *Statistical Procedures for Agricultural Research.* 2nd Edition. John Wiley & Sons, London, pp 7-29

Hamcoch, J. F., Pritts, M. P. & Siefker, J. H. 1984. Yield component of strawberries maintained in ribbons and matted rows. *Crop Research 24:* 37-43

Harper, J. L. 1977. *The Population Biology Of Plant. Academic Press, London.* p 151-89

Hill, J. E., de Datta, S. K. & Real, J. G. 1990. *Echinochloa* competition in rice. A comparison of studies from direct-seeded and transplanted flooded rice. *Biotrop Scientific Publication 38:* 115-29

Holm, L. G., Plucknett, D. L., Pancho, J. V. & Herberger, J. P. 1977. *The World's Worst Weeds. Distribution and Biology.* The University Press of Hawaii, Honolulu, U. S. A., p 32-295

Jordan, N. 1989. Path analysis of growth differences between weed and nonweed populations of poorjoe (*Diodia teres*) in competition with soybean (*Glycine max*). *Weed Science 37*: 129-36

Jones, D. B. & Snyder, G. H. 1987. Seeding rate and row spacing effects on yield and yield components of drill- seeded rice. *Agronomic Journal 79:* 623-26

Kim, Y. J. 1986. Analytical studies on rice yield components and yield in Southern region of Korea III. Variation in rice yield components and yield under different planting densities. *Korean Journal Of Crop Science 31:*104-11

Kropff, M. J. & Spitters, C. J. T. 1991. A simple model of crop loss by weed competition from early observation on relative leaf area of the weed. *Weed Research 31:* 97-105

Kropff, M. J., Weaver, S. E. & Smith, M. A. 1992. Use of ecophysiological model for crop-weed interference: relations amongst weed density, relative leaf area and yield loss. *Weed Science **40**: 296-301*

Kwon, S. L., Smith Jr., R. J. &. Talbert, R. E. 1991. Interference duration of Red Rice (*Oryza sativa*) in rice (*O. sativa*). *Weed Science **39**:* 363-8

Li, C. C. 1975. *Path Analysis- A Primer.* Boxwood Press Pasific Grone, CA., 347 p

Loehlin, J. C. 1987. Latent Variable Models: An introduction to factor path and structural analysis. L. Erlbaum Associates, Hillsdale, NJ., 273 p

Noda, K. 1973. Competition effect of barnyard grass (*Echinochloa crus-galli*) on rice. p 145-50. In: *Proceeding of the Fourth Asian Pacific Weed Science Society conference.*

Nyarko, K. A. & de Datta, S. K. 1991. *A Handbook For Weed Control In Rice.* International Rice Research Institute (IRRI), 102 p

Ooi, G. H. C. 1988. *Ischaemum rugosom* - The menace weed in Sungai Manik area. p 315-318. In: *Proceedings of the National Seminar and Workshop on Rice Field Weed Management (Lam, Y. M., Cheong, A. W. & Azmi, M., eds.) 1988.*

Pantone, D. J. & Baker, J. B. 1991a. Reciprocal yield analysis of Red Rice (*Oryza sativa*) competition in cultivated rice. *Weed Science **39**:* 42-7

Pantone, D. J. & Baker, J. B. 1991b. Weed-crop competition models and responce-surface analysis of red rice (*Oryza sativa* L.) competition in cultivated rice: *A Review of Crop Science **31**:* 1105-110

Pantone, D. J., Baker, J. B. & Jordan, P. W. 1992. Path analysis of Red Rice (*Oryza sativa* L.) competition with cultivated rice. *Weed Science **40**:* 313-19

Reddy, D. S. 1986. Effect of nitrogen and plant population on yield components of Jaya rice under recommended irrigation practice. *Madras Agricultural Journal **73***:321-24

SAS Institute. 1985. In: *SAS User's Guide: Statistics Version, 5th edition.* SAS Institute. Inc, Cary, NC., pp. 73-875

Satorre, E. H. & Snaydon, R. W. 1992. A comparison of root and shoot competition between spring cereals and *Avena Fatua* L. *Weed Research **32***: 45-55

Smith Jr, R. J. 1974. Competiton of barnyard grass with rice cultivars. *Issue 22:* 423-26

Smith Jr., R. J. 1968. Weed competition in rice. *Weed Science **16** :* 252-55

Sokal, R. R. & Rohlf, F. J. 1981. *Biometry 2nd Edition.* W. H. Freeman, New York, 642-61 p

Spitters, C. J. T. 1983. An alternative approch to the analysis of mixed cropping experiments. I. Estimation of competition effect. *Netherland Journal of Agricultural Science **31***: 1-11

Spitters, C. J. T. 1984. A simple simulation model for crop weed competition, p 353-66. In: *Procedings Of 7th International Symposium On Weed Biology, Ecology And Systematics,* COLUMA-EWRS, Paris.

Spitters, C. J. T. 1989. Weed: Population dynamics, germination and competition. pp.182-216 In: *Simulation Monographs (R. Ragginge, S. A. Word & H. H. van Laar, eds.)* Pudoc, Wageningen;

Spitters, C. J. T., Kropff, M. J. & de Groot, W. 1989. Competition between maize and *Echinochloa crus-galli* analyzed by a hyperbolic regression model. *Annals of Applied Biology **115**:* 541-51

van Bruggen, A. H. C. & Arneson, P. A. 1986. Path coefficient analysis of effect of *Rhizoctonia solani* on growth and development at dry beans. *Phytopathology **76***: 874-78

Weaver, S. E., Kropff, M. J. & Groerene H., R. M. W. 1992. Use of ecophysiological model for crop-weed interference: The critical period of weed interference. *Weed Science **40**:* 302-07

Wells, B. R. & Faw, W. F. 1978. Short-statured rice response to seeding and N. rates. *Agronomic Journal **70**:* 477-80

Williams, W. A., Jones, M. B. & Demnent, M. W. 1990. A conscise table for Path Analysis statistic. *Agronomic Journal **82**:* 1022-24

Wright, S. 1934. The method of path coefficients. *Annals Mathematical Statistics **5**:* 161-215

Yamada, N. 1961. On the relationships between yield and spacing in rice. *Agriculture Horticultura **36**:* 311-16

Yoshida, S. & Parao, F. T. 1976. Climatic influence on yield components of lowland rice in tropic. p 471-9 p. *Climate and Rice.* International Rice Research Institute, Los Baunos, Philippines

Zimdahl, R. L. 1980. *Weed-Crop Competition A Review.* International Plant Protection Center., Oregon State University, Corvallis, OR., p 83-93

GIS Application for Weed Management Strategy in Korea

K.H. Park[1] , K. Moody[2] , S.P. Kam[2] , Y.J. Oh[3] , and Y.C. Ku[1]

1 Rice Production Division, National Crop Experiment Station, RDA, Suwon 441-100, Korea
2 International Rice Research Institute, P.O.Box 933, Manila, Philippines
3 National Yeongnam Agriculture Experiment Station, Milyang 627-130,Korea

Abstract. GIS(Geographical Information Systems) was applied to determine a spatial analysis of weed community changes in lowland rice field in Korea. Based on vegetative analysis such as absolute and relative density, absolute and relative frequency, importance value, and summed dominance ratio(SDR) with weed number and dry weight of weed survey in lowland rice field, the most predominant weed species was *Eleocharis kuroguwai*, followed by *Sagittaria trifolia, Echinochloa crusgalli, Monochoria vaginalis, Sagittaria pygmaea, Cyperus serotinus* over the whole country in 1992. In particular, perennial weed species, *E. kuroguwai* was the most problem weed in this survey which was not so serious weed species in 1981. Although this species was the most predominent in the vegetative analysis over the whole rice field, there was a great population change in regional distribution through GIS analysis. The most predominant site of this weed species was at the southern area of Gyeonggi province. Meanwhile, spatial analysis of *Echinochloa crusgalli* through the GIS was resulted in highly different distribution with province. These data was highly significant in statistical analysis between cultural practices including different regions and weed occurrence.

Key words: GIS, weed, lowland rice field, vegetative analysis, *Eleocharis kuroguwai*

Introduction

Weed community has been highly shifted in lowland rice field due to change in water and fertilizer management associated with planting method like infant seedling and direct seeding and control method including herbicide. In particular, most of farmers would be depended upon weed control method with a major herbicide application. Thus, there was a gradual build-up of perennial weeds rather than annual weeds. In 1971, dominant weed species was annuals such as *Ratala indica, Monochoria vaginalis, Cyperus difformis, Echinochloa crusgalli* and *Lindernia procumbens* except *Eleocharis acicularis*, perennial weed. However, perennial weed species of *Sagittaria pygmaea, Potamogeton distinctus, Sagittaria trifolia* and *Cyperus serotinus* in 1981 was predominant in lowland rice field over the whole country. In 1992 *E. kuroguwai* and *S. trifolia*, perennial weeds were most dominant species. In addition, annual weed, *E. crusgalli* was the problem weed which was not involved in 10 major dominant weeds. Thus, weed control strategy would be introduced with an integrated method rather then one control measure like herbicide application. GIS may play a role in spatial analysis which would give an idea with specific interpretation in control measure against problem weed infestitation.

Materials and Methods

The weed data used was collected in 1992 with nationwide survey in lowland rice field of Korea as shown in Table 1. Vegetative analysis was done in terms of the summed dominance ratio(SDR) based on absolute and relative density, absolute and relative fequency, and importance value. The weed data was analyzed statistically in Genstat 5 (Release 3.1) in order to determine a significant difference between cultural practices and weed occurrence. A major weed species, *Eleocharis kuroguwai* was mapping with different sampling sites using by GIS software, SPANS (Spatial Analysis System, version 5.2, INTERATYDAC, 1991).

Results and Discussion

Vegetative Analysis. As a criteria of dominance in weed occurrence, vegetative analysis has been mainly used in which summed dominance ratio (SDR) would be calculated by absolute and relative density, absolute and relative frequency, and importance value.

Its parameter was calculated in the following formulars:

$$\text{Absolute density} = \text{Total number of each weed species}$$

$$\text{Relative density} = \frac{\text{Total number of each weed species}}{\text{Total weed number}} \times 100$$

$$\text{Absolute frequency} = \frac{\text{Number of quadrat in each species}}{\text{Sum of sampling quadarat}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Absolute frequency of each species}}{\text{Sum of absolute frequency in all weed species}} \times 100$$

$$\text{Importance valuce} = \text{Relative density} + \text{Relative frequency}$$

$$\text{Summed dominance ratio (SDR)} = \frac{\text{Importance value}}{2}$$

Based on the above formulars, dominance in weed population may represent as a summed dominance ratio in this weed survey. In 1992 the most predominant weed species was a perennial, sedges, *Eleocharis kuroguwai* in lowland rice field of the Korea as shown in Figure 1.

This species in 1981 was not so important weed in terms of vegetative analysis in the nationwide weed survey of lowland rice field. This weed belonged to a problem weed due to perennial and propagative organ of tubers and thus it is difficult to control with hand, mechanical and chemical control measures. Rice growing countries particularly in temperate area such as Korea and Japan have been heavily used with a specific herbicide in lowland rice field so as to control annual weeds in which continuously used for the long time since these chemicals were available in the market. Thus, weed population in 1981 and 1992 was highly shifted from annuals to perennials in the weed survey of the

lowland rice field in Korea. These changes were a serious problem in the rice paddy because most perennials have a tuber and/or rhizome as a propagative organ which is much more difficult to control with any control measures so far.

Statistiacl Analysis. In a nationwide weed survey, it is difficult to analyze statistically a relationship between cultural practices and weed occurrence because of a huge data structure. However, as long as statistical analysis may be calculated, a specific parameter such as region, planting time, soil texture, planting method, tillage time and kinds of herbicide would be proven as a major factor in weed occurrence and further it will be applied as a tool for integrated weed management. In this statistical analysis, most parameters were statistically significant as shown in Table 2 but some interactions were not significant statistically. This means that a specific factor in cultural practices would influence with a particular problem weed occurrence. Based on this analysis, farmer can effectively introduce a control measure against a problem target weed.

GIS Application. GIS may offer capability for monitoring the successful weed management programs for cultural paractices and herbicide targetting with spatial-referenced data. In vegetative analysis of the weed survey data in 1992, SDR was major parameter for the determination of problem and dominant weed species. When SDR of each weed species may report, most of farmers and researchers might be understood that the most predominant and problem weed in 1992 was E. kuroguwai in lowland rice field over the whole country of the Korea. Thus, most of researchers may suggest or recommend that farmer should apply a specific herbicide and/or control measures against this particular weed species in rice growing area. In addition, rice farmers in whold country would understand that *E. kuroguwai* was the most problem weed in rice field and thus farmers may introduce a control strategy against this target weed. However, in GIS mapping, there was highly difference in regional distribution of *E. kuroguwai* as shown in Figure 2. This weed was highly infested in the southern region of Kyunggi province due to much higher number and biomass of this weed per unit land area. Thus, farmers in this particular region should introduce a specific control measure enable to control this target weed not in other farmers' fields. Thus, farmers make a strategy to control this particular weed in individual field. In weed management, a suitable control measure against a target weed should be operated in terms of an effective weed control as well as environment safe.

References

1. Park, K.H., Ku, Y.C. and Oh, Y.J. 1992. Changes of weed communities in lowland rice fields in Korea. In Proceedings I of the 14th Asian-pacific weed conference, Brisbane, Australia. P. 349-352.

2. Song, Y.H. and Heong, K.L. 1993. Use of geographical information systems in analyzing large area distribution and dispersal of rice insects in South Korea. Korean J. Appl. Entomol. Vol 32(3): 307-316.

Table 1. Description of cultural practices in weed survey in 1992

Parameters	Description
Region	1. Plain 2. Intermediate 3. Mountainous 4. Others
Planting time	1. May 25 (early) 2. June 10 (optimum) 3. June 25 (late) 4. Others
Soil texture	1. Ordinary paddy 2. Water paddy 3. Sandy soil 4. Saline soil 5. Others
Cropping system	1. Single cropping (Rice only) 2. Double cropping
Planting method	1. Hand transplanting 2. Machine transplanting I (35 days seedling) 3. Machine transplanting II (8-10 days seedling) 4. Water seeding 5. Dry seeding
Tillage time	1. Autumn 2. Spring
Tillage method	1. Cow 2. Machine cultivator 3. Tractor
Herbicides	

Table 2. Statistical analysis of weed dry weight in weed survey, 1992

Parameters	d.f	s.s	m.s	v.r.
Region	3	11.0116	3.6705	19.49**
Planting time	3	49.2881	16.4294	87.24**
Soil texture	4	8.7075	2.1769	11.56**
Croipping system	2	15.8863	7.9431	42.18**
Planting method	5	17.6306	3.5261	18.72**
Tillage time	2	5.4710	2.7355	14.53**
Tillage method	3	7.1512	2.3837	12.66**
Herbicde	1	2.1127	2.1127	11.22**

** : Significantly different at the 1% level

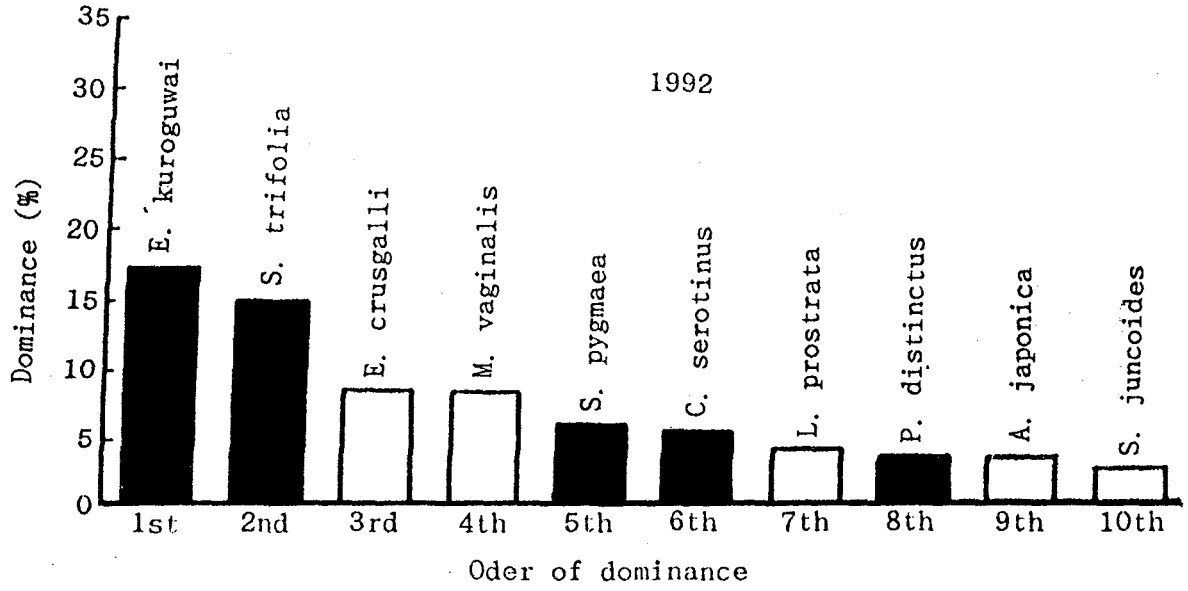


Figure 1. Major dominant weed species in lowland rice fields of Korea in 1995.

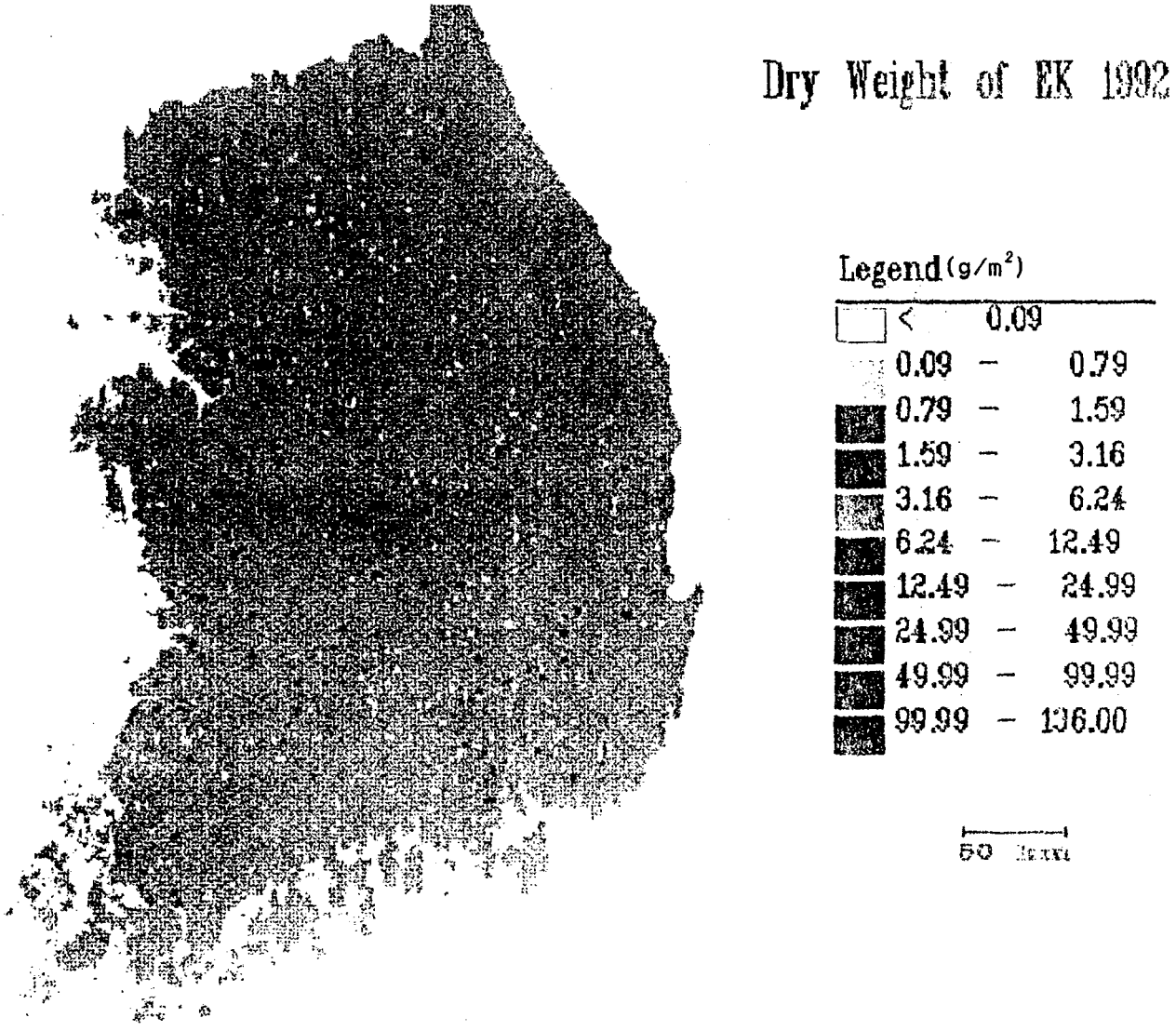


Figure 2. Spatial distribution of *Eleocharis kuroguwai* in lowland rice fields of Korea in 1995.

Control of Tuber Growth of Purple Nutsedge (*Cyperus rotundus*): Effects of Drying and Depth of Burying

B. MARAMBE, U.R. SANGAKKARA and S.K. RATNAYAKA
Department of Crop Science, Faculty of Agriculture
University of Peradeniya, Sri Lanka.

Abstract: Purple nutsedge (*Cyperus rotundus*) is a problematic weed in upland crop production systems of Sri Lanka. Chemical and biological techniques have failed to control this weed due to vigorous tuber growth. Pot experiments were conducted to test the effects of air- or sun-drying and depths of burying of tubers of nutsedge on tuber germination and plant growth (leaf area, and shoot, root and tuber dry weights). Burial at depths less than 10 cm or air drying for 60 days did not affect germination of tubers or plant growth. Poor growth was observed when buried at 10-30 cm depths, and tubers did not germinate at a depth of 40 cm. Sun-drying for 7 days reduced the moisture content and germination of tubers by 55-60% and plant growth by 65-75% when compared to the non-dried control. Tubers were completely dried and did not germinate when sun-dried for 14 days. The results indicated that sun-drying for 14 days or burying at depths greater than 40 cm would be effective in controlling tuber growth of purple nutsedge.

Key words: *Cyperus rotundus*, sun-drying, burying, tubers, moisture content

Introduction

Purple nutsedge (*Cyperus rotundus*), which is considered the world's worst weed (Holm et al. 1977) is found in many upland cropping systems in the tropics. Grichar et al (1992) reported that purple nutsedge continue to increase in severity under crop cultivation mainly due to vast use of selective herbicides that kill grasses and broadleaves. Apart from the chemical methods (Brecke 1991; Wiley et al 1991), several non-chemical methods (Ray and Wilcox 1969) were also reported to be partly successful in controlling purple nutsedge. The high vegetative ability of the weed and the difficulties faced by farmers to kill the numerous tubers have rendered the control of this weed more difficult. Therefore, this research was conducted to investigate the effect of different drying techniques and depth of burying on growth and development of nutsedge tubers in order to develop a possible mechanical technique for the effective control of this weed.

Materials and Methods

Selection of planting material:

Tubers of *Cyperus rotundus* were collected at flowering stage of the plant from a uniformly grown natural vegetation at the experimental station, University of Peradeniya, Sri Lanka. Tubers were detached from the mother plant, washed in running water, and dried on paper towels. Tubers of 50-60 mg fresh weight (44-45% moisture) were selected for the experiment.

Treatments:

The harvested tubers were pooled and devided into two lots. The first lot was air dried under 50% shade conditions and the second lot was sun-dried for 9-10 h/day for a period of 0 to 2 months. The tubers were then planted in tubular polyethelene bags

at depths ranging from 0-60 cm, filled with 2.5 kg of a 1:1 mixture of top soil:river sand. Each bag contained three tubers and a treatment was represented by six bags in each replicate. All the bags were watered once a week up to the field capacity and did not receive any commercial fertilizer.

Measurements:

Daily variation in the sun light intensity and temperature were recorded. The tubers were protected from rains during the drying period. The fresh and dry weights of tubers were measured before and after treatments. The dry weight was measured after oven- drying the tubers at 80°C for 48 h. Tuber moisture content was estimated by the difference in fresh and dry weights. Sampling was done at weekly intervals to measure tuber germination %, leaf area, shoot and root dry weights.

The experiment was conducted as a three factor factorial in a complete randmomize design with four replicates. The treatment means were compared using Duncan's New Multiple Range Test (DNMRT) or by "t" test at p=0.05.

Results and Discussion

Tuber moisture content

Sun drying tubers for 3 days resulted in a significant reduction of tuber moisture (Table 1). The tubers lost 50-55% moisture (fresh weight basis) after the first 7 days. Interestingly, tubers lost almost all its moisture after 14 days of sun-drying. Drying in shade did not produce any marked reductions in tuber moisture (Table 1). The high temperature that prevailed during the sun-drying period (Fig. 1) may have caused the rapid loss of moisture observed in these tubers when compared to the air-dried tubers.

Table 1. Tuber moisture content after drying treatments.

Treatments	Duration of drying (days)				
	0	3	7	14	28
Sun-drying	44.6 a	32.4 a	22.6 a	0.1 a	0.1 a
Air-drying	44.4 a	43.8 b	42.0 b	41.9 b	40.8 b

In each column data represented by the same letter are not significantly different by the t test at p=0.05.

Tuber germination

All tubers in the non-dried control showed 100% germination when buried at depths less than 20 cm (Table 2). However, the rate of emergence was delayed with increasing depth (data not shown). Burial at depths greater than 40 cm completely inhibited tuber germination in the non-dried control. The physical pressure exterted on the tubers by deep burial in soil may have caused the low tuber germination. Drying tubers under shaded conditions up to 60 days showed a similar pattern of germination to that of the non-dried control at different depths of burying (data not shown). These tubers also contained moisture similar to the levels found in

non-dried tubers. For sorghum seeds, Marambe et al (1991) reported that germination would not take place unless there is an optimum moisture level in the seeds. Similarly, the results of the present experiment indicate that high moisture retention in tubers is required subsequent germination and growth.

Table 2. Germination of tubers buried at different depths.

Duration of drying (days)	Depth of burial (cm)					
	5	10	20	30	40	50
Control	100 a	100 a	100 a	65 a	0 a	0 a
Sun-drying						
3	65 b	65 b	40 b	35 b	0 a	0 a
7	50 b	35 c	20 c	20 c	0 a	0 a
14	0 c	0 d	0 d	0 d	0 a	0 a
28	0 c	0 d	0 d	0 d	0 a	0 a
Shade-drying						
3	95 a	100 a	100 a	70 a	0 a	0 a
7	100 a	90 a	100 a	65 a	0 a	0 a
14	85 a	95 a	95 a	70 a	0 a	0 a
28	100 a	85 a	85 a	70 a	0 a	0 a

In each column data represented by the same letter are not significantly different by the DNMR Test at p=0.05.

Sun-drying for 3 days reduced the tuber germination by 35% when compared to the control (Table 2) when planted at 10 cm depth. Germination was not observed at depths greater than 40 cm. Only 50% of tubers germinated after 7 days of sun-drying and when buried at 5 cm depth. All tubers lost viability after 14 days of sun-drying. Complete loss of moisture observed in the tubers after drying may have resulted in poor germinability. Germination of tubers dried under shade followed a similar pattern to that of the non-dried control although they took longer time to germinate and emerge (data not shown).

Shoot growth

Sun drying of tubers for 7 days resulted in poor growth (Table 3). The leaf area of these plants buried at 0 or 10 cm depth, were 62-65% less than the control after the first month and 68-70% less after the second month. The results suggest that less vigorous seedlings produced by the sun-dried tubers had poor growth.

Increasing the depth of burial of the non-dried or dried tubers, reduced the growth potential of the nutsedge plants. The shoot dry weight of these plants followed a similar pattern to that of leaf area (Table 3). The results are in agreement with Squire (1990) who reported that high leaf area produces high leaf dry matter due to its photosynthetic capacity. A drastic reduction in leaf area and shoot growth of plants from non-dried dried tubers were observed when they were buried at depths greater than 20 cm. The physical pressure exerted on the emerging seedlings from tubers buried at deeper soil layers may be responsible for the less vigorous seedlings. The shoot growth of the tubers sun-dried for 3 days also showed poor growth when compared to the control, but the

growth of plants from shade-dried tubers were similar to that of the control (data not shown).

Table 3. Leaf area and shoot dry weight of 1 and 2 month old nutsedge plants following 7 days of sun-drying of tubers

Depth of planting (cm)	Leaf area (cm2/plant)				Shoot dry weight (mg/plant)			
	1 month		2 months		1 month		2 months	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
0	71.3	27.1	136.5	43.7	294	132	1151	460
10	72.0	25.3	134.1	40.8	301	125	1109	448
20	69.1	18.2	126.8	24.6	286	78	1024	126
30	44.2	6.6	79.6	8.6	149	28	470	43
40	NA	NA	NA	NA	NA	NA	NA	NA
50	NA	NA	NA	NA	NA	NA	NA	NA
CV%	8.1	5.3	10.1	9.8	14	11	13	11

NA - data not available due to no or very low germination %.

Root Growth:

The root dry weight also showed a similar pattern to that of leaf area and shoot dry weight (data not shown).

Conclusions

The results of the present experiment indicate that sun-drying for 14 days or burying at depths exceeding 40 cm could be effective in suppressing growth and development of tubers of purple nutsedge.

References

Brecke, B.J. (1991): Purple nutsedge (*Cyperus rotundus*) control in peanuts. Proc. South Weed Sci. Soc. 44: 139.

Grichar, W.J., Nester, P.R. and Colburn, A.ED. (1992): Nutsedge (*Cyperus* spp) control in peanuts (*Arachis hypogaea*) with Imazethapyr. Weed Technol. 6: 396-400.

Holm, L.G., Plukenett, D.L., Pancho, J.V. and Herberger, J.P. (1977): The world's worst weeds: Distribution and Biology. University Press, Hawaii, Honolulu, pp 609.

Marambe, B., Ando, T. and Kouno, K. (1991): Effect of inorganic cations in animal-waste composts on water and cation absorption

in germinating sorghum (*Sorghum bicolor* Moench) seeds. Soil Sci. Plant Nutr., 37: 487-495.
Ray, B.R. and Wicox, M. (1969): Chemical fallow control of nutsedge. Weed Res. 9: 86-94.
Squire, G.R. (1990): The Physiology of Tropical Crop Production. CAB International, Wallingford, UK. pp 236.
Wiley, G.L., Muzyk, K.R. and Walls, F.R. (1991): Methods of control of nutsedge in peanuts with imazethapyr. Proc. South. Weed Sci. Soc., 44: 141

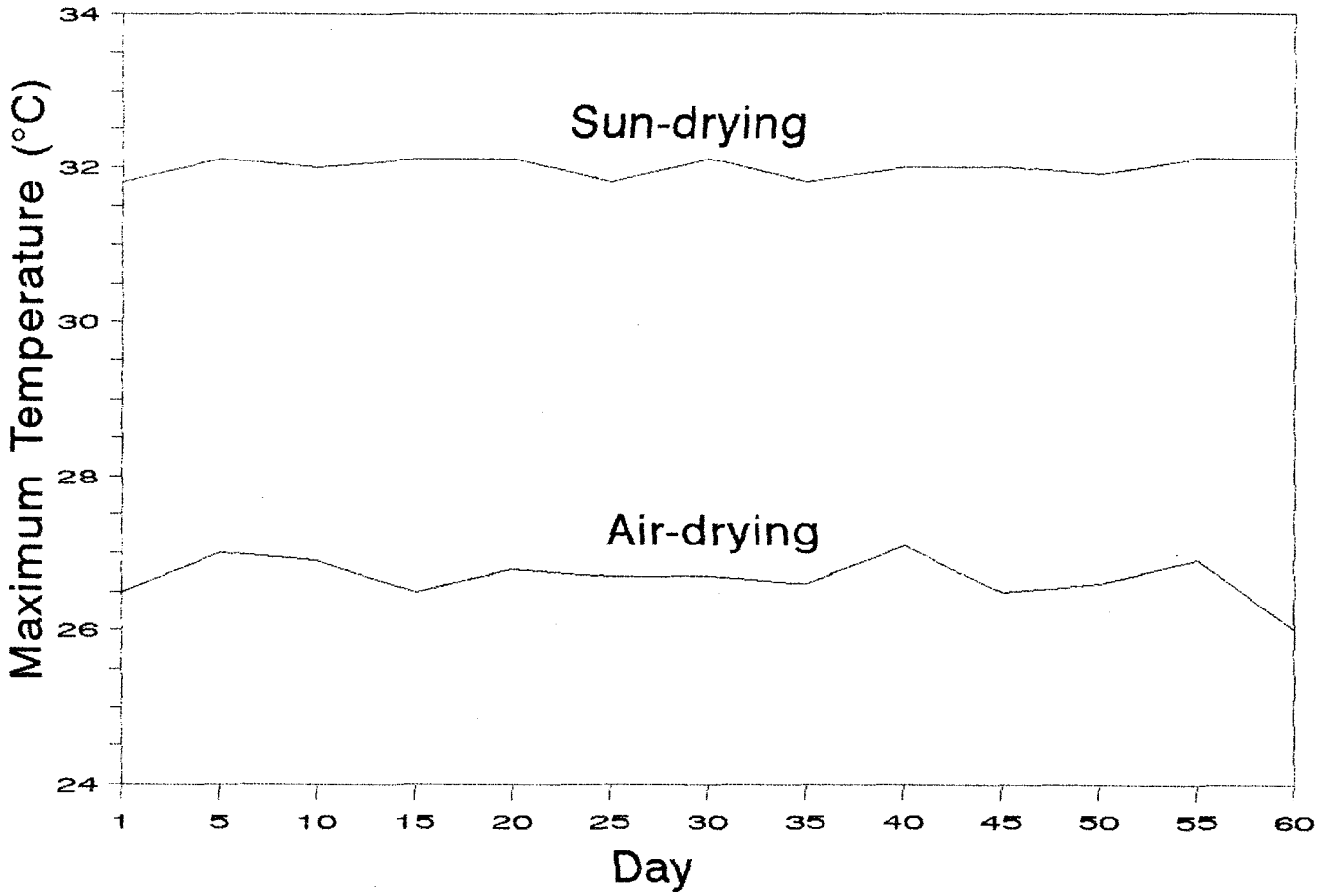


Fig. 1 Variations in maximum day Temperature during drying

Intraspecific Variations in Tubers and Herbicide Susceptibility in Arrowhead (*Sagittaria trifolia*) in Southern Japan

H.Morita*
National Agriculture Research Center, Tsukuba 305,Japan

Abstract. Arrowhead (*Sagittaria trifolia* L.) is a noxious paddy weed in southern Japan where its infestation has become serious because of difficulty in its herbicidal control. To identify the factors affecting the fluctuation in herbicidal efficacy, intraspecific variations in the tuber formation, sprouting and susceptibility to several herbicides were investigated with 19 clones of arrowhead collected from paddy fields in Kyushu, Japan. By principal component analysis with 12 characters, 19 clones were classified into four groups (A,B,C and D) among which the composition of tuber sizes was conspiciously different. Percentage sprouting at two weeks after placement in tap water was higher in tubers of the B group than that of A. A significant multiple regression formula with three factors, average dry weight per plant(X_1), average fresh tuber weight(X_2) and percentage sprout of tuber (X_3), was obtained through the differences in susceptibility(Y) to bensulfuron-methyl among nine clones from the A, B and C groups.
Key words. Arrowhead (*Sagittaria trifolia L.*), Intraspecific variation, Tuber formation, Sprouting, Bensulfuron-methyl.

Introduction

Arrowhead (*Sagittaria trifolia* L.) is a perennial weed in paddy fields widely distributing in Japan(Ito:1989 and Yamakawa:1991). Although the infestation is not so serious than in the north-eastern districts, arrowhead has become a noxious weed even in the southern Japan because effective herbicides are not available to control it as well as *Eleocharis kuroguwai* Ohwi. It has been reported that arrowhead has wide genetic variations in the traits and its variations may cause the fluctuation in herbicidal efficacy(Yamakawa:1991). To clarify the role of genetic variation on the herbicidal efficacy, intraspecific variations in the tuber formation(Morita et al.:1991), sprouting and susceptibility to several herbicides were investigated with 19 clones collected from paddy fields in Kyushu, Japan.

Materials and Methods

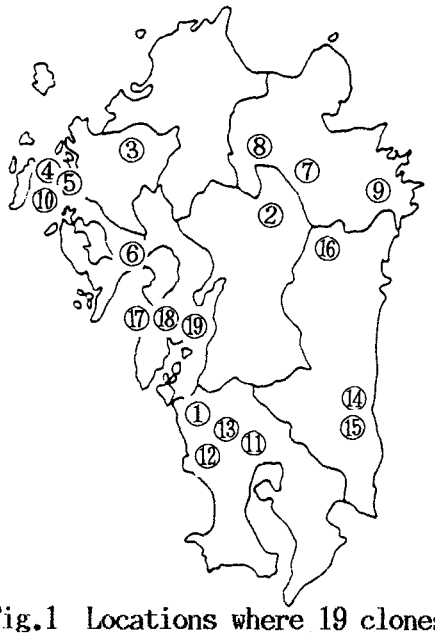
Samples of arrowhead were collected in paddy fields at 19 different locations of six prefectures of Kyushu district, southern Japan as shown in Fig.1. They were propagated vegitatively at Kyushu Natianl Agricultural Experiment Station, Chikugo, Fukuoka and given clone No.1 to 19.

Environmental variation in tuber formation

Sprouted tubers of clone No.3 were planted in a 1/2,000a Wagner pot filled with paddy soil. Following treatments were combined with two repetitions; amount of fertilizer applied: 0,1 and 3g of N, P_2O_5 and K_2O, plant density: 1,3 and 5 plants per a pot and planting time: June 7, june 29 and July 20,1989. Tubers were collected in November to classify with fresh weight into SS:~79mg, S:80~199mg, M:200~399mg, L:400~599mg and LL:600mg~.

Variations among clones

Three sprouted tubers were planted in a 1/2,000a Wagner pot filled with paddy soil fertilized with 1g of N, P_2O_5 and K_2O, on June 21,1990, with



1.Sendai City, Kagosima Pref.
2.Ichinomiya Town, Kumamoto Pref.
3.Mitsuze Village, Saga Pref.
4.Tabira Town(1), Nagasaki Pref.
5.Tabira Town(2), Nagasaki Pref.
6.Moriyama Town, Nagasaki Pref.
7.Kuju Town, Oita Pref.
8.Kokonoe Town, Oita Pref.
9.Saeki City, Oita Pref.
10.Sechibaru City, nagasaki Pref.
11.Kedouin Town, Kagoshima Pref.
12.Ijuin Town, Kagoshima Pref.
13.Tsuruta Town, Kagoshima Pref.
14.Miyazaki City(1), Miyazaki Pref.
15.Miyazaki City(2), Miyazaki Pref.
16.Hinokage Town, Miyazaki Pref.
17.Hondo City(1), Kumamoto Pref.
18.Hondo City(2), Kumamoto Pref.
19.Ariake Town, Kumamoto Pref.

Fig.1 Locations where 19 clones of arrowhead were collected in Kyushu.

three repetitions for every 19 clones. Size of tenth sagittate leaf was measured during July and August and formed tubers were collected in November after the aerial part died off. Tubers were classified as mentioned above.

Variation in tuber sprouting

Tubers of 18 clones stored at 10℃ since November,1990, were classified into 4 sizes, a:20~80mg, b:100~180mg, c:260~340mg and 450~550mg in fresh tuber weight. 30 tubers were placed in 250ml of tap water in a plastic case with three repetitions on January 31,1991. Sprouted tubers were counted every week till 14 weeks after placement in the incubator controlled at 30 ℃, 14 hours illumination.

Fluctuation of herbicidal efficacy

Seedling of arrowhead was planted in 1/2,000a Wagner pot in June, 1991 to produce tubers under submerged condition. Clones tested were 6,8,16 of the A, 1,2,10,15 of the B and 3,13 of the C group. Tubers emerged under 5cm depth of flooding after the soil was paddled and fertilized with 1g of N,P2O5 and K2O , May 18,1992. Bensulfuron-methyl(0.17% a.i. granule) and pyrazolate(10% a.i. granule) were applied 14 days after paddling with the rate of 5.1g and 300g a.i./ha respectively. Herbicidal efficacy was measured 51 days after application.

Results and Discussions

Environmental variation in tuber formation

Effects of amount of fertilizer applied, plant density and planting time on the tuber formation in clone No.3 were shown in Table 1, as the results of analysis of variance. Variations in the component of tuber size SS and S were not or less significant through three treatments, while there were significant differences in the number of tubers formed per pot or plant.

Component of tuber size is considered as a stable trait in arrowhead which is hard to fluctuate through the environmental factors as reported the tuber formation under the different fertilized nitrogen levels (Yamakawa *et al.*:1986).

Variations in tuber formation among clones

The result of principal component analysis is shown in Table2 and Fig. 2 based

Table 1 Result of analysis of variance for environmental variation in tuber formation of clone No.3 of arrowhead.

Trait & Factor	d.f.	No.of tuber/pot Mean square	F	No.of tuber/plant Mean square	F	% of SS tubers Mean square	F	% of S tubers Mean square	F
Repetition	1	32757.4	5.72*	6188.7	2.30	4.1	0.06	0.5	0.02
Planting time	2	55058.9	9.62**	20541.2	7.63**	264.5	4.21*	124.6	5.53*
Fertilizer	2	377743.6	65.99**	120921.6	44.89**	143.1	2.27	27.4	1.21
Density	2	8461.8	1.48	228404.6	84.87**	82.7	1.31	71.9	3.19
P*F	4	68878.7	12.03**	26077.5	9.69**	33.1	0.52	31.2	1.38
P*D	4	18749.4	3.28*	10614.1	3.94	39.4	0.62	19.4	0.86
F*D	4	20916.8	3.65*	40765.6	15.15**	10.7	0.17	3.0	0.13
P*F*D	8	15751.8	2.75*	24630.6	9.15**	20.2	0.32	9.9	0.43
Error	26	5724.4		2691.3		62.8		22.5	

*,** means significance at 5 and 1% level, respectively.

Table 2 Eigenvalues and accumulated contributions (%) for principal components based on 12 characters of arrowhead in Kyushu.

Character		Z1	Z2	Z3	Observed value Max.	Min.	Mean	
Plant height	X1	0.50	0.65	-0.40	43.2	25.8	36.5	cm
Upper lobe length	X2	0.02	0.66	-0.45	18.6	10.6	14.0	cm
Upper lobe width	X3	0.76	-0.43	-0.07	5.1	1.2	2.3	cm
No. of tubers	X4	-0.82	-0.19	-0.38	292	9	88.5	
Average tuber weight	X5	0.96	-0.20	-0.10	713	68	250.9	mg
Total tuber weight	X6	-0.20	0.58	-0.33	42.4	6.2	16.3	g
Maximum tuber weight	X7	0.92	0.04	-0.12	2735	684	1633.6	mg
SS size tubers (%)	X8	-0.95	-0.20	-0.21	75.5	6.4	34.1	%
S size tubers (%)	X9	-0.08	0.56	0.76	38.1	18.1	29.9	%
M size tubers (%)	X10	0.71	0.50	0.24	33.2	4.9	18.4	%
L size tubers (%)	X11	0.95	-0.17	-0.08	23.1	0.9	7.5	%
LL size tubers (%)	X12	0.95	-0.24	-0.13	38.3	0.5	9.8	%
Eigenvalues		6.52	2.14	1.34				
Accumulated contributions		54.5%	72.3%	83.5%				

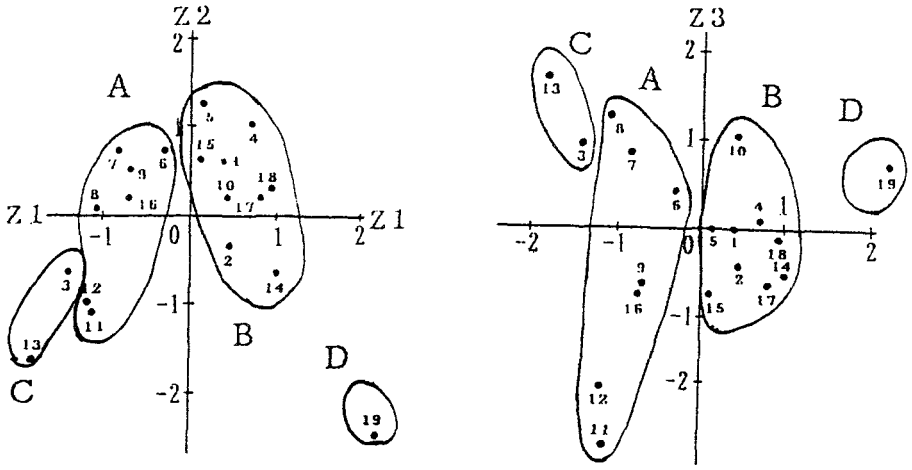


Fig.2 Results of principal component analysis based on 12 characters of plant size and tuber formation on 19 clones of arrowhead in Kyushu.

on 12 characters including plant height (petiole length of tenth sagittate leaf), size of leaf blade, total number and fresh weight of formed tubers, percentages of tuber size for SS to LL to the total number. The first, second

and third principal component were considered to mean average tuber weight and percentage of tuber size except S and M, traits of aerial part and percentage of S size tubers, respectively, with accumulated contribution of 82.5%. 19 clones were divided into four groups depending on the result. Composition of tuber size is summarized as follows and Fig.3 for each group.

A:Ratio of SS size tuber is high, that decreases for L and LL sizes.
B:Ratio of L and LL is high, those of smaller tubers are low
C:Ratio of SS is higher than A group, LL tubers are rare.
D:Ratio of LL is high

the D group was identified as cultivated arrowhead (*Sagittaria trifolia* L. var. *sinensis* Makino) escaped(Morita and Doi:1981).

Intraspecific variation in tuber size in arrowhead has been reported on the clones of Hokkaido (Morita and Doi:1981) and Kinki(Yamakawa *et al.*:1983) districts. It has been pointed out that clones which have small plant size and occur in highland paddy field, produce a large number of small tubers while those with large plant size distributing in lowland paddy fields produce few number of large tubers(Yamakawa:1991). In this study, no clear relationship is recognized between the number and size of tubers and habitat among 19 clones.

Variation in tuber sprouting

Changes in percentage sprouting at two and 14 weeks after placement differed among the A,B and C group as shown in Fig.4.Variation in percentage sprouting after two weeks was less than 35% and that was conspicuous 14 weeks after for clones of the A group. As for clones of the B group, percentage sprouting after two weeks ranged from 35% to 97%, and variation was inconspicuous 14 weeks after. Clones of the C group showed low percentage sprouting. Almost of large size tubers sprouted till 14 weeks through the groups, while variation among the groups was observed in smaller size tubers as shown in Fig.5.

Sprouting of tuber is affected by soil moisture condition and rice cropping season(Ito and Miyahara:1989) and amount of nitrogen applied(Yamakawa et al.:1986) in paddy field in arrowhead. Large tuber has deeper dormancy than small size tuber formed in well drained paddy field(Ito and Miyahara:1989). In this study, it is considered

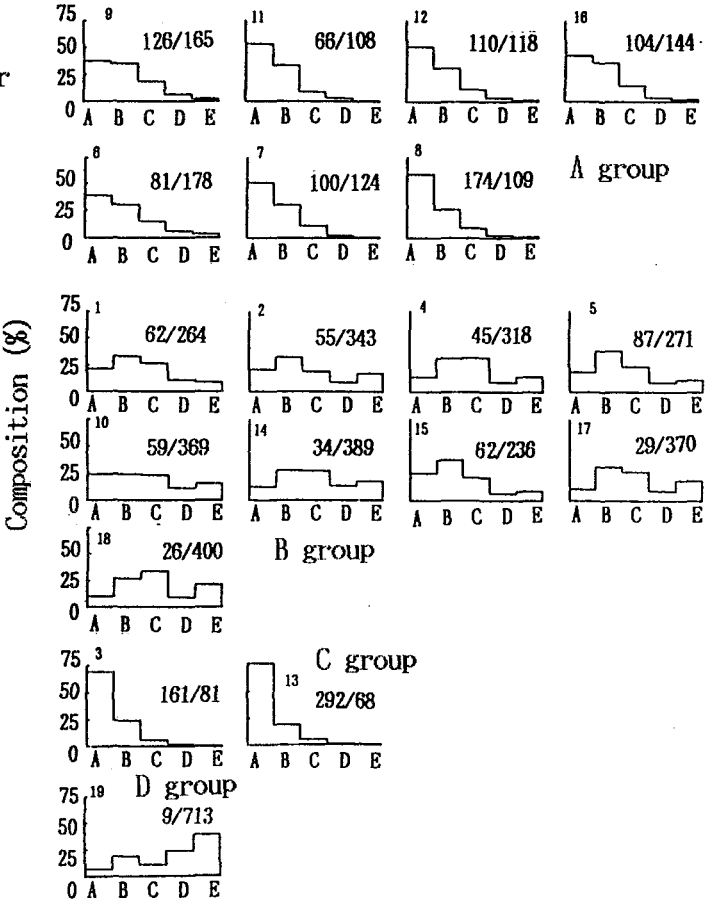


Fig.3 Variation in distribution of tuber size in 19 clones of arrowhead.
1) Tuber size;A:~79mg,B:80~199mg,C:200~399mg,D:400~599mg,and D:600mg~
2) Figure means (number of tuber per plant/average fresh weigt, mg)

that the type of dormancy breaking in tubers except large size is related to the group which each clone belongs to.

Fluctuation of herbicidal efficacy

Susceptibility to bensulfuron-methyl and pyrazolate in nine clones of the A, B and C groups is given in Fig.6. Fluctuation in herbicidal efficacy among clones was more conspicuous in bensulfuron-methyl than in pyrazolate. A tendency that susceptibility to bensulfuron-methyl was high in the clones of the B group, and low in the clones of the C group was recognized except clones No.6 and 15 in which herbicidal efficacy was not detected.

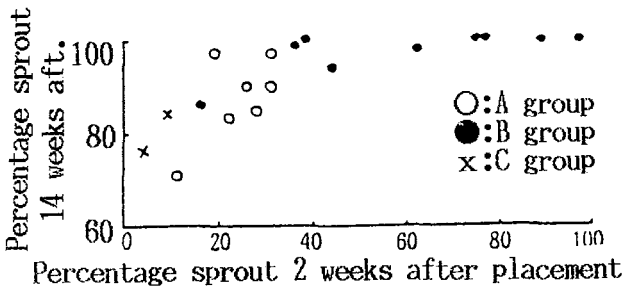


Fig.4 Relationship between percentage sprout at 2 and 14 weeks after placement in water in tubers of 18 clones of arrowhead.

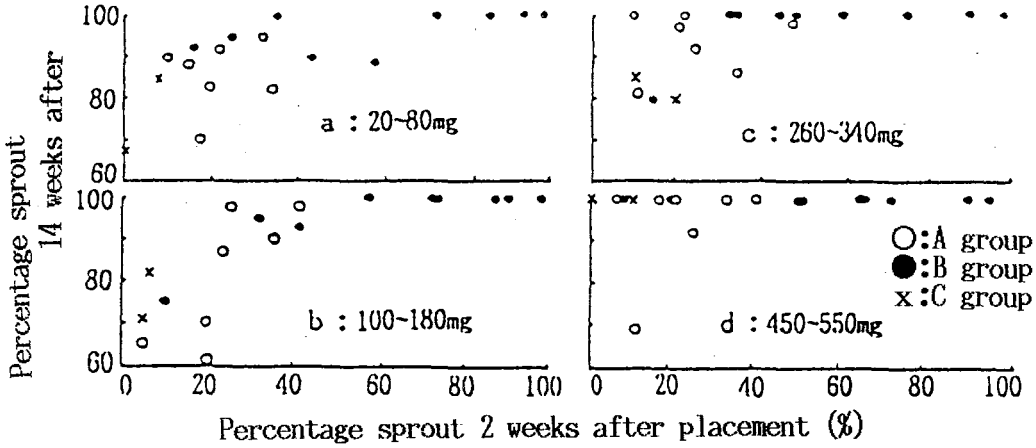


Fig.5 Relationship between percentage sprout at 2 and 14 weeks after placement in tuber sizes (a~d) in 18 clones of arrowhead.

A significant multiple regression formula with 3 factors, average dry weight per plant(X_1), average fresh tuber weight(X_2) and percentage sprout of tuber (X_3), was obtained through the differences in susceptibility(Y%) to bensulfuron-methyl among nine clones from the A, B and C groups as shown in Fig.7.

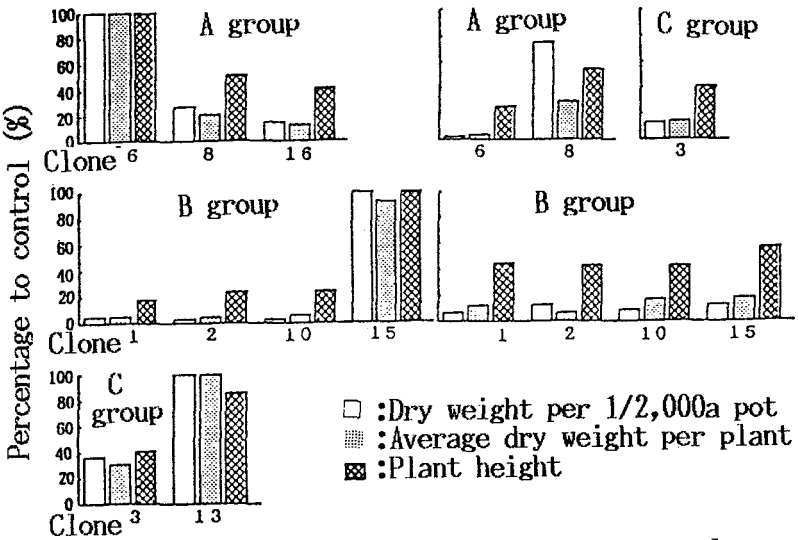
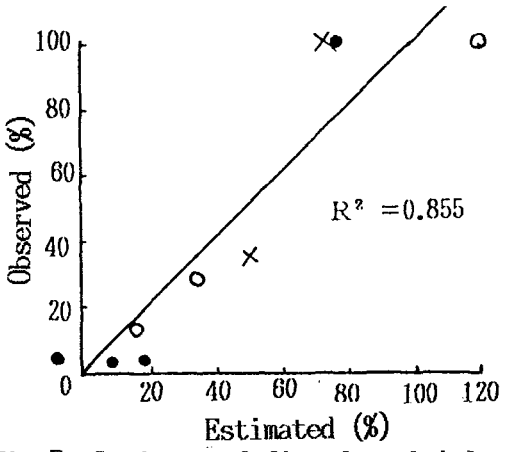


Fig.6 Fluctuation in herbicidal efficacy among clones and groups in arrowhead by bensulfrom-methyl(5.1g a.i./ha:left) and pyrazolate(300g a.i./ha:right).

These results suggest that the intraspecific variation in susceptibility to herbicides can be explained by combination of the ecological traits in arrowhead in southern Japan. Further studies are necessary to clarify the role

of physiological variation such as herbicide tolerance which is another significant factor for the fluctuation in herbicidal efficacy to arrowhead.



Y=-0.48X1 +0.32X2 +4.29X3 -196.85
Y:Dry weight of survived plant per 1/2,000a pot(% control).
X1:Average dry weight per plant in control plot, mg.
X2:Average fresh tuber weight(mg)
X3:Percentage sprout of tuber 14 weeks aft.(%)

○:A group
●:B group
x:C group

Fig.7 Goodness of fit of mmultiple regression formula for susceptibility to bensulfuron-methyl(5.1g a.i./ha) in clones and groups of arrowhead.

References

1) Ito,K. and M.Miyahara 1986 Intraspecific variation in propagule formation and emergence from tubers in arrowhead. Weed Res.,Japan31(Suppl.):117-118.
2) Ito,K. 1989 Ecology and control of arrowhead, *Sagittaria trifolia* L., a perennial paddy weed. Weed Res.,Japan34(2):101-106.
3) Ito,K. and M.Miyahara 1989 Ecological studies on the conditions of emergence of propagules in *Sagittaria trifolia* L., a perennial paddy weed IV. Time of dormancy breaking of tubers in paddy fields. Weed Res.,Japan 34(2):154-162.
4) Morita,H. and Y.Doi 1981 Some characteristics of arrowhead and cultivated arrowhead escaped in Hokkaido. Weed Res.,Japan26(Suppl.):57-58.
5) Morita,H., S.Nakayama and Y.Kawana 1991 Variations in traits of tuber formation in arrowhead, a perennial lowland weed in Kyushu district. Weed Res., Japan36(Suppl.I):108-109.
6) Yamakawa,S., K.Ide, H.Kobayashi and K.Ueki 1983 Intraspecific variation in arrowhead -Growing behavior in clones collected in Kinki district-. Weed Res., Japan28(Suppl.):13-14.
7) Yamakawa,S., K.Ide, H.Kobayashi and K.Ueki 1986 Clonal variation in the effect of nitrogen application on growth and propagule production of arrowhead, *Sagittaria trifolia* L.. Weed Res.,Japan31(1)41-50.
8) Yamakawa,S 1991 Intraspecific variation of *Sagittaria trifolia* L., a perennial paddy weed. Weed Res.,Japan36(1):8-16.

NEW APPLICATION METHODS OF WATER DISPERSIBLE GRANULE(WG) FOR PADDY RICE HERBICIDES IN JAPAN

Kenji Wakayama *[1)], Masayuki Hamada[1)], Tsutomu Nawamaki[1)]
Naoki Matsumoto[2)], Hirokazu Kamatani[2)], Toshiyuki Kokabu[2)]
[1)]Herbicide Group, Shiraoka Research Station of Biological Science,
Nissan Chemical Industries, Ltd., Saitama, Japan
[2)]Division of Agrochemicals of the Research, Central Research Institute,
Nissan Chemical Industries, Ltd., Chiba, Japan

Abstract. NC-355(commercial name : DIEHARD, pyrazosulfuron-ethyl 3.5% a.i.+ CH-900 50% a.i.) WG(water dispersible granule) and other WG are being developed as herbicides for paddy rice in Japan. Greenhouse pot experiments checking WG efficacy and crop safety were comparable to those for granule formulations. Selected field trials in paddy rice plots were also evaluated. In pot experiments, WG had high levels of herbicidal activity and crop safety. These results were reflected in field trials. Drop treatment of WG dissolved uniformly. Various application methods of WG, including drop treatment in water entrance during irrigation and direct application, provided effective weed control and crop safety. These methods could reduce farm labor and application time of herbicides.

Key words ; water dispersible granule, pyrazosulfuron-ethyl, CH-900, application method, paddy field

INTRODUCTION

Herbicides with 30kg/ha granular formulations are generally used in Japan for paddy rice, and are broadcasted by hand or machine onto the flood water. Recently, owing to a shortage of farm labor, aging farmers and increase of large scale paddy field management, it is desirable that the application of herbicides would be easy to handle, labor-saving and low input. NC-355(commercial name : DIEHARD, pyrazosulfuron-ethyl 3.5% a.i. + CH-900 50% a.i.) WG(water dispersible granule 0.6kg/ha) and other WG(0.42-2.5kg/ha) are being developed as herbicides for paddy rice fields by Nissan Chemical Industries, Ltd.. The purpose of the present work is to WG for paddy fields using new application methods, especially for NC-355 WG.

MATERIALS AND METHODS

Formulation studies. Dispersibility of conventional and enhanced formulation was measured for water ranging in temperature from 10C° to 28C°. Each 12g of NC-355(commercial name : DIEHARD, pyrazosulfuron-ethyl 3.5% a.i. + CH-900 50% a.i.) WG was put into a test tube with 100ml water at desired temperature, and turned upside down 3 times. After that, each suspension was passed through 106 μ sieves, and the remains on the sieves were weighed. The data of dispersibility was calculated as a percentage of remains vs. initial weight.

Greenhouse studies. Pot studies were conducted to verify herbicidal activity and crop tolerance of NC-355 WG and granule formulation under different conditions. Light clay soil(sand 28.2%, silt 43.5%, clay 28.2%, total carbon 0.73%, pH 7.1) was paddled and leveled in plastic pot. Weed seeds and tubers

were planted. Rice(*Oryza sativa L. var. Nihonbare*) seedlings at 2.5-3.0 leaf stage were transplanted, two plants per pot. Diluted suspension of NC-355 WG and the same technical granular formulation (pyrazosulfuron-ethyl 0.07% a.i. + CH-900 1.0%a.i.) were applied to flood water 2-4 days after transplanting . Water levels were maintained at a depth of 4cm during tests. Under the same conditions, activity of NC-355 WG on different leaf stages of *E. crus-galli* was checked. Visual assessment of herbicide activity and crop tolerance was made on 2-4 weeks after treatment. The assessment was based on comparison of treated plants which were rated from 0(no effect) to 9(complete kill). Two weeks after treatment, fresh weight of *E. crus-galli* and rice were measured, and % of control was calculated. All pot studies were replicated 3 times.

Field trials. From 1993 to 1995 seasons, field trials were carried out at the Shiraoka research station of Biological Science of NISSAN CHEMICAL and Ibaraki University in Japan. Three to five days after puddling, rice seedlings at 2.5 leaf stage were transplanted into the paddy field at a planting depth of approximately 3cm by transplanter.

Field trials. - Paddy injection studies -. Trials with a plot size of 1.8m² were block randomized with 2 replications. 60g of NC-355 WG was diluted in 500ml water. Desired application rates were achieved by injecting measured volumes of diluted NC-355 into the flood water. The granule formulation, used as reference, was cast by hand onto the flood water. The flood water in the test plots was maintained at 4 to 5 cm depth during trial period. Visual assessment of herbicidal activity and crop tolerance were evaluated in comparison with the untreated plots 1-4 weeks after application. 0.5cm of water depth loss a day was observed.

Field trials. - Dispersibility studies -. Trials with a plot size of 6m²(0.4X15m) were block randomized with 2 replications. NC-355 WG 60g was diluted in 500ml water, and the suspension was applied into the flood water at the short side of rectangular plots under the same conditions as the above mentioned field trial. At the check points, herbicidal activity and crop tolerance were evaluated in comparison with the untreated plots 1-4 weeks after application. TH-913ST(imazosulfuron 1.7% a.i., pyributicarb 12.0% a.i., daimuron 27.5% a.i.) SC(Suspension concentrate) were used as reference.

Field trials. - Drop treatment in water entrance during irrigation -. Trials with a plot size of 250m²(6.25X40m) were block randomized. NC-355 WG 60g was diluted in 500ml water, and the desired dose suspension was drop into the water during irrigation. The flood water was raised to 5-6cm depth. At the check points, herbicidal activity and crop tolerance were evaluated in comparison with the untreated plots 1-4 weeks after application.

Field trials. - Direct application of WG -. Trials with a plot size of 18m²(0.8X22.5m) were block randomized. Diluted NC-355 WG 60g in 500ml water and undissolved WG was applied at short side of rectangular plots into the flood water under above mentioned field trial conditions. At the check points, the soil was collected with a boller of 6.3cm diameter to 10cm depth. Collected soil was puddled and leveled in plastic pots with weed seeds after 1-2 weeks. Herbicidal activity was evaluated in comparison with the untreated plots and uniform application.

RESULTS AND DISCUSSION

Formulation. Enhanced formulation of NC-355 WG had a good dispersibility even at 10C° also at 28C°. (Fig.1)

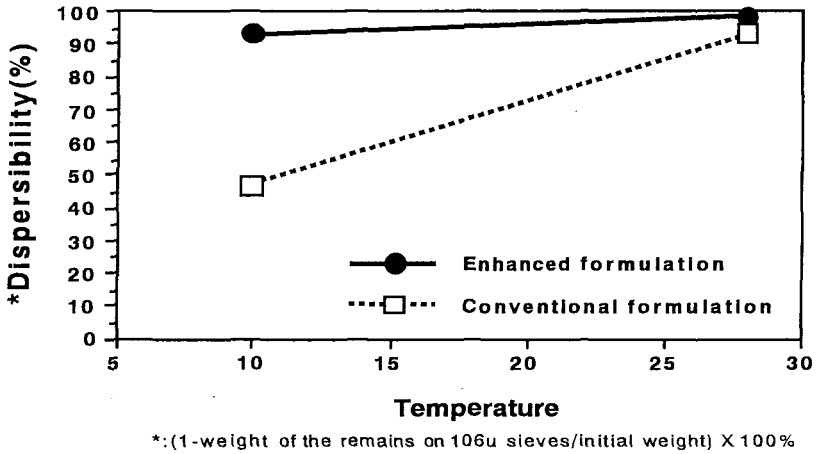


Fig. 1 Spontenius dispersibility of NC-355WG

Greenhouse studies. NC-355 WG at the rates 150 to 600g/ha provided excellent control of annual and perennial weeds and sedges, similar to granular formulation. Crop tolerance of WG was also good safe as granular formulation. (Table. 1)

Table 1. Weed control spectrum and crop tolerance of NC-355 WG compared with granular formulation

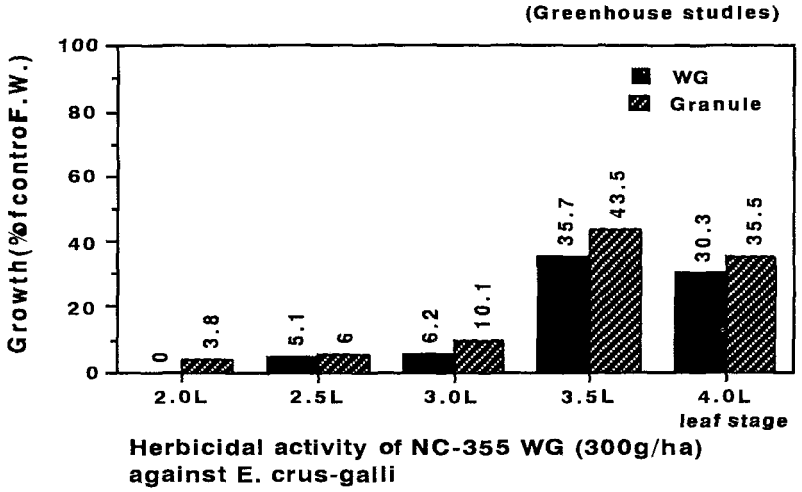
(Greenhouse studies 31DAT)

Formulation	Dose (g/ha)	pyrozosulfuron-ethyl +CH-900 Dose(g ai/ha)	ECHCR	SCPJU	MOOVA	ROTIN	SAGPY	CYPSE	RICE
NC-355 WG	150	5.25 + 75	9.0	8.0	8.0	7.0	8.0	9.0	0.0
	300	10.5 + 150	9.0	8.0	8.5	7.0	8.0	9.0	0.0
	600	21.0 + 300	9.0	8.5	8.5	8.0	8.0	9.0	0.0
NC-355 Granule	7500	5.25 + 75	9.0	8.0	8.0	7.0	7.0	9.0	0.0
	15000	10.5 + 150	9.0	8.0	8.0	8.0	8.0	9.0	0.0
	30000	21.0 + 300	9.0	8.5	8.5	8.0	8.0	9.0	0.0

Visual assessment : no effect(0) - complete kill(9)

ECHCR : *Echinochloa crus-galli* SCPJU : *Scrypus juncoides*
MOOVA: *Monochoria vaginalis* ROTIN : *Rotala indica*
SAGPY : *Sagitaria pygmaea* CYPSE : *Cyperus serotinus*

Activity of NC-355 WG on E. crus-galli. WG formulation of NC-355 gave excellent control of 2.5-3.0 leaf stage of *E. crus-galli* at 300 and 600 g/ha. WG formulation was superior to Granule formulation.(Fig. 2)



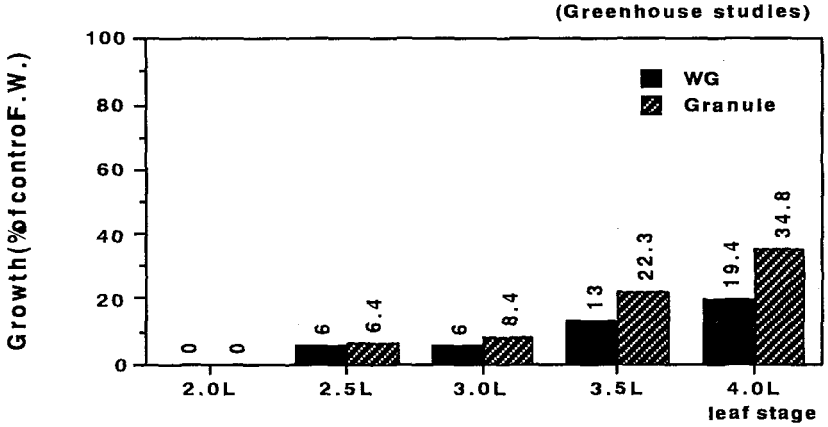


FIG. 2 Herbicidal activity of NC-355 WG(600g/ha) against E. crus-galli

Field trials. - Paddy injection studies -. Spreadability of NC-355 WG was seen to attack *E. crus-galli*, *Scrypus juncoides*, while maintaining an excellent crop safety. (Fig. 3)

Field trials. - Drop treatment in water entrance during irrigation -. During irrigation, dissolved NC-355 WG was dropped in water entrance. NC-355 WG controlled weeds perfectly up to 30m from water entrance, while maintaining crop tolerance.(Fig. 4)

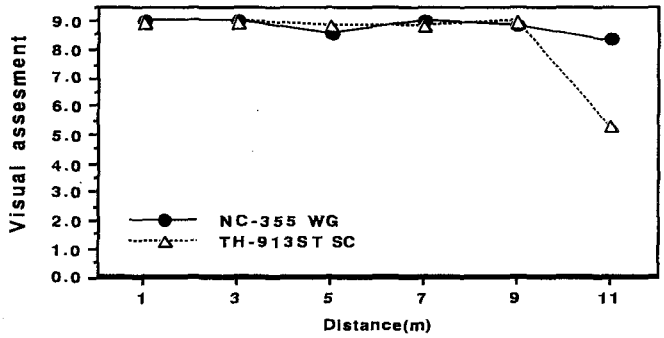


Fig. 3 Spread efficacy of NC-355 WG on E. crus-galli

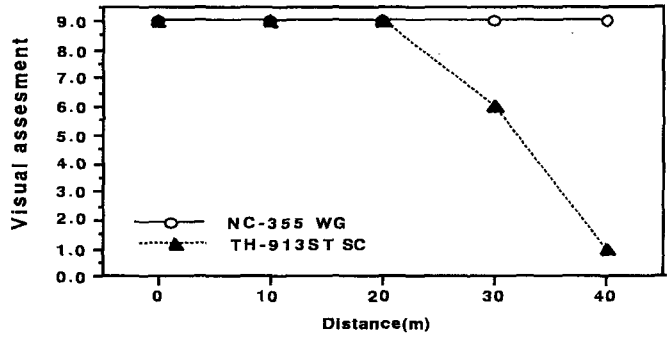
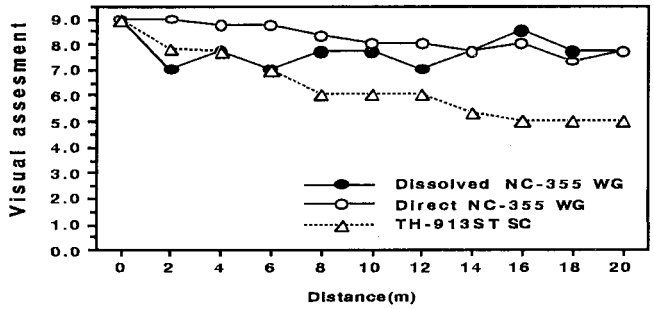
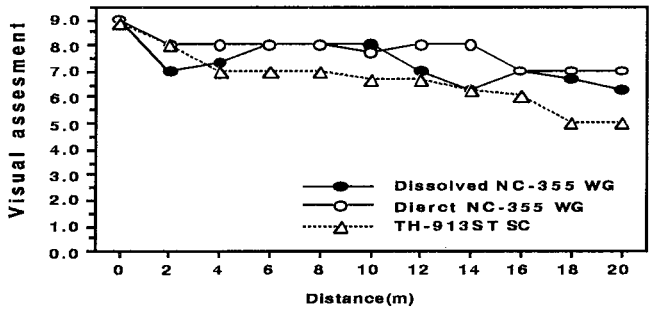


Fig. 4 Efficacy of NC-355 WG against E. crus-galli at drop treatment in water during irrigation

Field trials. - Direct application of WG -. Efficacy of direct application of NC-355 WG was excellent in controlling weeds up to 20m; as good as dissolved NC-355 WG. (Fig.5)



Efficacy of direct and dissolved application by dissolved and direct NC-355WG on E. crus-galli



Efficacy of dierct and dissolved application by NC-355 WG on Monochoria varginalis

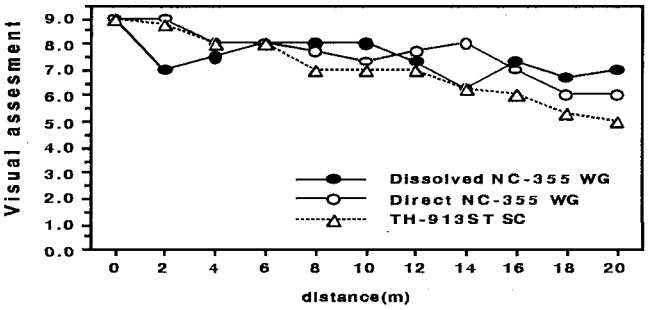


Fig. 5 **Efficacy of direct and dissolved application by NC-355 WG on Scrypus juncoides**

Acknowledgment : The authors gratefully acknowledge the contributions of Dr. R. Sago in Ibaraki University in the field trials for this paper.

Throw-in Type Formulation of Quinoclamine (ACN) Giant Foaming Tablet and Its Algicidal Mode of Action

Seigo Koura, Sigeji Ueda, Syuichi Usui, Kiyoshi Takasuka, Noriyoshi Katsuyama, Yukiharu Sato* and Ko Wakabayashi*
Agro-kanesho Co., Ltd. Research Center, 852 Shimoyasumatsu, Tokorozawa-shi, Saitama, Japan.
*Graduate School of Agricultural Science, Tamagawa University, Machida-shi, Tokyo, 194 Japan.

Abstract. ACN, 2-amino-3-chloro-1,4-naphthoquinone, is a well-known chemical which controls the growth of aquatic weeds and algae in paddy fields.[1, 3] The throw-in type formulation of ACN giant foaming tablet (now commercially known as ACN "Jumbo"-formulations [The Jumbo-formulation is massive formulation with spherical or disk shape, according to The Japan Association for Advancement of Phyto-Regulators]) is a new formulation to enhance weed control and to make the practices used in paddy fields more convenient. In this presentation, the algicidal mode of action of the ACN Jumbo-formulation will be introduced focusing on the following three items biologically and biochemically ; (1) Diffusion of the active ingredient into paddy water, (2) Effect of the Jumbo-formulations on algae and surface soil separation, (3) Algicidal mode of action of quinoclamine (ACN).

Key words' throw-in type formulation , ACN, giant foaming tablet, diffusion into paddy water, algicidal mode of action

INTRODUCTION

The development of Jumbo-formulation of ACN was requested by Japanese farmers, because of its simple application such as throwing it from the footpaths between paddy fields. It was sometimes recognized that the effect of the pesticide decreased when algae or soil surface exfoliation occurred at the time of application of the pesticide. So, it makes more effective to apply ACN Jumbo-formulation before the application of the pesticides. It becomes very much important to make clear the algicidal action of ACN Jumbo-formulation biologically and biochemically, in order to use the formulation environmentally safe. In this presentation, we will show also the developmental studies of ACN Jumbo-formulation.

MATERIALS AND METHODS

1, Diffusion of the active ingredient of ACN into paddy water from the ACN Jumbo-formulations

Paddy field experiments have been conduced since 1992 in Japan. These trials contained four (one time in 1992 and three times in 1993) replicates, with a plot size of

50 m^2 each. ACN Jumbo-formulations were applied from footpath between paddy fields with throwing.[6)]

2. Effect of the ACN Jumbo-formulations on algae and surface soil separation

Effect were observed and determined at the same time as the diffusion experiments of 1.[7)]

3. Algicidal mode of action of the active ingredient of the ACN Jumbo-formulations, ACN

Experiments using green micro-algae, exactly *Scenedesmus acutus*, were performed in order to confirm the real algicidal mode of action of ACN, and the active principle of the ACN Jumbo-formulations.[2)] Growth inhibition, chlorophyll decrease, accumulation of protoporphyrin-IX and ethane formation in the presence of ACN were investigated, comparing with those effects of ioxynil (photosynthetic electron transport inhibitor), dinoseb (uncoupler in photosynthetic ATP production), paraquat (superoxide producer in PS-I system)[8)] and chlorophthalim (peroxidizing herbicides).[10,11)] Growth inhibition and chlorophyll decrease were checked also using heterotrophic *Scenedesmus* cells, in order to determine the inhibition of chlorophyll biosynthesis. Algal culture and measurements of effects were done according to the method of Watanabe *et al.*[5,12)]

RESULTS AND DISCUSSION

1. Diffusion of the active ingredient, ACN, into paddy water from the ACN Jumbo-formulations

The under water diffusion of the active ingredient was very rapid so that ACN could diffuse over 50 m^2 within 24 hr after application in paddy water. The ACN exudation into the water from ACN Jumbo-formulations increased than the ACN guranule-formulation and disappeared very rapidly. The foaming ingredients in the Jumbo-formulation is considered to give an improvement for the underwater diffusion of ACN and to contribute to efficacy of the Jumbo-formulation (see Fig. 1 and 2)

2. Effect of the ACN Jumbo-formulation on algae and surface soil separation

Application of the ACN Jumbo-formulation (2 pieces/are) showed excellent algae control as well as very rapid prevention of the surface soil separation indicating no injury to transplanted rice plants. Its efficacy was stronger than that of the granule-formulation when they were applied at the early or middle growing stage of algae, and lasted for more than 3 weeks. From these results, it was confirmed that the ACN Jumbo-formulation was efficient enough to control algae and surface soil separation in paddy fields.

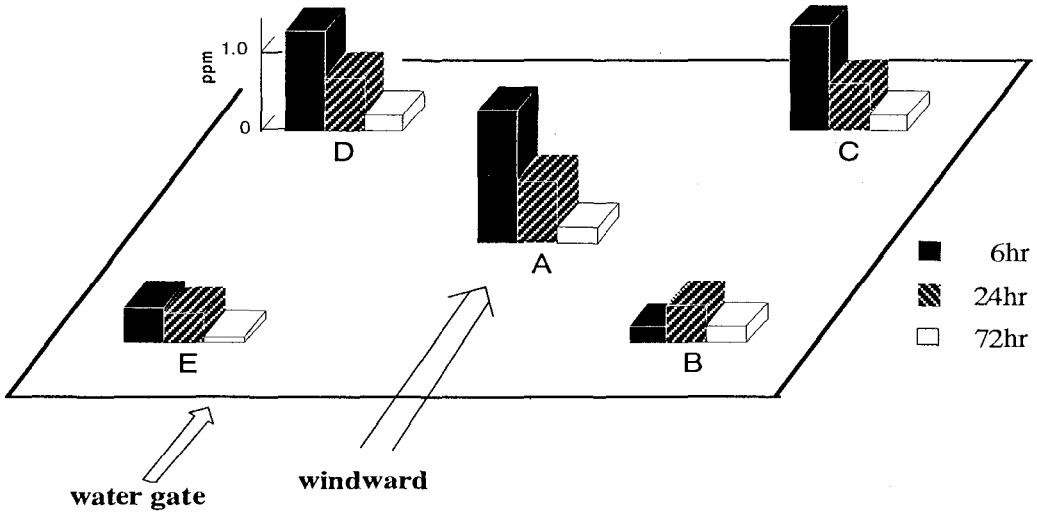


Fig. 1 Under water ingredient concentration of throw-in type formulation of Quinoclamine(ACN) giant foaming tablet

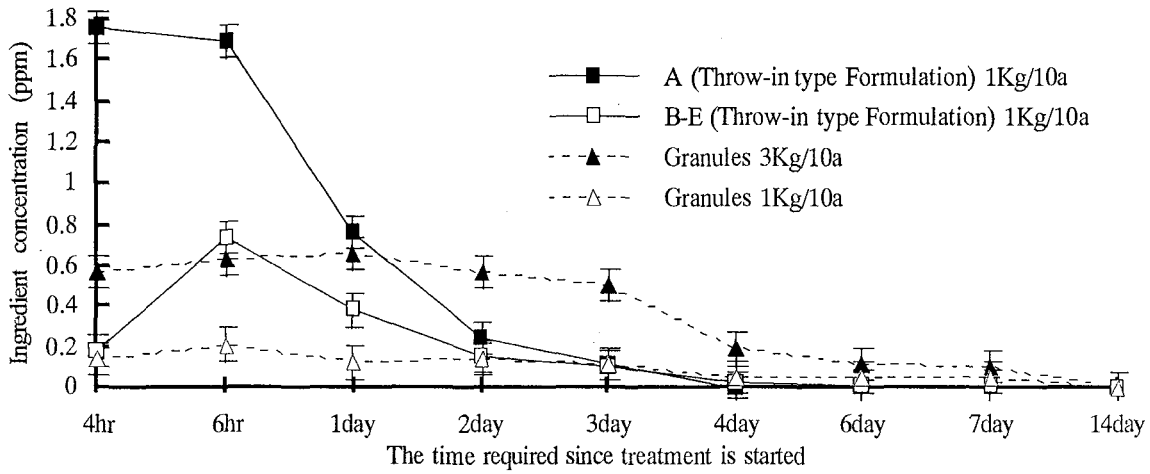


Fig.2 A change with passage of time on active ingredient under water

3. Algicidal mode of action of the active ingredient of the ACN Jumbo-formulations

ACN inhibited the *Scenedesmus* growth almost completely at the concentration of 10^{-5} M and the 50% inhibition was observed at 10^{-6} M both in autotrophic and heterotrophic conditions. This result is very similar to the peroxidizing herbicides, chlorophthalim (Table 1). Although ACN slightly affected the chlorophyll contents in *Scenedesmus* cells even at 10^{-4} M in the heterotrophic cultures, ACN greatly decreased chlorophyll contents at 10^{-6} M in the autotrophic cultures (Table 2). This phenomenon was quite different from phytotoxic symptoms exhibited by ioxynil, dinoseb and paraquat, but similar to the chlorophyll decrease by chlorophthalim in autotrophic *Scenedesmus* cultures. However, chlorophthalim decreased chlorophyll contents even in the heterotrophic condition at 10^{-6} M. These findings indicated that ACN inhibited the chlorophyll biosynthesis scarcely or very slowly.

Table 1 Effect of ACN on Chlorophyll content in autotrophic *Scenedesmus acutus* cells over 20hr of cultivation.

Compound	Concentration (M)	Growth (ml p.c.v./ml)	Chlorophyll (mg/ml p.c.v.)
At Start		2.2	5.95
Control		6.6	5.32
ACN	10^{-4}	2.1	0.00
	10^{-5}	2.6	0.07
	10^{-6}	4.2	3.67
Dinoseb	10^{-5}	5.3	5.91
	10^{-6}	6.6	5.50
Ioxynil	10^{-5}	5.7	5.82
	10^{-6}	6.5	5.71
Chlorophthalim	10^{-5}	2.4	0.17
	10^{-6}	2.8	0.25

Table 2 Incubation of heterophically grown *Scenedesmus acutus* cells with ACN : Growth inhibition and Chlorophyll content after a 20-hr incubation period.

Compound	Concentration (M)	Growth (ml p.c.v./ml)	Chlorophyll (mg/ml p.c.v.)
At Start		1.0	7.99
Control		1.5	6.52
ACN	10^{-4}	1.1	4.76
	10^{-5}	1.1	4.76
	10^{-6}	1.3	5.22
Chlorophthalim	10^{-5}	1.0	2.85
	10^{-6}	1.1	4.90
	10^{-7}	1.3	6.65

Although ACN didn't accumulate protoporphyrin-IX in *Scenedesmus* cells at the concentration of 10^{-6} M to 10^{-4} M. ACN evolved ethane, the useful indicator of peroxidizing effect of herbicides, at 10^{-5} M.[9] Ioxynil, dinoseb and paraquat didn't affect chlorophyll contents, protoporphyrin-IX accumulation nor ethane evolution in the cell. Chlorophthalim greatly accumulated protoporphyrin-IX and evolved in ethane in *Scenedesmus* cultures. These data indicated that the algicidal mode of action of ACN was quite different from the photosynthetic electron inhibition, uncoupling mechanism of dinoseb and photosynthetic inhibition of paraquat (Table 3 and 5).[4] Algicidal mode of action of ACN was a little different from the peroxidizing mechanism exhibited by chlorophthalim, because ACN didn't accumulate protoporphyrin-IX in the experiments in this study. If ACN has a peroxidizing herbicidal action on algae, it may accumulate another unidentified photosensitizer except protoporphyrin-IX or protoporphyrin-IX itself in a very short period before the first detection time (Table 4). Finally, it was confirmed that 5 x 10^{-6} M (the lowest concentration for perfect control of *Scenedesmus* within 24

hr) of ACN showed perfect inhibition after soaking for 3 hr. Therefore ACN Jumbo-formulation was expected that it become an available algicide with immediate and enough effect on algae and surface soil separations by low concentration (Table 6).

It has been known that ACN was easily metabolized to the water-soluble materials (the half-life period : ca. 3 days), and the major metabolite was also 1,4-dihydroxy-naphthalene. The naphthalene was considered to conjugate with natural substances from rice, rat and soil to form nontoxic conjugates against animals and plants. Therefore, through the investigations mentioned above, the ACN throw-in type formulation (Jumbo-formulation) become an efficient algicide to control algae and surface soil separations, without any worse effects to environments.

Table 3 Effect of ACN on Protoporphyrin-IX accumulation and Ethane evolution in autotrophic *Scenedesmus acutus* cells cultivation.

Compound	Concentration (M)	Protoporphyrin-IX (nM/ml p.c.v.)	Ethane evolution (nM/ml p.c.v.)
Control		2.0(1hr)	0.28(24hr)
ACN	10^{-4}	3.5	10.43
	10^{-5}	2.0	7.51
	10^{-6}	2.0	0.23
Dinoseb	10^{-5}	2.0	0.34
	10^{-6}	1.5	0.18
Ioxynil	10^{-5}	2.0	0.30
	10^{-6}	2.5	0.22
Chlorophthalim	10^{-6}	23.5	8.77

Table 4 Several pI_{50}-values obtained using *Scenedesmus acutus* cells.

Compound	pI_{50}-values		
	Growth	Chlorophyll	Ethane
ACN	6.08	5.82	4.09
Dinoseb	4.65	< 4.00	< 4.00
Ioxynil	< 4.00	< 4.00	< 4.00
Chlorophthalim	5.97	5.61	6.21

Table 5 Effect of ACN and Paraquat on Protoporphyrin-IX accumulation and Ethane evolution in autotrophic *Scenedesmus acutus* cells cultivation.

Compound	Concentration (M)	Growth (ml p.c.v./ml)	Chlorophyll (mg/ml p.c.v.)	Protoporphyrin-IX (nM/ml p.c.v.)	Ethane evolution (nM/ml p.c.v.)
Control		3.60	4.07	8.76(1hr)	0.13 (20hr)
Paraquat	10^{-5}	2.90	3.17	4.34	0.30
	10^{-6}	1.3	2.98	6.57	0.15
ACN	10^{-5}	1.35	0.12	8.91	5.47
	10^{-6}	1.82	2.32	8.04	0.40

Table 6 Effect of soaking time on Growth inhibition and Chlorophyll content in autotrophic *Scenedesmus acutus* cells over 24-hr of cultivation.

Concentration (M)	Soaking time (min. or hr.)	Growth (ml p.c.v./ml)	Chlorophyll (mg/ml p.c.v.)
At Start		2.2	8.54
Control		7.4	7.54
ACN	5 min	6.4	6.30
(5×10^{-6})	10 min	5.8	5.78
	20 min	5.5	5.18
	30 min	5.3	4.06
	1 hr	4.5	3.58
	2 hr	4.2	2.38
	3 hr	3.2	0.38
	5 hr	2.3	0.00
	24 hr	2.2	0.00

'Acknowledgment' : We gratefully acknowledge the help and advice by N. Yoshizawa (JAPR), M. Kamoi (JAPR), Prof. Dr. K. Ishizuka (Univ. of Tsukuba), Prof. Dr. S. Iwahori (Univ. of Tsukuba), Prof. Dr. P. Böger (Univ. of Konstanz), Dr. H. Watanabe (Mitsubishi Chemical Corporation) and Dr. H. Kohno (Tamagawa Univ.), for the present study.

References

1) Black, C. and L.Myers 1966. Some biochemical aspects of the mechanism of herbicidal activity. Weeds. 14 (4) : 331
2) Böger, P. and G. Sandman. 1990. "Chemistry of plant protection", ed. by W, B. Bowers. 6 : 173-216.
3) Hagimoto, H. 1970. The herbicidal activity of 2-amino-3-chloro-1,4-naphthoquinone Part 3 Mode of action and invitation. Weed Res. 10 (3) : 187.
4) Koura, S. C.Ogino., K.Wakabayashi. 1993. Abstracts of papers, 18th Annual Meeting of Pesticide Science Society of Japan, Tokyo, March, p.81.
5) Koura,S. K.Takasuka, N.Katuyama, C.Ogino, Y.Sato, and K.Wakabayashi. 1994. Mode for Herbicidal Action of 2-Amino-3-chloro-1,4-naphthoquinone (ACN). Biosci. Biotech. Biochem., 58 (7) : 1210-2.
6) Koura,S. K. Noritake, M.Kamoi, T.Tanaka, C.Ogasawara, S.Ueda, H.Kondo, E. Taniguchi, T. Kataoka. 1994. Diffusion form of quinoclamine (ACN) giant foaming tablets in paddy field water, Weed Res. 39 (2) : 91-95.
7) Koura, S. K.Noritake, M.Kamoi, T.Tanaka, C.Ogasawara, S.Ueda, Y.Takahashi, Y.Seki, T.Kataoka. 1994. Effect of quinoclamine (ACN) giant foaming tablets on the control of surface soil separation and green algae. Weed Res. 39 (2) : 96-101.
8) Sandman,G. and P.Böger. 1982. Plant Sci. Lett.. 24 : 347-352.
9) Sumida,M. S.Niwata, S. Iwata, T. Tanaka, K. Wakabayashi, and P. Böger. 1993. in "Pesticide
/Environment : Molecular Biological Approaches," ed. by T. Mitsui, F.Matsunaka, and I.Yamaguchi,
Pestic. Sci Society of Japan, Tokyo. : 255-262.
10) Wakabayashi,K. 1989. Plant Protection. 43 : 575-584.
11) Wakabayashi,K. 1978. Advances in Pesticide Science, Part 2"ed. by Geissbuhler, H. : 256-260.
12) Watanabe, H. Y.Ohori, G.Sandman, K.Wakabayashi, and P.Böger. 1992. Pestic. Biochem. Physiol. 42 : 99-109.

APPLICATION OF FLOWABLE HERBICIDE ON IRRIGATION INLET OF LARGE PADDY FIELD

R.Tanaka* and H.Takahashi
Miyagi Pref. Agr. Res. Cent., Takadate Natori 981-12
and Furukawa Agr. Exp. St., Suwa Furukawa 989-61,Japan

Abstract:To reduce labor in weed control on large paddy fields, since uniform application of granular formulations is difficult on fields with short sides longer than 30m, we tested flowable herbicides poured into irrgation inlets and carried by water to these large fields(0.3～1.4ha). The result was successfull and there was no illeffect on rice. This is thus a more efficient technique than broadcasting granules.

Key words:large paddy fields, flowable herbicides, irrigation inlets, labor-saving application

Introduction

Recently in northeastern district of Japan, size of paddy fiels are made more larger by the agricultural land reformation year by year. But uniform application of granular fertilizes or chemichals on the large fields with short sides longer than 30m is very difficult from ridges.[1] The Aapplication of the flowable though the spillway could be performed more ready and in a shorter period than brordcasting of granules.[2] Then we tested flowable herbicides poured or dripped into irrigation inlets and carried by water to these large fields for a labor-saiving method.

Materials and Methods

We tested some bottled flowable consisting combination of Pyributicarb, Bromobutide and Benzofenap(TSM-612), combination of Imazosul-furon, Pyributicarb and Dymron(TH-913ST), combination of Pyributicarb and Bensulfuron methyl(TDS-888) and combination of Thenylchlor, Pyrazoxfen and Bromobutide(SL-970). These herbicides are on the market as early weed controlers at onece time using.

We selected some large paddy field(0.5～1.4ha) with short sides longer than 30m and applicated flowable herbicides by pouring or dripping into irrgation inlets and carried by water to these large fields. We have known the irrgation rate of each fields before application and then we controled dripp rate and stoped irrigation at just suitable water level(3~5cm) and then We kept the level duaring 4 to 6 days after.

Dominant species on these fields are Early watergrass(Echinochloa oryzicola Vasing), Kuroguwai(Eleocharis kuroguwai Ohwi), Japanese bulrushand(Scirpus juncoides Roxb.var.hotarui Ohwi) and annual weeds including Common falsepimpernel(Lindernia procumbens(Krock.)Borbas), Indian toothcup(Rotala indica(Wiid.)Koehne var. uliginosa(Miq.)Koehne) and Smallflower umbrella sedge(Cyperus difformis L.) etc.

We investgated weed emergency and rice injury at 40days later from planting and rice growth and yield at hervest period.

Results and Discussion

Time of application of flowable herbicides poured or dripped into irrigation inlets of large fields(0.3～1.4ha) was depend on the amount of herbicide and number of inlets and yet less than fifteen minutes. After dripping or pouring, irrigation was continuted some hours until the water level was up to 4～5cm. But we don't have to operate anything during the irrigation time.

And the flowable herbicides poured or dripped into the irrigation inlets were smoothly carried and spreaded to the whole fields by the water.

Effects of weed control were successfuly completed and there were no influence on growth and yeild of the rice. But, a little number of annual weeds on the shallow plots emerged again aerlier than on the deep plots of the same fields.

Then we guess this way is a more labor-saving technique than broadcasting granules on the large paddy fields made plainly. The pouring way needs no dripping tools and has same effects for weed control compared with the dripping way on the condition with enough irrigation water.

Acknowledgement:We wish to thank Farm Advisers of Kahoku, Tukidate, Furukawa,Watari,Nakanida,Hazama and Ishinomaki Agricultural Extension Station for their assistance in the test. We also thank Mitubisiyuka Co., Sankyo Co.,Takeda Chemichal Ind. Co. and ISK Biosiences K.K. for offering of experimental herbicide.

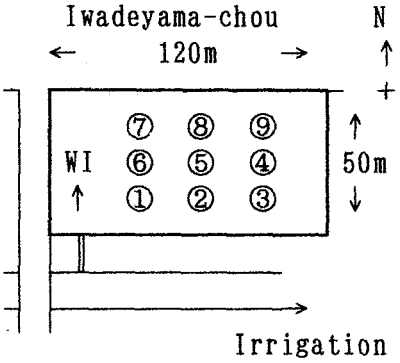
References

1)Ryo TANAKA,Hiroaki TAKAHASHI,Kouichi SANO 1993. Application of Herbicide and Fertilizer to Paddy Field through the Auto-irrigation System. 3. Pouring Flowable Herbicide on Irrigation Inlet. Tohoku Agric. Res. 45 :107-108,Japan

2)Nobumasa ICHIZEN,Makoto KONNAI and Testuo TAKEMATSU 1990. Studies on the Development of Weed Control Method for Paddy Rice through the Apprication of Flowable Formulations. Weed Research 35:(3)261-267,Japan

Table 1. Distribution of weeds on the large paddy field poured flowable herbicide into a water inlet(1993)

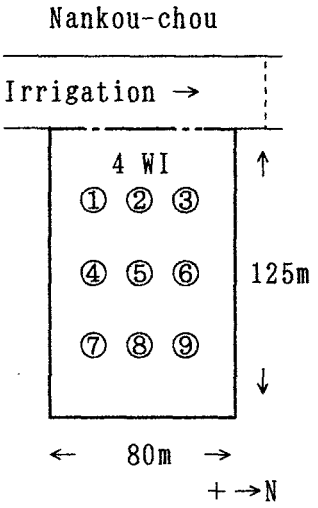
Plot No	E.watergrass (n)	(dw)	Kuroguwai (n)	(dw)	Annual weeds (n)	(dw)
①	0.1	t	0.0	0.0	0.1	t
②	0.1	t	0.0	0.0	0.1	t
③	0.5	t	0.0	0.0	0.0	0.0
④	0.8	t	0.2	t	0.0	0.0
⑤	0.6	t	0.1	t	0.0	0.0
⑥	0.5	t	0.3	0.1	0.0	0.0
⑦	0.4	t	0.1	t	0.0	0.0
⑧	0.2	t	0.1	t	0.0	0.0
⑨	1.0	0.1	0.0	0.0	0.0	0.0



Note:a) n stans for Number. dw stands for g/㎡ of dry weight. t stans for trace(<0.1). We Investigated at 45days later from planting.
b) WI stand for water inllet. Plot ⑨ and ④ are little upland.
c) Fllowable herbicide is TSM-612.

Table 2. Distribution of weeds on large paddy field dripped flowable herbicide on water inlets(1992)

Plot No	E.watergrass (dw/㎡)	(%)	J.bulrush (dw/㎡)	(%)	Kuroguwai (dw/㎡)	(%)
①	0.0	0	0.0	0	0.0	0
②	0.0	0	0.0	0	0.0	0
③	0.0	0	0.0	0	0.0	0
④	0.0	0	0.0	0	0.3	25
⑤	t	t	0.0	0	0.1	8
⑥	0.0	0	t	t	0.1	8
⑦	t	t	0.0	0	0.2	17
⑧	0.0	0	0.0	0	0.1	8
⑨	0.0	0	0.0	0	t	t
Total	t	t	t	t	0.8	67
No weeding	10.2	100	5.6	100	1.2	100



Note:a) t stans for trace(under0.04). % stads for Percentage of dw against no weeding plots. We Investigated at 45days later from planting.
b) Fllowable herbicide is TMS-612.

table 3. Result of tests of flowable herbicide dripped on water inlets of large paddy fields(1992,1995)

Place	Iwadeyama	Monou	Nangou	Toyosato
area	60a(120x50m)	48a(120x40m)	100a(125x80m)	60a(120x50m)
Flowable	TMS-612	TMS-612	TMS-612	TDS-888(1995)
Period	17 May(+8)	9 May(+7)	21 May(+11)	15 May(+7)
Amount	6ℓ,(1ℓ)	5ℓ,(1ℓ)	12ℓ(1.2ℓ)	3ℓ,(0.5ℓ)
Inlets	1	1	4	2
Drip device	Lonfriend	Lonfriend	Petbottle	Lonfriend
rate	15mℓ/minute	30mℓ/m	38mℓ/m	30mℓ/m
Time	6.5hours	2.5h	5.2h	1.5h
Irrigation	4cm water depth	4.5cm	3.5cm	5cm
Operation	Good	Good	A little good	Good
Weeds	No weeds	No weeds	No applicable weeds	No weeds
Rice injury	Nothing	Almost nothing	Yellow leaves on near inlet	Nothing

Note a) Parenthesis in period of application is number of day from planting.
b) Parenthesis in amount of Flowable is rate of application per 10a.
c) Lonfiend is an autodriper on the market. Petbottle is a handmaking driper with a exit tube.

table 4. Result of tests of flowable herbicide poured into water inlets of large paddy fields(1992,1993)

Place	Monou	Iwadeyama	Ishinomaki	Nakanida
area	48a(120x40m)	60a(120x50m)	100a(125x80m)	50a(125x40m)
Flowable	TMS-612(1992)	TH-913ST	SL-970	TDS-888
Period	12 May(+7)	11 May(+5)	7 May(+5)	19 May(+5)
Amount	5ℓ,(1ℓ)	2.5ℓ,(0.5ℓ)	10ℓ(1ℓ)	2.5ℓ,(0.5ℓ)
Inlets	2	1	2	2
Pouring time	15mimutes	5m	5m	3m
Irrigation	1.5hours	2.5h	2h	3h
Water depth	4cm	5cm	4cm	5cm
Field flatness	A little good	A little good	Good	Good
Water reduction	1cm/day	2cm/day	2cm/day	2cm/day
Operation	Good	Good	Good	Good
Weeds	A little weeds on upland spots	A little weeds on upland spots	No weeds	No weeds
Rice injury	Nothing	Nothing	Nothing	Nothing

fig.1 Dripping a bottle of flowable herbicide on a irrigation inlet

(1995, Toyosato, 60a field)

fig.2 Pouring flowable herbicides on a irrigation inlet

(1995, Natori, 30a field)

CYHALOFOP BUTYL: FORMULATION TECHNOLOGY ON CYHALOFOP BUTYL GRANULE

N. Kondo, T. Matsumoto, K. Matsuya, H. Katahashi, Y. Imai
DowElanco Division, Dow Chemical Japan Limited,
Tennoz Central Tower, 2-24, Higashi Shinagawa 2-chome, Shinagawa-ku, Tokyo 140, Japan

Abstract. Cyhalofop butyl, [R-(+)-n-butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propionate] is a new selective rice graminicide, which can control up to 5-6 LF stage of *Echinochloa crus-galli* by foliar application. Considering the Japanese market, the granule formulation development of cyhalofop butyl was essential. However, the conventional type extruded granule of cyhalofop butyl showed unacceptable bioefficacy. In order to improve bioefficacy of granule formulation on cyhalofop butyl, different commercial ester products on KCℓ core granule were evaluated in green house trials and field trials in Japan. Among them, di-tridecyl phthalate enhanced the efficacy of significantly over the efficacy of water treatment of cyhalofop butyl EC formulation. Employment of di-tridecyl phthalate contributed to the high concentration of cyhalofop butyl on the water surface, prolonged half life of cyhalofop butyl on hydrolysis and enhanced uptake of cyhalofop butyl into the plants. These results suggested that cyhalofop butyl granule containing di-tridecyl phthalate would be effective way to maximize bioefficacy of cyhalofop butyl.
Key words: cyhalofop butyl, *Echinochloa crus-galli*, granule, di-tridecyl phthalate, oil granule technology.

Introduction

Cyhalofop butyl, Japanese code no. DEH-112, is being developed by DowElanco to control *E. crus-galli* in rice paddy. The granule formulation development of cyhalofop butyl was essential considering of the Japanese market. However, conventional type extruded granule of cyhalofop butyl showed poor bioefficacy, contrary to our expectations. In order to improve bioefficacy of granule formulation on cyhalofop butyl, "oil granule technology" was applied to cyhalofop butyl through considering mode of action, such as foliar uptake. This report covers evaluations of oil granule formulations for increasing the efficacy of cyhalofop butyl on rice.

Materials and Methods

Green house trial 1

1% conventional type extruded Gr of cyhalofop butyl was prepared and its bioefficacy was evaluated in comparison with bioefficacy of water application of 30% EC formulation of cyhalofop butyl under green house condition at Gotemba laboratory of DowElanco. The pot used for the trial was 1/5000 are Wagner's pot and water level was maintained at 3 cm. *E. crus-galli* at 1.8 - 2 leaf stage was treated with cyhalofop butyl Gr and EC. Visual assessment of herbicide activity and crop tolerance was made 4 weeks after application with 3 replications (0%: no effect - 100%: complete kill).

Green house trial 2

1% oil Gr of cyhalofop butyl was prepared employing 9% di-tridecyl phthalate and KCℓ core Gr for cyclosal insecticide developed by Nippon Kayaku and its bioefficacy was evaluated in comparison with one of water application of 30% EC formulation of cyhalofop under green house condition at Gotemba laboratory of DowElanco. 1/5000 are Wagner's pot was used for the trial and water level was maintained at 3 cm. *E. crus-galli* at 1.5 - 2 leaf stage was treated cyhalofop butyl Gr and EC. Visual assessment of herbicide activity and crop tolerance was made 4 weeks after application with 3 replications (0%: no effect - 100%: complete kill).

Abbreviations: EC, emulsifiable concentrate; Gr, granule; LF, leaf stage; a.e., acid equivalent; a.i., active ingredient; WAA, weeks after application; Conc., concentration; HAT, hours after treatment.

Chemical assay
0.1 g each of 1% conventional type extruded Gr and 1% oil Gr were applied into 1 ℓ distilled water of 3 ℓ beakers. At 6, 24 and 48 hours after application, cyhalofop butyl concentration in water was analyzed using HPLC method. In addition, 1 and 48 hours after application, cyhalofop butyl concentration on surface of water was also analyzed using HPLC method.

Fate of cyhalofop butyl
Fate of cyhalofop butyl in a laboratory paddy rice: Explanatory studies were conducted using ^{14}C-labelled cyhalofop butyl EC formulation and oil solution for oil Gr formulation in the laboratory of The Dow Chemical Company, U.S.A. Soil/water experiments and sand/water/*E. crus-galli* experiments were conducted for half life of cyhalofop butyl on hydrolysis and uptake of cyhalofop butyl by the plants, respectively. The *E. crus-galli* plants were used at the 2.5 - 3 leaf stage. The oil solution simulated the field use of oil Gr impregnated with 9% di-tridecyl phthalate.

Field trial 1
0.6% oil Gr of cyhalofop butyl formulations containing 4 - 9% of di-tridecyl phthalate on KCℓ core Gr were prepared. Field trials on those formulations were carried out at Fukuoka field station of DowElanco. The plots were separated by corrugated plastic boards. The plot size of the trials was 2 m^2 and the water of the test plot was maintained at 3 cm depth during trial period. *E. crus-galli* at 3 leaf stage was treated cyhalofop butyl Gr formulations. Weed control efficacy and crop injury were visually evaluated in comparison with the untreated plots at 2 weeks after application and 1 week after application, respectively with 3 replications (0%: no effect - 100%: complete kill).

Results and Discussion

Green house trial 1: Bioefficacy of 1% cyhalofop butyl extruded Gr formulation
1% cyhalofop butyl conventional extruded Gr formulation showed poor bioefficacy to 1.8 - 2 LF stage of *E. crus-galli* even at 480 g a.e./Ha, while water application of 30% cyhalofop butyl EC formulation showed excellent - good bioefficacy to 1.8 - 2 LF stage of *E. crus-galli* at 480 - 240 g a.e./Ha (Table 1).

Table 1. Cyhalofop butyl formulation - efficacy on *E. crus-galli* and injury on rice plant (green house trial/Gotemba)

		control/injury at 4WAA	
Formulation	Rate g a.e./Ha	% control of *E. crus-galli*	% injury of rice plant
Cyhalofop butyl 1% extruded Gr	480	35	0
	240	7	0
(conventional)	120	0	0
	60	0	0
Cyhalofop butyl 30% EC	480	99	0
	240	88	0
(water application)	120	40	0
	60	20	0
Leaf stage at the application		1.8 - 2.0 LF	3.5 LF

Green house trial 2: Bioefficacy of 1% cyhalofop butyl oil Gr
1% cyhalofop butyl oil Gr formula-tion exhibited excellent control on 1.5 - 2 LF stage of *E. crus-galli* at 480 - 240 g a.e./Ha and it was superior to the bioefficacy of water application of 30% cyhalofop butyl EC at 480 - 240 g a.e./Ha (Table 2).

Table 2. Cyhalofop butyl formulation - efficacy on *E. crus-galli* and injury on rice plant (green house trial/Gotemba)

control/injury at 4WAA

Formulation	Rate g a.e./Ha	% control of *E. crus-galli*	% injury of rice plant
Cyhalofop butyl 1% oil Gr	480	99	0
	240	94	0
	120	83	0
	60	75	0
Cyhalofop butyl 30% EC	480	90	0
	240	77	0
(water application)	120	60	0
	60	47	0
Leaf stage at the application		1.5 - 2.0 LF	3.5 LF

Chemical assay: Release performance of cyhalofop butyl Gr

1% cyhalofop butyl oil Gr showed extremely high concentration of cyhalofop butyl on the surface of water even 1 hour after application while 1% cyhalofop butyl extruded Gr showed quite low concentration of cyhalofop butyl on the surface of water at 1 hour after application (Table 3).

Table 3. Release performance of cyhalofop butyl on cyhalofop butyl Gr

Formulation	Conc. in water (ppb)			Conc. on water surface (ppb)	
	6 hrs.	24 hrs.	48 hrs.	1 hr.	48 hrs.
Cyhalofop butyl 1% oil Gr	169	148	149	912	907
Cyhalofop butyl 1% extruded Gr (conventional)	168	386	493	60	469

Fate of cyhalofop butyl

The half life for hydrolysis of cyhalofop butyl in a soil/water mixture is about 1 hour or >2 days for application as the EC formulation or the oil solution for oil Gr, respectively (Table 4). In the sand/water/*E. crus-galli* experiments, uptake of ^{14}C activity by the plants after water treatment with EC - formulated [^{14}C] cyhalofop butyl is the 0.2% of the applied activity at 2 - 48 hours and on the other hand, uptake of ^{14}C activity by the plants after water treatment with the oil solution for oil Gr of [^{14}C] cyhalofop butyl is 36% of the applied activity at 24 hours after treatment (Table 5).

Table 4. Time course of the fate of [^{14}C] cyhalofop butyl (EC formulation and oil solution) in a soil/water system[a].

HAT	EC formulation [^{14}C] components in water phase (% cyhalofop butyl)	HAT	Oil solution [^{14}C] components in water phase (% cyhalofop butyl)
0.5	67	0.5	99
2	14	3	95
4	13	5	94
9	<1	9	88
26	<1	26	75
51	N.D.	51	61

a. Application data: 290 g a.e./Ha for EC formulation
312 g a.e./Ha for oil solution

Table 5. Time course of the fate of [^{14}C] cyhalofop butyl (EC formulation and oil solution) in a sand/water/*E. crus-galli* system[a].

HAT	Distribution of recovered activity of EC formulation plant extract	HAT	Distribution of recovered activity of oil solution plant extract
0	<0.1%	0	5%
2	0.2%	1	30%
5	0.4%	5	24%
23	0.1%	24	36%
49	0.3%		

a. Application data: 250 g a.e./Ha for EC and oil solution

Field trial 1 - oil optimization for cyhalofop butyl oil Gr
All 0.6% cyhalofop butyl oil Gr formulations containing 4 - 9% of di-tridecyl phthalate as an oil on KCℓ core Gr exhibited excellent control on 3 LF stage of *E. crus-galli* and no injury on rice at 180 g a.i./Ha (Table 6).

Table 6. Cyhalofop butyl 0.6% G (180 g a.i./Ha) - oil optimization study (field trials/Fukuoka)

Formulation	Content of di-tridecyl phthalate (%)	Rate g/Ha	*E. crus-galli* control at 2WAA (%)	Rice injury at 1WAA (%)
Cyhalofop butyl 0.6% Gr	4	1200	98	0
	5	1500	97	0
	6	1800	98	0
	7	2100	100	0
	8	2400	97	0
	9	2700	98	0
Leaf stage at the application			3 LF	4.5 - 5 LF

Present status of herbicide use in Vietnam

Duong Van Chin
Cuulong Delta Rice Research Institute, Omon, Cantho province, Vietnam

Abstract. Rice is the most important crop in Vietnam. There are two major rice areas, i.e., Mekong river and Red river deltas with 3.96 million hectares, which are 62% of all planted areas in the country. The main crop seasons for rice are Winter-Spring, Summer-Autumn and Autumn-Winter. Lowland rice accounts for 94% of all the rice area in Vietnam. The traditional method of transplanting has shifted to direct seeded system due to the availability of short duration varieties suitable for direct seeding, wide spectrum herbicides with reasonable prices and the shortage of labor during the peak period of transplanting. The shift is taking place faster in the Mekong River than in Red River delta. In direct seeded systems, weeds emerge at the same time and compete seriously with the rice crop. Weed infestation in transplanted and water seeded rice is lower than that of other methods of crop establishment due to the suppressive effect of water. About 400 weed species of 73 families have been reported in rice fields in Vietnam. Two important families are Poaceae and Cyperaceae having 42% of weed species with 21% each. Yield loss due to weeds in the Mekong delta is about 46%. The hand weeding method is still prevalent in Vietnam's rice fields. A few studies on weed control in rice by mechanical, cultural, physical and biological means are reviewed. In recent years, chemical method have increasingly been adopted. The popular herbicides with large scale application in Vietnam are: butachlor, 2,4-D, fenoxaprop-ethyl, oxadiazon, pretilachlor, pyrazosulfuron-ethyl, thiobencarb+propanil.

Key words. weed control, direct seedee rice, Mekong River Delta, crop season, herbicidal activity

Introduction

Total land area of Vietnam is 33.169Mha in which the arable land and land under permanent crops account for 6.697Mha. There are 4.211Mha for rice production sharing 76.5% of arable land in the whole country. Rice is the most important crop in Vietnam with 6.475Mha planted area and the production of 21.59MT in 1992(3). Rice is grown in all eight ecological zones of Vietnam but two main zones for rice production are the Mekong River Delta and the Red River Delta. These two zones have 3.96Mha constituent 62% of rice planted area of the whole country. The other zones are North mountain, Midland, Central coast of northland, Central coast of southland, Central highland, North-east of southland. The south has advantageous climatic condition as compared to the North but it has some other constraints such as drought and flood. Three crops of rice can be grown in the South.

With regard to the above background, this paper outlines rice production and weed control system mainly in the Mekong River Delta in Vietnam.

Rice production in Vietnam

The Mekong delta is the most important rice area in Vietnam. There are three rice cropping seasons in the region ; Winter-Spring season, Summer-Autumn season, Autumn-Winter season. Winter-Spring rice is established at the end of rainy season(November, December) or at the beginning of dry season and harvested in the middle of dry season. Rice duration in Winter-Spring season prolongs only 3.5 to 4 months, and rice yield under this season is highest as compared to others. Summer-Autumn rice is sown or transplanted at the beginning of rainy season and harvested in the middle of the season mainly in the South. The crop duration is about 3.5 to 4 months and yield is lower than that of Winter-Spring season. Autumn-Winter rice is established when rainy season starts and harvested at the end of wet season or early next year. Both modern high yielding and local photosensive varieties are used. Planted areas under this season tend to decrease and

yield is lowest as compared to the other two seasons. The difference in yield is about 1 to 1.5T/ha due to some constraints such as low solar radiation, drought, flood and low yield potential of local varieties(Table 1).

Rice cultivation type in Vietnam

The traditional method of transplanting has shifted to direct seeding system due to the availability of short duration varieties suitable for direct seeding, wide spectrum herbicides with reasonable prices and the shortage of labors during the peak period of transplanting. The shifting is faster in Mekong River Delta as compared to other regions. Both transplanting and direct seeding exist in Central coast of North and Southland. In Red River Delta transplanting rice is popular. There are only 20,000ha of rice established by direct seeding in four years during 1991-1994 in the delta. On the other hand, 94% of planted area in Mekong Delta is direct seeded of which 54% is wet seeding(1). There are four types of the wet seeding ; Traditional wet seeding: pregerminated seeds are sown on well puddled saturated soil. Water seeding: pregerminated seeds are sown on puddled soil under water. Minimum tillage wet seeding: land is plowed and harrowed in dry condition. Fields are watered to reach saturation and pregerminated seeds are broadcasted on the soil surface. Zero tillage wet seeding: rice straw of the previous rice crop is scattered throughout the field and burnt. Fields are watered and pregerminated seeds are sown on moist soil surface(Table 2).

Weed ecology

Weed infestation in transplanted and water seeded rice is lower than that of other rice cultivation methods due to suppressive effect of water. Aquatic weeds are popular in transplanted and water seeded rice. In wet seeded rice cultivation, weeds emerge at the same time and compete seriously with rice crop and cause substantial yield loss. About 400 weed species of 73 families have been reported in lowland and upland rice fields in Vietnam(Table 3)(6). Two most important families are Poaceae and Cyperaceae having 42% of weed species with 21% each. Other important families are: Asteraceae(26species), Scrophulariaceae(18species), Fabaceae(14species), Lythraceae(10species) and Lamiaceae(9species). Predominant weed species observed in lowland rice fields in Mekong delta are: *Echinochloa crus-galli, Echinochloa colonum, Leptochloa chinensis, Paspalum distichum, Cyperus difformis, Cyperus iria, Fimbristylis miliacea, Eleocharis dulcis, Monochoria vaginalis, Marsilea minuta, Ludwigia octovalvis* and *Sphenoclea zeylanica*(1). Yield loss due to weeds in the Mekong delta reaches at about 46%. Some predominant weed species observed in lowland rice fields in Red river delta are: *Echinochloa crus-galli, Echinochloa colonum, Cyperus rotundus, Panicum spp, Heleocharis equisetinea, Eclipta alba, Rotala indica, Marsilea quadrifolia, Monochoria vaginalis* and *Jussiaea repens*(Table 4)(4).

Weed control

Prevention method

One way of weed infestation in direct seeded rice in Vietnam is the contamination of weed seeds into rice seeds. Farmers had the tradition of keeping part of their product to use as seeds for the next season. Trading or exchange rice seeds among farmers cause spreading of weeds though contaminated seeds. Certified seeds have not been produced with large amount by companies to meet the demand of farmers resulting in using low quality seeds for rice production. Securing good quality seed is the first step to ensure the high yield.

Physical weed control

Water management is one successful method for weed control in transplanted rice. Most of grasses can be suppressed with the depth of water about 5cm or above but sedges and broadleaf weeds need more water to be controlled(7). Weed infestation in water seeded rice in Mekong Delta is lower than that of wet seeded rice due to low weed germination and emergence caused by water. Weed density in Summer-Autumn season is higher than that of Winer-Spring season due to the fluctuation in rainfall and the shortage of river water.

On the other hand in Winter-Spring season, plenty of water in rivers after peak period of flooding allows farmers to keep their field flooded to suppress.weeds. A pot experiment on weed control by water management was conducted at the Cuulong Delta Rice Research Institute(CLRRI). Water at the depth of 5cm or above can control weeds especially grasses and it caused reduction in weed dry weight(Table 5).

Chemical weed control

A few studies of biological control have been conducted, no one, however, provides on established means to control weeds in the paddy fields. As of today, chemical weed control is the most reliable and economical tool, especially in large scale rice cultivation. In Vietnam, the tendency of an increase in use of herbicides has been observed. Major herbicides registered in Vietnam for weed control in rice is listed in Table 6. A main part of weed control experiments has been conducted in rice in Mekong Delta. Few studies were carried out in Red River Delta and other regions. In Mekong Delta, treatments of granular thiobencarb gave high efficacy in controlling *Echinochloa* spp in Winter-Spring season, whereas thiobencarb + propanil showed better efficacy in Summer-Autumn season in well puddled and irrigated fields(5). Du et al reported that pyrazosulfuron-ethyl at 25gai/ha, pretilachlor at 400gai/ha, butachlor at 1000gai/ha, thiobencarb at 1800-2000gai/ha resulted in increasing yields as compared to weedy check. Yield under pyrazosulfuron-ethyl was higher than that of hand weeding twice(2).The popular herbicides with large scale application are butachlor, 2,4-D, fenoxaprop-ethyl, oxadiazon, pretilachlor, pyrazosulfuron-ethyl, thiobencarb+propanil. CLRRI studied on these herbicides in four experiments during Winter-Spring and Summer-Autumn season of 1993-1994. Results are shown Table 7 and 8. All treatments resulted in increasing yields as compared to weedy check.

Discussion

The chemical method of weed control is one important parts of integrated weed management. In Vietnam, the shifting from transplanted to direct-seeded rice system has been taken place and the fast shifting is observed. Weed infestation under direct-seeded rice is severer as compared to the transplanted rice cultivation. The amount of rice herbicides is expected to increase in the future. The integrated weed management, including cultural, physical, mechanical, biological and chemical methods, should be studied. In three cropping types of rice in Vietnam, Summer-Autumn rice usually faces high infestation of weeds due to lacking of water from rain and in the rivers. The effect of herbicides and water management should be studies to minimize yield loss due to weeds in this particular season. The effect of rice herbicides on non-target organisms in paddy fields, which will have become a social concern, requires further studies in the future in Vietnam.

References

1. Chin. D.V.and Sadohara. H. 1994. Weed problem and weed control in direct seeded rice in Mekong Delta, Vietnam. Weed research, Japan Vol. 39.

2. Du.P.V., Cuong.N.L. and Chau.L.M. 1991. Effect of herbicide to *Echinochloa* spp in Mekong Delta. Monthly Journal of Science, technology and economic management. 30(7), p312-314.

3. General Statistical Office-Department of Agricultural, Forestry and Fishery. 1994. Statistical data of agricultural, forestry and fishery. Hanoi, Vietnam, p195.

4. Khiem. T. 1992. Grass control in direct sowed rice fields by watering. Monthly journal of science, technology and economic management. 31(6), p221-223.

5. Kim.H.M. 1988. On the control of *Echinochloa* spp on rice fields by herbicides. Agricultural science and techniques monthly journal. 27(3), p112-115.

6. Moody. K. 1989. Weeds reported in rice in south and southeast Asia. International Rice Research Institute, p442

7. Reissig. N.V. and Heinrichs. E.A., Litsinger. J. A., Moody. K., Fiedler. L., Mew. T.W. 1986. Illustrated guide to integrated pest management in rice in tropical Asia. IRRI.

Table 1 Main crop season of irrigated direct seeded rice in Mekong Delta

Number of crops	Crop season(month)															
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4
2 crops/year			←	SA		→					←	WS	→			
3 crops/year				←	SA		→	←	AW		→	←	WS		→	
3 crops/year		←	Soy		→	←	SA		→		←	WS	→			

Remarks: SA: Summer-Autumn season rice, AW: Autumn-Winter season rice, WS: Winter-Spring season rice, Soy: Soybean

Table 2 Cultivation types in irrigated direct seeded rice in Vietnam.

Type	Land preparation	Seed preparation	Soil condition at sowing
1. Traditional	Puddled	pregerminated	saturated
2. Water seeding	puddled	pregerminated	flooded
3. Minimum tillage	No puddled (plowed & harrowed)	pregerminated	saturated
4. Zero tillage	No tillage previous rice's straw were scattered and burnt	pregerminated	moist soil with ash of straw

Table 3 Weeds reported to occur in rice in Vietnam(adapted from Moody, 1989)

Family	Number of species	Percentage
Poaceae	84	21.0
Cyperaceae	83	21.0
Asteraceae	26	6.5
Scrophulariaceae	18	4.5
Fabaceae	14	3.5
Lythraceae	10	2.5
Lamiaceae	9	2.3
Amaranthaceae	7	1.8
Onagraceae	7	1.8
Aizoaceae	7	1.8
Acanthaceae	6	1.5
Commelinaceae	6	1.5
Convolvulaceae	6	1.5
Hydrocharitaceae	6	1.5
Others	110	27.6
Total	399	100.0

Table 4 Predominant rice weeds in Mekong and Red River Deltas.
(adapted from Chin and Sadohara 1994 and Khiem 1992)

Mekong River Delta	Red River Delta
Echinochloa crus-galli	*Echinochloa crus-galli*
Echinochloa colonum	*Echinochloa colonum*
Leptochloa chinensis	*Panicum spp.*
Paspalum distichum	*Cyperus rotundus*
Cyperus difformis	*Heleocharis equisetinea*
Cyperus iria	*Eclipta alba*
Fimbristylis miliacea	*Rotala indica*
Eleocharis dulcis	*Marsilea quadrifolia*
Monochoria vaginalis	*Monochoria vaginalis*
Marsilea minuta	*Jussiaea repens*
Ludwigia octovalvis	
Sphenoclea zeylanica	

Table 5 Effect of water depth on weed and rice yield.
(CLRRI, Vietnam, 1993-1994 Winter-Spring season)

Water depth	Weed dry weight(g/m2)* at 55DAS			Yield
	Grasses	Sedges	Broadleaf weeds	g/pot
0 cm(till 6DAS)	3.2 ab	5.9 a	6.0 a	39.6 a
0 cm(till 12DAS)	13.2 b	8.2 a	10.8 ab	40.6 a
2.5cm	1.8 a	11.2 a	11.1 ab	40.0 a
5.0cm	0.7 a	7.2 a	12.7 ab	55.0 c
7.5cm	0.7 a	4.9 a	14.0 b	46.0 ab
10.0cm	0.7 a	5.5 a	9.3 ab	42.6 ab
12.5cm	1.4 a	5.3 a	8.9 ab	49.0 bc

* Transformed data $\sqrt{X+0.5}$

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

Table 6 Major herbicides in wet seeded rice in Vietnam.

Name	Dosage (ga.i./ha)	Time of application
Butachlor	960-1280	1 - 7 DAS
2,4-D	920-1100	15-20DAS, 40DAS
Fenoxaprop-ethyl	37	20-30 DAS
Oxadiazon	250	2-3 DAS
Pretilachlor	300	1-3 DAS
Pyrazosulfuron-ethyl	20-30	3-7 DAS
Thiobencarb + Propanil	1200+600 - 1600+800	5-10 DAS

Remarks: DAS : days after sowing

Table 7 Effect of some pre-emergence herbicides on weeds and yield of wet-seeded rice in Winter-Spring season in 1993-1994.

Treatment	Dosage (ga.i./ha)	Application timing (DAS)	Weeds dry weight(g/m2)* at 55 DAS	Yield (T/ha)
Oxadiazon	250	3	0.7	5.06
Pretilachlor	400	3	1.7	5.30
Butachlor	960	5	2.1	5.20
Pyrazosulfuron-ethyl	20	6	3.2	5.63
Thiobencarb	1500	6	4.7	4.86
Untreated control	-	-	14.5	3.33
Hand weeding	twice	20&30	2.8	5.06
(LSD 0.05)			2.4	0.45

* Transformed data $\sqrt{X+0.5}$

DAS :days after sowing

Table 8 Effect of some post-emergence herbicides on weeds and yield of wet-seeded rice in Winter-Spring season in 1993-1994.

Treatment	Dosage (ga.i./ha)	Application timing (DAS)	Weeds dry weight (g/m2)*at 55 DAS	yield (T/ha)
Propanil	3000	15	4.74	4.31
Propanil + 2.4-D	1200+400	15	4.38	4.02
Butachlor + 2,4-D	560+400	15	4.69	4.65
Fenoxaprop-ethyl + 2,4-D	45+800	15	2.30	4.45
Thiobencarb + propanil	1200+600	15	4.98	4.80
Fenoxaprop-ethyl	45	20	4.50	4.31
2.4-D	800	20	3.69	2.24
Untreated control	-	-	8.91	2.98
Hand weeding	twice	20&30	3.30	5.04

* Transformed data $\sqrt{X+0.5}$

DAS :days after sowing

PRESENT STATUS AND PROSPECT'S OF WEED CONTROL IN RICE IN VIETNAM

HA MINH TRUNG , N.T.TAN, H.A.CUNG
National Institute of Plant Protection,Chem - Tuliem - Hanoi

ABSTRACT:

During the last few years in the course of the country's economic renovation, agriculture production, particularly rice production has recorded very important progress, and has been able to meet the demands of domestic consumption and export. Rice production in 1993 was 21.9 million tons which is 38% more than 1985. The average yield per hectare was 3.43 tons, 26% more than 1985.

Weeds are considered the major constraint in rice production and weed control is an important factor in the progress of rice production. About 50 weed species have been identified in Vietnam, out of which Echinochloa crus- galli, Echinochloa colonum, Cyperus difformis, Cyperus sp. Leptochloa sinensis, Fimbristylis diphylla, Paspalum scrobiculatum, Marsilea quadrifolia,Heleachoris chactalia considered economically important. The most common weed control practises are chemical and manual. Chemical control is common in about 2.5 million ha where direct sowing is practiced. The most common herbicides used are Sofit, Rifit, Butachlor, Ronstar, Saturn and 2,4D. Manual weed control takes 40 - 50 mandays per ha. In recent years due to changes in socio-economic structure the area under direct sowing has been increased as a consequence, the quantity of herbicide usage will also be increased. Effective strategy for weed management under the framework of IPM are discussed.

1.INTRODUCTION

Vietnam is an agricultural country with abundant agricultural resources and over 70 million inhabitants , of whom 50 million are in agricultural households. Stretching from 8 oN to 23 oN with a variable topography and climatic conditions the country is devised into seven agroecological regions : North mountain and Midland, Red River Delta, North Central Coast, South Central Coast, Central Highlands, East Cochinchine and Mekong River Delta (Please see Figure 1 and Tab.1). There are 33 million ha of land, of which 7 million ha are used for agricultural purpose. Rice is grown on 6,3 million ha, maize 0.43 million ha, sweet potato 0.35 million ha, cassava 0.27 million ha, peanut 0,2 million ha and sugarcane 0.15 million ha. There is a possible expansion of 1 - 2 million ha in Red River Delta, Mekong River Delta and Central Highlands.

Abundant water resources and well developed irrigation systems

make it possible to grow rice throughout the year. Four major types of rice crops are grown : Winter - Spring (November - May), Spring (February - May), Summer - Autumn (April - August) and Monsoon or Summer (June - October). Vegetable, pulses, oil seeds and other cereal crops are usually grown in intercrops with rice. The main cropping pattern in rice - based farming system are as follows :

+ Monsoon rice - Winter non rice crops - Spring rice
+ rice - Spring rice - Summer Autumn rice
+ Monsoon rice - Winter spring rice.

During the last few years in the course of country's economic renovation rice production has recorded very important progress and has been able to meet the demands of domestic consumption and export. Rice production in 1993 was 21.9 million tons which is 38% more than 1985. The average yield per hectare was 3.43 tons, 26% more than 1985 (see Tab.2)

One of the important factor enhancing this progress in rice production is the larger use of intensive farming, new high-yielding varieties, increase of number rice crops per year and larger dose of fertilizers. However, the course of intensive farming often break up the biological balance between rice crops and their pests resulting in their emergence and damages in a bigger way .Modern varieties erect leaves, therefore more light penetrates the crop canopy and more weeds emerge and survive. The high fertilizer rates applied to high-yielding varieties worsen weed problems. Therefore weed control is a crucial measure, contributing to sustainable rice production.

2. MAJOR WEEDS PESTS

Weeds were surveyed in Red River Delta, Mekong Delta and some areas of upland rice in mountain and Midlands region of North Vietnam. The results of field surveys showed that there are about 60 weed species in upland rice fields, 50 species in water seeded rice, and 37 species in transplanting rice. The major weed pests are given in Table 4. Their distribution is varied depending on rice ecosystems and soil types. The dominant weed pests in irrigated rice land are Echinochloa spp, Leptochloa sinensis, Digitaria setigera and Cynodon dactylon. Upland rice fields are dominated by Imperata cylindrica, Panicum repens, Cyperus rotundus, Eleusine indica, Amaranthus spinosus and Mimosa pudica.

3. WEED DENSITY AND YIELD LOSS

The density of major weeds in rice fields is varied depending on cropping seasons and cultivation practices (please see Tab.4).

Seeded rices is common in Mekong River Delta. There are three seeding method practiced :

+ Sowing onto puddled soil. Pregerminated seeds are sown on the well puddled soil with adequate water surface.

+ Sowing with minimum land preparation. Seeds are broadcast on the soil surface with out puddling.

+ Zero tillage wet seeding. No plowing, harrowing and puddling is conducted before sowing. Weed density is usually higher in the fields with the two last seeding methods. A survey on fields with zero tillage wet seeding in spring 1993 revealed that the density of Echinochloa, Leptochloa, Cyperus, Fimbristylis and Monochoria were 16-384; 58- 291; 39- 1045 and 5 - 175 plants/m2 relatively.

During their growth the rice crops and weeds compete for water, nutrients and light. Weeds usually grow faster than the rice crops, therefore they absorb greater quantities of nutrients and water and inhibit rice growth, resulting in considerable yield loss. Some data of yield loss due to Echinochloa is given in Table 5. The average yields loss due to major weeds is estimated about 5 - 15% for transplanting and 19 - 30% for seeded rice.

WEED CONTROL.

The most common weed control practices are manual and chemical. Manual weed control is dominant in North of Vietnam, where transplanting rice is more practiced. Manual weeding is conducted two time per season at 15 - 20 and 30 - 35 days after transplanting. Beside of hand pulling of weeds farmers also use some primitive implements for weed control such as hoes and rotary weeders. Hand weeding usually takes 70 - 80 mandays per hectare.

In South of Vietnam where direct seeding is more practiced, manual weed control is more time-consuming. Farmers usually conduct three hand weeding per season : at 10 - 15, 25 - 30 and 40 - 45 days after sowing. Totally it takes about 180 - 200 mandays per ha to keep rice crops free of major weeds. In upland rice fields, where the water surface is absent weeds often make manual control much more labor inputs (250 and more mandays per ha).

Chemical control is common in about 2.5 million ha of seeded rice. The most common herbicides used are Sofit, Butachlor, Ronstar, Rifit, Saturn and 2,4D. Generally herbicides are effective and reliable means for controlling weeds, particularly some major species such as Cyperus, Echinochloa and Leptochloa. Herbicides are normally used in combination with hand weeding, because chemical control alone is usually not effective against all the types of weed present. Herbicides occupied about 20 25% of total amounts of pesticides used in 1993.

Chemical control has clear advantages but also may cause some negative environmental effects.

To avoid this undesirable effect an integrated weed control is recommended for irrigated rice as follows :

1. Conducting land preparation with tillage
2. Flooding, where it is possible
3. Using pre-emergence herbicide
4. Hand weeding : 15 - 20 and 35 - 40 days after transplanting or sowing
5. Ploughing down rice field immediately after harvesting.
6. Training farmers on safe application of herbicides.

CONCLUSION AND PROPOSAL :

In future as the cost of labor hand weeding has been increased, the quantity of herbicide usage will also be expected to increase. To improve weed control studies on the water-tillage- weed interaction should be conducted with the aim reducing water use and reducing herbicide dependency. Studies on the use of natural pathogens for biological weed control and the use of allelopathy in rice cultivars would be initiate too.

REFERENCES CITES

1.Annual Report of NIPP 1976, 1980, 1985

2. HA MINH TRUNG. 1994. Pests and diseases : major constraint to agricultural production in the Red River Delta of Vietnam. Report of the VN - IRRI Project EPPD IRRI, 16 p.

3. Jan H.Oudejans. 1991. Agropesticides : properties and functions in integrated crop protection. ESCAP publication 263 - 291.

4. K. Moody,C.E.Munroe, R.T.Lubigan,E.C.Paller. 1984. Major weeds of the Philippines.

5. R.Barker, Y.Hayami, 1983. Weeds control practices as a component of rice production system. In Proc. of the Conference on weed control of rice, 31 Aug - 4 Sept. 1981, IRRI - IWSS 37 - 47

Table 1 : Agricultural profile of agroecological regions in Vietnam

Region	Natural land(mil ha)	Sown area of rice (mil.ha)	Elevation metter above sea level	Annual rainfall (mm)	Annual Tempera-ture(oC)	Soil type	Wet season	No rice crops per year
North moun-tain and Midland	9.8	0.821	100-3100	1600-2500	22(with cold peri-ode Nov.-Feb.)	poor infertile light coloured soil	Mid Apr early Nov	1-2
Red River Delta	1.0	1.024	3-8	1700	23 with a cold peri- ode Dec.-Feb.)	Alluvial soil	Apr-early Nov.	2
Central coast of Northland	2.3	0.678	100-2710	2890	25.3	Alluvial, sundy soil light coloured soil	Apr. Dec.	2
Central coast of Southland	4.6	0.472	100-2000	1000	26.0	do	Aug-Feb	2
Central highlands	5.5	0.181	1000	2280	21-23.0	Basalt soil light coloured soil	Apr-Oct	1-2
East cochinchine	2.3	0.317	400	2000	26	do	Apr-Oct	1-2
Mekong River Delta	4.0	2.9	10	2000	26- 27	Alluvial soil	Apr-Nov.	2-3

Table 2 : Progress in Rice production

Criteria	1985	1990	1991	1992	1993
Yield (ton/ha)					
Average	2.78	3.19	3.09	3.33	3.43
Winter - Spring rice	3.50	3.78	3.15	4.01	3.88
Summer - Autumn rice	3.33	3.38	3.48	3.39	3.57
Summer (Monsoon) rice	2.22	2.65	2.85	2.73	2.94
Production (million tons)					
Throughout Vietnam	15.87	19.22	19.42	21.54	21.90
The Red River Delta	3.09	3.61	3.11	4.00	4.51
The Mekong River Delta	6.85	9.48	10.35	11.00	10.74

(Statistical data of Agr. Forest. and Fishery, 1985 - 1993)

Table 3 : Ranking of major weeds of rice

Species	Species
Echinochloa crus - galli	Leptochloa sinensis
Echinochloa colonum	Fimbristylis diphylla
Cyperus difformis	Imperata cylindrica
Cyperus serotinus	Panicum repens
Cynadon dactylon	Digitaria setigera
Eleusine indica	Paspalum scrobiculatum
	Marsilea quadrifolia
	Mimosa pudica
	Heleachoris chactala
	Amaranthus spinosa
	Rotala indica

Table 4 : The density of major weeds in RRD (1990)

Planting method	Weed	Density (plants/m2)	
		Spring rice	Monsoon rice
Transplanting rice	Echinochloa spp.	7 - 986	5 - 72
	Cyperus spp.	18 - 867	9 - 402
	Broad leaf weeds	114 - 720	19 - 304
Seeded rice	Echinochloa spp.	5 - 156	-
	Cyperus spp.	21 - 673	-

Table 5: Yield loss of rice due to Echinochloa (NIPP 1990)

Density of weeds (plants/m2)	Yield reduction (%)
0-5	0
5-10	7-13
15-35	23-27

RECENT DEVELOPMENT AND PROBLEMS OF CHEMICAL CONTROL OF WEEDS IN CHINA

SU SHAO QUAN

(Laboratory of Weed Science, Northeast Agricultural University)
Harbin 150030, Heilongjiang Province, China

This paper discuses the present situation and problems of chemical weed control, and the future development of herbicides in China

THE DEVELOPMENT OF CHEMICAL CONTROL OF WEEDS

The application of organic selective herbicides in China began from 1956 when 2,4,5–T was experimented in paddy field and 1960 when 2,4–D was experimented in spring wheat field. Before 1970s The herbicides industry was not well developed, the species of herbicides were limitted. So the area of chemical weed control increased very slowly. For example, in 1967 the total area of chemical weed control was only 330 thousand ha, The main herbicides applied were nitrofen、propanil、2,4–D and MCPA, Most of them were used in Heilongjiang province.

The situation of chemical weed control changed greatly in 1978. In that year China imported trifluralin, alachlor, paraquat and linuron at batches from U.S., British and Italian. These played important role in speeding the development of Chinese herbicide industry and chemical weed control. Since 1980s more and more foreign herbicides have been imported, the quantity of imported herbicides has increased consecutively, the national herbicide industry has greatly developed. The species and quantity have notably increased, so the chemical weed control has advanced quickly. The total area of chemical weed control was about 23500 thousand ha in 1990, and 33000 thousand ha

in 1994. In 1995 they well neach 35700 thousand ha. Now the area of chemical weed control is over 800 thousand ha every year in Heilongjiang、Jilin、Liao Ning、Jiang Su、Guang Dong、Yun Nan、He Bei、He Nan、Shan Dong、An Hui、Zhe Jiang、Hu Bei provinces. Because of grain production occupies a very important place in Chinese agriculture, most of the herbicides are used for grain crops whether they are imported or home produced. The home produced herbicides with largest annual output are butachlor、acetochlor、2,4–D、glyphosate、bentazon etc. and a variety of new chemical compounds are going to be produced. Now, we have about 30 species of herbicides, with an annual output of about 30 thousand tons. More than 100 herbicides have been improted, 25 of them are most important. It is estimated that by the year 200, China will need about 50–60 thousand tons of herbicides, most of them are new herbicides for cotton、soybean 、corn and vegetables. The herbicides mostly used now are:

Rice: butachlor、pyrazosulfuron–ethyl、bensulfuron–methyl、molinate、quinclorac、oxadiazon、bentazon、oxyfluorofen、simetryn.

Wheat: 2,4–D、MCPA、chlorsulfuron、metsulfuron–methyl、triallate、difenzoquat、chlorotoluron、fenoxaprop.

Soybean: trifluralin、vernam、acetochlor、metolachlor、metribuzin、acifluorfen、fomesafen、imazethapyr、dimethazone、lactofen、bentazone、chlorimuron–ethyl、sethoxydim、fluazifop–butyl、haloxyfop–methyl、quizalofop–ethyl.

Corn: atrazine、metribuzin、acetochlor、2,4–D.

Peanut: acetochlor、sethoxydim、fluazifop–butyl.

Rape: acetochlor、trifluralin、sethoxydim、fluazifop–butyl、ethametsulfuron.

Flax: MCPA、chlorsulfuron、sethoxydim、fluazifop–butyl.

Vegetable: acetochlor、metolachlor、pendimethalin、napropamide、trifluralin、sethoxydim.

Sugar cane: atrazine、metribuzin.

Orchard、tea and rubber plantation: glyphosate、paraquat、atrazine、simazine.

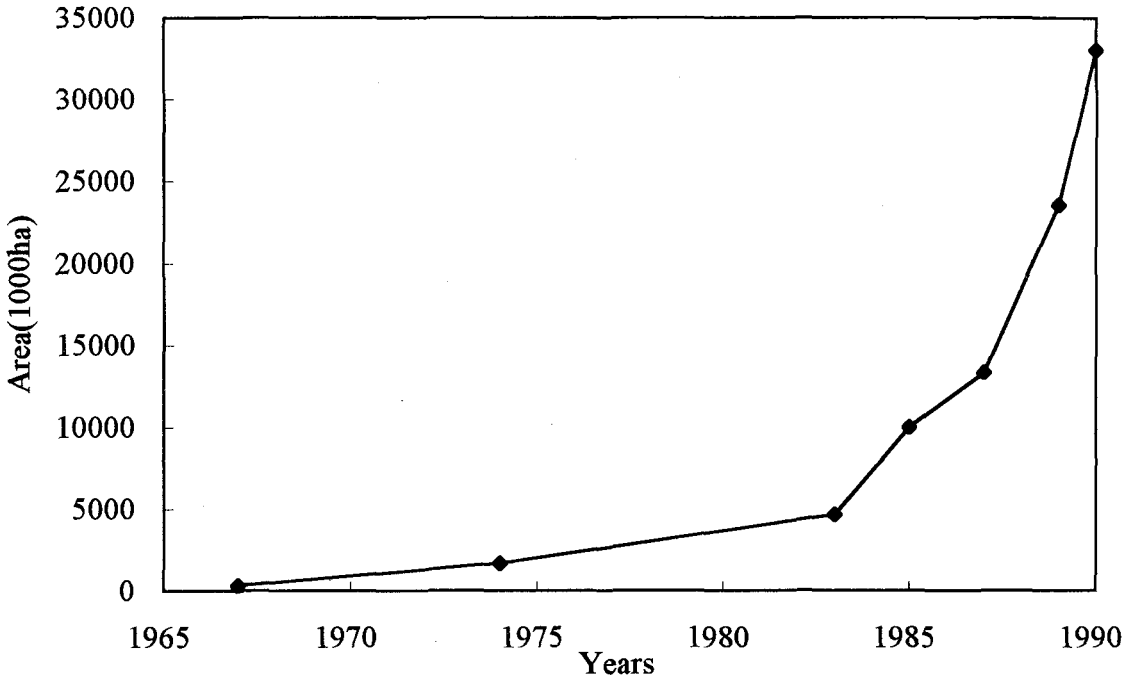


Fig. 1 The change in area of cropped land treated with herbicides in China

Topics requiring further study are: (1) The chemical control of weeds in cotton、corn、melon and crops grown under plastic cover; (2) Weed control in nutrition pots of cotton and vegetables; Weed control in sugar beet planted with seedlings in paper pots; Weed control in tobacco seedling beds; (3) Setting up systems of chemical control of weeds in notillage cultivation; (4) the chemical control of perennial weeds in upland and paddy fields; (5) the chemical control of weeds in forest seedling nurseries, city lawns and park.

PROBLEMS IN CHEMICAL CONTROL OF WEEDS IN CHINA

THE DAMAGE OF CROPS CAUSED DIRECTLY BY HERBICIDE

An early problem when herbicides were first used in China was that 2,4–D droplet evaporation and drift from wheat fields damaged sensitive crops and trees. In aerial spraying, 2,4–D droplet drift reached 4,000 m away causing serious damage to soybean, sunflower, vegetable and trees.

In application of herbicides, except 2,4–D. Herbicides, also cause damage of crops (Table 1). Reasons for the damage are: (1) Not using properly; the time of application is either too early or too late. For example, 2,4–D was used after jointing of winter wheat in 1984 in Shan Xi province, dicamba was used at shooting period of winter wheat in 1986 in Jiang Su province, they all caused the damage of wheat in a large area and serious loss of harvest; (2) The effect of weather condition; under the conditions of low temperature, excess rain and less light, the tolerance of the crops will to herbicides reduce, and they are easy to be hurted. In the Yang–Zhi Basin, the main reason of damage of winter crops (rape) and early rice is the low temperature and excess rain in winter and spring period, while in the Northeast such as Heilongjiang、Jilin and Liao Ning, there is usually a climate of low temperature and excess rain in spring, which cause the damage of herbicides to rape、soybean and rice.

Table 1 The herbicides which damaged crops directly in China

Herbicides	Damaged crops	Area(ha)	year	province	Reason
quinclorac	rice (directly seeding, seedling bed)	50–60	1992	Jilin	small seedling low temperate
oxyfluorofen	rice (transplanting)	150–200	1991	Jilin	small seedling
ethametsufuron	rape (directly seeding、transplanting)	1000–1500	1993	Jiangsu Zhejiang	weak seedling low temperature and rainy weather
butachlor	rice (seedling bed, transplanting)	1500–2000	1986	Jilin	〃
2,4–D	winter wheat	1000–1500	1984	Shanxi	the time of application is too late
dicamba	winter wheat	1500–2000	1986	Jiangsu	〃
benthiocarb	rice (seedling bed)	10–15	1962	Heilongjiang	poor quality of product
butachlor+2,4–D	rice (transplanting)	300–500	1992	Guangdong	low temperate

THE PROBLEMS WITH HERBICIDE RESIDUE

Since 1990s, the sulfonylurea and imidazolinone herbicides have been

used in large areas. Because their high activity, small quantity of application, wide spectrum of weed control and low cost, they are welcome by the farmers, so the area treated with herbicides enlarged swiftly. The total amout used and the application area in 1994 can be seen in table 2.

Table 2 The total amount used and application area of long residue herbicides in China (1994) (After Wang Huan Min, 1995)

Herbicide	chlorsulfuron (25% wp) wheat	metsulfuron -methyl (25% wp) wheat	chlorimuron -ethyl (20% wp) soybean	ethametsul- furon (25% wp) rape	dimethazone (40% EC) soybean	imazethapyr (5% AS) soybean
Amount (t)	60	92	45	3	150	950
Area (1,000ha)	1,000	1,000	460	60	8	547

With the application of the long residue herbicides, the residue from previous years cause the damage of the next crops in rotation, and leads to loss of crops (Table 3).

Table 3 The injury of next crops in rotation caused by the long residue herbicides from previous years

Herbicide	crop applied	crop injured	area (ha)*	province	year
imazethapyr	soybean	Sugar beet	20	Heilongjiang	1992
chlorsulfuron	wheat	sugarbeet corn soybean	30	Heilongjiang	1993、1994
ethametsulfuron	Winter rape	early rice	20,000	Jiangsu Zhejiang	1994
metsulfuron-methyl	Wheat	early rice	500	Sichuan	1994
metsulfuron-methyl	wheat	corn cotton	2,000	Sichuan	1994
metsulfuron-methyl	wheat	cotton	20,000	Hubei	1994

* The area is estimated.

In fact, the half life of sulfonylurea and imidazolinone herbicides in soil

is not very long, analysis from all over the world has proved that the half life of the longest residue chlorsulfuron is only 4–6 weeks, but the problem is that sulfonylurea and imidazolinone are more special herbicides. Especially the sulfonylurea underges hydrolysis in aqueous media at a rate which is a function of pH and temperature (Table 4), These data clearly show that hydrolysis is much more rapid under acidic conditions. Since sulfonylureas are weak acids, they exist primarily in anionic form in aqueous solutions at neutral and basic pH's. In this form the herbicides are much less subjected to hydrolysis.

Table 4 The Hydrolysis Half–lives of Sulfonylurea Herbicides as a Function of pH and Temperature (Beyer, E. M. et al., 1988)

Temperature (℃)	Herbicide	Half–life in days			
		pH 5	pH 6	ph 7	ph 8
25	metsulfuron–methyl	33	–	–	–
	sulfometron–methyl	18	–	–	–
	bensulfuron–methyl	11	–	143	–
35	chlorsulfuron	6	53	256	208
	chlorimuron–ethyl	2.4	17	64	89
	sulfometuron–methyl	1.8	6	15	20
45	chlorsulfuron	1.7	14	51	58
	chlorimuron–ethyl	0.6	4	14	18
	sulfometuron–methyl	0.4	1	6	7
	metsulfuron–methyl	2.1	–	33	–
55	chlorsulfuron	0.5	4	10	12
	chlorimuron–ethyl	0.2	1	3	4
	sulfometuron–methyl	0.1	0.3	2	2

We had determined the soil residue of chlorsulfuron using gas chromatograpphy. The data (Table 5, Fig. 2) showed that residue increased not apparently in the wheat–rice rotation in the southern region of China where the application continued successively within three years, the residue did not reach 4.26 ppb needed to cause rice injury when the total amount applied was 63g ai / ha (15+18+30) in three years.

Table 5 The soil residue and accumulation of chlorsulfaron in wheat–rice rotation (Kunshan, Jiangsu)

Dosage (g ai / ha)	Residue in 0–30cm layer of soil (ppb)		Mean residue (ppb)
	1	2	
90	6.60	7.22	6.91
75	5.43	5.79	5.61
60	3.94	4.58	4.26
30	0.40	0.46	0.43
15	0.32	0.36	0.34
15+18+30	0.72	0.79	0.77
15+18+0	<0.30	<0.30	<0.30
15+0+0	Not determined	Not determined	–

In the northeastern region of China, where one crop per annum is grown, soybean、corn、sugarbeet and rape are grown after spring wheat. As the climate is cold and dry, the hydrolysis process is slow, the soil residue of chlorsulfuron is a problem to some sensitive crops. Under normal dosage (15g ai / ha), the residue is 0.34 ppb after 160 days, this quantity is 34 times of the level causing injury of sugarbeet.

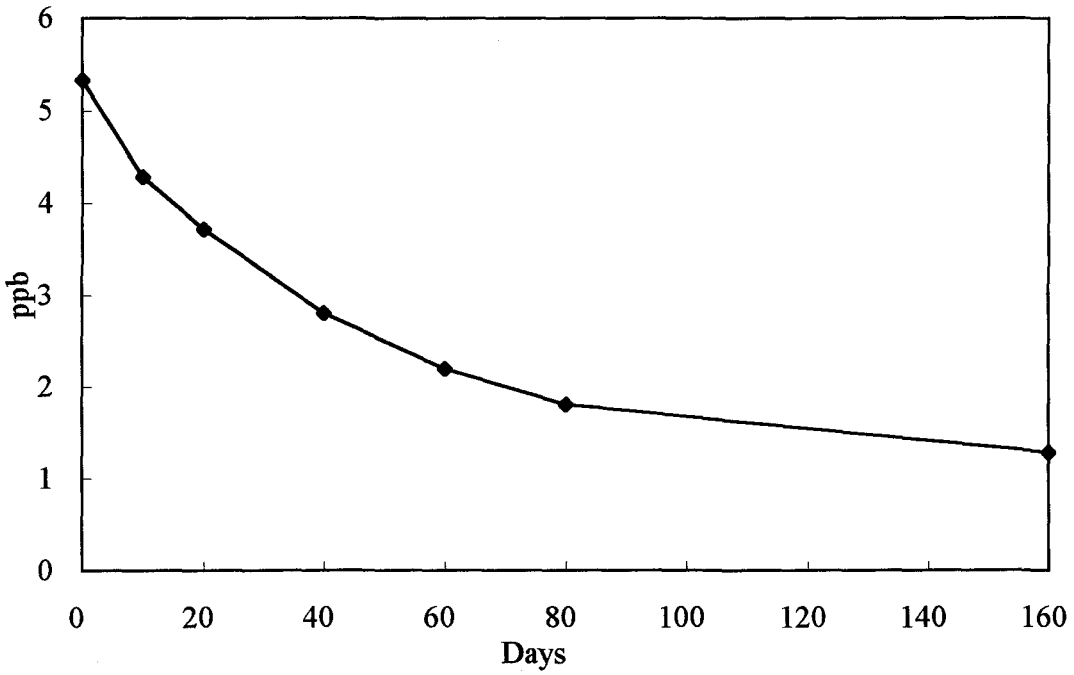


Fig. 2 The Degradation of chlorsulfuron in Field of Spring wheat (Harbin, 1992)

The soil residue of chlorimuron–ethyl and imathezapyr also injure some sensitive crops. When pre–emergence application is 15–30g ai / ha or post–emergence treatment 9–13g ai chlorimuron–ethyl per hectare, the crops planted next year such as sugarbeet、 rape、 vegetable、 melon and potato will severely injured. The sulfonylamide also has the same problem.

HERBICIDE RESISTANCE IN WEEDS

Of all the agricultural chemicals, herbicides are developed and applied much later compared with others, and renewing of species is comparatively fast in China, so we paid little attention to the problem of herbicide resistance. In fact, early problems were due to selectivity of weed species, with more resistant weeds appeared from competition with other weeds now controlled by herbicides. From 1970 onwards, there have been increasing reports of the evolution of resistant biotypes of previously susceptible weed species as shown by their ability to survive herbicide treatment at higher than normal doses. The number of species involved has now reached more than 100.

Butachlor has been used for nearly 20 years in China. It is the most common paddy herbicide. But now it is found that barnyardgrass [*Echinochloa crus–galli* (*L.*) Beauv.] has produced obvious resistance to it when it is applied 8–12 years successively. The resistance level shows an increasig tendency from north to south and the resistance in the double cropping paddy fields is higher than that in the single cropping fields. Resistance in the single–double cropping paddy fields is intermediate. Trifluralin is the main herbicide of soybean. It has been used for 17 years in state farms of HLJ province since 1978. Recently it has been found that its effectiveness of controlling the gramineous weeds is obviously decreased. In the eastern areas of Jilin province s–triazines herbicide has been used for a long period. Because the application of atrazine in successively planted corn fields has been continued for nearly 10 years, the resistance of some broadleaf weeds has been increased markedly.

From 1990, chlorsulfuron has been used in wheat and flax fields in the middle and lower reaches of Yang–Zhi River and HLJ province. Imazethapyr and chlorimuron were used in soybean field of HLJ in 1990 and 1993. The applied quantity of the first in 1994 is about 950 ton, and that of the later is

about 50 ton. Successive application of these single target herbicides in large scale will speed the development of resistance, and bring serious problem to weed control.

The speed of resistance formation caused by different kinds of herbicides is notablely different. When the target of herbicide is single, the resistance can be quickly formed. For example, when the sulfonylurea herbicides, their targets are ALS, are successively used for 4–5 years to cause resistance formation. In China, there is a large number of different herbicides used in agricultural production for more than 10 years, such as 2,4–D, trifluralin, vernam, acetochlor, butachlor, sethoxydim, fluazifop–butyl, atrazine etc. Especially in recent years, herbicides having single target are begining to be used in paddy field and upland crops, we should pay more attention to herbicide resistance.

Resistance leads to a substantial reduction of herbicide efficacy when applied at field doses. Cross–resistance is a very important practical consequence which may occur with the formation of resistant biotypes. The diversity of cross–resistance causes serious problems for practical control of resistant biotypes.

We regard the herbicide resistanted as a potential problem in China, but we also regard it as a manageable problem. We're going to find more resistance in more weeds to more herbicides through a range of modes of action. If however, we can retain some of our older chemistry and if we pratcice good resistance management now, we can put off the problem, if not avoid it at least on a wide–scale basis.

There is a perceived public need to reduce some user's reliance on herbicides. This will the implementation of complementary weed control practices such as cultivation, biological control and crop rotation. No single strategy will likely whip the resistance challenge by itself. But by adopting carefully selected comprehensive strategies, we can avoid the problem.

THE FUTURE DEVELOPMENT OF HERBICIDE IN CHINA

Agriculture plays a very important role in chinese national economy.

With the development of reform and the county enterprises, there are three trends of the agriculture:

(1) With the development of county enterprises and the market economy, a lot of rural labours move to the second and the third industry. In the south of Jiangsu province, where the economy is highly developed, labours released from agriculture have occupied 40–50% of the whole rural labours. This trend also occurs in the under developed areas. In 1993, the scales and speed of rural laboure loss in Guang Xi province broke the highest record in history, 11710 thousand persons were moved which in 60% more than 1992. With the large–scale move of rurial labours, the number of farmers will decreas in the future.

(2) The cultivated lands have gradually concentrated to fewer persons, the scope of management will enlarge, many big grain growers appear. Each household cultivates hundread ha, even thousand ha of farm land.

(3) Labor saving cultivation; minimum tillage and zero tillage has developed. In the provinces of Yang–Zhi Basin, area of zero tillage on wheat and rape expand quickly and is now extending to other crops.

Based on the above reasons, Chinese agriculture will have an urgent need of herbicides and will be a wide potential market for herbicides. With the steady increase of imported herbicides, we should speed the development of our national herbidede industry. The most important problem is to screen and develop new herbicides. Because grain crops、cotton and oil crops occupy a great proportion of Chinese agriculture production (Table 6), they should be the target for developing new herbicides.

Table 6 The area of field crops in China (1991)

crops	area (1,000ha)	crops	area (1,000ha)
rice	32590.1	millet	2080.9
wheat	30947.9	sorghum	1387.7
corn	21574.3	sesame	679.5
cotton	23471.8	flax	105.5
soybean	7041.0	sugar cane	1163.7
peanut	2879.9	sugar beet	783.5
rape	6133.3	tobacco	1804.0
potato	2879.3	sunflower	790.5

To meet the demand of herbicides, solubility of the chemicals should be medium, they should have low vapour pressure, their persistance in soil should be 60–80 days. They should have also a wide spectrum of weed control and a wide selective spectrum. Both special and common types are needed especially those herbicides which can effectly control the dayflower (Commelina communis L.) 、 common reed (Phragmites communis Trin.)、purple nutsedge (Cyperus rotundus L.)、Bermuda grass (Cynodon dactylon (L.) Pers.)、Common hemp–nettle (Galeopsis bifida Boenn.)、crested elsholtzia (Elsholtzia patrinii Garcke) and flatsedge (Cyperacea) in paddy field. In accordance with the change of weed flora under the selection pressure of herbicides, developing new herbicides which can control perennial weeds is very important.

REFERENCES

1. Elmo M. Beyer, Jr., Michael J. Duffy, James V. Hay, and David D. Schlueter, Sulfonylureas, In "Herbicides, Chemistry, Degradation, and Mode of Action", Volume 3. 117–189 (1988), edited by P.C. Kearney and D.D. Kaufman, MARCEL DEKKER INC., New York and Basel.

2. Gary Mangels, Behavior of the Imidazolinone Herbicides in Soil——A Review of the Literature, In "The Imidazolinone Herbicides", 191–209 (1991), edited by Dale L. Shaner and Susan L. O'Connor, CRC Press INC.

3. Ronaldo G. Hart, Edward M. Lignowski and Fred R. Taylor, Imezethapyr Herbicide, In "The Imidazolinone Herbicides", 247–259, (1991), edited by Dale L. Shaner and Susan L. O'Connor, CRC Press INC.

New Weed Control Approaches in Yunnan Province of China

Sun Xizhi　Guo Yiqing　You Chengli

Yunnan Academy of Agricultural Sciences, Yunnan　650205 China

Abstract　The focal point of this article is to set forth the types, infestation and progress on control of field weeds in Yunnan Province. Investigation has shown that Yunnan has various kinds of weeds, about 102 families with 629 species, and has complex communities of about 359 coenotypes. The area of weed infestation is 3,000,000 ha. and the yield loss per year is 5.1—14.4%. The control methods are different depending on the crops planted in the field: for paddy rice, upland rice and sugarcane fields, the primary method is chemical control supplemented cultivation; for maize and tobacco fields, the use of plastic film has a striking effect and cuts down expenses; the integrated control system of weeds in economic wooden garden and the introduced weed Croftonweed represents "chemical+substitution" or "chemical+substitution+biological control". Discussion of trends and countermeassures to the field weeds in Yunnan is offered.

Key words:　*Control*, *weeds*, *chemical Control*, *integrated control system*

Yunnan is situated in South—west part of China, within 21—29.°of North latitute and 97—106° of East longitute. Located in low latitute plateau, the topography of Yunnan slops from the northeast to the southwest. The Ailao Mountains streches from the north to the south at the central Yunnan and cut the province into east and west parts. Owing to the complex topography and vast altitude differences there is a variety of climatic types, namely the temperate, the torrid and the frigid zones. The agricultural area is from 80m above sealevel at Hekou County to 3600m above sealevel at Deqing County, the difference of height is 3500m with 25℃ difference in temperature; the difference of latitute is 8°with 19℃ difference in temperature. The crops are suitable for different areas and has tipical distribution on vertical and horizon level with solid agricultural characteristics. Under such completed natural ecological envirement and culturing conditions, weed has various types and serious infestation, meanwhile it is so difficult to control.

I. Field Weeds and Infestation

According to investigation, there are 102 families with 629 species of field weeds in Yunnan, among it the amount of paddy rice field weeds is 224 species, of dry farm weeds is 405 species. Most of the paddy rice field weeds are higher autophyte angiospern. The dry farm weeds consists of lower plants, higher autophyte and some paraphyton and epiphyte. Most weeds in the economic wooden garden are perennial weeds. Through preliminary research there are various communities such as 146 coenotypes of paddy rice field weeds community, 143 coenotypes of dry farm

weeds community and 70 coenotypes of economic wooden garden community.

Nowadays the weed infestation is still seriously in Yunnan Province. The area of weed infestation above middium extend is about 3,000,000 ha. accounts for 70% of total planted area. The yield losses of the cereal crops is 8.4—14.4% and losses of the economic crops is 5.1—7.3%(See Table I)

Table I Weeds Infestation for Major Crops

Crop	Paddy rice	Upland rice	Wheat	Maize	Sugarcane	Tea	Tobacco	Fruit trees
Planted Area 10000ha	102.61	20.00	56.96	98.99	14.16	15.87	23.55	9.33
Area of weed Infestation 10000ha	78.6	14.33	43.75	66.02	9.27	11.72	15.87	6.9
Field losses (%)	11.2	14.4	11.8	9.4	8.0	5.1	7.3	9.1

According to the extend of infestation, the scope of distribution and difficulty of control, we appoint 40 species of weeds as the major weeds need to be controlled in Yunnan(See Table II).

Table II. Major Weeds in Yunnan

Type	Standard	Paddy Rice Field Weeds	Dry Farm Weeds
One	Serious infestation; Wide distribution difficult for control	Bernyardgrass (*Echinochloa crusgalli*) Bulrush (*Scirpus planiculmis*) Pondweed (*Potamogeton distinctus*)	Polypogon (*Polypogon fugax*) Lambsquarter(*Alopecurus Aequalis*) Wild Oat (*Avena fatua*) Crabgrass(*Digitaria sanguinalis*) Nepal Knotweed (*Polygonum nepalense*) Tropic Ageratum(*Ageratum conyzoides*) Smallflower Marsilea(*Galinsoga parviflora*) Cogongrass(*Imperata cylindrica*) Sour Paspalum (*Paspalum Conjugatum*)

Type	Standard	Paddy Rice Field Weeds	Dry Farm Weeds
Two	Serious infestation in some areas; infesting in large area; a little bit diffcult for control	Umbrellaplant (*Cyperas diffomis*) Sheathed Monochoria (*Monochoria Vaginalis*) Fourleaf Marsilea (*Marsilea quadrifolia*) Rush — like Bulrush (*Scirpus juncoides*) Needle Spikesedge (*Eleocharis Yokoscensis*) Climbing Seedbox (*Ludwigia adscendens*) Broadleaf Blainvilla (*Blainvillea aemella*)	Lyrate Hemistepta (*Hemistepta Iyrata*) Yellow Sweetclover (*Melitotus Officinalis*) Water Mouse — ear Chickweed (*Stellaria aquaticum*) Chickweed (*Stellaria media*) Railway Beggarticks (*Bidenspilosa*) Bermudagrass (*Cynodon dactylon*) Yellow Bristlegrass (*Setaria Lutescens*) Raygrass (*Leptochloa panicea*) Beardless Barnyardgrass (*Echinochloa crusgalli*) Slimleaf Goosefoot (*Chenopodium sirotinum*) Copperleaf (*Acalypha brachystachya*) Itchgrass (*Rottboella exalata*) Horsetils Fleabane (*Erigeron canadensis*) Nutgrass Galingale (*Cyperus rotundus*) Chinese Pennisetum (*Pennisetum alopecuroides*) Aciculate Chrysopogon (*Chrysopogon aciculatus*) Tropical carpetgrass (*Axonopus compresus*) Elsholtzia (*Elsholziaciliata*) Gardon Euphorbia (*Euphorhia hirta*) Fragrant Eupatorium (*Eupatorium odoratum*) Fodder Vetch (*Vicia sativa*)

Ⅱ. New Approaches on Weeds Control

It started in the middle of 1960s in Yunnan Province that to use herbicides to control weeds and had got great progress since 1980s. The area already enlarged to more than 1,000,000 ha and accounts for 30% of total agricultural area. At recent years the integrated control has been used which is considered the chemicals control as the major method while suplimented with other technologies. (Table Ⅲ).

Table Ⅲ Integrated Control System for Field Wees

System / Object	Chemical Control	Biological Control	Substitution Control	Covered Control	Others
Paddy rice	Butachlor Molinate Bensulfuron Goal Kunming N0. 1				Rotation Water—depth Purifyirg grains
Upland rice	Butachlor Chlorto luron Goal 24 — D + Benvil				
Maize Tabacco	Goal Prometryn Atrazine			Plastic Film	Hilling soil
Sugarcane	Goal Diuron Atrazine Asulam				Intercropping Hillirg Soil
Economic Wooden Garden	Glyphosate 24 — D + Diesel oil		Legumes crop	Straw	
Croftonweed	Glyphosate 24 — D + Diesel oil	Proce- cidochares utilis	Legumes crop Huashang Pines	Straw	

Ⅱ. I Weeds Control in Paddy Rice Field

All the paddy rice are being transplanted after seeding for 40 days in Yunnan. To control the weeds in paddy rice field the major method is to use chemicals to treat the field that will be transplanted to, suplimented with rotation between Paddy Rice and Upland Crops, mannaging the water—depth and purifying the grains. For the paddy rice field which has the bernyardgrass usually

use the herbicides like butachlor and molinate. For the paddy rice field has both bernyardgrass and broad—leaf weeds we introduced and extended some sulfonylureas such as Bensulfuron, molinate and so on. In recent years some institutions like Kunming Station of Plant Protection has started to do research on using mixing herbicides and already got the new type of herbicide like Kunming No. 1. It is used for one time treatment to the paddy rice field which will be transplanted to and can effectively control the bernyardgrass, broad—leaf weeds and flatsedge. It has high activity, long persistence and insuring security and has been extended for large area in Yunnan.

Ⅱ. Ⅱ Weed Control in Dry Farm

Ⅱ. Ⅱ. Ⅰ Weed Control in Upland Rice Field

Most of the upland rice are being planted in the muggy area in south part of Yunnan. In that area the weeds have many species, high density and serious infestation. The method to control the weed is to treat the soil at the period between seeding and seedling with the herbicides such as butachlor, acetochlor, diuron, chlortoluron, prometryn, goal and oxadiazon. Another word, it is to apply the mixture of herbicides and soil after soaking rain and before seedling. At the 3—5 leaves age of weed we use 2. 4—D+Chlortoluron or 2. 4—D+Benvil to treat the stalk and blade that can control the flatsedge and broadleaf weeds.

Ⅱ. Ⅱ. Ⅱ Weed Control in Maize and Tabacco Field with Plastic Film

Plastic film has been used for planting several crops such as maize, tabacco and vegetables. Under the condition of film covering the growing and disappearing of weeds has got some changes. It is caused by the euthermic and moist ability of film that the weeds can grow fast and the amount will be increased and can break out the film while getting some bad results. The field we use the film usually has the annotinous which peaked at 10—20 days after irrigating or soaking rain. After finishing the research that to do comparison among using the colored film, herbicides film, hyaline film and hyaline film+pre—emergence herbicides, it shows that the weeding efficiency is like this:

black film > blue film >hyaline film;

hyaline film+pre—emergence treatment herbicides>herbicides film

Among them the black film is the most effective material for cotrolling weeds which can save labour and herbicides and increase yield and has spred for large area of maize and tabacco field.

Ⅱ. Ⅱ. Ⅲ Weed Control in Sugarcane Field

Sugarcane is one of the important economic crops in Yunnan and mainly spread in tropical and subtropical area. The weeds inevst sugarcane through the whole mature period: in the early maturity stage is the dogstail; broad—leaf weeds in middle maturity ;in late maturity is heterotype that is rarely invest sugarcane. The weeds in the dry farm is influenced by the rain and usually sprout in the rain season (June—August) and accounts for 70—90% of the whole year. To the irregated area it not only the rain but also the water applying in early maturity can influence the happening of the weeds. So the weeds could reach two peak times: first time is after the

second water applying time and accounts for 20—40% of the total; the second time is in the rain season and accounts for 60—80% of the total. The cotrol method is that: intercropping with other crops, hilling up and applying pre—emergence treatment herbicides like goal and diuron. For the sugarcane field which has the perennial weeds like the bermudagrass and sourpaspalum is usually use Asulam to treat stalk and blades.

Ⅱ. Ⅱ. Ⅳ Weeds Contral in the Economic Wooden Garden

The economic plants encludes tea, mulberry, fruit and rubber trees and about 90% is in the hilly area. The ecological condition in the economic wooden garden is relatively stable and the perennial weeds is the major problem. The control method is: "chemicals+substitutions". It is that to use herbicides first of all and then plant legumes or put crop straw on the ground of the garden. (Table Ⅳ)In recent years after doing research we already selected 20 species of legume that is suitable for Yunnan(Weed control effectivity is 75—95%) and already extended in large area.

Table Ⅳ The Eeffect of Substitution Control on Orchard Weeds

Item	Substitution Control	Routine Farming	Effect(%)
Weeds amount (plant/m^2)	4.1	121	96.61
Gein(%)	2.7	1.8	+50.00
Soil erosion (kg/ha/year)	1062	7979.5	−86.69
Soil humidity (%)	18.5	13.7	+35.04
Natural enemy for vermin (head)	289.5	184.5	+56.91
Yield of apple (kg/ha)	27195	23580	15.33
Diameter of Young Plant Stem (CM)	5.6	4.2	+33.33

Ⅱ. Ⅲ Control the Introduced Weed——Croftonweed

The Croftonweed(*Eupatorium adenophorum Spreng*) was orrigionally from Mexico and introduced into Yunnan in 1930s and already creep—spred for large area(more than 24,000 square

kilometers) and caused serous infestation. To control it is to use the integrated control system with "chemicals+substitution+biological control". First of all use the glyphossate or 24—D+ Diesel Oil to treat the stalks and leaves at early maturity of Croftonweed community. (Figure I) The second is to plant legumes or Huashang Pines to get rid of the Croftonweed in this area (Table V Figure Ⅱ). Meanwhile use the *Procecidochares utilis* to control the growth of the Crofton by sterilizing it.

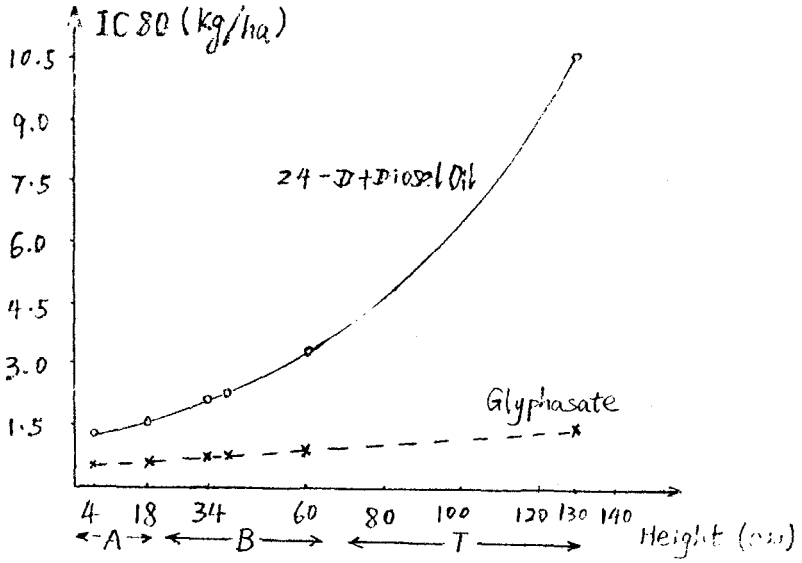


Figure I. The IC80 of Varied Aged Communities
A—annual B—biemial T—triennial

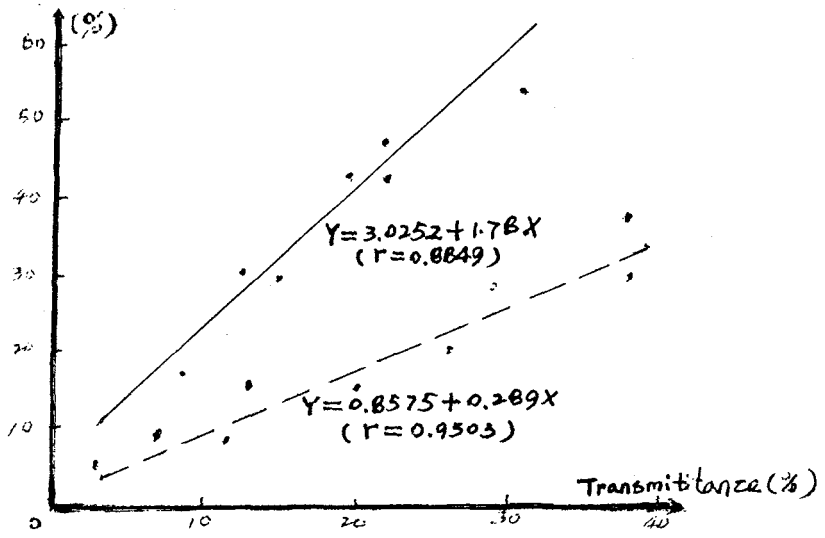


Figure Ⅱ. Effect on Influencing the Germination and Survival ratio of Crofton weed
—Germination ————Survival ratio

Table V The Effect of Integrated Control on Croftonweed

Chemical Control		Covered by Legumes crops	
Dosage (kg ai/ha)	Effect(%)	Transmittance (%)	Eeffect for preventing from reencroaching(%)
Glyphosate 1.75	89.12	5.17	87.86
Glyphosate 2.25	100.00	12.91	77.24
24—D+Diesel oil 2.0	75.28	26.16	64.24
24—D+Diesel oil 3.0	90.76	35.48	53.28

Ⅲ. Trends and Countermeasures in the Future

Ⅲ. Ⅰ. With saving labour power and increasing yield the chemical control is still the main method for weed control. In recent years with the development of the countryside economy and the adjustment of the industrial stucture, the chemical control has increased quickly and covered 30% of the total agricultural area and keeping go up steadily. In order to make chemical control for further developing and to make full use of it in the sustainable agriculture we should do more research on the technology of using chemicals. According to the community structure of weeds to arrange the herbicides in resonable groups and to apply to the field scientifically and to develop the technology of totally control the weeds in one time per season. Meanwhile to supervise the dynamic trends of the weeds population after using the herbicides and to do research on the community succession and resistant of the weed.

Ⅲ. Ⅱ The integrated control of weeds is the efficiency method that is on the foundation of ecological principles to make full use of the self coordinate mechanism of the biotic interations and artificial step so that to control the weed more effectively and to improve the soil producbility. In recent years we adhere to this rule on control weeds that is to make chemicals as key method with developing new technology such as biological, substitutional and plastic film control as while as to pay enough attention on combining these methods organically in order to set up an integrated control system. The practice of integrated control on the weeds of the orchards and Croftonweed is the tippical example for combining control the harmful organisms with make full use of natural resources and full prooved that the integrated control has great vitality and shall be considered as the leading direction on weeds control.

Ⅲ. Ⅲ Enhancing the fundamental research, improving weeds control technology. In order to arrange the herbicides in resonable groups, to apply to the field scientifically and more effectively, we should do research on the actional characteristics of the herbicides, for example, on the relation between the activity of the soil applied herbicides and the three—phases of soil (liquid phase, solidoid and gas phase). To set up an integrated control system should be on the base of full knowing on the relations and principles among the organisms species in the farmland ecologi-

cal system. So that we shall enhance on this fundamental research, make full understanding to the inherent relations among the organisms species in the farmland ecological system especially between crop to crop and between crops to weeds, in order to provide scientific basis for setting up a realistic integrated control system to the weed and necessary technology.

Improving Performance and Reliability of Soil Applied Herbicides in New Zealand Soils

A. Rahman and T.K. James
AgResearch, Ruakura Agricultural Research Centre, Hamilton, New Zealand

Abstract. This paper reviews the research done in New Zealand on predicting and optimising the activity of cropping herbicides, particularly those applied to the soil. Because of the high weed density and diversity, and more importantly the different nature of New Zealand soils, many of which are of volcanic origin and contain very high levels of organic matter, considerable research effort has been necessary to define herbicide rates for cost effective and reliable weed control. Our results confirm the significant relationship between herbicide phytotoxicity and certain soil properties, but show that the influence of increasing organic matter levels diminishes at the higher end of the range. This is in contrast with results of many theoretical studies. Using bioassay methods it has been established that if initial application rates for herbicides are adjusted according to the soil characteristics, the residue carryover and environmental contamination problems would be minimised, the desired weed control achieved and crop damage avoided. In addition to soil organic matter and soil texture, the influence of soil moisture, soil compaction and addition of major nutrients to the soil on the activity, persistence and mobility of several soil applied herbicides has been investigated. In recent years many investigations have been carried out to determine the behaviour of herbicides belonging to the sulfonylurea group in New Zealand soils and on ways to improve their performance over a range of soil and environmental conditions.

Key words: Soil applied herbicides, phytotoxicity, reliability, persistence, herbicides.

INTRODUCTION

As the number of soil applied herbicides has increased, the knowledge of edaphic factors which affect their bioactivity, behaviour and persistence in the soil has assumed greater importance. New Zealand, for its size, has an unusually wide range of soils. Soil organic matter levels are usually high, particularly in areas cultivated out of highly productive pastures. Most agricultural soils have an organic matter content of higher than 5%, with an average for volcanic soils in the vicinity of 10 to 12% (5, 21). Further, most soils are acidic with a pH of between 5.5 and 6.5. A number of soils contain a high proportion of allophane clays, most of which are derived from volcanic materials. Soils containing allophane clays have many physical properties which are in striking contrast to those of other mineral soils (1, 18), including a high cation exchange capacity and thus a high adsorption capacity for anionic substances (3).

New Zealand is situated between 34° S and 47° S latitudes and is within the temperate zone, just south of the high pressure belt of the sub-tropics. The average precipitation in the agriculturally important areas is usually between 600 and 2000 mm. Mean annual temperatures at sea level range between 9.4°C in the south and 15°C in the north, but show considerable daily, seasonal and altitudinal variation. The winters are mild enough to allow sheep and cattle to stay outside all year round. A large part of the country has at least 2000 hours of sunshine per annum and the range is from 1700 hours in Southland to 2400 hours in the Nelson and Hawkes Bay. (50).

It is remarkable that in the relatively short history of intensive cropping in New Zealand most of the common European weed species have established and reached high levels of infestation (25). Weed populations are extremely high in cultivated soils, as peaks of 40×10^6 seedlings/ha and total viable seed populations of up to 250×10^6/ha have been recorded (25, 36). While a large number of annual species occur regularly, only a few dominate at any one time and account for most of the total weed population. However, if these are removed by cultivation or herbicide, then other species best suited to the conditions dominate (36). The major weed flush usually starts earlier than the optimum sowing time of most crops. The sequential emergence of weeds continues well into the summer in most years, and this makes the

selective control of all weeds not only extremely difficult but also expensive. New Zealand's maritime climate with its well distributed rainfall encourages this sequential emergence.

In view of New Zealand's specialised soil characteristics, favourable climate for herbicide degradation and dissipation and very high weed pressures in most cultivated soils, considerable research effort has been devoted to improving the performance and reliability of soil applied herbicides, while avoiding residue carryover and environmental contamination problems. The major topics studied and the significant results achieved from this research are discussed below.

CORRELATING THE HERBICIDE PHYTOTOXICITY WITH THE SOIL PROPERTIES

Since the soil is a complex dynamic system, herbicide behaviour in it is influenced by many factors. Many researchers have demonstrated that the soil organic matter fraction, as well as the percent and type of clay in the soil, strongly influence the availability of herbicides (2, 51, 56). However, other soil factors such as soil pH, cation exchange capacity, and various environmental factors such as the amount and intensity of rainfall, can also influence a herbicide's fate in the soil. Understanding which soil properties are more important is difficult because of the various interactions between the herbicide and soil which determine herbicide availability in the soil.

Herbicide - Organic Matter Interactions

Our initial glasshouse experiments showed a very strong positive relationship between the soil organic matter content and the phytotoxicity of soil applied herbicides. Depending on the herbicide, from 2 to 4 times more chemical was required for a 50% reduction in the growth of test species (GR_{50}) in a soil with 22.1% organic matter compared to one with only 8% organic matter (Fig.1). In general, the herbicides of relatively high water solubility were more effective in higher organic matter soils than chemicals of low water solubility (19, 20). These results were confirmed through several field trials by comparing the relationship between soil organic matter content and the herbicide rates required for 80% weed control in oats and soya beans (31). Two to three times more herbicide was required for a similar level of weed control in the highest organic matter soil compared with the soil lowest in organic matter (Fig. 2). The relationship between the phytotoxicity of a herbicide and the soil organic matter was similar between the two crops used in the field and between the field and glasshouse experiments.

Field experiments also showed large differences in the phytotoxicity of several herbicides between peat and mineral soils (30). Herbicide activity was much lower in the peat soil, but the reduction in weed control was not of the same magnitude as the difference in organic matter levels of the two soils. This could be due to different chemical and physical characteristics of the organic matter, or it may be due to differences in various other properties of the two soils such as clay mineral composition, pH, type and amount of exchangeable cations. Interestingly, if compared on the basis of weight of organic matter (peat had only about two thirds the bulk density of mineral soil), a similar relationship between the organic matter and the phytotoxicity of herbicides was apparent in both peat and mineral soils.

Once the relationship between soil organic matter levels and the phytotoxicity of a herbicide has been established, the question then arises as to whether this relationship holds true for other members of the same chemical group. To answer this question, the influence of soil organic matter on thirteen *s*-triazine herbicides was investigated in glasshouse experiments. Results showed that the GR_{50} values for all these triazine herbicides were highly and positively correlated with the soil organic matter (49). It appeared that some indication could be obtained about the relationship for other members of the chemical group, bearing in mind the water solubility of the compounds. However, such projections must be limited to chemically and structurally related compounds.

Herbicide - Clay Interactions

The amount and type of clay mineral influences the adsorption of many herbicides since the soils containing high surface area, expanding lattice type clays are more adsorptive than those containing non expanding clays with low surface area (10, 52). Identification of the importance of soil characteristics other than organic matter has been difficult because of the high correlation between organic matter and

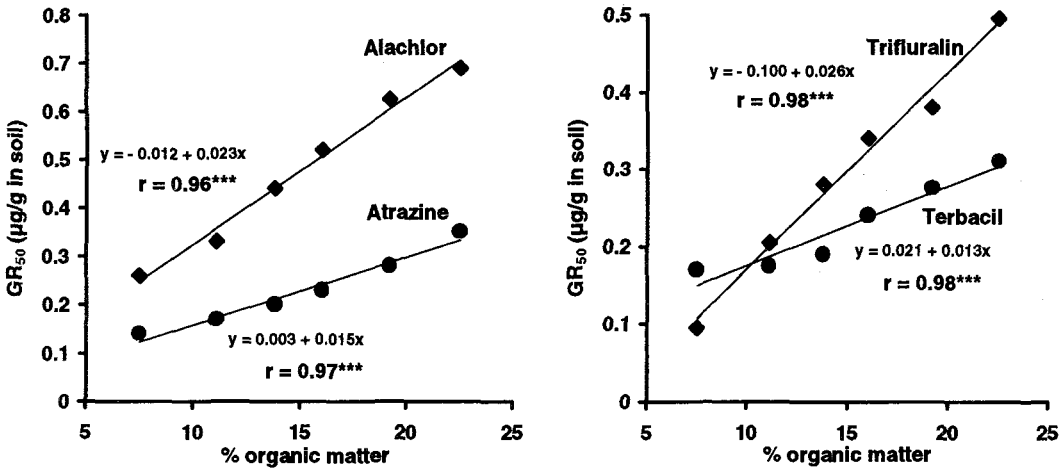


Figure 1: **Effect of soil organic matter on the phytotoxicity of various herbicides in glasshouse studies (adapted from Ref. 20).**

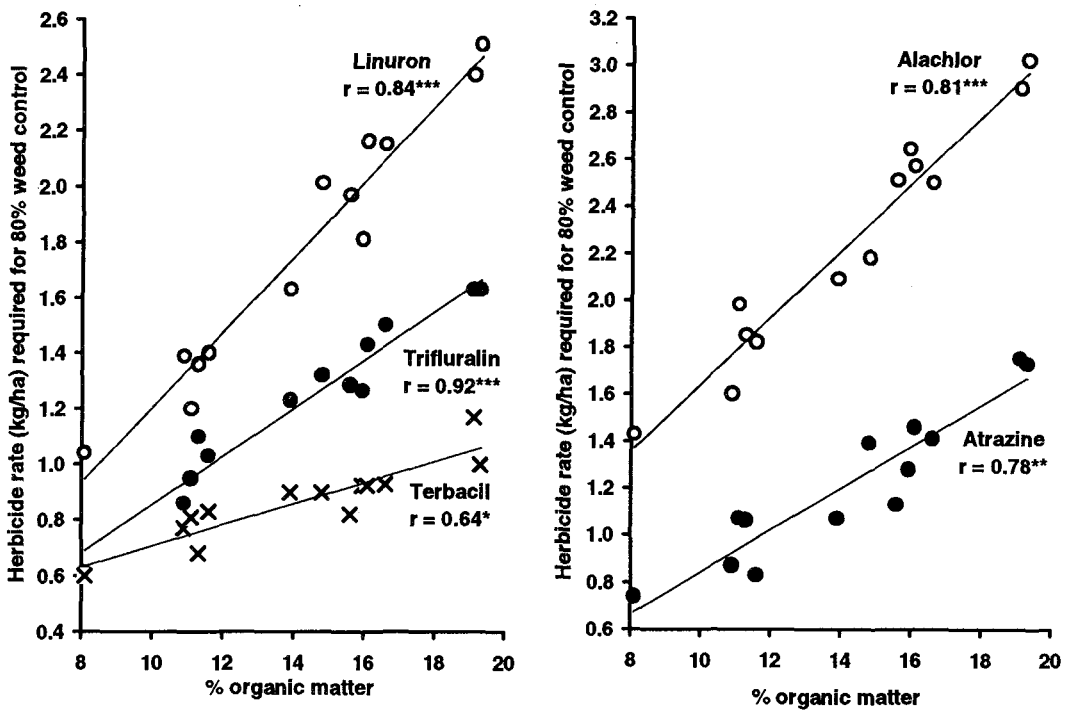


Figure 2: **Relationship between soil organic matter content and the herbicide rate required for 80% weed control in oats (adapted from Ref. 31).**

herbicide phytotoxicity. In a glasshouse study we used seven different soils containing organic matter levels between 11.1 and 11.6% but varying widely in their texture, parent material and other physico-chemical properties. Results showed that GR_{50} values of atrazine and trifluralin were correlated positively with clay and cation exchange capacity, and negatively with the sand content of the soil (46). However, none of these soil properties were related as highly to the herbicide activity as the organic matter content of the soil (20).

The above described and many other related studies gave a clear indication that the herbicide recommendations developed in countries overseas would not be suitable for New Zealand soils. These studies also enabled us to define rates for many herbicides in different soils for cost effective and reliable weed control, without causing crop damage or herbicide residue carryover problems. Much of this information has been transferred on to the herbicide labels, and commercial services are now available to New Zealand farmers for determining soil organic matter levels at reasonable costs.

Herbicide - Nutrient Interactions

The adsorption of herbicides by soil colloids depends on a number of factors, including the concentration of various ions in the soil. The widespread use of fertilisers, especially superphosphate, in New Zealand soils could be expected to have some effect on the performance of soil applied herbicides. Additions of phosphorus to the soil have been reported to influence the phytotoxicity of some herbicides, including certain triazines and dinitroanilines (52, 53). Through several glasshouse experiments we investigated the interaction between additions of phosphorus (as calcium monophosphate) to the soil and the phytotoxicity of several herbicides including atrazine, ethofumesate and trifluralin. In the presence of relatively high, but non toxic levels of phosphate in the soil, the phytotoxicity of herbicides to bioassay test species was increased significantly (23, 45, 47). This effect was not observed at sub-lethal concentrations of atrazine nor at phosphate levels below 150 µg/g, the kind of rates used in the field situations (Fig. 3). Phosphorus concentrations in plant shoots increased with increments in phosphorus levels in the soil but was unaffected by the herbicide at low rates. At high herbicide concentrations some significant increases in phosphate levels of plant shoots were recorded, although such increases tended to vary directly with the size of the shoot. From a practical point of view these studies suggest that the light to moderate rates of phosphorus application, which are commonly used in New Zealand, should have no marked effect on the phytotoxicity of soil applied herbicides. However, at high rates of application a significant positive relationship could be expected between the phosphorus levels and herbicide activity.

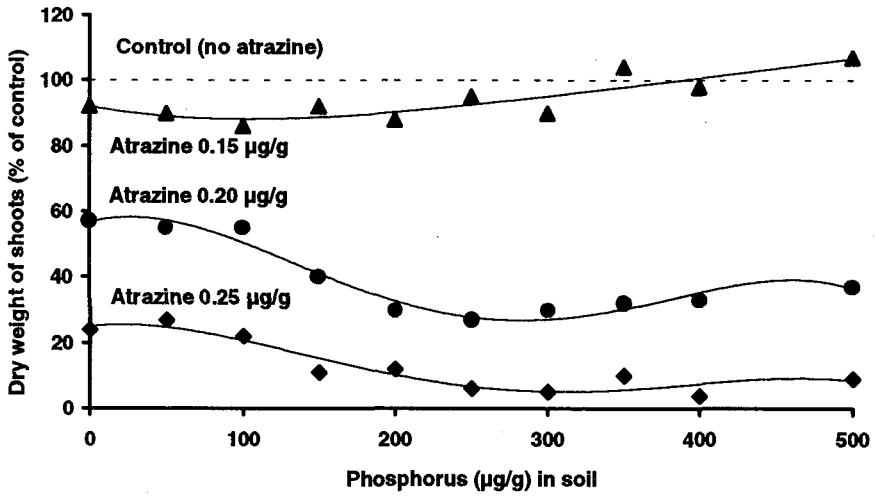


Figure 3: Influence of phosphorus and atrazine on the shoot dry weight of turnips (adapted from Ref. 45).

Effects of Soil Compaction

The tendency to use heavier and more powerful farm machinery for improved operational efficiency has often resulted in increased compaction of the soil. In a large field study we investigated the effect of three levels of soil compaction on the activity and persistence of atrazine and trifluralin. The moderate and heavy compactions used in this study increased the soil bulk density by 24.7% and 35.1% respectively, while the normal compaction had negligible effect on physical properties of the soil compared to the freshly cultivated ground (28). Results showed that initial phytotoxicity of both herbicides was increased significantly by the heavy compaction treatment. Moderate soil compaction also enhanced the initial activity of atrazine but not that of trifluralin (Table 1). These results have implications for minimum tillage, reduced tillage or no tillage systems where the activity and persistence of some herbicides may differ from those measured in conventionally tilled systems due to differing levels of soil compaction.

Table 1. Effect of soil compaction on herbicide activity against oats in the field. Dry matter yield as % of control for each compaction treatment (adapted from Ref. 28).

Herbicide	Compaction treatment		
(kg ai/ha)	Normal	Moderate	Heavy
Atrazine			
0.25	63a*	59a	56a
0.50	43a	29b	20b
1.00	30a	9b	0c
1.50	8a	3ab	0b
Trifluralin			
0.25	56a	65a	51a
0.50	47a	54a	40a
1.00	36a	35a	17b
1.50	21a	21a	9b
Control			
(yield kg/ha)	4770a	4190ab	3560b

* Duncan's multiple range test (P=0.05).

Effects of Soil Moisture

The compaction study mentioned above was not able to pinpoint the mechanisms involved and the factors responsible for enhanced activity of herbicides in compacted soils. It was postulated that in part it could be due to its influence on physical properties of the soil such as soil structure and moisture content. More evidence has since been obtained on the influence of soil moisture status before or after herbicide application on the phytotoxicity of several herbicides. These glasshouse investigations with atrazine (26), a number of grass herbicides (13), and nine different sulfonylurea herbicides (35) show that, in general, moisture stress around the time of application significantly reduced the phytotoxicity of herbicides. There was a highly significant reduction in damage to plants from all herbicides when the soil moisture was reduced to 55% field capacity compared to the pots watered to 100% field capacity. Some herbicides showed a marked reduction in phytotoxicity also with a drop in soil moisture to 70% field capacity. The effect of moisture stress was relatively independent of the herbicide rate and could be due to reduced translocation of herbicides within the plant or changes in the soil-root interface. Thus adequate soil moisture at the time of treatment appears necessary for optimal effectiveness of many herbicides.

PREDICTING HERBICIDE PERSISTENCE AND MOBILITY IN THE SOIL

A soil acting herbicide must have a residual life which is adequate to maintain an effective concentration for the period during which weed control is required, but it must not persist excessively or pose a hazard to a succeeding crop. This is perhaps an over-simplification since many would regard a compound which persisted significantly beyond the life of the crop as being undesirable even if the following crop was tolerant. In practice therefore, there are some differences of emphasis in the evaluation of the significance of persistence between the agricultural and environmental view points (9). The farmer

needs precise information, preferably on a field by field basis, while those concerned with the assessment of environmental impact often hope to achieve only a general impression.

A considerable amount of research was done in the early years with atrazine (27, 29), alachlor, metolachlor, acetochlor, dimethenamid, linuron (29, 38, 48), terbacil, trifluralin (21, 46), ethofumesate (22), hexazinone (24), EPTC (6, 33), simazine and many other triazines (32, 49) and a number of grass herbicides (14, 40, 42). All these studies led to two main conclusions. Firstly, the length of persistence of toxic residues in New Zealand soils was in general less than those reported in overseas soils. Secondly, the persistence of most herbicides was influenced appreciably by the soil organic matter and soil texture. In some cases even the type of clay mineral was suggested to have a significant influence on the length of residual activity (27).

All the above investigations utilised bioassays for measuring phytotoxic levels of herbicides in the soil. It was evident that the residual toxicity from any given concentration of a herbicide was negatively correlated with both the organic matter and the clay content of the soil. However, it must be borne in mind that lower herbicide rates are required for a significant reduction in the growth of bioassay plants in a low organic matter or a sandy soil than in a high organic matter or a heavy soil. Thus if the initial rate was related to the GR_{50} concentration of the herbicide, the residual activity did not vary appreciably between soils. Therefore, an important practical point to come out of this work is that if initial rates used for weed control in different soils are adjusted according to soil characteristics, then the residual toxicity to sensitive crops in the rotation will be reasonably comparable.

RECENT RESEARCH WITH SULFONYLUREA HERBICIDES

The sulfonylurea herbicides have emerged as a major new class of herbicide and an important advance in chemical weed control technology. They are highly active against a wide spectrum of broadleaf and grass weeds. With their unprecedented herbicidal activity, field use rates have fallen dramatically resulting in application rates of grams rather than kilograms per hectare. The broad spectrum and reliability of weed control, low dose rates, ease of handling and the favourable toxicological profiles of these compounds have contributed to the rapid increase in their use. Although some members of the sulfonylurea group are known to have a very short half life in the soil, others such as chlorsulfuron and triasulfuron persist much longer in the soil. Chemical hydrolysis and microbial breakdown are the principal modes of their degradation which is dependent on soil pH, soil texture, moisture and temperature and can vary widely between different soils (4).

We initiated a programme to develop a sulfonylurea herbicide for broadcast application to control weeds in pastures. Most of these chemicals result in some damage to the two main constituents of our pastures viz. perennial ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)*, especially if applied in autumn (44). The time for which these herbicides persist in the soil is therefore of particular importance as this will have serious implications for the long term health of these pasture species. The level of soil activity and persistence of metsulfuron, triasulfuron and primisulfuron was studied in different soils and climatic conditions (15, 39, 43). In general the length of residual activity was similar to or less than reported in the literature, but both the initial injury and long term damage to pasture species caused by these compounds was unacceptable. Research work then concentrated on two compounds with much shorter residual activity - viz., tribenuron (41) and thifensulfuron (44). Based on nearly a decade of investigations the herbicide thifensulfuron has now been registered in New Zealand for control of docks (*Rumex* spp) and some buttercups (*Ranunculus* spp) as a broadcast treatment in pasture.

To predict the impact of residual levels of compounds on an existing or a succeeding crop, the challenge was to develop a practical assay method which was sensitive enough to detect extremely low residues in the soil and to correlate this with the sensitivity of the crop concerned. Several laboratory and glasshouse plant bioassays have been used by researchers to study the behaviour of persistent sulfonylureas, e.g. chlorsulfuron, but most of these are not sensitive enough for compounds like thifensulfuron which have low activity through soil and undergo rapid breakdown. We therefore developed sensitive bioassay procedures for quantitative determination of several sulfonylurea herbicides and investigated their activity and mobility in different soils (18, 34, 35). Suitable chemical analysis procedures have also been

developed using gas chromatographic analysis for some sulfonylurea compounds (16) and high performance liquid chromatographic methods for other members of the group (17). A simple bioassay method has also been developed to measure the concentration of sulfonylurea compounds in water or leachate samples using sunflowers *(Helianthus annus)* as test plants (7).

Simultaneous or sequential applications of herbicides and other pesticides are commonly used by farmers to combat weed, insect and disease problems. Our field studies in maize have shown that combinations of certain insecticides, especially terbufos and phorate, should not be used with sulfonylurea herbicides when the insecticides are applied in the furrow at the time of planting. Although not leading to yield reduction, the crop damage was too severe in many instances to be commercially acceptable. No damage resulted from combinations with chlorpyrifos (37).

In the latest series of investigations we are studying the influence of temperature and soil moisture on the degradation of sulfonylurea herbicides. Analysis of the herbicides in the soil is done by gas chromatography or high performance liquid chromatography methods. In the case of sulfonylurea herbicides studied so far, it appears that temperature is the main factor influencing the rate of their degradation. Influence of soil water content on the stability of the compounds is less evident at moderate to high moisture levels, although most compounds degrade considerably more slowly under low moisture conditions viz 40% of maximum water holding capacity (11, 12)

CONCLUSIONS

It is clear from the information presented in this paper that a close relationship exists between certain soil properties and the phytotoxicity and persistence of soil applied herbicides. In addition to confirming the findings of some previous researchers, our results show that this relationship continues to exist in soils with very high levels of organic matter and/or large amounts of clays. The effect of increasing organic matter levels, however, diminishes at the higher end of the range and this is in contrast to theoretical studies conducted by many researchers. Using bioassay methods we have shown that although the residual activity of a compound from a given concentration varies greatly between soils, it is fairly similar if the initial concentration is adjusted to the GR_{50} value of these soils. Research effort should therefore be directed to accurately define the initial application rates of herbicides for different soils. If the rates are adjusted according to the soil characteristics, this would minimise the residue carryover and environmental contamination problems, in addition to providing the maximum weed control and avoiding crop damage.

REFERENCES

1. Allbrook, R.F., 1983. Some physical properties of allophane soils from the North Island, New Zealand. *New Zealand Journal of Science 26*: 481-492.
2. Bailey, G.W. and J.L. White, 1970. Factors influencing the adsorption, desorption and movement of pesticides in soil. *Residue Reviews 32:* 29-92.
3. Birrell, K.S. and M. Fields, 1952. Allophane in volcanic ash soils. *Journal of Soil Science 3*: 156-166.
4. Brown, H.M., 1990. Mode of action; crop selectivity, and soil relations of the sulfonylurea herbicides. *Pesticide Science 29*: 263-281.
5. Burney, B., A. Rahman, G.A.C. Oomen and J.M. Whitham., 1975. The organic matter status of some mineral soils in New Zealand. *Proceedings of the New Zealand Weed and Pest Control Conference 28*: 101-103.
6. Ghani, A., R.F. Allbrook and A. Rahman, 1986. Influence of several soil properties on the degradation of EPTC in some New Zealand soils. *New Zealand Journal of Agricultural Research 29*: 469-474.
7. Günther, P., W. Pestemer, A. Rahman, and H. Nordmeyer, 1993. A bioassay technique to study the leaching behaviour of sulfonylurea herbicides in different soils. *Weed Research 33*: 177-185.
8. Günther, P., A. Rahman and W. Pestemer, 1989. Quantitative bioassays for determining residues and availability to plants of sulphonylurea herbicides. *Weed Research 29*: 141-146.

9. Hance, R.J., 1984. Herbicide residues in the soil : some aspects of their behaviour and agricultural significance. *Australian Weeds 3*(1): 26-34.
10. Harrison, G.W., J.B. Weber and J.V. Baird, 1976. Herbicide phytotoxicity as affected by selected properties of North Carolina soils. *Weed Science 24*: 120-126.
11. James, T.K., P. Klaffenbach, P.T. Holland and A. Rahman, 1995. Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. *Weed Research 35* : (in press).
12. James, T.K., A. Rahman, D.R. Lauren and P.T. Holland, 1995. Degradation and Persistence of flazasulfuron in a Volcanic Soil. *Proceedings of the 15th Asian-pacific Weed Science Society Conference 15* : (in press).
13. James, T. K. and A. Rahman, 1990. Soil mobility and moisture studies with some grass herbicides. *Proceedings of the New Zealand Weed and Pest Control Conference 43*: 146-149.
14. James, T.K., A. Rahman and J. Mortimer, 1988. Persistence and movement of quizalofop in two soil types. *Proceedings of the New Zealand Weed and Pest Control Conference 41*: 49-52.
15. James, T.K., A. Rahman and T.M. Patterson, 1988. Residual activity of triasulfuron in several New Zealand soils. *Proceedings of the New Zealand Weed and Pest Control Conference 41*: 21-25.
16. Klaffenbach, P. and P.T. Holland, 1993. Analysis of sulfonylurea herbicides by gas-liquid chromatography. 2. Determination of chlorsulfuron and metsulfuron-methyl in soil and water samples. *Journal of Agriculture and Food Chemistry 41* : 396 - 401.
17. Klaffenbach, P and P.T. Holland, 1993. Analysis of sulfonylurea herbicides by gas-liquid chromatography. 3. Mass spectrometry and multiresidue determination. *Biological Mass Spectrometry 22* : 565 - 578.
18. Maeda, T., H. Takenaka and B.P. Warkentin, 1977. Physical properties of allophane soils. *Advances in Agronomy 29*: 229-264.
19. Nishimoto, R.K. and A. Rahman, 1985. Effect of incorporation depth and soil organic matter content on herbicidal activity. *New Zealand Journal of Agricultural Research 28*: 531-535.
20. Rahman, A. 1976. Effect of soil organic matter on the phytotoxicity of soil-applied herbicides-glasshouse studies. *New Zealand Journal of Experimental Agriculture 4*: 85-88.
21. Rahman, A. 1977. Persistence of terbacil and trifluralin under different soil and climatic conditions. *Weed Research 17*: 145-152.
22. Rahman, A., 1978. Effect of climatic and adaphic factors on the persistence and movement of ethofumesate. *Proceedings 1978 British Crop Protection Conference - Weeds* : 557-563.
23. Rahman, A., 1979. Enhancement of ethofumesate phytotoxicity by phosphorus. *Proceedings of the Seventh Asian-Pacific Weed Science Society Conference 7* : 463-466.
24. Rahman, A., 1981. Persistence and movement of hexazinone in some New Zealand soils. *Proceedings of the Eighth Asian-Pacific Weed Science society Conference 8* : 475-479.
25. Rahman, A., 1982. The agrestal weed flora and vegetation of the world: Chapter 27, New Zealand. pp. 299-308. *In*: Biology & Ecology of Weeds. ed. W. Holzner & M. Numata. Dr W. Junk Publishers, The Hague.
26. Rahman, A. 1987. Effect of soil moisture and organic matter on the activity of atrazine. *Proceedings of the New Zealand Weed and Pest Control Conference 40*: 179-181.
27. Rahman, A. and N.S. Brown, 1977. Atrazine residues in the Gisborne Plains and Waikato regions. *Proceedings of the New Zealand Weed and Pest Control Conference 30*: 19-24.
28. Rahman, A., B. Burney and B.E. Manson, 1978. Effect of soil compaction on phytotoxicity and persistence of soil-applied herbicides. *Weed Research 18*: 93-97.
29. Rahman, A., B. Burney and B..E. Manson, 1978. Persistence of some herbicides used for weed control in maize. *Proceedings of the New Zealand Weed and Pest Control Conference 31*: 25-29.
30. Rahman, A., B. Burney, J.M. Whitham and B.E. Manson, 1976. A comparison of the activity of herbicides in peat and mineral soils. *New Zealand Journal of Experimental Agriculture 4*: 79-84
31. Rahman, A., C.B. Dyson and B. Burney, 1978. Effect of soil organic matter on the phytotoxicity of soil - applied herbicides - field studies. *New Zealand Journal of Experimental Agriculture 6*: 69-75.
32. Rahman, A. and P.T. Holland, 1985. Persistence and mobility of simazine in some New Zealand soils. *New Zealand Journal of Experimental Agriculture 13*: 59-65.
33. Rahman, A. and T.K. James, 1983. Decreased activity of EPTC+R-25788 following repeated use in some New Zealand soils. *Weed Science 31*: 783-789.

34. Rahman, A. and T.K. James, 1989. Comparative mobility of nine sulfonylurea herbicides in soil columns. *Proceedings of the Twelfth Asian-Pacific Weed Science Society Conference 12*: 213-217.
35. Rahman, A., and T.K. James, 1991. Influence of soil moisture on the post-emergence activity of nine sulfonylurea herbicides. *Proceedings of the Thirteenth Asian-Pacific Weed Science Society Conference 13*: 331-337.
36. Rahman, A. and T..K. James. 1993. Patterns of weed seedling emergence in two New Zealand soils. *Proceedings of the 8th EWRS Symposium, Braunschweig 1993*: 665-672.
37. Rahman, A. and T.K. James, 1994. Enhanced activity of nicosulfuron in combination with soil applied insecticides in corn (*Zea mays*). *Weed Technology 7*: 824-829.
38. Rahman, A. and T.K. James, 1994. Field persistence of some maize herbicides in two soils. *Proceedings of the New Zealand Plant Protection Conference 47*: 6-10.
39. Rahman, A., T.K. James, P. Martin and D.K. Fullerton, 1991. Persistence of metsulfuron in different soils. *Proceedings of the New Zealand Weed and Pest Control Conference 44*: 99-104.
40. Rahman, A., T.K. James and J. Mortimer, 1985. Residual activity of some newly developed post-emergence herbicides for grass weeds. *Proceedings of the New Zealand Weed and Pest Control Conference 38*: 61-65.
41. Rahman, A., T.K. James and J. Mortimer, 1988. Persistence and mobility of the sulfonylurea herbicide DPX-L5300 in a New Zealand volcanic soil. *Proceedings of the International Congress of Crop Protection 53/36: 1463-1470.*
42. Rahman, A., T.K. James and J. Mortimer, 1988. Efficacy, persistence and movement of clethodim in two soils. *Proceedings of the New Zealand Weed and Pest Control Conference 41*: 39-43.
43. Rahman, A., T.K. James and T.M. Patterson, 1990. Soil activity and persistence of the sulfonylurea herbicide primisulfuron. *Proceedings of the New Zealand Weed and Pest Control Conference 43*: 142-145.
44. Rahman, A., T.K. James, P. Sanders and K. Nicholson, 1993. Tolerance of perennial ryegrass / white clover pastures to five sulfonylurea herbicides. *Proceedings of the Fourteenth Asian - Pacific Weed Science Society Conference 14* : 310 - 314.
45. Rahman, A., B.E. Manson and B. Burney, 1976. Effect of phosphorus on phytotoxicity and residual activity of atrazine. *Proceedings of the New Zealand Weed and Pest Control Conference 29*: 124-128.
46. Rahman, A., B.E. Manson and B. Burney, 1979. Influence of selected soil properties on the phytotoxicity of soil applied herbicides. *Proceedings of the Sixth Conference of the Asian-Pacific Weed Science Society 6*: 579-586.
47. Rahman, A., B.E. Manson, B. Burney and L J Matthews, 1976. Effects of phosphorus on the phytotoxicity and residual activity of trifluralin. *Proceedings of the Fifth Asian-Pacific Weed Science Society Conference* 5 : 162-166.
48. Rahman, A., B.E. Manson, B. Burney and J.M. Whitham, 1975. Residual activity of atrazine, alachlor and linuron under different soil and climatic conditions. *Proceedings of the New Zealand Weed and Pest Control Conference 28*: 104-108.
49. Rahman, A. and L.J. Matthews 1979. Effect of soil organic matter on the phytotoxicity of thirteen *s*-triazine herbicides. *Weed Science 27*: 158-161.
50. Robertson, N.G., 1959. A descriptive atlas of New Zealand. Government Printer, Wellington.
51. Stevenson, F.J., 1972. Organic matter interactions involving herbicides in soil. *Journal of Environmental Quality 1*: 333-343.
52. Stolp, C.F. and D. Penner, 1973. Enhanced phytotoxicity of atrazine-phosphate combinations. *Weed Science 21*: 37-40.
53. Wilson, H.P., F.B. Stewart and T.E. Hines, 1976. Effect of temperature on response of tomatoes to several dinitroaniline herbicides and phosphorus. *Weed Science 14*: 115-119.
54. Wilson, J.S. and C.L. Foy, 1992. Influence of various soil properties on the adsorption and desorption of ICIA-0051 in five soils. *Weed Technology 6*: 583-586.
55. Weber, J.B., 1970. Mechanisms of adsorption of *s*-triazines by clay colloids and factors affecting plant availability. *Residue Reviews*, 32:93-130.
56. Weber, J.B. and S.B. Weed, 1974. Effects of soil on the biological activity of pesticides. pp 223-256 *In* : Pesticides in soil and water. ed. W.D. Guenzi. Soil Science Society of America Inc. Madison, Wisconsin.

Degradation and Persistence of Flazasulfuron in a Volcanic Soil.

T.K. James*, A. Rahman, D.R. Lauren, S. Baskaran and P.T. Holland
AgResearch and HortResearch, Ruakura Research Centre, Hamilton, New Zealand

Abstract. The degradation and persistence of flazasulfuron in soil were determined under both field and controlled environment conditions. In the degradation study spiked soil samples were maintained at each of five temperature/soil moisture regimes, viz, 22°C/80% maximum water holding capacity (mwhc), 22°C/60% mwhc, 22°C/40% mwhc, 30°C/60% mwhc and 10°C/60% mwhc, and these were periodically sampled for analysis by high performance liquid chromatography (HPLC). Persistence in two field experiments was measured both by bioassays using white mustard (*Sinapis alba* L.) and forage sorghum (*Sorghum bicolor* (L.) Moench), and by HPLC. Flazasulfuron was applied at 50 g ai/ha and 100 g ai/ha to a sandy loam soil of volcanic origin (7.3% organic carbon, pH of 5.7). The limits of detection were, 1 µg/kg and 5 µg/kg for bioassays with white mustard and forage sorghum respectively, and 0.5 µg/kg for HPLC. The controlled environment study showed that the degradation of flazasulfuron was strongly affected by temperature but only slightly by the soil moisture content. Based on the white mustard bioassay, flazasulfuron was found to persist for 49 and 56 days for the 50 g ai/ha and 100 g ai/ha rates respectively in both field experiments. Comparatively, residues were not detectable after 35 and 42 days using HPLC analysis. Degradation was faster in the field than extrapolated for equivalent controlled environment conditions.
Key words: flazasulfuron, persistence, degradation, bioassay, SL-160.

Introduction

Flazasulfuron is a sulfonylurea herbicide which was discovered by Ishihara Sangyo Kaisha Ltd. It has a broad spectrum of weed control including both monocots and dicots, and both annuals and perennials (4, 5, 7). Some of the important weed species controlled include barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), green foxtail (*Setaria viridis* (L.) Beauv.), sow thistle (*Sonchus oleraceus* L.), chickweed (*Stellaria media* (L.) Vill.), annual mouse-ear chickweed (*Cerastium glomeratum* Thuill.), shepherds purse (*Capsella bursa-pastoris* (L.) Med.), fathen (*Chenopodium album* agg.), couch (*Elytrigia repens* (L.) Beauv.), purple nut sedge (*Cyperus rotundus* L.), horned oxalis (*Oxalis corniculata* L.), white clover (*Trifolium repens* L.) and sorrel (*Rumex acetosa* L.). Flazasulfuron is both foliar and root absorbed, and like other sulfonylurea herbicides it inhibits the enzyme acetolactate synthase (ALS). Weeds treated with flazasulfuron stop growing immediately and show discolouration of the developing leaves within a few days. This is followed by leaf necrosis, desiccation and plant death. Tolerance to flazasulfuron is gained by the rapid metabolism of the herbicide in tolerant species. Flazasulfuron has good crop selectivity when applied either pre or postemergence to perennial turf, vines, coffee, sugarcane and tomato (1). The product used in these studies was formulated as a 25% a.i. water soluble granule.

In New Zealand flazasulfuron is being investigated for selective weed control in tomato (*Lycopersicon esculentum* Mill.), kumara (*Ipomoea batatas* (L.) Poir) and pasture. It is also being evaluated in combination with glyphosate for control of certain scrub weeds. In these circumstances it is important to know the persistence of phytotoxic residues in the acidic, high organic matter soils of New Zealand. Many sulfonylurea herbicides have been shown to be quite persistent in alkaline soils (10, 13). Most of the sulfonylurea herbicides are degraded both by chemical hydrolysis and by soil micro-organisms, with the former particularly important in low pH soils (2). Degradation is also dependant on temperature and soil-water content.

Low application rates of sulfonylurea herbicides make chemical analysis very difficult. Methods based on high performance liquid chromatography (HPLC) (14, 15) and gas chromatography (GC) (8) have been reported. The lack of a cost-effective chemical assay method of sufficient sensitivity for determination of sulfonylurea herbicide residues in the soil has led to a dependence on bioassay techniques (3, 11). Bioassays have the advantage of a high sample throughput combined with a relatively high sensitivity, but they require large amounts of soil.

In this study the effects of temperature and soil moisture content on the degradation of flazasulfuron were investigated in controlled environments. Also, bioassay and chemical methods of analysis were compared for determining the persistence of this herbicide in the field over a two year period.

Materials and Methods

Bioassay calibration experiments

For the 1993/94 season three glasshouse experiments were initiated on 23.12.93, 20.1.94 and 21.2.94 to establish calibration curves for the activity of flazasulfuron in the soil on the two bioassay species, viz. forage sorghum (*Sorghum bicolor* (L.) Moench) and white mustard (*Sinapis alba* L.). The test soil was a Horotiu sandy loam freshly taken from a site at the Blands Horticultural Research Station near Hamilton, New Zealand. This soil is of volcanic origin and has 61% sand, 23%silt, 14% clay, 7.3% organic carbon, a bulk density of 0.71 kg/L, a CEC of 31.6 m.e./100 g, a field capacity of 41.6% and a pH of 5.7. A range of concentrations of flazasulfuron between 0.5 and 500 µg/kg was used to establish a standard calibration curve for each of the test species. The herbicide standard solutions were prepared by mixing the formulated product with water in volumetric flasks. The herbicide solutions were added to moist soil at the rate of 20 mL/kg of oven dry soil equivalent. This soil was thoroughly mixed by shaking in a polythene bag and then allowed to stand overnight to equilibrate before being used for potting out four replicates of the test species. Visual observations of plant damage were made throughout the duration of the experiments and the plant foliage was harvested for dry matter yields 3 to 4 weeks after planting. For the 1994/95 season two experiments were initiated on 9.11.94 and 21.2.95 and completed as above.

Controlled environment degradation study

The bulk sample of Horotiu sandy loam soil described for the bioassay studies was also used for the degradation study. Aliquots of soil (50 g dry-weight) in conical flasks (250 mL) were individually treated after being adjusted to the appropriate soil-water content. The soil was spiked with an aqueous solution (1 mL) of the formulated product (7.1 mg a.i./L), equivalent to the 100 g ai/ha rate used in the field if it was incorporated to a depth of 75 mm. The flasks were maintained at one of five soil-water content/temperature combinations, viz. 60% of maximum water holding capacity (m.w.h.c.) maintained at either 10, 22 or 30°C and 40 and 80% of m.w.h.c. maintained at 22°C. Water was added once a week to bring the flasks up to the predetermined weight. At each sampling date two flasks were taken from each soil-water content/temperature combination for each herbicide and stored at -20°C until analysed by HPLC method.

Chemical analysis

After thawing 50 g soil samples were extracted three times with 100 mL of 0.1 M $NaHCO_3$ solution. The mixture was sonicated (2 min) and then centrifuged (10 min, 3500 rpm) and the aqueous phase decanted through glass wool. The combined extracts were acidified to pH 2 (HCl) and aspirated through a C18 Solid Phase Extraction (SPE) disk. After drying (20 min) the disks were eluted with two aliquots of ethyl acetate (10 mL). The eluate was dried with anhydrous sodium sulphate, rotary evaporated to near dryness and redissolved in 1.6 mL cyclohexane/ethyl acetate (1:1) for gel permeation chromatographic (GPC) cleanup (9). After evaporation, the GPC fraction was redissolved in 1 mL of methanol/water (1:1) for HPLC analysis with UV detection. The HPLC was run isocratically with a mobile phase of methanol/0.5% v/v aqueous acetic acid (52:48) at 1 mL/min and a Zorbax ODS C18 column (150 x 4.6 mm) held at 35°C. Sample injection size was 100 µL and detection wavelength was 250 nm. This method has a detection limit of 0.2 µg/kg. Results were corrected for recovery of fortified control soils (65% at 200 µg/kg)

Persistence of flazasulfuron in the field

Two experiments were done during spring and summer of 1993/94 and 1994/95 on the same Horotiu sandy loam site as described for the bioassay calibration experiments. Individual plots were 10 m x 2 m and were replicated four times in a randomised block design. Flazasulfuron was applied at 50 and 100 g a.i./ha to freshly cultivated ground on 23.11.93 (Trial 1) and 3.11.94 (Trial 2) with a CO_2 powered precision sprayer delivering 300 L/ha at 200 kPa. Soil samples from the top 75 mm were collected from each plot the day after treatment and at one or two weekly intervals thereafter until three samplings after the last residues were detected by the bioassay method. The soil samples were thoroughly mixed and then used to plant out one pot of each of the bioassay species and placed in a glasshouse. Subsamples were taken for soil moisture determination (20 g) and chemical analysis (50 g). The latter were stored at -20°C prior to analysis. The bioassay plants were grown and assessed as for the bioassay calibrations above. Average soil temperature (100 mm depth) and average rainfall for the intervals between samplings for the field study are presented in Figure 1. For Trial 1 the rainfall was adequate to maintain soil-water content at 70-85% of field capacity but for Trial 2 the soil-water content was lower than 60% of field capacity for much of the duration of the experiment.

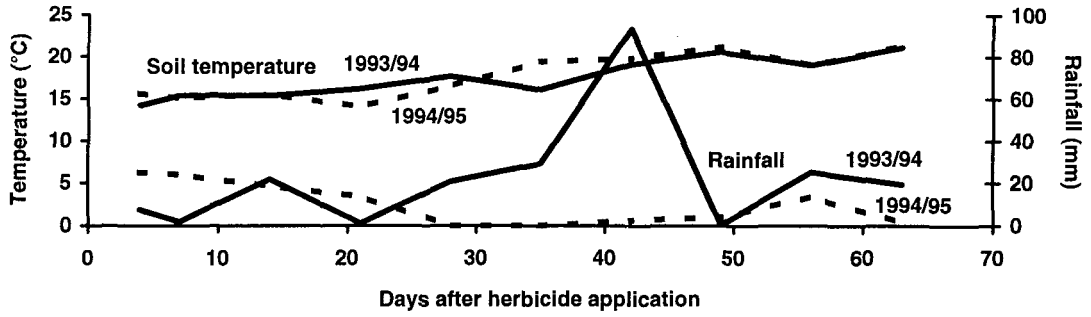


Figure 1: **Average soil temperature (100 mm depth) and average rainfall for the intervals between samplings for the two field experiments**

Results and Discussion

The combined results from the three bioassay calibration experiments for 1993/94 are presented in Figure 2. White mustard was more sensitive to flazasulfuron than was forage sorghum, with significant dry matter reductions recorded from the 1 µg/kg and 5 µg/kg concentrations for the two species respectively. The results from the two bioassay calibration experiments in 1994/95 were very similar to those shown in Figure 2 so the data are not presented.

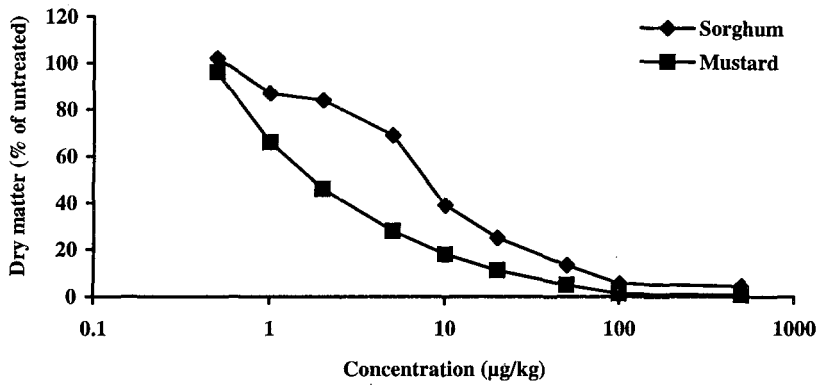


Figure 2: **Effect of known rates of flazasulfuron on bioassay species grown in the glasshouse - dry shoot weight as percent of untreated.**

The chemical method of analysis for flazasulfuron based on HPLC determination proved sensitive and reproducible. The GC method (8) validated for six other sulfonylureas proved unsuitable for flazasulfuron as a stable dimethyl derivative could not be formed with a good yield.

The results of the controlled environment degradation study are presented in Figure 3. Under these conditions the degradation of flazasulfuron was not constant. The rate of degradation in the first 4 weeks was considerably faster than in the subsequent weeks. This is probably due to reduced microbial activity with increasing duration of the experiment as Visser *et al* (12) has demonstrated reduced microbial activity with increasing time in conditions similar to our study. However, this reduced degradation did not effect the overall relativities of the degradation rates under the different soil moisture/temperature regimes. The soil moisture content had very little effect on the degradation rate of flazasulfuron. Initially, degradation in the soil with high moisture content was slightly faster but after the first 2 weeks the degradation rate slowed, allowing the other soil moisture regimes to catch up.

Temperature, however, had a highly significant effect on the degradation rate of this herbicide. At 30°C residues fell below detectable levels after only 4 weeks, whereas at 10°C residues were still present after 26

weeks. The half-life of flazasulfuron in the soil for the three temperature regimes (first 4 weeks only) were 3.5, 7 and 12 days for the 30, 22 and 10°C regimes respectively.

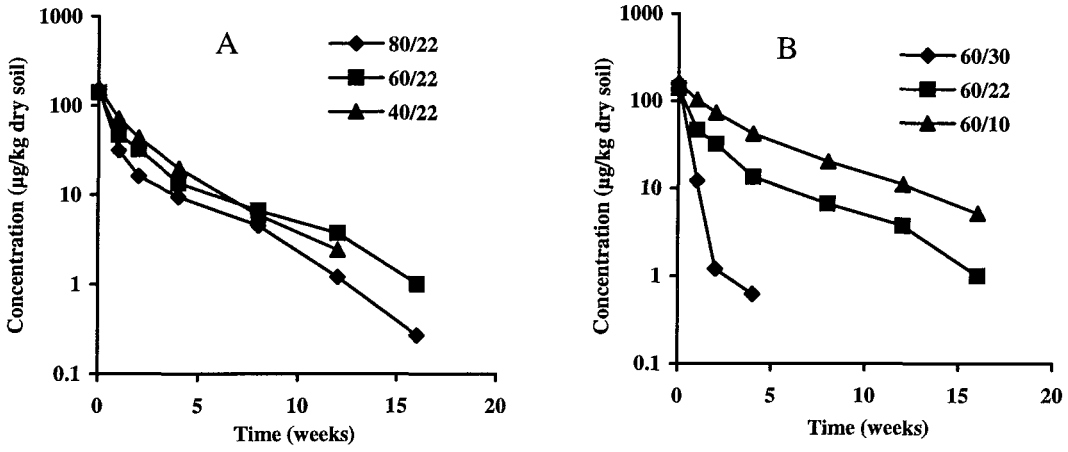


Figure 3: **Effect of (A) moisture and (B) temperature on the degradation of flazasulfuron in the soil.**

In the field experiments flazasulfuron was slightly less persistent than would have been predicted from the degradation rates determined in the controlled environment study (Tables 1 and 2). The white mustard bioassay was the most sensitive, detecting measurable residues up to 49 and 56 days for the 50 and 100 g ai/ha rates respectively in both experiments. The forage sorghum bioassay detected residues for only 21 and 35 days for the same rates in Trial 1 and for 42 and 49 days in Trial 2. The chemical analyses measured residues up to 35 and 42 days for the 50 and 100 g ai/ha rates respectively in both experiments. Although the chemical analysis method has a lower detection limit than the white mustard bioassay method it appears that the plants were more efficient at extracting the herbicide from the soil than the chemical method. This is probably due to the flazasulfuron becoming more strongly adsorbed with time, and more forcing methods of chemical extraction would therefore be required to recover these residues.

Table 1: **Flazasulfuron residues in 0-75 mm depth of Horotiu sandy loam soil determined by both bioassay and chemical analysis - Trial 1.**

Rate	Days after spraying										
(g a.i./ha)	1[a]	4	7	14	21	28	35	42	49	56	63
	Mustard bioassay (dry shoot weight as % of untreated)										
50	0	13[b]	18	22	25	40	43	45	77	93	107
100	0	1	1	9	22	21	35	37	58	77	106
	Sorghum bioassay (dry shoot weight as % of untreated)										
50	11	18	22	25	85	96	126	108			
100	6	10	11	21	52	47	92	102			
	Chemical analysis (µg/kg dry soil)										
50	53	41	18	12	5.5	2.5	0.6	n.d.[c]			
100	113	86	38	24	10	5.4	2.5	0.8	n.d.		

a Sampled 24 h after spraying .
b Generally s.d.'s are < 25% for values below 50 and < 15% for values above 50.
c n.d., not detected at 0.2 µg/kg.

The similarity of the lengths of persistence in field experiments over two growing seasons reinforces the results of the controlled environment studies. The average soil temperatures for the duration of the field experiments were similar, but the rainfall figures were quite different (Figure 1). This difference in rainfall, and consequently in the soil moisture content, did not result in significant differences in the persistence of flazasulfuron over the two growing seasons. These results also suggest that leaching may not be a significant factor in the case of flazasulfuron.

Table 2: Flazasulfuron residues in 0-75 mm depth of Horotiu sandy loam soil determined by both bioassay and chemical analysis - Trial 2.

Rate	Days after spraying										
(g a.i./ha)	1[a]	4	7	14	21	28	35	42	49	56	63
	Mustard bioassay (dry shoot weight as % of untreated)										
50	0	0	7[b]	2	17	45	61	71	79	122	113
100	0	1	1	2	15	30	44	67	67	88	98
	Sorghum bioassay (dry shoot weight as % of untreated)										
50	5	15	21	24	22	34	58	88	97	106	
100	0	15	18	13	16	28	39	73	97	103	
	Chemical analysis (µg/kg dry soil)										
50	49	35	20	8.8	8.6	2.9	0.6	n.d.[c]			
100	104	67	29	23	17	3.5	2.4	1.2	n.d.		

a Sampled 24 h after spraying.
b Generally s.d.'s are < 25% for values below 50 and < 15% for values above 50.
c n.d., not detected at 0.2 µg/kg.

Semilogrithmic plots of the residue levels determined in the field experiments were close to straight lines ($r^2 >$ 95%) demonstrating that under these conditions there was a constant rate of degradation with a soil half-life of 6 days for flazasulfuron. With average soil temperatures of 14 to 17°C over the period, the degradation rate should have been intermediate between the 10 and 22°C treatments of the controlled environment regimes, ie about 9 to 10 days. The shorter persistence is probably due to higher microbial degradation in the field as a result of the more favourable environment. Results of this study show that flazasulfuron is less persistent than metsulfuron and primisulfuron which have previously been evaluated under similar conditions (6). The degradation of both these herbicides was also more influenced by temperature than by soil moisture.

It would appear that under normal cropping conditions in New Zealand, on high organic matter soils of volcanic origin, flazasulfuron had sufficient residual activity for initial weed control but residues should not carry over to cause problems in subsequent crops.

Acknowledgments

The authors wish to thank Y. Lu, C. Maley and N. Childerhouse for assistance with the extraction and analysis of flazasulfuron.

References

1 Anon., 1988. Technical Information Report - SL-160 a Selective Herbicide. Ishihara Sangyo Kaisha Ltd.

2 Beyer, E.M., M.J. Duffy, J.V. Hays, D.D. Schlueter, 1988. Sulfonylurea Herbicides, in: Herbicide: Chemistry, Degradation and Mode of Action, Vol 3. (Eds P.C. Kearney and D.D. Haufman), Marcel Dekker Inc.: New York, pp 117-189.

3 Günther, P., A. Rahman, W. Pestemer, 1989. Quantitative bioassays for determining residues and availability to plants of sulfonylurea herbicides. *Weed Research* 29: 141-146.

4 Haga, T., Y. Tsujii, K. Hayashi, F. Kimura, N. Sakashita, K. Fujikawa, 1991. Trifluoromethylpyridenes as building blocks for new agrochemicals. Discovery of a new turf herbicide. *ACS Symposium Series* 443: 107-119.
5 Hasihizume, B., 1990. Flazasulfuron, a new turf herbicide (Shibagen). *Japan Herbicide Information* 57: 27-30.
6 James, T.K., P. Klaffenbach, P.T. Holland, A. Rahman, 1995. Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. *Weed Research* 35: in press
7 Kimura, F., N. Sakashita, S. Honzawa, H, Yoshi, 1989. SL-160, a new selective herbicide for warm-season turf. *Proc. 6th International Turfgrass Research Conf.*: 285-287.
8 Klaffenbach, P., P.T. Holland, D.R. Lauren, 1993.Analysis of sulfonylurea herbicides by gas-liquid chromatography. 1. Formation of thermostable derivatives of chlorsulfuron and metsulfuron-methyl. *J. Agric and Food Chem.* 41: 388-395.
9 Klaffenbach, P., P.T. Holland, 1993. Analysis of sulfonylurea herbicides by gas-liquid chromatography. 2. Determination of chlorsulfuron and metsulfuron-methyl in soil and water samples. *J. Agric and Food Chem.* 41: 396-401.
10 Nicholls, P.H., A.A. Evans, A. Walker, 1987. The behaviour of chlorsulfuron and metsulfuron in soils in relation to incidents of damage to sugar beet. *Proc. 1987 British Crop Prot. Conf. - Weeds*: 549-556.
11 Rahman, A., 1989. Sensitive bioassays for determining residues of sulfonylurea herbicides in soil and their availability to crop plants, in Environmental Bioassay Techniques and Their Application. (Eds Munawar *et al*) *Hydrobiologia* 188/189: 1365-1375.
12 Visser, S., J. Fujikawa, C.L Griffiths, D. Parkinson, 1984. Effect of topsoil storage on microbial activity, primary production and decomposition potential. *Plant and Soil* 122: 41-50.
13 Walker, A., E.G. Cotterill, S.J. Welch, 1989. Adsorption and degradation of chlorsulfuron and metsulfuron-methyl in soils from different depths. *Weed Science* 29: 281-287.
14 Zahnow, E.W. 1982. Analysis of the herbicide chlorsulfuron in soil by liquid chromatography. *J. Agric and Food Chem.* 30: 854-857.
15 Zahnow, E.W. 1985. Analysis of the herbicide sulfometuron in soil by liquid chromatography. *J. Agric and Food Chem.* 33: 479-483.

Herbicidal Property and Soil Behavior of A New Herbicide, Azimsulfuron

J. C. Chun[1], S. Y. Ma[2], and Y. C. Hahn[3]
[1]Department of Agricultural Chemistry, Jeonbug National University, Jeonju 560-756, Korea
[2]Department of Life Resource Science, Woosuk University, Wanju 565-800, Korea
[3]DuPont Korea Ltd., Seoul 135-082, Korea

Abstract. Azimsulfuron [1H-pyrazole-5-sulfonamide,N-(((4,6-dimethoxy-pyridine-2-yl- aminocarbonyl-4-(2-methyl-2H-tetrazole-5-yl)] is a new sulfonamide herbicide that selectively controls a wide range of weeds in lowland rice (*Oryza sativa*). It effectively controlled *Cyperus serotinus*, *Eleocharis kuroguwai*, *Sagittaria pygmaea*, *S. trifolia*, and *Scirpus juncoides* at 7.5-30 g ai/ha. In the tolerance test on grasses carried out in a nutrient solution containing 0.3 - 30 ppm of azimsulfuron, greater inhibition occurred in roots of both rice and barnyardgrass (*Echinochloa crus-galli*) than in shoots. However, rice root was approximately 5-fold more tolerant than barnyardgrass. The downward movements as determined by 50% growth inhibition of *S. juncoides* were 4-cm in clay loam and 6.5-cm in sandy loam soil with 3-cm/day leaching for 3 days. When incubated at 20 and 30°C, the residual effect in clay loam soil lasted for 30 and 21 days, respectively. In a soil column applied at 15 g/ha of azimsulfuron followed by 3-cm/day leaching for 3 days, dry weights of *S. trifolia* emerging at 5, 10, and 15-cm depth were reduced to 87, 85, and 79% of the corresponding untreated control, respectively. Susceptibility of *S. trifolia* to azimsulfuron did not greatly vary with the emergence depth.

Key words: Azimsulfuron, Herbicidal property, Soil behavior

INTRODUCTION

Azimsulfuron is a new pre-emergence sulfonamide herbicide being developed by E. I. DuPont de Nemours Company for control of a wide range of dicotyledonous and sedge weeds[1)]. In Korea change in dominant weed population from annual to perennial weeds has occurred due to very extensive use of herbicides effective to annual weeds[2)]. This has required an introduction of herbicides which are effective to perennial weeds. In spite of wide use of the present sulfonylurea herbicides, however, such perennial weeds as *E. kuroguwai* and *S. trifolia* are difficult to satisfactorily control.

Our preliminary field trials with azimsulfuron have shown good activity against the major troublesome weed species. The herbicide can be applied at rates of 7.5 to 30 g/ha within 10 to 15 days after the rice is transplanted. Based on the results obtained, azimsulfuron is considered to be a new promising herbicide which can effectively control the perennial weeds that are tolerant to the present sulfonylurea herbicides. To obtain more information on azimsulfuron to develop a new rice herbicide, we determined differential responses of rice and various weed species to azimsulfuron and some soil behaviors.

MATERIALS AND METHODS

Growth response.

Granular formulated azimsufuron (0.05%) was applied at rates of 7.5 to 30 g/ha into the 3-cm standing water of plastic pots containing 2 leaf stage of rice and dormancy-broken weed seeds and tubers. The pots were placed in a greenhouse maintained at a 30°C day/23°C night temperature regime and 14-h photoperiod. Water was not added to the pots for 2 days after azimsulfuron application, after which flood level was maintained at 3-cm water depth. Rice injury and herbicidal activity were determined 30 days after application by recording shoot dry weight reduction of the treated plants compared with untreated control plants.

To compare differential susceptibility of rice and barnyardgrass to azimsulfuron, thirty pre-germinated seeds of rice and barnyardgrass were transferred to a hydroponic nutrient solution containing azimsulfuron ranged from 0.3 to 30 ppm. They were placed in a dark chamber at 25°C and 70% relative humidity. Shoot and root lengths of the plants were measured 10 days after herbicide application.

Persistence.

Azimsulfuron at the rate of 15 g/ha was applied to two types of soils (clay loam and sandy loam) in plastic pots maintained at 3-cm water depth. The pots were kept in growth chambers of 20 and 30°C. Seeds of *S. juncoides* were sown at the interval of 5-day and harvested 2 weeks after seeding.

Growth inhibition was determined as percent of the untreated controls on the basis of shoot dry weight. Half-life was calculated on the basis of the day required to cause 50% growth inhibition of *S. juncoides*.

Downward movement.

Fifteen-cm stacked-cylinder columns were prepared from 12-cm diameter plastic pipe which had been cut into 1-cm sections. The columns were packed with soils and flooded at 1-cm water depth. Azimsulfuron at the rate of 15 g/ha was applied to the columns. Leaching at the amounts of 1, 2, and 3-cm/day was initiated 5 hours after herbicide application. One-cm water depth in the columns was always maintained during the leaching process by watering from top of the columns. Upon completion of the leaching process, the soil column was divided into 1-cm sections and the section soil was transferred to a plastic pot. *S. juncoides* was sown into the pot and grown in the growth chamber described above. Dry weight of the shoots was measured 20 days after seeding. The experiment was replicated three times.

Susceptibility of *S. trifolia* and *E. kuroguwai* emerging from different soil depths to azimsulfuron was determined in relation to downward movement of azimsulfuron. Unless stated, the above experimental procedure was followed. Tubers of the two weeds were planted at 5, 10, and 15 cm depth of the clay loam soil column. After application of azimsulfuron at 15 g/ha, water was leached at the amount of 3-cm/day for 3 days. Growth inhibition on the basis of the dry weight was determined as percent of the corresponding untreated control at each planting depth.

RESULTS AND DISCUSSION

Susceptibility difference.

There was a great difference in susceptibility to azimsulfuron between rice and broadleaf and sedge weeds studied (Fig. 1). Rice transplanted at 2-leaf stage tolerated to azimsulfruron applied at rates of 7.5 to 30 g/ha. At 30 g/ha of azimsulfuron growth inhibition of rice reached only about 10% of the untreated control, while about 90% growth inhibition was obtained for all the weed species tested. As decreasing the application rate, however, susceptibility difference between the weed species occurred. At 7.5 and 15 g/ha of azimsulfuron a relative tolerance was greater in *S. pygmaea* than in *S. trifolia*. A similar effect was also found with the sedges, that is, *C. serotinus* was more tolerant than *E. kuroguwai* and *S. juncoides*.

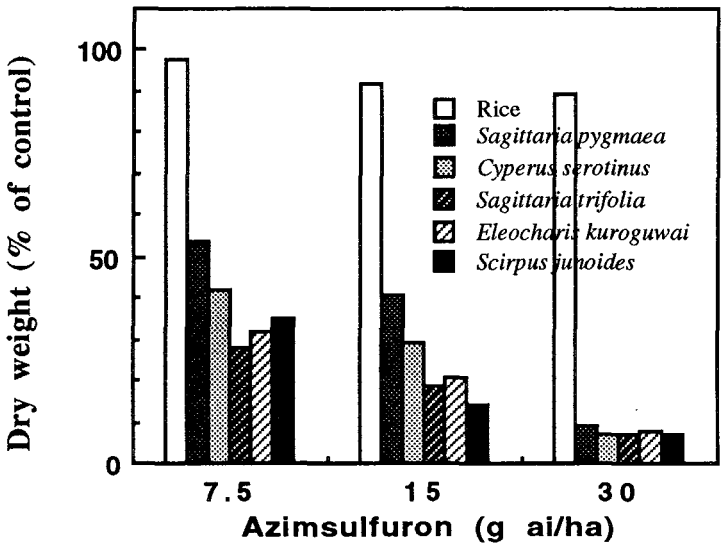


Fig. 1. Growth response of rice and perennial paddy weeds to azimsulfuron

Susceptibility difference to azimsulfuron was also found between rice and barnyardgrass. In the tolerance test carried out in a nutrient solution containing 0.3 - 30 ppm of azimsulfuron, greater inhibition occurred in roots of both plants than in shoots (Fig. 2). However, rice root was approximately 5-fold more tolerant than barnyardgrass. Concentration of azimsulfuron required to cause 50% growth inhibition of root was 5.5 ppm for barnyardgrass and 28 ppm for rice.

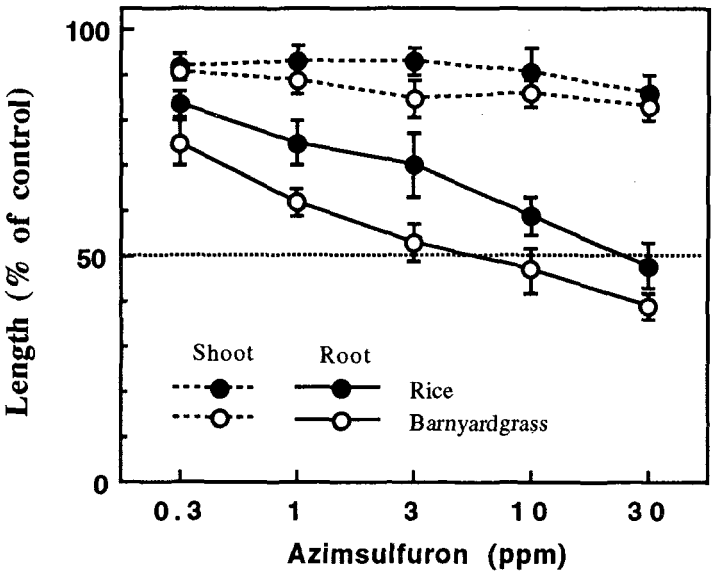


Fig. 2. Differential response of rice and barnyardgrass to azimsulfuron

Persistence.

Persistence of azimsulfuron in soil did not vary with soil type, but was affected by temperature. There was no significant difference in the soil half-life between clay loam and sandy loam soils (Table 1). However, the half-life was longer when incubated at 20°C than at 30°C. In both the soils, azimsulfuron persisted for 30 to 32 days at 20°C and for 21 to 22 days at 30°C.

Table 1. Persistence of azimsufuron in different soil types at two temperature regimes.

Soil texture	Temperature (°C)	Half-life (day)[1]
Clay loam	20	30 a
	30	21 b
Sandy loam	20	32 a
	30	22 b

[1]Means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Downward movement.

The downward movement was expressed by the soil depth where 50% growth inhibition of the test plant, *S. juncoides* occurred. Mobility of azimsulfuron varied with soil type and amount of leaching (Table 2). The movement was greater in sandy loam soil than in clay loam soil and increased as increasing the leaching amount. The results indicate that azimsulfuron exhibits a relatively high mobility potential in the soils.

Effect of emergence depth on sensitivity of *Sagittaria trifolia* and *Eleocharis kuroguwai* to azimsulfuron.

In a soil column applied at 15 g/ha of azimsulfuron followed by 3-cm/day leaching for 3 days,

Table 2. Downward movement of azimsulfuron under different soil types and leaching grades.

Soil texture	Mobility (cm)[1]		
	Leaching amount (cm/day)		
	1	2	3
Clay loam	2 d	2.5 d	4 bc
Sandy loam	3 cd	4.5 b	6.5 a

[1]Means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

dry weights of *S. trifolia* emerged at 5, 10, and 15-cm depth were reduced to 87, 85, and 79% of the corresponding untreated control, respectively (Fig. 3). The growth reduction of *E. kuroguwai* was obtained with 85, 80, and 57% at 5, 10, and 15-cm depth, respectively. For the both species, the growth inhibition decreased with increasing the emergence depth. This was due probably to decrease in concentration of azimsulfuron at deeper soil depths. As shown in the above mobility experiment, azimsulfuron leached to about 4-cm depth under the same condition given to this experimental system. On the other hand, difference in susceptibility between emergence depths was greater in *E. kuroguwai* than in *S. trifolia*. The fact that there is no great difference in susceptibility of *S. trifolia* between the emergence depths indicates that azimsulfuron can control satisfactorily *S. trifolia* emerging at various soil depths.

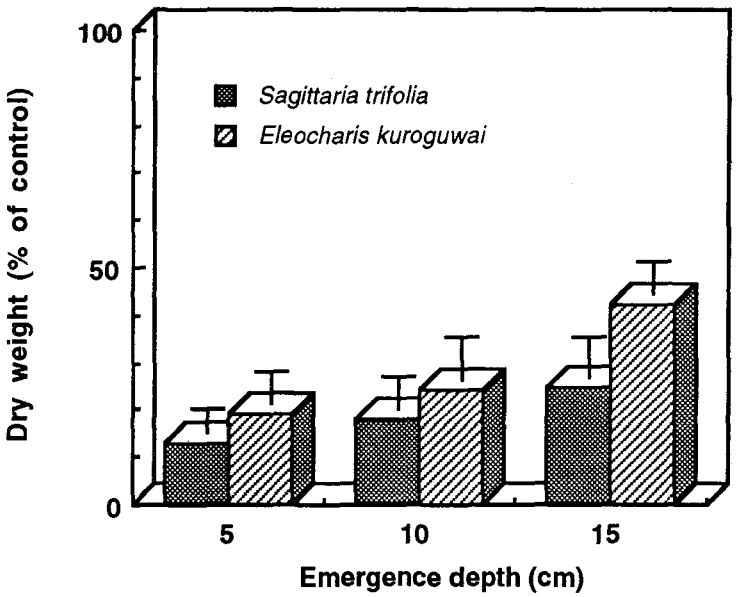


Fig. 3. Growth response of *Sagittaria trifolia* and *Eleocharis kuroguwai* emerging at different soil depths to azimsulfuron

REFERENCES

1. Anonymous. 1989. DPX-A8947 and development. AG-4224, Rev. 8/89. Agricultural Products, DuPont.
2. Choi, C. D., S. C. Kim, and D. Y. Hwang. 1989. Effect of repeated use of same herbicide on weed growth in lowland rice. Kor. J. Weed Sci. 9:39-45.

Post-Application Attenuation of Molinate and Thiobencarb in Rice-Field Water

Hassan Said* and Mineko Yukimoto**
* MARDI Rice Research Center, 13200 Kepala Batas
Penang, Malaysia
** Japan International Research Centre For Agriculture Science, Tsukuba, Ibaraki 305, Japan

Abstract Molinate (S-Ethyl hexahydro-1H-azepine-1-carbothiote) and thiobencarb [S-(4-Chlorobenzyl)-N,N-diethyl thiocarbamate] have been widely used to control grassy weeds in direct seeded rice in Malaysia. Knowledge of herbicidal residues after their application is useful for maintenance of a safe rice ecosystem. Studies on molinate and thiobencarb attenuation were conducted in fibre-glass troughs as well as in the rice-field at MARDI Rice Research Center, Penang, Malaysia. Gas chromatography was used to measure herbicide concentration. Preliminary results showed that both herbicides attenuated faster in the field than in the trough. After one week, the concentration of the herbicides had decreased by more than 90% of the original concentration applied in the field, suggesting their non-persistency in rice-field water. Several environmental factors affecting the fate of herbicides in rice-field water were also discussed.

Key words: molinate, thiobencarb, rice-field water.

Introduction

The adoption of direct seeding method for rice crop establishment in Malaysia had resulted in serious weed infestations, particularly the grassy weeds, in the paddy field. *Echinochloa crussgali* had been known to cause yield loss as high as 41% (Azmi 1988). Hence the use of herbicide for weed control is considered inevitable under critical condition.

Molinate and thiobencarb were found to be effective to control grassy weeds particularly *Echinochloa spp.* and less phytotoxic to rice plants (Cheong and Azmi 1988). Molinate was reportedly used in the MUDA area (a major rice growing area in Malaysia) since 1983 and had been widely used until 1992 (Ho and Zainuddin 1995). When used as post-emergence, these herbicides were normally applied into rice field with standing water. Indiscriminate used of these herbicides resulted in its discharge into the paddy field drains and the build up residues may pollute the rice ecosystem. Both herbicides are toxic to fish. The LC_{50}(48h) for molinate to trout is at 1.8 ppm and 3.4 ppm is the TLm(48h) for thiobencarb to carp (Anon,1993). Since molinate and thiobencarb were widely used in Malaysia, monitoring the environmental effects due to their application are thus vital. Their attenuation properties is one of the criteria in evaluating their fates in the environment. This study therefore was conducted to monitor the attenuation of molinate and thiobencarb in rice field water after their application to determine their persistence in the rice field ecosystem.

Materials and Methods

Trough Experiment

A Thapto-Histic Tropic-Fluvaquent clay soil was placed in a 250 x 100 x 30 cm fibre glass trough up to 15cm depth. The soil was then flooded, puddled and incubated for a week for equilibrium. The water level was maintained at 10 cm. Molinate (Odram 8EC, 90.9%) or thiobencarb (Saturnil EC, 40%) was applied onto the flooded water. The amount used were 1.8 and 1.2 g a.i./trough respectively (double the recommended rate i.e 3.0 and 2.5 kg a.i/ha respectively). The flood-water

was then randomly sampled with a 5 ml pippete to a volume collected (200 ml). Prior to the sampling, water level and temperature were recorded.

Field experiment

The experiment was conducted at MARDI Rice Research Centre, Penang, Malaysia with the same soil type used in the trough experiment. The rice crop was direct seeded. An experiment plot of 200 x 500 cm was separated with PVC sheet. The herbicide was sprayed onto the field water at the application rates of 3.0 kg a.i/ha molinate and 2.5 kg a.i./ha thiobencarb. The field water was sampled as in the trough experiment mentioned above.

Analytical method

A water sample was filtered through a glass wool prior to the clean-up procedure. The filtrate was then cleaned by a solid phase extraction method using a Millipore tC18 Sep-Pak cartrigde. Before using, the cartridge was conditioned with 10 ml methanol and 10 ml distilled water. The sample was filtered through the cartridge at a flow rate of 4 ml/min, after which herbicide in the sample was loaded into the cartridge. Prior to the extraction procedure, the cartridge was washed with 3 ml of 1:1 water:methanol solution. Acetone was then used for extraction . Almost 100% of molinate was extracted with 3 ml acetone as compared to only 85% of thiobencarb. Therefore molinate and thiobencarb was extracted with 5 ml and 10 ml of acetone respectively. The extract was then injected into a gas chromatography Shimadzu GC-9A equiped with flame-ionization detector(FID). A glass-column with 1.6 m length and 32 mm diameter containing 2% OV-101 on 80-100 mesh,Chromosorb W was used for thiobencarb and a column containing 3% OV-17 on 60-80 mesh, Gaschrome Q was used for molinate. The N_2 flow rate was at 50 ml/min, H_2 pressure at 0.7 kg/cm^2 and air pressure at 0.5 kg/cm^2.

Results and discussion

Molinate and thiobencarb concentration in rice field water detected after the herbicides application were summarized in *Fig.1a* to *Fig.1d*. The molinate concentration in the trough reached its peak on the next day of sampling probably due to the time taken for complete dissolution. In a laboratory observation, molinate required about 18 hours for complete dissolution from its granular formulation (Soderquist et al 1977). However, the sample from the field experiment reached its peak in the 4th hour sample. Molinate and thiobencarb concentration were less than 10% of their original concentration 144 hours after application, thus suggesting their non-persistence in the rice field water.

Both herbicides showed rapid losses from the field experiment compared to the trough experiment. Major dissipation path of molinate from rice field water according to Soderquist et al.(1977) was through volatilization (75-85%) and photolysis (5-10%). The rate of losses through volatilization was negligible at 15°C but was very rapid at the typical field of 28°C. The Malaysian rice field water is prone to such losses due to the high temperature condition (*Fig.2a*), long sunshine hours and high solar radiation (*Fig.2b*).

Malaysian rice field water hydrochemistry was identified as having precipitation dominance mechanism which means that the water chemistry is predominantly controlled by the dilution effect of rainfall (Sani 1991). *Fig.2c* shows the rainfall pattern for year 1994. Vegetatative stage of rice crop normally coincide with high rainfall period. The herbicides applied during this time were therefore strongly subjected to the dilution effects of the rainfall.

Field monitoring showed that concentration of molinate and thiobencarb were seasonal i.e early vegetative stage. The concentration detected ranges from undetected to 17 ppb for molinate and thiobencarb at a detection limit 0.1 ppb (Cheah and Kum 1994). The soil residue however, recorded the amount of molinate at 2.851mg/kg at 10 days after treatment and 1.875 mg/kg at 30 days after treatment (Nashriyah et al.1994). Molinate has also been reported to be persistent in anaerobic soil (Tanji et al. 1974).

From this preliminary finding, we feel that a detail understanding on environmental impacts of commonly used herbicides in Malaysian rice field is needed for managing our safe rice ecosystem.

Acknowledgement: The authors wishes to thank MARDI for the support given in the project and JIRCAS for the collaboration and financial support. Thanks are also extended to Mr Sani Kimi for his critical reading of the early version of this manuscript.

References

Azmi, M.(1988). Weed competition in rice production *In* Lam, Y.M., Cheong, A.W. and Azmi, M. (1988). *Proceeding of the National Seminar and Workshop on Rice Weed Management*, 7-9 June,1988 Penang,Malaysia: MARDI

Anon. (1993). *A Guide to Crop Protection*; ACM Sdn Bhd.

Cheah, U.B. and Lum, K.Y.(1994). Pesticide residue and microbial contamination of water resources of rice ecosystem in MUDA area. *The paper presented at Seminar on Impact of Pesticide on the Rice Ecosystem*, 12-13 December 1994, Penang, Malaysia: MUDA-MINT

Cheong, A.W. and Azmi, M. (1988). Notes on Weed Management Option *In* Lam, Y.M., Cheong, A.W. and Azmi, M. (1988). *Proceeding of the National Seminar and Workshop on Rice Weed Management*, 7-9 June,1988 Penang,Malaysia: MARDI

Ho, N.K. and Zainuddin, Z. (1995). Integrated weed management (IWM) on dry-seeded riced in MUDA area, Malaysia. *The paper presented at the Agriculture Extension Meeting*, 14th March 1995, Alor Star, Malaysia.

Nashriyah, M., Ho, N.K. and Azimahtol, H.L.P. (1994). The residue level of molinate in soil and its effect on population of equatic flora and fauna under recycling and non recycling practices in MUDA area. *The paper presented at seminar on impact of pesticide on the rice agrosystem in MUDA area.* 12-13 December 1994. Penang,Malaysia: MUDA-MINT

Sani, K. (1991). Hydrochemistry of MUDA Irrigation water. *MARDI Res.J.* 19(1):143-158

Sodarquist, C.J.,Bower, J.B. and Cosby, D.G. (1977). Dissipation of molinate in a rice field. *J.Agric. Food Chem.*,vol. 25(4):940-945

Tanji, K.K., Biggar, J.W., Mehran, M., Cheng, M.W. and Henderson, D.W. (1974). *Calif. Agric.*, 28(5): 10

Fig.1a: Molinate concentration in treated water

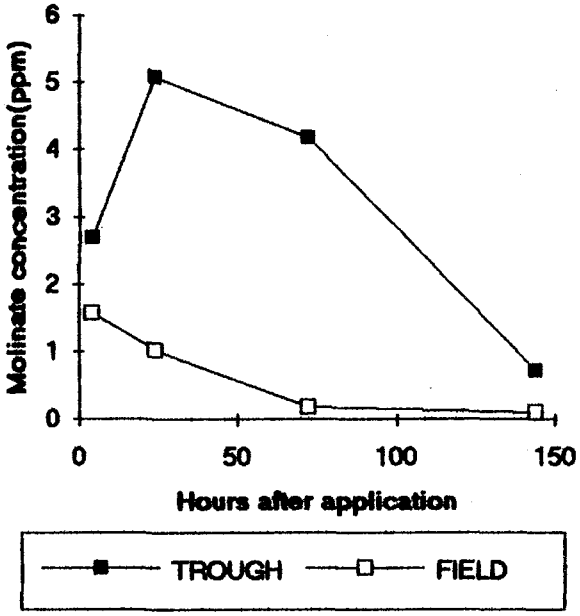


Fig.1b: % molinate concentration in treated water

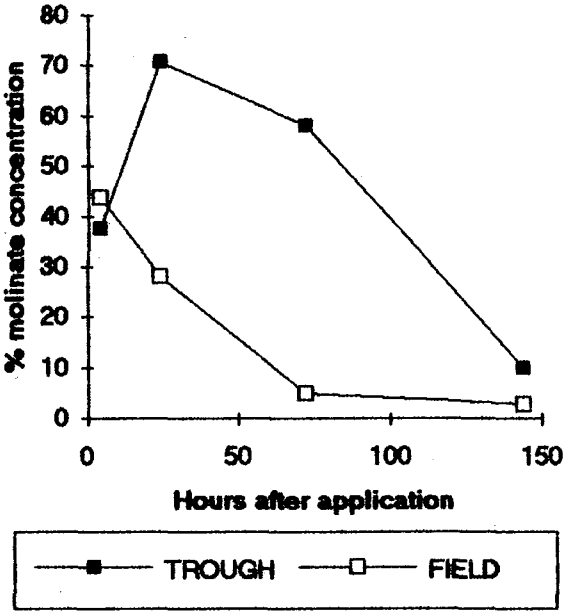


Fig.1c: Thiobencarb concentration in treated water

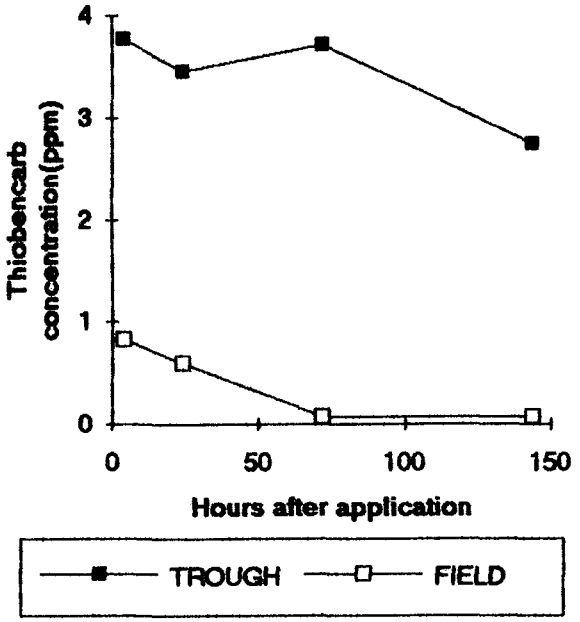


Fig.1d: % thiobencarb concentration in treated water

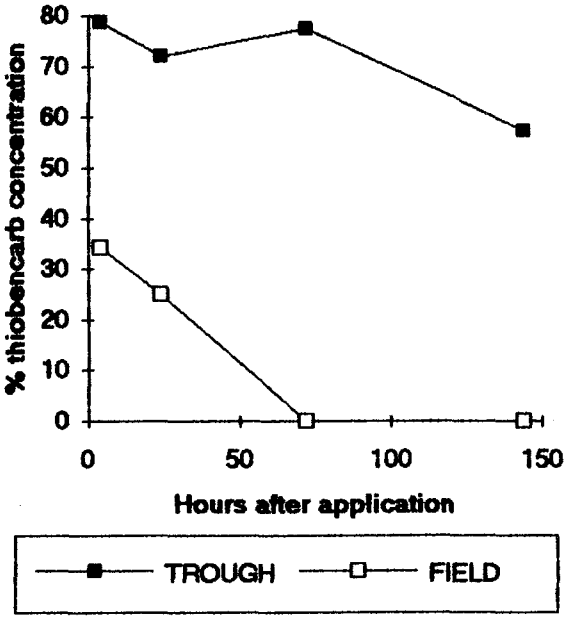


Fig.2a: Means of ten daily air temperature (1994)

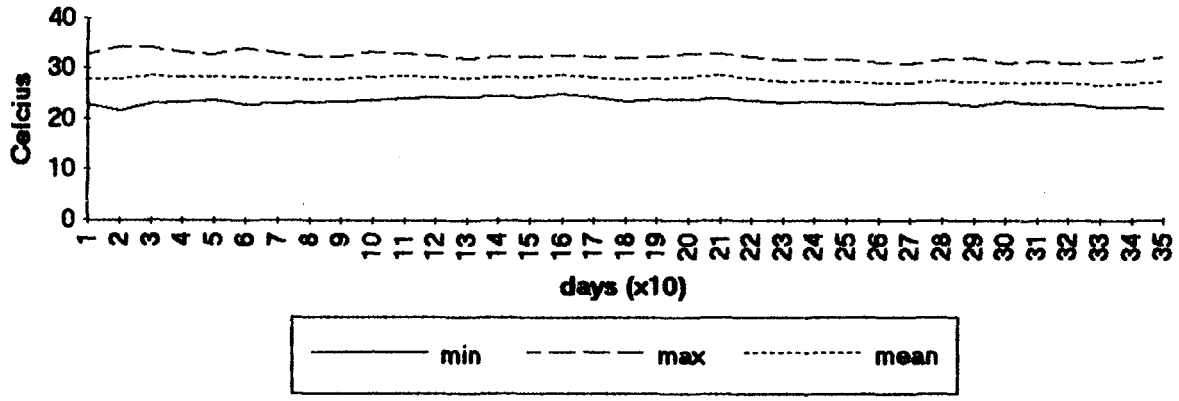


Fig.2b: Ten daily sunshine hours and solar radiation (means daily- 1994)

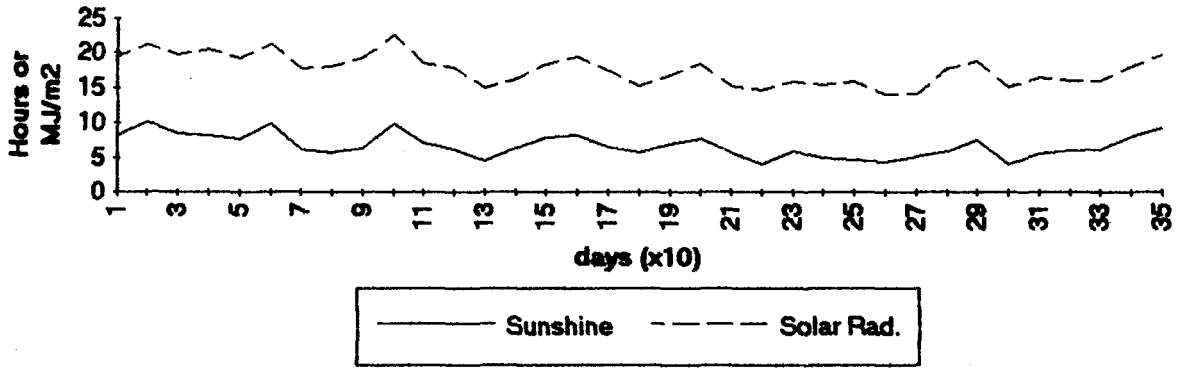
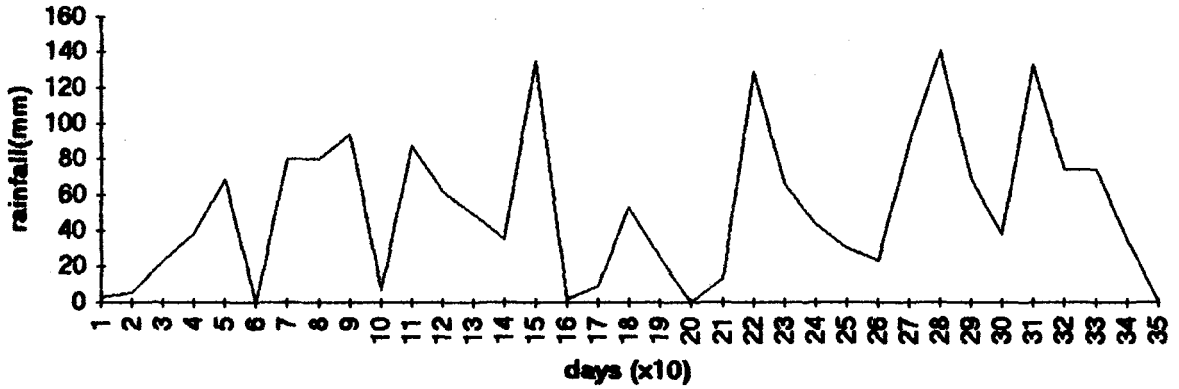


Fig.2c: Amount of ten daily rainfall (1994)



Inhibited Function of Ammoniac Bacteria in Paddy by Butachlor *

Yu Liuqing[1] Wu Linfu[1] Li Yanghan[2] Ying Cunshan[1]
(1.China National Rice Research Institute, Hangzhou 310006, China; 2. Nanjing Agricultural University, Nanjing 210095, China)

Abstract. pot experiments and soil microbe analysis were conducted in the greenhouse and laboratory to determine the response of soil ammoniac bacteria to herbicides and the reduction of nitrogen loss in rice production. Soil samples were collected from a treatment of butachlor and a control pot with nine replication at a soil depth of 0-15 cm, and diluted 10^5 times with sterile distilled water. Colonies of bacteria were counted after 2 days of incubation by plane culture. *Sporosarcina ureae* (Beijerinck) Kluyver et van Niel were inoculated in the extract agar containing butachlor at different rates. The colony diameters of *S.ureae* were measured at 2 days after treatment.The concentration of ammoniac bacteria in soil was decreased significantly with the increase of butachlor rate from 0.9 kg ai./hm^2 to 1.35 kg ai./hm^2 at 7 days after treatment. Butachlor at 500 ppm inhibited the reproduction of *S. ureae* significantly. The mixture of butachlor plus MCPA has the same function as butachlor alone. Butachlor applied to control weeds in a rice field reduced the loss by urea because the urease action decreased after treatment of this herbicide. However, quinclorac, dual, goal, londax, NC-311, MCPA and basagran did not affect the growth of *S. ureae*.

Key words: Butachlor; Ammoniac bacteria; *Sporosarcina ureae*; Rice filed.

Introduction

The utilization ratio of urea for rice is only 30% to 35% because the urea is dissolved into NH_3 resulted from soil microorganisms. Reducing the losses of nitrogen is an important project to the sustainable agriculture. Mandal (1987) applied nitrofen, goal, thiobencarb and butachlor in the direct-seeded rice field as pre-emergence treatment and found the concentration of bacteria were decreased in the paddy soil at 4 ~18 days after treatment. Dodda Byregowda (1989) showed that applications of propanil or pendimethalin plus urea reduced the nitrogen losses after taking analysis the concentration of NO_2, NO_3 and NH_4^+. Yu (1993-1) reported that treatments of butachlor plus urea inhibited the reproduction of ammoniac bacteria in the soil and provided higher grain yield.

* This is a project of national Natural Science Foundation of China started in 1993.

S. ureae dissolve the urea seriously resulted from the function of their urease, therefore, there are considerable meaning on studies of effect of herbicides on *S.ureae*. However, nobody has reported about *S.ureae* responses to herbicides.

The objectives of these experiments were to assess the influence of butachlor on the activity and relative size of the total ammoniac bacteria in the conditions of green house and field, and to determine the response of herbicides on *S.ureae* in controlled condition of laboratory.

Materials and Methods

1.The effects of butachlor at various concentrations to soil ammoniac bacteria

The pots experiment was conducted in the green house with the replicates of nine. Butachlor was obtained commercially, the rate of 0.9, 1.13 and 1.35 kg ai./hm^2 of butachlor was applied respectively at 5 days after rice (*Oriza sativa* L.) transplanted in the plastic bucket of 5 litters with the silt clay of pH6.2 and 3.7%organic matter. Soil samples were collected at 7 days after treatment at a soil depth of 0~15 cm, and diluted 10^5-fold with sterile distilled water.

Ammoniac bacteria were enumerated on the selective medium of beef broth albumen peptone agar with their pH adjusted to 7 in NaOH solution. Portions (1 ml) from each dilution of each sample were placed into culture dishes of 9 cm diameter , then puls the medium of 10 ml. The bacterial colonies were counted while the cultures were incubated for 2 days in the cultural chamber of 28℃.

2. The effects of butachlor in repeated applications on soil ammoniac bacteria

Rice was grown with *Oryza sativa* indica for first crop, *O. sativa* japonica for second crop each year at the Seed Multiplication Farm of Dongshan, Fuyang during two years of 1991~1992. Butachlor of 1.2 kg ai./hm^2 was treated in the mixture of urea 112.5 kg/hm^2 at 5 days after transplanting the rice. The experiments were arranged in a randomized complete block with three replications. Soil samples were collected at 7, 14 and 28 days after treatment at a soil depth of 0~15 cm respectively. Bacteria were analyzed following the same method as the pots experiment . Weeds control showed by fresh weight of plants and rice grain yield were taken each crop.

3. The response of *S. ureae* to butachlor

S. ureae was separated from the soil in the fallow paddy field , and identified according to the traditional methods. *S.ureae* were incubated in a test tube (18×180mm) with 5 replications at 28℃for 5 days and kept in a refrigerator of 1~3℃ . Butachlor was prepared at different concentrations diluted with sterile distilled water. The bacteritic generations of *S. ureae* were dissolved with sterile distilled water of 1ml before application. Butachlor solution of 0.5 ml was moved into the culture dish before plus the medium of 10 ml with four replications. Inoculation holes were drilled in the plate with an iron bar after burning on a spirit lamp. *S. ureae* were inoculated into the hole in the plate with twelve replications. The colony diameter of *S. ureae* were measured after incubating in culture chamber of 28℃ for 5 days.

Results and Discussion

1.The effects of butachlor at various concentrations on soil ammoniac bacteria

Soil samples taken from the butachlor at 1.35 kg ai./hm^2 treated pots showed an 42% less in the ammoniac bacteria population as compared to the control (Table 1). There was no significant difference in the numbers of bacteria from the control and other treatments of butachlor of lower rate. Mandal (1987) reported that butachlor applied pre-emergence at 3 kg ai./hm^2 in direct-seeded rice resulted in a sharp fall in bacteria flora at 4~18 days after treatment, and recovered at 25 days after application. Yu (1993-1) has demonstrated reproductions of ammoniac bacteria recovered at 28 days after treatment of granule of butachlor plus urea.

Table 1. Effects of butachlor at various concentrations on soil ammoniac bacteria

treatment (kg ai./hm^2)	bacteria concentration[1] (Organisms/g dry soil)
control	659×10^5a
butachlor 1.35	381×10^5b
butachlor 1.13	533×10^5a
butachlor 0.90	597×10^5a

1. values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. Response of *sporosarcina* ureae to butachlor

treatment (mg/kg)	diameter of colony[1] (mm)
0	4.4a
6.5	4.2a
13	5.5a
130	4.2a
500	0 b
1000	0 b

2. The effects of butachlor in repeated application on soil ammoniac bacteria in paddy field

The concentrations of ammoniac bacteria obtained from the treatment of butachlor plus urea at 1.2 kg ai./hm^2 plus 112.5 kg/hm^2 were lower than the control of urea at 112.5 kg/hm^2 (Fig. 1). However, there was no reduction of ammoniac bacteria as compared the results of the last crop in two years of repeated use butachlor plus urea for four crops with the data of first crop in 1991. Inhibited function of butachlor on ammoniac bacteria was more satisfactory in first crop than in the second crop during the rice early growth period each year.

The analysis time of soil samples affected the concentrations of ammoniac bacteria, the last analysis time at 28 days after treatment resulted in more ammoniac bacteria than the first one taking at 7 days after treatment each crop because of plants grew more densely at late growth period which included rice plants in the treatment of herbicide and rice associated weeds in the control.

The concentrations of ammoniac bacteria related to the climatic temperatures ,during the period of higher temperatures in the second crop the reproduction of bacteria developed more rapidly than the time with lower temperature in the first crop. However, this result did not reappear in next year.

3. Weeds control and rice grain yields obtained from the treatments of butachlor in different formulations.

The treatments of butachlor in emulsifiable concentrate of 1.2 kg ai./hm^2 and granule of butachlor plus urea of 1.2 kg ai./hm^2 plus 112.5 kg/hm^2 have performed excellent weed control and more rice grain yields than the untreated check of urea (Fig. 2). The granule formulation of butachlor plus urea gave better weed control and rice grain yield than it's emusifiable concentrate alone. This result shows that the treatment of butachlor plus urea may occur as a synergistic function. Previous research (Yu, 1993-2) indicated that this mixture of butachlor and urea gave the synergistic effects in controlling barnyardgrass (*Echinochloa crusgalli* (L.) Beauv. var mitis (pursh) petern) when it was applied at germination stage of barnyardgrass seeds. However, there was no any synergistic function in weed control when the mixture was applied at one heart stage of barnyardgrass. So that the granule of butachlor plus urea must apply at the period of barnyardgrass germination.

4. The response of *S. ureae* to butachlor

The results of response of *S. ureae* to butachlor were presented in Table 2. Application of butachlor at 500 mg/kg resulted in a decrease in the colony diameter of *S. ureae*. However, the rates of butachlor at equal or less than 130 mg/kg didn't affect the reproduction of *S. ureae* as compared with control. *S. ureae* is an urea bacterium of releasing urease strong which promotes decomposition of urea and reduces the utilization ratio of nitrogen fertilizer (Yu, 1994). The suppression function of butachlor to *S. ureae* is an important finding because the application ratio of nitrogen has been improved when butachlor used in granule of the herbicide plus urea.

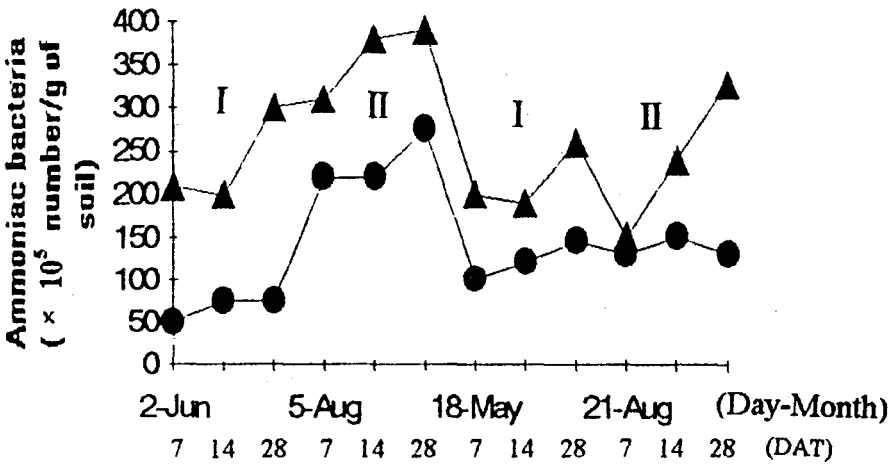


Fig.1 Effects of butachlor in repeated application on soil ammoniac bacteria in paddy field. (▲) Urea 112.5 kg/hm^2; (●) Butachlor+Urea 1.2 kg ai./hm^2+112.5 kg/hm^2; (Ⅰ) First crop; (Ⅱ) Second crop; (DAT) Days after treatment.

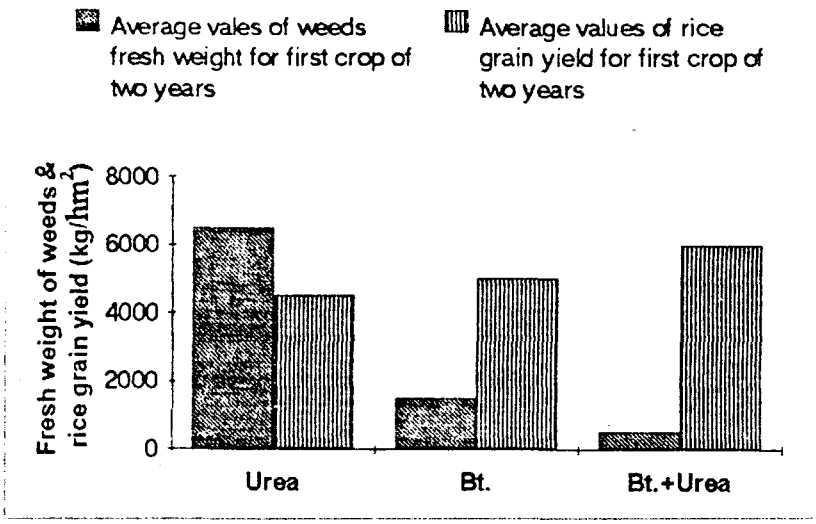


Fig.2 Weeds control and rice grain yields obtained from the treatments of butachlor (Bt.) in different formulations .

Literature Cited

Mandal, B.B. et al. 1987, Effect of some pre-emergence herbicides on micro-flora in direct seeded rice, Indian Agriculturist 31(1): 19~23.

Doodad Byregowda, R.S. et al. 1989, Studies on nitrification in flooded soils as influenced by some herbicides, Current Research --Univ. of Agricultural Science 18(4):49~52.

Yu Liuqing, Zhang Lei et al. 1993-1, Limit action on soil microbe and effect on rice production by butachlor-fertilizer, Chinese J. Rice Sci. 7(1): 55~57.

Yu Liuqing, Wu Linfu et al. 1993-2, Study on synergistic effects of treatments with mixture of herbicide and urea, J. of Weed Sci. 7(4): 27~31.

Yu Liuqing, Wu Linfu, 1994, Inhibition of *sporosarcina ureae* by herbicides used in rice field, Proceedings of the 5th Weed Science Conference of China, WSSC, P94~97.

WEED MANAGEMENT IN WET-SEEDED RICE IN ASIA

Aurora M. Baltazar
National Crop Protection Center
University of the Philippines at Los Banos, College, Laguna 4031, Philippines

Abstract: Adoption of wet-seeded rice has been steadily increasing in Asia due to savings in labor, time, and production cost. Two major constraints to a successful wet-seeded rice crop are obtaining good rice stands and weed control problems. Saturated moisture conditions at seeding promote growth of semi-aquatic grass weeds, which pose greater competition and selectivity problems than broadleaf weeds and sedges. The major weed control methods are cultural and chemical methods but chemical control is usually a more cost-effective option. Crop production practices like water and nutrient management, land leveling, seed handling, crop rotation, choice of cultivar and seeding rate are important factors which help wet-seeded rice compete better with weeds. Factors which would alleviate weed control problems in wet-seeded rice include: 1) better facility for land leveling, water control and seeding method; 2) identification of more competitive cultivars for wet-seeding; 3) better understanding of the biology and ecology of weeds infesting wet-seeded rice, particularly weed seeds and propagules, and 4) the role of soil moisture, which is a principal determinant of weed growth in wet-seeded rice in tropical Asia.

Introduction

Wet-seeded rice (broadcasting pregerminated seed into puddled soil) has been an increasingly attractive alternative to transplanted rice in Asia due to high cost and unavailability of labor for seedling establishment and transplanting. Its labor requirement is about 27 days/ha less than that of transplanted rice, which cuts production costs by as much as 50% and increases cropping intensity by reducing turn-around time (De Datta et al, 1989a). Long term studies have shown that it yields as well as transplanted rice (De Datta and Nantasomsaran, 1991). The largest wet-seeded rice areas are in South and Southeast Asia led by Sri Lanka (60%) and Malaysia (50%). It is also increasing in Thailand, Philippines, and India (about 30%). In temperate East Asia, (Japan, Korea, China) rice is still predominantly transplanted (>90%) but wet-seeding is gaining recognition.

The two major constraints to adoption of wet-seeded rice are obtaining good rice stands and greater weed control problems. For the past 30 years or so, research on rice weed control in Asia has focused on transplanted rice. Increasing adoption of wet-seeding dictates a need to develop weed control technology suited to wet-seeded rice because each culture has unique production and weed control conditions. This paper will discuss the nature of weed problems in wet-seeded rice in Asia and how they are controlled.

Growing Conditions and Dominant Weeds

Where temperature is not limiting, soil moisture is the principal factor determining weed growth and habitation (Arai et al, 1955; Tanaka, 1976; Kusanagi, 1981). In wet-seeded rice, moisture levels or flooding depths at certain periods result in different species dominating a particular growth stage. At seeding soil moisture is kept at field capacity to saturation to enhance seed germination and seedling emergence which is highly critical at this stage to obtain a good stand. Unfortunately, this also promotes growth and dominance of terrestrial and

semi-aquatic weeds, mostly grass weeds. About 7 to 10 days after seeding when seedlings have rooted and are well-anchored, the field is flooded to a depth of 5 to 15 cm. Flooding promotes growth and dominance of aquatic weeds, predominantly broadleaf and sedge weeds.

The shift of dominant weeds from aquatic broadleafs/sedges in continuously flooded transplanted rice to semi-aquatic grass weeds in wet-seeded rice due to moist or saturated soil at seeding has been most prominently observed in Malaysia (Lo and Cheong, 1994; Ho and Zuki, 1988). The most significant implication of this shift is that most of the grass weeds that grow with wet-seeded rice during the critical competition stage are C4 plants (*Echinochloa* spp.) which have low moisture requirements and higher resource (N. water, photosynthesis) use efficiencies than rice which is a C3 plant (Nishida and Kasahara, 1975; Tanaka, 1976; Matsunaka, 1983; Pamplona et al, 1990). Aquatic broadleafs/sedges (*Monochoria vaginalis, Cyperus* spp) are C3 plants with lower resource use efficiencies, thus not as competitive as the C4 grass weeds.

Grasses are thus more difficult to control in wet-seeded rice because: 1) they emerge at the same time as, or even earlier than, rice thus there is greater competition for similar growth requirements; 2) similar age, size, morpho-physiology and vulnerable stages to control pose selectivity problems in both chemical and even cultural control methods.

Control Methods

Herbicides

Herbicides used in transplanted rice are also used in wet-seeded rice. However, grass herbicides have narrower margin of selectivity in wet-seeded rice because of close similarity of rice and grass weeds.

The most widely used preplant or preemergence herbicides (2 days before seeding to 6 days after seeding) are the chloroacetamides (butachlor, pretilachlor, mefenacet) and thiocarbamates (thiobencarb, molinate, dimepiperate). Due to their nature of germination, grasses are more susceptible than broadleaf weeds, thus preemergence herbicides are used mainly for grass control. They also pose the greatest selectivity problems in wet-seeded rice because rice seedlings are also most susceptible at this stage (germination to one-leaf stage). The safest times of application have been determined at 2 days before seeding or at 6 to 8 days after seeding when absorbing organs of grasses, the mesocotyl-coleoptile region of rice have emerged or are away from herbicide-treated layer (Chun and Moody, 1985; Arceo and Mercado, 1981). Later applications are safer to rice but will be ineffective against grass weeds. Flooding due to unexpected rainfall at time of treatment enhances rice injury (Bernasor and De Datta, 1983). Butachlor and pretilachlor have safened formulations for use in wet-seeded rice.

The most widely used postemergence grass herbicides are propanil applied at 2-4 leaf stage, and fenoxaprop, applied at 3-5 leaf stage. Propanil has adequate selectivity to rice because rice can metabolize it while grass weeds do not. But it should not be applied within 7-10 days of carbamate or oraganophosphate insecticide application. Propanil also controls certain broadleafs and sedges. Fenoxaprop controls only grasses. Rice younger than 3 to 4 leaf stage is susceptible. Selectivity is obtained by fine-tuning application rates and timings most selective to rice yet still provide adequate grass control (Baltazar et al, 1993). Flooding and N application within 7 to 10 days of fenoxaprop application enhances injury to rice (Smith et al, 1993).

Postemergence broadleaf herbicides are the phenoxys (2,4-D, MCPA) applied 20-30 days after seeding and sulfonylureas (bensulfuron, pyrazosulfuron) applied 6-20 days after seeding. They all have adequate selectivity to rice although phenoxys may injure rice when applied at certain stages. Rice and grasses metabolize sulfonylureas.

Other herbicides used in wet-seeded rice are quinclorac, oxadiazon, anilofos, piperophos, and bentazon.

Herbicides maybe the best option in broadcast-seeded rice where absence of rows make hand or mechanical weeding tedious or impractical. However, herbicide injury to rice, lack of technical knowledge and high cost to small farmers in Asia limit their widespread use. Also, they are not effective on weed seeds and propagules, the most important survival mechanisms of weeds (Yamasue and Ueki, 1983).

Hand or Mechanical Weeding

The most common practice in many parts of Asia is to pass an animal-drawn spike-toothed harrow or rotary weeder over the broadcast-seeded field at 5 to 15 days after emergence to kill weeds and to make rows as well as thin out the rice seedlings (Mukhopadhyay, 1983; Moody, 1992). Handweeding is also done at 30 days after seeding as follow-up to a herbicide treatment (Casimero et al, 1994).

Tillage and Land Leveling

Plowing, harrowing, and puddling provides optimum conditions for seedling emergence and establishment. A tillage operation for leveling is also important in wet-seeded rice to provide good drainage and maintenance of uniform water depth. This prevents low spots which may submerge seedlings and high spots which enhances weed growth. In the absence of precise land leveling, farmers in Asia dig shallow ditches to drain excess water (Takashima, 1984).

Seeding Method

Coating seeds with a slurry of cattle mature is done in India to make seeds heavier and allow them to sink into the soil for better root anchorage (Gogoi et al, 1994). Seed treatment with calcium peroxide to supply O_2 for better germination is also practiced in some countries (Gogoi et al, 1994). Availability of equipment for drill-seeding into puddled soil with precise seeding depth for better root anchorage would improve wet-seeded rice production.

Seeding Rate

Seeding rates higher than recommended (>100 kg/ha) are used to help control weeds and compensate for rat, bird and snail damage (Moody, 1992). Seeding rates should not be too low to provide open spaces for weed growth but not too high to result in thick stand, intraspecific competition, thin culms, loading.

Cultivar

The modern short duration cultivars with high seedling vigor, rapid early growth, high tillering capacity and sturdy culms that resist lodging are preferred for use in wet-seeded rice (De Datta and Nantasomsaran, 1991). Cultivars that can germinate well in flooded conditions by having low O_2 demand or high O_2 use efficiency will facilitate water-seeding. Water seeding suppresses growth of grass weeds and protects seeds from birds and rats. Anaerobic cultivars that can germinate, grow well, and compete better with C_4 grass weeds in flooded conditions are being evaluated (Pablico et al, 1994). Cultivars must also be resistant to lodging as wet-seeded rice tends to lodge if root anchorage is poor (Castillo, 1962).

Water Management

Flooding suppresses growth of terrestrial or semi-aquatic weeds and make them easier to control by cultural or chemical methods. It also enhances herbicide efficacy but excessive flooding can enhance rice susceptibility to herbicides. Flooding promotes growth of broadleaf aquatic and sedge weeds but these are easier to control with little selectivity problems and are not as competitive as the C4 grasses.

Good water control and drainage are important requirements of successful water management. Unfortunately, 80% of rice fields in South and Southeast Asia are rainfed wetlands where precise water control is not obtained due to highly uneven rainfall and poor irrigation and drainage facilities (De Datta, 1981). Soil moisture fluctuates from drought to floods in a single season. In Sri Lanka, an intricate system of drainage canals have been developed to facilitate uniform distribution of water (Pathinayake et al, 1991). In Malaysia, current irrigation system is designed for transplanted rice and there is a need to develop canal density, discharge capacity, etc. that are suited to wet-seeded rice (Lo and Cheong, 1994).

Fertilizer Management

Application timing (two- or three- split) for transplanted rice is also applicable to wet-seeded rice (De Datta and Nantasomsaran, 1991). The first dose is broadcast at 10 to 20 days after seeding and the second is top-dressed at panicle initiation. Weeds should be controlled before fertilizer application. Recommended rates range from 75 to 150 kg/ha (Ampong-Nyarko and De Datta, 1989; Anonymous, 1993). Very high rates enhance lodging which is not desirable in wet-seeding. In the Philippines, farmers apply higher rates in transplanted rice than in wet-seeded rice (Erquiza et al, 1990). Wet-seeding may be advantageous over transplanting in terms of lower total N losses (about 10% less) in the former (De Datta et al, 1989b).

Crop Rotation

Rotating rice with a broadleaf crop will 1) break the cycle of weeds associated with rice; 2) rotate herbicide use to avoid putting pressure on a single species which favors shifting of dominant species to a more difficult-to-control species; and 3) enable use of postemergence grass herbicide in the rotation crop which otherwise cannot be used in rice due to selectivity problems. In dry-seeded rice, rice rotation with soybean reduced red rice population and allowed use of postemergence grass herbicides for red rice control (Smith, 1989).

Preventive

Use of weed-free seeds and farm equipment will minimize spread of weed seeds and propagules. In Thailand, ducks eating *E. crusgalli* seeds disseminate seeds to other areas (Vongsaroj, 1994). ASEAN PLANTI has proposed to regulate rice seeds to not more than 1 weed seed per 50g of rice seed (Vongsaroj, 1994). It is also important to maintain clean fields and surroundings during fallow periods to prevent weeds from going into seed and adding to seed reserves in the soil. Proper seed handling will be an important factor in preventing spread of red rice which is now posing a threat to rice fields in Asia.

Points to Consider

Improvement of Rice Stands

Improvement of the following practices in Asia would greatly improve wet-seeded rice stands: 1) mechanism for improved land leveling, drainage, and water control; 2) facility for drill-seeding at precise seeding depth for better root anchorage; 3) identification of lodging resistant, vigorous cultivars and enhancing factors or practices; 4) more selective grass herbicides;

5) cold-tolerant seedlings in East Asia; 6) to facilitate water-seeding for better weed control, identification of cultivars that germinate anaerobically and enhancing factors.

Current and Future Weed Problems

1) Moisture conditions in wet-seeded rice favor dominance of C4 grass weeds, prominently *E. crusgalli, E. glabrescens* and *E. oryzoides.* In Malaysia and Philippines, another group of grasses, *Leptochloa chinensis* and *Ischaemum rugosum* dominate once barnyardgrass is controlled and in drier conditions (Lo and Cheong, 1994; Moody and Drost, 1981; Ahmed and Moody, 1982). In Malaysia, continuous use of molinate which selectively controlled barnyardgrass contributed to increase populations of *L. chinensis* (Lo and Cheong, 1994).

2) Red rice is beginning to appear in Malaysia and Philippines. Although it is more of a problem in dry-seeded rice, it also poses a threat to wet-seeded rice (Lo and Cheong, 1994).

3) Broadcast-seeding, currently the common practice of wet-seeding in Asia, favors use of chemicals over cultural methods. Repeated use of herbicides every cropping season could likely result in development of herbicide-resistant weeds. *Fimbristylis miliacea* and *Sphenoclea zeylanica* have developed resistance to 2,4-D used continuously for about 20 years in wet-seeded rice fields in Malaysia and the Philippines (Watanabe et al, 1994; Mercado et al, 1990). Rotation management programs with other herbicides and other kinds of control methods will prevent continuous use of any one single herbicide and minimize development of herbicide-resistant weeds.

4) There is a need to understand better the biology of weeds and the factors that favor specific weeds or weed groups in wet-seeded conditions. In the tropics, soil moisture is the principal determinant of weed growth. How to use this knowledge to predict emergence or dominance patterns may help identify what control measures are needed and when to apply them. Also, identifying ways to control the most important survival mechanism of weeds, which are the seeds and propagules, would be a giant step towards reducing weed problems.

Literature Cited

1. Ahmed, N. U. and K. Moody. 1982. Weeds in rainfed cropping systems as affected by landscape position. Paper presented at the International Conference on Plant Protection in the Tropics. Kuala Lumpur, Malaysia.
2. Ampong-Nyarko, N. and S. K. De Datta. 1989. Ecophysiological studies in relation to weed management strategies in rice. Proc. 12th Asian-Pacific Weed Sci. Soc. Conf. Seoul, Korea. 31-46.
3. Ampong-Nyarko, and S. K. De Datta. 1991. A handbook for weed control in rice. International Rice Res. Instit. Laguna, Philippines. 113p.
4. Anonymous. 1993. Rice Almanac. International Rice Res. Instit. Laguna, Philipp. 142p.
5. Arai, M., M. Miyahara, and H. Yokomori. 1955. Ecological studies of weeds on arable lands. IV. On the types produced by adaptation to the soil water of weeds [in Japanese, English summary]. J. Kanto-Tosan Agric. Exp. Stn. 8:56-62.
6. Arceo, L. M. and G. L. Mercado. 1981. Improving crop safety of butachlor in wet-seeded rice (*Oryza sativa* L.). Philipp. J. Weed Sci. 8:19-24.
7. Baltazar, A. M., S. K. De Datta, and M. A. Llagas. 1993. Efficacy and selectivity of fenoxaprop for postemergence grass control in rice (*Oryza sativa*). Philipp. J. Weed Sci. 20:45-55.

8. Bernasor, P. C. and S. K. De Datta. 1983. Integration of cultural management and chemical control of weeds in broadcast-seeded flooded rice. Proc. 9th Asian-Pacific Weed Sci. Soc. Conf. Manila, Philipp. 137-155.
9. Casimero, M. C., R. T. Cruz, and S. R. Obien. 1994. Weed management in wet-seeded rice in the Philippines. Paper presented at Int. Workshop on Constraints, Opportunities, and Innovations for Wet-Seeded Rice. Bangkok, Thailand. 20p.
10. Castillo, P. S. 1962. A comparative study of directly-seeded and transplanted crops of rice. Philipp. Agric. 45:560-566.
11. Chun, J. C. and K. Moody. 1985. Effect of butachlor on rice mesocotyl elongation. Proc. 10th Asian-Pacific Weed Sci. Soc. Conf. Chiangmai, Thailand. 33-40.
12. De Datta, S. K. 1981. Principles and practices in rice production. John Wiley and Sons. New York. 618p.
13. De Datta, S. K. and P. Nantasomsaran. 1991. Status and prospects of direct-seeded flooded rice in tropical Asia. Pages 1-16 in Direct Seeded Flooded Rice in the Tropics. Int. Rice Res. Instit. Manila, Philippines.
14. De Datta, S. K., P. C. Bernasor, T. R. Migo, M. A. Llagas, and P. Nantasomsaran. 1989. Emerging weed control technology for broadcast-seeded rice. Pages 133-146 in Progress in irrigated rice research. Int. Rice Res. Instit. Manila, Philippines.
15. De Datta, S. K., R. J. Buresh, W. N. Obcemea, M. I. Samson, and J. G. Real. 1989b. Comparison of ammonia volatization and denitrification losses in broadcast-seeded flooded and transplanted tropical rice. Page 236 in American Soc. Agron. Abstracts. Las Vegas, Nevada.
16. Erquiza, A., B. Duff, and C. Khan. 1990. Choice of rice crop establishment technique; transplatning vs wet-seeding. Int. Rice Res. Instit. Research Paper Series 139. 10p.
17. Gogoi, A. K., U. C. Upadhaya, and A. K. Roy. 1994. Status of weed management in wet-seeded rice in Assam, India. Paper presented at the Int. Workshop on Constraints, Opportunities, and Innovation for Wet-Seeded Rice. Bangkok, Thailand. 28p.
18. Ho, N. K. and I. Md. Zuki. 1988. Weed population changes from transplanted to direct-seeded rice in the Muda area. Proc. Nat. Seminar and Workshop on Rice Field Weed Mgmt. MARDI, Penang, Malaysia. 55-67.
19. Kusanagi, T. 1981. Ecological aspects of weeds on paddy field. Proc. 1981 Weeds and Weed Control in Asia. FFTC Book Series No.20. Taipei, Taiwan, ROC. 68-88.
20. Lo, N. P. and A. W. Cheong. 1994. Herbicide input restriction and usage limitation in wet-seeded rice. Proc. Int. Workshop on Constraints, Opportunities, and Innovations for Wet-Seeded Rice. Bangkok, Thailand. 23p.
21. Matsunaka, S. 1983. Evolution of rice weed control practices and research; world perspective. Proc. 1981 Conf. on Weed Control in Rice. Int. Rice Res. Instit. Laguna, Philippines. 5-17.
22. Mercado, B. L., S. K. De Datta, T. R. Migo, and A. M. Baltazar. 1990. Growth behavior and leaf morphology of Philippine strains of *S. Zeylanica* showing differential response to 2,4-D. Weed Res. 30:245-250.
23. Moody, K. 1992. Weed management in wet-seeded rice in tropical Asia. Proc. Int. Symposium Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. Tsukuba, Japan. 1-20.
24. Moody, K. and D. C. Drost. 1983. The role of cropping systems on weeds in rice. Proc. 1981 Weed Control in Rice. Int. Rice Res. Instit. Laguna, Philippines. 73-88.
25. Mukhopadhyay, S. K. 1983. Weed control technology in rainfed wetland rice. Proc. 1981 Weed Control in Rice. Int. Rice Res. Instit. Laguna, Philipp. 109-118.

26. Nishida, F. and Y. Kasahara. 1975. Experimental studies on the populations of weeds in fallow paddy fields [in Japanese]. Proc. 14th Annu. Meeting Weed Sci. Soc. Japan. 193-195.
27. Pablico, P. P., M. Yamauchi, T. P. Tuong, R. J. Cabangon and K. Moody. 1994. Crop establishment and weed competitiveness of direct-seeded anaerobic rice cultivar as influenced by seeding and water management systems. Paper presented at Int. Workshop on Constraints, Opportunities, and Innovations for Wet-Seeded Rice. Bangkok, Thailand. 21p.
28. Pamplona, R. R., M. Dingkuhn, K. Ampong-Nyarko, C. J. Moll, and S. K. De Datta. 1990. Tropical rice and weed competition for resources. Proc. 1990 Pest Management Counc. Philipp. Conf. Bacolod City, Philipp.
29. Pathinayake, B. D., L. Nugaliyadde and C. A. Sandanayake. 1991. Direct-seeding practices in Sri-Lanka. Pages 77-90 in Direct-Seeded Flooded Rice in the Tropics. Int. Rice Res. Instit. Manila, Philipp.
30. Smith, R. J., Jr. 1989. Cropping and herbicide systems for red rice (*Oryza sativa*) control. Weed Tech. 3:414-419.
31. Smith, R. J., Jr., A. M. Baltazar, and P. Nastasi. 1993. Effect of light, N, and water management on rice (*Oryza sativa*) tolerance to fenoxaprop. Proc. Arkansas Acad. Sci. 47:116-122.
32. Takashima, Y. 1984. Making drainage ditch by hauling a pot in germinated seed direct sowing paddy field. In Useful Farming Practices. Assoc. for Int. Coop. of Agric. and Forestry. Tokyo, Japan. 16-17.
33. Takana, I. 1976. Climatic influence on photosynthesis and respiration of rice. Pages 223-247 in International Rice Res. Instit. Proc. Symp. on Climate and Rice. Laguna, Philippines.
34. Vongsaroj, P. 1994. Weed control in wet-seeded rice in Thailand. Paper presented at Int. Workshop on Constraints, Opportunities, and Innovations for Wet-Weeded Rice. Bangkok, Thailand. 18p.
35. Watanabe, H., M. I. Zuki, and N. K. Ho. 1994. Infestation of 2,4-D resistant biotype of *F. miliacea* in direct-seeded rice fields in the Muda area, Peninsular Malaysia. Paper presented at Ann. Mtg. of Japan Weed Sci. Soc. Chiba Univ. April 1994.
36. Yamasue, Y. and K. Ueki. 1983. Biology of paddy weeds and their control in wetland rice. Proc. 1981 Weed Control in Rice Conf. Int. Rice Res. Instit. Laguna, Philipp. 227-242.

BARNYARDGRASS CONTROL IN WET SEEDED RICE

Paitoon Kittipong[1], Chaiyot Supatankul[1] and U-sup Chaimanit[1]

ABSTRACT

Experiments conducted at the Sanpathong Rice Experiment Station, Chaing Mai during seasons of 1991 and 1992 showed that the top 4 noxious weed species were *Echinochloa crusgalli* (L.) Beauv., *Sphenochlea zeylanica* Gaertn, *Scirpus juncoides* Roxb. and *Ammania baccifera* Linn. All tested compounds provided excellent control of *E. crusgalli* (L.) Beauv.) and other noxious species with no rice injury. Dry weight of weeds collected at 30 days after treatment indicated significant difference between treated plots. Height differences at 30 days after application of herbicides and at harvesting were found to be insignificant while tiller number at harvesting gave significant difference among treatments. Yield of rice showed significant difference among treated plots. Treatments which provided yield higher than untreated check at a significant level were bensulfuron methyl at 0.04 Kg(ai)/ha., quinclorac/oxadiazon at 0.25/0.5 Kg(ai)/ha, quinclorac/bensulfuron methyl at 0.25/0.02 Kg(ai)/ha, benthiocarb at 1.0 Kg(ai)/ha, and quinclorac/benthiocarb at 0.25/0.5 Kg(ai)/ha.

Introduction

The Kingdom of Thailand has a land area of approximately 320.7 million rai (51.3 million hectares), of which 41.5 percent are used for agriculture. Rice is the most important food and exported crop and being grown on 52.0 percent of agricultural land. The total annual production is about 20.0 million metric tones. (Office of Agri. Eco., 1994)

A serious problem of rice cultivation in Thailand and Southeast Asian countries was competition of grass weeds leading by barnyardgrass (*Echinochloe crugalli* (L.) Beauv.) a troublesome weed of rice throughout the world (Holm et al, 1991). Competition of barnyardgrass in direct-seeded rice and transplanted rice reduced grain yield to significant level (Noda et al, 1968; Smith, 1968). Rice culture in Thailand also affected by competitive power of this grass weed. Degree of yield loss from competition was high in both wet and dry-seeded rice where ecology was suitable for weed growth.

Effect of weed competition on rice was not only reducing grain yield but also decreasing grain quality due to weed seeds contamination as well. Application of many grass herbicides to rice as post-emergence gave satisfactory control of barnyardgrass but did not have enough residue to control other weeds. Then competitive power of broadleaf and sedges were still in effect. Treatment of compounds that have character of being pre and post-emergence along with grass killing action should be a good solution for the place that barnyardgrass was a dominant species. (Kearney and Kaufman, 1988.)

Materials and Methods

The experiments were conducted on Hang Dong silt loam soil at Sanpathong Rice Experiment Station, ChaingMai during growing season of 1991 and 1992 Experimental field was plowed and puddling as normal. Weeds and remnant portions were taken away during land preparation. Fertilizer (16-20-0) at 50 kg per rai (312.5 kg/ha) was applied as basal application prior to seeding of rice and barnyardgrass.

[1]Weeds Science Group, Botany and Weeds Science Div. Dept. of Agriculture

Rice cultivar was RD-7 which mature in 120 days. Clean seeds of RD-7 were broadcasted on top of well prepared wet soil at rate 8 kg per rai (50 kg/ha). Shortly after seeding all Experimental plots were flooded with irrigation water to the depth of 10 cm. Water was kept on the field for 48 hours to stimulate germination before being drained out. Seeds of barnyardgrass with 60 percent germination were soaked with water for 48 hours. The soaked seeds of weeds were broadcasted into each experimental plot at rate of 0.4-0.5 kg per rai (2.5-3.1 kg/ha) shortly after draining was completed to ensure a uniform grass infestation. Rice emerged within 2 days after draining same time as barnyardgrass.

All treated plots were sprayed with herbicides when rice and weeds were about four days old using a sprayer which pressure and flow rate of water can be controlled. Four herbicides were used in this study. They were bensulfuron methyl (Londax 10 % DF) (Methyl 2-(3-(4,6-dimethoxy-pyrimidin-2-yl) ureidosulfonylmenthyl) benzoate at 0.04 kg(ai)/ha), benthiocarb (Saturn 80 % EC) (S-(4-chlorobenzyl) N,N-diethyl-thiocarbmate) at 1.0 kg(ai)/ha, oxadiazon (Ronstar 25% EC.) (5-tert-butyl-3(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazone-2-(3H)-one) at 0.5 kg(ai)/ha, and quinclorac (Facet 50 % WP) (3,7-dichloro-8-quinoline carboxylic acid) at 0.5 kg(ai)/ha.

Plots were first flooded seven days after treatment of chemicals. Level of flooding water increased with rice age. Finally, water was maintained at the depth of 10 cm throughout growing season. Rice stand for each individual plot was about 120-140 plants per square meter while barnyardgrass population was around 24 plants per square meter in average. At 45 days after seeding all plots were applied once with 4 kg (N) per rai (25 kg/ha) from urea fertilizer as top-dressing treatment.

Experimental design was a randomized complete block with seven treatments and four replications. Plot size was 2.4 m x 4.8 m = 11.52 sq. m. Data taken on experiments included weeds profile, control efficacy, phytotoxicity, oven dry weight, height, tillers number and yield at 14 percent moisture content.

Weed profile was collected at 35 days after seedling from untreated check plots using a quadrate of 0.25 meter square sample. Control efficacy and phytotoxicity were recorded using visual observation at 30 days after treatment on 10 points scale basis with 0=normal, 1-3 = slightly control, 4-6 = moderately control, 7-9 = good control and 10 = completely control. Fresh weed samples which being collected from each individual plot with 0.25 meter square quadrate were oven dried for 3 days at 70°c for dry weeds weight study. Height and tillers were recorded at 30 days after treatment and at harvesting. Yield at 14 percent moisture content was recorded at kg per rai and kg per hectare respectively.

Appropriate statistical analysis were conducted separately for each year and combined over 2 years. Averages were grouped using Duncan's multiple range test.

Results and Discussion

Results obtained from experiments were summarized as following :

Weeds infestation

Weed species or weeds profile found at Sanpathong Rice Experiment Station, during growing season of 1991 and 1992 reported in Table 1. Data indicated that there were 10 paddy weed species found in the experimental area. The top four species which infested over 10 percent of total weed number were listed as : *Echinochloa crusgalli* (L.) Beauv., *Sphenoclera zeylanica* Gaertn., *Scirpus juncoides* Roxb., and *Ammania baccifera* Linn. Degree infestation of previous mentioned species ranging from 22.43, 18.69, 16.82 and 14.02 percent respectively. The rest six species were found to be minority with degree infestation ranging from 8.14 percent of *Cyperus difformis* Linn. at the top and downed to 1.87 percent of *Fimbristylis miliacea* (L.)Vohl. at the bottom. Average number to barnyardgrass infestation for this experiments were 24 plants per

square meter which considered to be highly infested when compared with 1-2 plants per square meter in farmers field while some other species such as *S.zeylanica* Gaertn., *S. juncoides* Roxb. and *A. baccifera* Linn. were found to be at 20, 18 and 15 plants per square meter.

Noxious weeds species in rice cultivation in Thailand was a mixed population of grasses, sedges, broadleaf and aquatics (Kittipong, 1983.). Infestation of grassy weed specially barnyardgrass was found to be a big problem for rice production in wet seeded rice (Smith, 1968).

Table 1. Weed species found at Sanpathong Rice Experiment Station 1991 and 1992.

Species	Plants/m^2	%
1. Echinochloa crusgalli (L.) Beauv.	24	22.43
2. Scirpus juncoides Roxb.	18	16.82
3. Cyperus difformis Linn.	9	8.41
4. Cyperus pulcherrimus Willd ex Kunth.	4	3.74
5. Fimbristylis miliacea (L.) Vohl.	2	1.87
6. Ammania baccifera Linn.	15	14.02
7. Sphenoclea zeylanica Gaertn.	20	18.69
8. Marsilea crenata Presl.	3	2.80
9. Rotala indica Linn.	4	3.74
10. Monochoria vaginalis (Brum.F) Presl.	8	7.48

Remark : Figures are average of four replications collected at 35 days after seeding.

Control efficacy and phytotoxicity

Phytotoxicity, control efficacy of general weeds and barnyardgrass and oven dry weeds weight were recorded in Table 2. Results suggested that all treated compounds provided excellent control of barnyardgrass with no toxicity to the young rice. Quinclorac at 0.5 kg(ai)/ha, quinclorac/benthiocarb at 0.25/0.5 kg(ai)/ha, quinclorac/ bensulfuron methyl at 0.25/0.02 kg(ai)/ha and quinclorac/oxadiazon at 0.25/0.5 kg(ai)/ha gave completely control of barnyardgrass while benthiocarb at 1.0 kg(ai)/ha and bensulfuron methyl at 0.04 kg(ai)/ha provided good control of barnyardgrass. Control efficacy of the same herbicides on other paddy weeds species beside barnyardgrass indicated that quinclorac by itself gave only moderate control of sedges and broadleafs species when compared to other products which provided satisfactory and good control of the paddy weeds. Dry weeds weight indicated that all treated plots provided low dry weeds weight when compared with untreated check. Application of single compound such as bensulfuron methyl gave lowest dry weeds weight of 4.38 percent when compared to benthiocarb and quinclorac that provided dry weeds weight equivalent to 16.47 and 27.49 percent. Herbicides which being applied in combination with quinclorac showed that quinclorac/bensulfuron methyl provided lowest amount of dry weeds weight of 0.60 percent quinclorac/oxadiazon and quinclorac/benthiocarb gave 2.72 and 14.50 percent compared to untreated check.

Quinclorac had been used for barnyardgrass control in paddy due to its high control efficacy and high selectivity between rice and grass (Kibler et al 1987). Under a mixed population of paddy weeds in farmers field of Thailand, application of single compound could not provide completely control of all species. Treatments of herbicides in combination seemed to provide a broader spectrum of control than single product under a similar conditions. (Goto., 1973). Herbicides that gave good control or completely control of paddy weeds would provide a less dry weeds weight when compared to untreated plot.

Tillers number and height of rice

Tillers number per plant and plant height of rice collected at 30 days after application of herbicides and at harvesting were reported in Table 3. Tillers number per plant collected at 30 days after treatment of chemicals indicated no significant difference among treatments. But tillers number collected at time of harvesting was found to be significant between treated plots and untreated check. Height collected at 30 days after treatment and at the end of growing season did not showed significant difference between treatments at all. Vegetative growth of rice in term of tillers number and height at early stage did not affected by benthiocarb, bensulfuron methyl, quinclorac, and oxadiazon because all four compounds possessed a low phytotoxic effect. A rapid growth of rice under weeds free conditions occurred after treatment of herbicides because ecology of rice field was suitable for the rice plants to grow. Rice cultivar could utilize all growth factors more effectively due to no competitive power from weeds. Tillers number were found to be more sensitive to good growth conditions more than plant height at later stage.

Rice yield

Grain yield of rice at 14 percent moisture content was recorded and reported in Table 4. Yield of rough rice showed significant difference among treated plots and untreated check. Bensulfuron methyl, quinclorac/oxadiazon and quinclorac/bensulfuron methyl provided highest yield of 4.77, 4.70 and 4.66 ton per hectare when compared with 3.79 ton per hectare of untreated plot. All treated plots gave production of rough rice over the untreated check ranging from 25.7 - 15.2 percent. Bensulfuron methyl alone yielded over check equivalent to 25.7 percent. Quinclorac/oxadiazon and quinclorac/bensulfuron methyl provided yield over check 23.9 and 22.9 percent. Benthiocarb by itself, quinclorac/benthiocarb and quinclorac alone gave yield over untreated check equivalent to 19.1, 18.7 and 15.2 percent respectively.

Degree of weed competition was more severe in dry seeded or wet seeded rice than in transplanted rice because growing condition of wet seeded rice cultivation favored weeds growth. Infestation of grass weeds especially barnyardgrass reduced yield of rice a lot more than broadleaf and sedges. (Smith, 1968). Elimination of grass weeds in early stage by herbicides treatments increased production over check to significant level. (Oelke and Morse, 1968.)

Application of herbicides as single product could not provide completely control of mixed population of paddy weeds due to narrow control spectrum of chemicals. But treatment of herbicides in combination provided a better control spectrum. In a complex weeds population which had barnyardgrass as a dominant species, application of single compound with specific grass control action could not provide completely control of all paddy weeds when compound with application of herbicides in combination(Smith and Khodayari, 1985)

Acknowledgment

We thank Mr. Utah Tanapanyo, chief of Sanpathong Rice Experiment Station, for helping to conduct field experiments. The following companies who supplied chemicals for this study; Du pont Thailand, BASF - Thailand and TJC - Chemical Company.

Literature Cited

Goto, M. 1973. The use of benthiocarb in rice cultivation. Proc. 4 th Asian-Pacitic Weed Sci. Soc. Conf. 1 :124 - 129.

Holm, L.G., D.L. Plucknett, J.V. Pancho and J.P. Herberger. 1991. The world's worst weeds, Distribution and Biology. Krieger ,Publishing Company,Malabar, Florida. p. 32 - 40.
Kearney, P.C. and D.D. Kaufman. 1988. Herbicides Chemistry, Degradation andMode of action. Vol. 3. p. 121.
Kibler, E., B.H. Menck and H.Rosebrck. 1987 Quinclorac a new Echinochloa Herbicide for rice and an excellent partner for broad spectrum rice herbicides. APWSS (11 th Conference) : 89 - 97
Kittipong, P. 1983. Weed control in farmer's fields in Thailand. Weed control in Rice IRRI and IWSS Publication : 193 - 200 p.
Noda. K., K. ozawa, and K. Ibaraki. 1968 Studies on the Damage to Rice Plant dueto Weed, Effect of Barnyardgrass Competition on Growth, Yield and Some Ecophysiological Aspects of Rice Plants. Kyushu Agri. Exp. Sta. Bull 13 : 345 - 367.
Oelke, E.A. and M.D. Morse. 1968. Propanil and molinate for control of barnyardgrass in water - seeded rice. Weed 16 : 235 - 230.
Office of Agricultural Economics Ministry of Agriculture and Co-operatives, Bangkok,Thailand 1994. Agricultural Statistics in Brief Crop Year 1993/94. Agricultiral Statistics NO. 7/1994. p. 1 - 21.
Smith, R.J., Jr. 1968. Weed competition in rice. Weed Sci., 16 : 252 - 255.
Smith, R.J. and K. Khodayari. 1985. Herbicide Treatments for Control of Weeds in Dry - Seeded Rice (*Oryza sativa*). Weed Sci., 33 : 686 - 692.

Table 2. Toxicty, barnyardgrass and general weeds control efficacy and dry weed weight from wet seeded rice experiments at Sanpathong Rice Experiment Station 1991 and 1992

Treatments	Rates kg(ai)/ha	Toxicity	Barnyardgrass control	General weeds control	Dry weed weight	
					gm/m²	%
1. benthiocarb	1.0	0.25	9.15	8.10	10.9 b	16.47
2. bensulfuron methyl	0.04	0.05	9.25	9.25	2.9 b	4.38
3. quinclorac	0.5	0.0	10.00	4.50	18.2 ab	27.49
4. quinclorac/benthiocarb	0.25/0.5	0.8	10.00	8.80	9.6 b	14.50
5. quinclorac/bensulfuron methyl	0.25/0.02	0.3	10.00	9.80	0.4 b	0.60
6. quinclorac/oxadiazon	0.25/0.5	1.0	10.00	9.75	1.8 b	2.72
7. Untreated	-	0.0	0.0	0.00	66.2 a	100.00
					*	

Remark : NS = nonsignificant
: * = significant
: Means followed by common letter are not significant at 5 % level.

Taber 3. Tillers number and height colleced from wet seeds rice experiments at Sanpathong Rice Experiment Station 1991 and 1992

Treatments	Rates kg(ai)/ha	Tillers / plant				Height (cm.)			
		30 Days		Hervesting		30 Days		Hervesting	
		plant	%	plant	%	cm.	%	cm.	%
1. benthiocarb	1.0	3.33	98.81	6.04 a	113.32	49.52	100.59	105.33	100.88
2. bensulfuron methyl	0.04	3.39	100.59	6.10 a	114.45	49.43	100.41	104.61	100.19
3. quinclorac	0.5	3.32	98.52	5.98 a	112.20	48.16	97.83	105.11	100.60
4. quinclorac/benthiocarb	0.25/0.5	3.48	103.26	6.35 a	119.14	48.18	97.87	106.08	101.60
5. quinclorac/bensulfuron methyl	0.25/0.02	3.54	105.05	6.54 a	122.70	48.25	98.01	105.41	100.90
6. quinclorac/oxadiazon	0.25/0.5	3.43	101.78	6.56 a	123.08	48.85	99.23	104.47	100.05
7. Untreated	-	3.37	100.00	5.33 b	100.00	49.23	100.00	104.41	100.00
		NS		*		NS		NS	

Remark : NS = nonsignificant
: * = significant
: Means followed by common letter are not significant at 5 % level.

Table 4. Yield collected from wet seeded rice experiments at Sanpathong Rice Experiment Station 1991 and 1992.

Treatments	Rates kg/(ai)/ha	Yield		
		kg/Rai	kg/ha	%
1. benthiocarb	1.0	723.96	4524.75 ab	119.14
2. bensulfuron methyl	0.04	763.54	4772.13 a	125.66
3. quinclorac	0.5	700.00	4375.01 bc	115.20
4. quinclorac/benthiocarb	0.25/0.5	721.18	4507.38 ab	118.68
5. quinclorac/bensulfuron methyl	0.25/0.02	746.88	4668.00 a	122.91
6. quinclorac/oxadiazon	0.25/0.5	753.13	4707.06 a	123.94
7. Untreated	-	607.64	3797.75 c	100.00
		*	*	

Remark : NS = nonsignificant
: * = significant
: Means followed by common letter are not significant at 5 % level.

Seed Soaking in Herbicide Solution for Controlling *Ischaemum rugosum*, a Rice Seed Contaminant

A.N. Rao and K. Moody*
International Rice Research Institute
P.O. Box 933, Manila, Philippines

Abstract. *Ischaemum rugosum* Salisb. which is spread as a rice seed contaminant is emerging as a major weed in direct-seeded rice in south and southeast Asia. The objective of this study was to determine the effect of soaking rice seed contaminated with *I. rugosum* seed in herbicide solutions on weed control and rice growth. Butachlor, butachlor + safener and pretilachlor + safener were tested at four concentrations (2.5, 5, 10, and 25 ppm), for two periods of soaking in herbicide solution (1 h and 24 h) and two washing regimes (seeds washed with water and unwashed after soaking). Soaking in pretilachlor + safener at all concentrations was least effective in reducing seedling survival of *I. rugosum*. Soaking in butachlor at 10 and 25 ppm for 24 h reduced seedling survival of *I. rugosum*, but resulted in reduced rice seedling survival. Soaking in 10 ppm of butachlor + safener for 24 h without washing resulted in least (2%) seedling survival of *I. rugosum* without significant reduction in rice seedling survival. Further tests need to be conducted to confirm the suitability of this method of weed control.

Key words: Rice seed, *Ischaemum rugosum*, herbicides, weed seed contamination, seed treatment, novel herbicide application

Introduction

Contamination of crop seed with weed seeds is a source of perpetuation in crop fields year after year (Edward *et al.*, 1963). Numerous authors including Camus (1921), Senaratna (1943), Baksha *et al.* (1979), Kaul (1986) and Rao and Moody (1990) have reported that weed seeds are disseminated as contaminants of crop seed.

I. rugosum which causes major yield and quality losses is becoming increasingly important as a rice weed in the Philippines, Thailand and Malaysia (Moody, 1993). Moody (1991) reported that *I. rugosum* is spread as a seed contaminant.

Peudpaichit *et al.* (1985) and Mabbayad and Moody (1992) noted that seed treatment with bensulfuron-methyl and pretilachlor + safener, respectively, appeared to be a promising method of herbicide application for controlling weeds in wet-seeded rice. Using this technique to prevent weed seed germination in contaminated rice seed has not been tested previously. Hence, a study was conducted to try to control *I. rugosum* contaminating rice seed by soaking the seed in herbicide solution.

Materials and Methods

The experiment was conducted in a greenhouse of the International Rice Research Institute, Los Baños, Philippines in September and October 1994. A split-plot design was used with herbicides as main plots and seed treatments as subplots. There were three replications. Butachlor, butachlor + safener and pretilachlor + safener were tested at four concentrations (2.5, 5, 10 and 25 ppm). Two periods of soaking in herbicide solution (1 h and 24 h) and two washing regimes (washed and unwashed) were tested. The washing treatment was included to remove the herbicide from the surface of the seed so that farmers would not have direct contact with the herbicide when broadcasting the seed. Washing was done by rinsing the seed in tap water three times for 15 sec each. An untreated check was included for comparison purposes. The rice cultivar used was IR 74.

For each treatment, 50 seeds of *I. rugosum* and rice were soaked, separately, in 10 ml of the different herbicide solutions or water for 24 h and incubated for 48 h. After incubation, the seeds were

broadcast on the surface of puddled saturated soil in pots containing 1.7 kg soil. A lowland soil with 41% clay, 38% silt and 21% sand was used. It had a pH of 6.9, 0.128% N and 38 mg/kg available P, 1.17 meq/100 g exchangeable K, 17.8 meq/100 g exchangeable Ca and 12.0 meq/100 g of exchangeable Mg.

The number of seedlings which survived was recorded 15 days after seeding (DAS) and seedling survival as a percentage of the untreated check was determined. Rice and *I. rugosum* seedling height and dry weight (g/pot) were determined at 15 DAS. The data were analyzed using the methods described by Gomez and Gomez (1984).

Results and Discussion

Soaking in 25 ppm of pretilachlor for 24 h with or without washing resulted in 81.7% and 84.7% reduction in survival of *I. rugosum* seedlings, respectively (Table 1), without deleterious effect on rice seedling survival (Table 1) and height and dry weight of rice seedlings (Table 3). This treatment resulted in 43.1% and 63% reduction in plant height and 84.2% and 97.4% reduction in dry weight of the surviving *I. rugosum* plants, respectively (Table 2).

Soaking in 25 ppm of butachlor for 24 h with or without washing resulted in 55.3% and 61.7% reduction in rice seedling survival (Table 1), 31.8% and 25.71% reduction in height of the surviving rice seedlings (Table 3) and 41.7% and 38.8% reduction in rice seedling dry weight (Table 3), respectively. Mercado-Noriel (1980) observed increased inhibition of shoot length with increasing butachlor concentration. However, Mabbayad and Moody (1992) reported that shoot length of rice cv. IR 36 was not affected by butachlor at 25 ppm. This may be due to differences among cultivars in susceptibility to herbicides. Moody and Madrid (1983) reviewed rice cultivar tolerance to herbicides and provided a number of references for cultivar tolerance to butachlor.

Butachlor + safener was most effective in controlling *I. rugosum* (Tables 1 and 2). Complete kill was obtained when the seeds were soaked in 25 ppm for 24 h whether they were washed or not. However, rice seedling survival was significantly reduced compared with the untreated check (Table 1). Soaking in 10 ppm of butachlor + safener for 24 h without washing resulted in 98% kill of *I. rugosum* seedlings (Table 1) without significant reduction in rice seedling survival (Table 1), height and dry weight (Table 3). Differences in response to the various treatments may be due to differential absorption of the herbicides by rice and *I. rugosum* seeds.

Seed treatments are usually very cost effective. Because they are applied directly to the site where they have to function they are effective, provided that there is small variation about the mean target dose. Possible injurious effects to non-target organisms such as the applicator, birds and soil-inhibiting mammals need to be carefully examined (Hislop, 1993).

Soaking rice seed in 10 ppm of butachlor + safener for 24 h may be a useful technique to control *I. rugosum*, a common rice seed contaminant. Farmers usually soak rice seed in water equivalent to two to three times the volume of seed. Therefore the cost of soaking 100 kg seed in 300 l of 10 ppm butachlor + safener ($21.20/l) solution is only $0.08. The possibility of using all or portion of the remaining herbicide solution to control weeds in the field after planting by pouring the solution directly into the flood water in the field has yet to be tested. Good weed control can be achieved by pouring concentrated herbicide directly into standing water in the field (Peudpaichit *et al.*, 1985; Mabbayad and Moody, 1992).

Further studies are needed to confirm that this is a suitable method for controlling weed seed contaminants as well as weeds in the field after planting.

Acknowledgements: Thanks are due to Ms. Maria Santos for providing soil analysis data. A.N. Rao conveys his thanks to Dr. K. Lampe and Dr. K.S. Fischer of International Rice Research Institute for the encouragement extended.

References

Baksha, M.M., A.M. Huq and M.S. Khan. 1979. Taxonomic studies in the monocotyledonous weeds of the paddy fields of Dacca. Dacca Univ. Studies 27:147-160.

Camus, J.S. 1921. Rice in the Philippines. Bull. No. 37. Bureau of Agriculture, Department of Agriculture and Natural Resources, Manila, Philippines.

Edward, J.C., R.N. Srivastava and R.B Singh. 1963. Weeds of the Allahabad Agricultural Institute Campus. Allahabad Farmer 37:1-44.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. Wiley, New York, USA.

Hislop, E.C. 1993. Application technology for crop production: an introduction. Pages 3-11 *in* G.A. Matthews and E.C. Hislop, eds., Application technology for crop protection. CAB International, Wallingford, Oxon, U.K.

Kaul, M.K. 1986. Weed flora of Kashmir valley. Scientific Publications, Jodhpur, India.

Mabbayad, M.O. and K. Moody. 1992. Herbicide seed treatment for weed control in wet-seeded rice. Trop. Pest Manage. 38:9-12.

Mercado-Noriel, L.R. 1980. Selectivity of butachlor in rice (*Oryza sativa* L.). M.S. thesis, University of the Philippines at Los Baños, College, Laguna, Philippines.

Moody, K. 1991. Crop weed complexity in lowland rice. Pages 15-26 *in* Proceedings of 13th Asian-Pacific Weed Science Society Conference, Jakarta, Indonesia.

Moody, K. 1993. Postplanting weed control in direct-seeded rice. Pages 249-265 *in* A.A. Hamid (ed.) Proceedings for the National Workshop on Direct Seeding Practices and Productivity. MARDI, Penang, Malaysia.

Moody, K. and M.T. Madrid, Jr. 1983. Rice cultivar tolerance to herbicides. Tech. Bull. 76. Food and Fertilizer Technology Center, Taipei, China.

Peudpaichit, S., G.S. Ide, C. Poola-or and P. Tipyasothi. 1985. DPX-F5384 herbicide application flexibility for broadleaf weed control in direct seeded rice - Thailand. Pages 114-121 *in* Proceedings of 10th Asian-Pacific Weed Science Society Conference, Chiangmai, Thailand.

Rao, A.N. and K. Moody. 1990. Weed seed contamination in rice seed. Seed Sci. Tech. 18: 139-146.

Senaratna, J.E. 1943. *Cyperus procera* Rottboell, an indigenous plant liable to be troublesome weed of paddy lands. Trop. Agric. 99:212-213.

Table 1. Effect of different treatments on percentage seedling survival of rice and *Ischaemum rugosum*.

Herbicide concentration (ppm)	Soaking duration (h)	Butachlor + safener		Butachlor		Pretilachlor + safener	
		Rice	*I. rugosum*	Rice	*I. rugosum*	Rice	*I. rugosum*
Check-water		100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Washed							
2.5	1	93.0 a	97.4 ab	94.3 a-e	94.2 ab	94.7 a	92.2 ab
5.0	1	94.0 a	73.0 bc	95.0 a-d	71.4 bc	97.3 a	90.3 ab
10	1	100.0 a	35.3 d-g	96.7 abc	68.7 bc	87.0 a	72.0 bcd
25	1	92.7 a	28.7 efg	88.3 a-f	24.6 ef	96.3 a	38.3 ef
Unwashed							
2.5	1	96.7 a	75.3 abc	93.7 a-e	88.1 ab	97.0 a	67.7 bcd
5.0	1	90.0 a	55.8 cd	93.0 a-e	78.8 ab	91.0 a	92.2 ab
10	1	94.7 a	38.4 def	97.7 ab	50.6 cd	84.7 a	49.8 cde
25	1	93.0 a	18.9 e-h	54.0 g	38.3 de	89.0 a	69.6 bcd
Washed							
2.5	24	99.3 a	86.7 ab	82.7 b-f	80.1 ab	97.0 a	46.6 de
5	24	85.0 ab	29.2 efg	80.3 c-f	29.3 def	93.3 a	54.0 cde
10	24	88.7 ab	17.7 fgh	78.0 ef	9.0 f	99.3 a	29.7 ef
25	24	74.3 b	0.0 h	45.7 gh	7.5 f	91.7 a	18.3 f
Unwashed							
2.5	24	100.0 a	44.6 de	79.0 def	23.0 ef	88.0 a	54.3 cde
5.0	24	99.3 a	10.7 gh	76.3 f	15.8 ef	96.0 a	75.7 abc
10	24	93.0 a	2.0 h	76.3 f	3.9 f	84.0 a	36.0 ef
25	24	73.3 b	0.0 h	38.3 h	3.6 f	98.3 a	15.3 f

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

Table 2. Effect of different treatments on the height (cm) and dry weight (g/pot) of surviving *Ischaemum rugosum* seedlings.

Herbicide concentration (ppm)	Soaking duration (h)	Butachlor + safener		Butachlor		Pretilachlor + safener	
		Height	Dry weight	Height	Dry weight	Height	Dry weight
Check-water		21.4 a	0.38 a	21.4 abc	0.38 a	21.4 abc	0.38 a
Washed							
2.5	1	14.8 b	0.23 bc	22.7 ab	0.28 ab	23.5 a	0.30 abc
5	1	14.8 b	0.12 cd	18.9 bcd	0.18 bcd	20.7 abc	0.26 abc
10	1	12.8 b	0.11 cd	15.8 cde	0.14 cde	21.7 abc	0.19 cde
25	1	12.9 b	0.05 d	14.7 de	0.15 b-e	12.8 de	0.06 f
Unwashed							
2.5	1	14.0 b	0.26 b	26.6 a	0.35 a	20.6 abc	0.33 ab
5	1	12.6 b	0.22 bc	20.7 a-d	0.35 a	19.9 abc	0.24 bcd
10	1	13.0 b	0.13 bcd	18.0 b-e	0.20 bc	15.5 bcd	0.09 ef
25	1	10.8 bc	0.14 d	16.8 b-e	0.14 cde	10.6 de	0.10 ef
Washed							
2.5	24	15.2 b	0.22 bc	17.1 b-e	0.20 bc	15.3 cd	0.13 def
5	24	12.4 b	0.06 d	16.3 cde	0.13 cde	15.9 bcd	0.07 ef
10	24	13.0 b	0.04 d	15.0 de	0.05 de	13.1 de	0.08 ef
25	24	0.0 d	0.00 d	5.2 f	0.02 e	12.2 de	0.02 f
Unwashed							
2.5	24	14.3 b	0.11 cd	23.1 ab	0.09 cde	23.3 a	0.29 abc
5	24	14.4 b	0.02 d	16.4 cde	0.10 cde	19.8 abc	0.26 abc
10	24	5.5 c	0.02 d	12.0 e	0.07 cde	13.0 de	0.04 f
25	24	0.0 d	0.00 d	2.1 f	0.03 e	7.9 e	0.01 f

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

Table 3. Effect of different treatments on the height (cm) and dry weight (g/pot) of surviving rice seedlings.

Herbicide concentration (ppm)	Soaking duration (h)	Butachlor + safener		Butachlor		Pretilachlor + safener	
		Height	Dry weight	Height	Dry weight	Height	Dry weight
Check-water		19.8 ab	1.03 a-f	19.8 ab	1.03 b	19.8 ab	1.03 abc
Washed							
2.5	1	19.0 a-d	1.06 a-e	18.1 a-d	0.95 bc	23.0 a	1.29 a
5	1	18.1 a-d	1.04 a-f	17.3 b-e	1.01 b	20.0 b	1.06 abc
10	1	17.8 a-d	0.91 b-f	16.8 c-f	0.91 bcd	18.8 bc	0.99 abc
25	1	16.9 cd	0.74 f	16.3 def	0.93 bc	18.1 bc	1.01 abc
Unwashed							
2.5	1	20.4 a	1.23 a	19.6 ab	0.88 bcd	18.9 bc	0.96 bc
5	1	20.2 a	1.17 ab	18.6 a-d	0.90 bcd	18.6 bc	0.96 bc
10	1	19.4 abc	1.11 a-d	18.8 a-d	0.83 bcd	18.7 bc	1.03 abc
25	1	20.0 a	0.83 c-f	13.7 g	0.67 cd	17.1 c	0.98 abc
Washed							
2.5	24	18.0 a-d	0.93 a-f	19.1 abc	0.86 bcd	19.6 bc	1.29 a
5	24	17.8 a-d	0.83 c-f	15.1 efg	0.87 bcd	18.4 bc	1.08 abc
10	24	17.3 bcd	0.83 c-f	15.1 efg	0.84 bcd	18.6 bc	0.87 c
25	24	16.5 d	0.77 ef	13.5 g	0.64 d	18.2 bc	0.91 bc
Unwashed							
2.5	24	19.0 a-d	0.99 a-f	19.9 a	1.30 a	20.0 b	1.20 ab
5	24	18.0 a-d	0.83 c-f	19.1 abc	1.03 b	19.2 bc	1.07 abc
10	24	17.8 a-d	1.17 abc	18.4 a-d	0.84 bcd	19.0 bc	1.00 abc
25	24	16.9 cd	0.80 def	14.7 fg	0.63 cd	18.5 bc	0.98 abc

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

Farmers' Rice Seed Cleaning Methods in Nueva Ecija, Philippines and Their Effectiveness

F.F. Fajardo* and K. Moody
International Rice Research Institute
P.O. Box 933, Manila, Philippines

Abstract. Many weeds important in rice culture are not native to the area but have been introduced as impurities in rice (*Oryza sativa* L.) seed. During harvest, weed seeds are harvested and processed together with rice seed. Thus, seed to be used for planting the next crop is contaminated. This study was conducted to determine the effectiveness of farmers' seed cleaning methods in Nueva Ecija, Philippines. Most farmers (41.4%) cleaned their seeds before planting by winnowing. Flotation, usually in water, the next most popular method, was used by 17.9% of the farmers. Flotation was superior to winnowing in removing weed seeds. Different combinations of methods were used by 22.8% of the farmers to achieve superior seed cleaning. The remainder of the farmers (17.9%) did no seed cleaning before planting thus adding to existing weed populations in their fields.

Introduction

Contamination of crop seeds with weed seeds is a source of weed perpetuation in crop fields (Edward *et al.*, 1963). During harvest, weed seeds are harvested and processed together with rice. Rice seed to be used for planting the next crop is, thereby, contaminated. In the southern United States, many weeds are spread almost entirely in rice seed; even cleaned rice seed may contain weed seeds because they are difficult to remove during cleaning (Smith *et al.*, 1977). In California, with the exception of *Echinochloa crus-galli* (L.) P. Beauv., all the introduced species of rice weeds have been brought into the state in contaminated rice seed (Fuller and Barbe, 1983).

Previous studies in the Philippines (Rao and Moody, 1990; Fujisaka *et al.*, 1993) reported that processed rice seed was contaminated with weeds seeds. After processing, seeds of *Echinochloa* spp., *Ischaemum rugosum* Salisb., and, to a lesser degree, various sedges remained as major rice seed contaminants.

Seshu and Dadlani (1989) noted that chaff, straw, clay particles, broken or damaged seed or seed parts and other inert matter can be effectively separated from rice seed by winnowing and flotation in water or salt solution.

Advantages of using clean seed include higher yield, maintenance of seed purity (elimination of mixtures of cultivars), higher germination percentage resulting in better (more uniform) stand establishment and less weed and disease problems.

This study was conducted to determine the effectiveness of farmers' seed cleaning methods in Nueva Ecija, Philippines.

Materials and Methods

One hundred and sixty-two randomly-selected farmers in Nueva Ecija province, Philippines were interviewed in July and August 1993 to determine how they processed rice seed to remove weed seeds and other contaminants before planting. Rice seed samples (approx. 1 kg each) were collected using standardized procedures (ISTA, 1976) before and after processing from 25 farmers to determine the efficiency of their seed cleaning methods. Weed seed, undesirable rice grains and other debris were separated from the rice seed and the efficiency of cleaning determined.

Twenty-five farmers were asked to clean rice seeds (15 kg) contaminated with varying amounts of *Echinochloa glabrescens* Munro ex Hook. f. seeds corresponding to 500, 1,000 and 1,500 weed seeds per kilogram of rice seed using their seed cleaning methods. One-kg samples were taken after processing to determine the efficiency of cleaning.

Results and Discussion

Most farmers (41.4%) cleaned their rice seed before planting by winnowing; 23.5% used wind winnowing (*pahangin*) allowing the seed to drop from a height, usually arms length above their heads, to the ground (Table 1). In the process, some of the weed seeds and debris were removed by the force of the wind. Winnowing (*tahip*) which consists of tossing the rice seed into the air from large trays (*bilao*) and catching it again in the tray was used by 8.0% of the farmers. This uses both arm action and the force of the wind to separate the debris and the weed seeds from the rice seed. Some (9.9%) used artificial wind sources, such as electric fans, rice thresher blowers or other motor-driven blowers, for winnowing.

Flotation (*palutang*), the next most popular method, was used by 17.9% of the farmers. This usually consisted of placing rice seed into drums containing water; unfilled grains, light weed seeds and debris floated to the surface of the water and were removed. Four farmers (2.5%) added salt (approx. 1 kg/50 kg rice seed) to the water to improve the efficiency of the system. As the specific gravity of the solution increases, more impurities should be removed. Some cleaned their seed in flowing streams, a fine mesh net was used to prevent rice seed loss.

Different combinations of cleaning methods were used by 22.2% of the farmers to achieve superior seed cleaning and reduce the possibility of sown weed seeds with rice seed.

Almost 18% of the farmers did not clean their rice seed. Reasons given included most of the debris was removed during threshing (8 farmers), seeds were selected carefully at harvest (4), the debris will be blown away or float during seeding (3) and, seed were purchased from a certified seed producer, therefore, it is clean (2). Data presented by Rao and Moody (1990) and Fujisaka *et al.* (1993) refute a number of these claims. Farmers in northern Mindanao, Philippines reported that some seed sold as certified seed did not meet quality standards (Ramos, 1987).

Weed seed contaminants included *Echinochloa* spp. [*E. crus-galli* ssp. *hispidula* (Retz.) Honda, *E. glabrescens* and *E. oryzoides* (Ard.) Fritsch.], *I. rugosum*, *Fimbristylis miliacea* (L.) Vahl, *Ludwigia octovalvis* (Jacq.) Raven, *Scirpus juncoides* Roxb., *Cyperus iria* L., *Leersia hexandra* Sw., *Monochoria vaginalis* (Burm. f.) Presl, *Echinochloa colona* (L.) Link and *Scleria tessellata* Willd., the commonest being *Echinochloa* ssp. (found in 92% of the samples) and *I. rugosum* (in 84% of the samples).

All samples contained unfilled, partially filled and broken dehulled grains. Forty-four percent of the samples contained red-hulled rice seed ranging in amount from 0.07 g - 12.29 g/kg rice seed.

Seven of the 25 farmers who were preparing their rice seed for planting did no cleaning. The efficiency of the cleaning methods used by the other farmers varied from farmer to farmer, by method and by contaminant. Five farmers out of 16 succeeded in totally removing seeds of *Echinochloa* spp., 8 of 15 removed all *I. rugosum* seeds while *L. octovalvis* and *F. miliacea* seeds were completely removed from the 5 rice samples that they contaminated.

A combination of winnowing + flotation was usually superior to either winnowing or flotation (Table 2). Four of the five farmers who used winnowing + flotation removed nearly all the weed seeds (99.5 - 100%) and other debris (89.9 - 100%) indicating that it is not necessary to include an additional step (sieving) in the cleaning process. However, the other farmer who used this technique removed only 24.9% of the weed seeds and 20% of the other debris.

An average of 79.3% of the other debris was removed by the various cleaning procedures. In contrast, only 35.8% of the undesirable rice grains were removed. The closer the undesirable material is in dimensions and weight to the desired rice grains, the more difficult it is to remove. Use of certified seed from reputable sources or careful hand sorting are the only ways to ensure that rice seed used for planting is free of off-types, red-hulled rice and discolored (diseased) grains.

Of the 25 farmers who were asked to clean rice seed which had been contaminated with known amounts of *E. glabrescens* seeds, 18 used some form of cleaning, 7 did nothing. Again the efficiency of the cleaning methods varied among farmers and by method (Table 3). Winnowing removed an average of 58.3% (range: 11.5 - 91.5) of the weed seeds, flotation removed 86.8% (range: 74.9 - 99.4) while a combination of winnowing + flotation removed 95.1% (range: 83.5 - 99.8), with winnowing, in this combination, removing 57.5% of the weed seeds. There was little effect of the level of weed seed contamination on the cleaning efficiency, an average of 76.9% (range: 74.6 - 81.2) was removed by the different methods. However, there was variation in the cleaning efficiency being obtained by different farmers, the best being 0.2% (range: 99.7 - 99.9) and the worst 41.7% (range: 8.5 - 50.2).

Thus, by using combinations of seed cleaning methods, farmers are capable of removing most of the weed seed contaminants in rice seed. This will prevent, to a large extent, the perpetuation of weeds in a field and the introduction of new weed species.

References

Edward, J.C., R.N. Srivastava and R.B. Singh. 1963. Weeds of the Allahabad Agricultural Institute Campus. Allahabad Farmer 37(2):1-44.

Fujisaka, S., R.A. Guino, R.T. Lubigan and K. Moody. 1993. Farmers' rice seed management practices and resulting weed seed contamination in the Philippines. Seed Sci. & Technol. 21:149-157.

Fuller, T.C. and G.D. Barbe. 1983. Taxonomy and ecology of some rice weeds of California. Pages 60-65 *in* Proceedings of the 9th Asian-Pacific Weed Science Society Conference, Manila, Philippines.

ISTA (International Seed Testing Association). 1976. International rules for seed testing. Seed Sci. & Technol. 4:1-180.

Ramos, L.A. 1987. Utilization of certified seeds among rice farmers in northern Mindanao. M.S. thesis, University of the Philippines at Los Baños, College, Laguna, Philippines. 160 p.

Rao, A.N. and K. Moody. 1990. Weed seed contamination in rice seed. Seed Sci. & Technol. 18:139-146.

Seshu, D.V. and M. Dadlani. 1989. Role of women in seed management with special reference to rice. IRTP Tech. Bull. 5. International Rice Research Institute, Los Baños, Laguna, Philippines. 24 p.

Smith, R.J., Jr., W.T. Flinchum and D.E. Seaman. 1977. Weed control in U.S. rice production. Agric. Handb. 497, United States Department of Agriculture, Washington, D.C., U.S.A. 78 p.

Table 1. Farmers' rice seed cleaning methods in Nueva Ecija, Philippines.

Cleaning method	Farmers	
	Number	(%)
Wind winnowing (*pahangin*)	38	23.5
Flotation (*palutang*)	29	17.9
No cleaning	29	17.9
Winnowing (artificial wind)	16	9.9
Winnowing (*tahip*)	13	8.0
Wind winnowing + winnowing	13	8.0
Wind winnowing + flotation	7	4.3
Winnowing + flotation	6	3.7
Wind winnowing + sieving	5	3.1
Winnowing + manual selection	3	1.9
Winnowing + sieving + flotation	2	1.2
Sieving	1	0.6

Table 2. Undesirable materials in farmers' rice seed (g/kg seed) before and after cleaning.[a]

Cleaning method[b]	Rice grains[c]		Other debris[d]		Weed seeds	
	Before	After	Before	After	Before	After
Winnowing (8)	66.1	43.6 (34.0)	0.88	0.23 (73.9)	0.65	0.29 (55.4)
Flotation (4)	106.4	81.7 (23.2)	0.97	0.16 (83.5)	0.29	0.06 (79.3)
Winnowing flotation (5)	104.8	56.2 (46.4)	0.54	0.07 (87.0)	0.38	0.13 (65.8)
Sieving + winnowing + flotation (1)	36.1	16.2 (55.1)	1.26	0.25 (80.2)	1.10	0.03 (97.3)
Average removal (%)	35.8		79.3		65.4	

[a]Percentage removed indicated in brackets.

[b]Number of farmers indicated in brackets.

[c]Unfilled, partially filled, broken, discolored and red-hulled rice.

[d]Straw, rachis and other plant parts.

Table 3. Efficiency of farmers' seed cleaning methods in removing *Echinochloa glabrescens* from contaminated rice seed.

Cleaning method[a]	% Weed seeds removed[b]			
	Low[c]	Medium[c]	High[c]	Mean
Winnowing (8)	54.8 (10.1-91.2)	56.0 (4.9-95.3)	64.1 (19.6-94.5)	58.3 (11.5-91.5)
Flotation (4)	85.4 (66.0-100)	80.2 (53.5-98.7)	94.7 (85.5-100)	86.8 (74.9-99.4)
Winnowing + flotation (6)	58.1 35.7 93.8 (82.8-99.7)	56.8 39.9 96.7 (85.9-99.9)	57.7 37.2 94.9 (81.7-99.8)	57.5 37.6 95.1 (83.5-99.8)
Mean	74.6	74.9	81.2	76.9

[a]Number of farmers indicated in brackets.

[b]Range indicated in brackets.

[c]Level of weed seed contamination. Low - 500/kg; Medium - 1,000/kg; High - 1,500/kg.

No-Till Irrigated Transplanted Rice - A New Cropping System Using Glyphosate Herbicide

W. Hermawan1), M. Utomo2), W.S. Ardjasa3) and S. Abdulrachman3)
1) Monsanto Co., PT Monagro Kimia, Jl.M.H. Thamrin 57, Jakarta
2) The University of Lampung, Bandar Lampung
3) Research Institute for Food Crops of Taman Bogo, Lampung and Sukamandi, West Java, INDONESIA

Abstract. Three series of two consecutive rice *(Oryza sativa L.)* season trials were completed November 1993 through June 1994. Each trial emphasized a different aspect in the development of the no-till system in irrigated transplanted rice (NTR) with the use of two glyphosate based herbicides. The NTR was compared to the conventional farmer tillage (FTR). The trial in Sukamandi showed that no-tilled soil with bulk density around 0.98 g/cm3, porosity 63.35% and moisture content 61.15% was muddy enough for common hand transplanting and good plant establishment. The trial in Trimurjo under loamy soil demonstrated that the redox potential (Eh) was not much different i.e. 0.21 mV (NTR) and 0.25 mV (FTR). Field lysimeter measurement showed that 11-week water requirement of FTR was 846 mm while the NTR was 573 mm (32% lower). In the trial in Taman Bogo, the soil porosity and the soil C.E.C. increased. Results also showed that the period from herbicide application to transplanting of 12-17 days provided less weed coverage than the period of 19-24 days. In cost, the B/C ratio of FTR farming was 1.94 while the NTR was 2.53. In yield, the NTR was similar to or higher than the FTR.

Key words. Glyphosate, lysimeter, no-till system, transplanted rice, conventional farmer tillage.

Introduction

The rate of increase of world wide rice production is slowing down, and if the trend is not reversed, severe food shortages will occur in the next century (3). For Indonesia, eventhough they have been succeeding to be selfsufficient in rice for almost 20 years, but to keep the productivity in pace with the population growth is very costly and succeptible. Major increases in the area planted are unlikely while conversion in prime ricelands to non-agricultural purposes cannot be controled easily. In the last five years, the lost of arable land is around 50,000 hectares where rice used to plant twice a year.

Water available for rice production will also decrease especially during the dry season. Labor shortages due to higher pay in the manufacturing industry, better education, lessening enthusiasm for hard field work, low profitability, increasing production cost and the slow adoption of mechanized farming are other critical issues involved in rice production in Indonesia.

The lowland rice as the most contributor for national productivity is in fact the highest water demanding crop. The higher water consumption is not only due to the evapotranspiration, percolation and seepage, but substantially is required for land preparation, which is also aimed to reduce weed problem. This operation takes about 28% of total water requirement (4), uses 30% of total labor (1) and spends about 30% of total cropping time. By plowing and puddling, nutrient and soil sediment can also be flushed away into the downstream.

An alternative tillage system which can control weed and prepare the good growth media for the seedling, and on the other hand can sustain soil and water resource base, is then urgently needed.

These experiments were undertaken to study the long-term effects of no-till system in irrigated transplanted rice (NTR) on the soil, the crop itself, and its economical benefits to the farmers.

Methods

These experiments arranged in apropriate experimental designs and conducted in two consecutive wet and dry seasons during November 1993 to June 1994. Three locations with different soil texture were selected and each trial emphasized a different aspect in the development of the no-till system in irrigated tranplanted rice (NTR). The experiment in Trimurjo, Lampung on loamy soil emphasized on water requirement and soil characteristics. The experiment in Sukamandi, West Java on clayish soil studied the soil biology and characteristics, while the experiment in Taman Bogo, Lampung on sandy clay soil observed the herbicide application timing and weed control, soil characteristics and economic value of NTR farming There were two glyphosate-base herbicides used i.e. glyphosate 180 g/l ai under the trade name of Polaris 240 AS, and glyphosate 120 g/l ai as Spark 160 AS. Several rates of each herbicide were tested as a pre-plant spraying applied post-emergent with backpack sprayer as commonly used by farmer. At certain period (7-14 days) after spraying, the field was flooded and submerged for certain period (5-10 days) before 21-day old seedling being transplanted manually. All treatments were compared to that farmer conventional tillage (FTR) practice.

Results and Discussion

From Trimurjo. One common question arise with NTR is how to transplant the seedling by hand into untilled soil. This experiment on loamy soil showed that with 5-day flooding, the soil strength at 0-5 cm depth was close to zero atm., and similar to conventional tillage (FTR) at 5-10 cm depth. Under flooded condition, acid Ultisol soil changed to reduced state. However, soil pH and redox between tillages three weeks after transplanting were not much different. At 0-5 cm depth, soil pH average in NTR and FTR was still similar. The oxidation -reduction potential (Eh), which is a useful parameter for measuring predominant reactions taking place in reduced state, also not much different, i.e. 0.21 mV for NTR and 0.25 mV for FTR. In NTR, in situ straw from past crop left in the field and it is not intended for protecting soil from erosion, rather, maintaining soil organic carbon and releasing plant nutrients in a long-term (Tabel 1).

Table 1. Soil Properties of Lowland Rice in Different Tillage Systems, Trimurjo, Lampung, WS 1993/94.

No.	Treatment	Rate kg a.i./ha	Organic-C (0-20 cm)	Soil Strength (5-10 cm)	Redox (0-5 cm)	pH (0-5 cm)
			--- % ---	--- atm ---	-- mV --	
1.	NTR+G-12%	0.48	0.684	0.306	+0.203	6.3
2.	NTR+G-12%	0.96	0.761	0.357	+0.217	6.2
3.	NTR+G-18%	0.54	0.672	0.306	+0.213	6.2
4.	NTR+G-18%	1.08	0.733	0.306	+0.200	6.3
5.	Min-till (w/o herb)		0.744	0.408	+0.221	6.3
6.	FTR (conv-till)		0.802	0.255	+0.252	6.2

Note : NTR : no-till transplanted rice system
FTR : farmer/conventional tillage system
Min-till : weeds are removed by manual hoeing
G-12% : glyphosate herbicide 12% (SPARK 160 AS)
G-18% : glyphosate herbicide 18% (POLARIS 240 AS)

The current water requirement of lowland rice with plow-based system is approximately 800-1200 mm for a 4-month rice crop season. Besides for evapotranspiration, percolation and seepage, 25-30% of those figures is used during the land preparation (4). Based on field measurement of lysimeter, the 11-week water requirement of NTR was 573 mm compared to 846 mm for FTR, or 32% lower (Table 2). This tremendous reduction is mainly due to no-water need for plowing and puddling. The only water requirement is for softening soil before transplanting in 5-day submersion.

Table 2. The Average of 11-week Water Consumption for Lowland Rice in Different Tillage Systems, Trimurjo, Lampung, WS 1993/94.

No.	Water Usage	Tillage System		
		Full	Minimum	No-till
		<----- mm H2O ----->		
1.	Growth media preparation			
	- plowing and puddling	305	0	0
	- submersion	0	47	46
2.	Evapotranspiration	356	340	346
3.	Percolation and seepage	185	168	181
	TOTAL	846	553	573

Note: Minimum tillage was done by manual hoeing

From Sukamandi. The soil physical characteristics observed at up to transplanting showed that bulk density of NTR on clayish soil at wet season was around 0.92-1.04 g/cm3, while at dry season it was ranging from 0.81-1.17 g/cm3. The soil porosity was around 62-64% and 58-65%, while the soil moisture was around 60-62% and 56-61% at the wet and dry seasons, respectively (Table 3). In this conditions, the transplanting could be done as usual by hand. The increasing of bulk density in NTR was followed by the reducing in its porosity from wet to dry season. It was not caused by the herbicide it self but as the consequence of the submersion and the high clay content (72%). The increasing bulk density had not impeded the crop establishment. It is reported that rice will be physically obstructed its growth if the bulk density is above 1.4 g/cm3 (2).

Table 3. Soil Bulk Density, Porosity and Moisture Content in Lowland No-till Rice, Sukamandi, W. Java, WS 1993/94 and DS 1994.

T r e a t m e n t	Time of Assessment (DAP)		
	- 28	- 14	0
	Bulk Density: WS/DS (g/cm3)		
1. NTR with herbicide	0.96/1.13	0.97/1.15	1.04/1.15
2. NTR without herbicide	0.97/1.17	0.97/1.15	0.95/1.15
3. FTR/conventional tillage	0.95/1.02	0.86/1.03	0.92/0.81
	Porosity: WS/DS (%)		
1. NTR with herbicide	64/61	63/60	62/59
2. NTR without herbicide	64/60	64/60	64/58
3. FTR/conventional tillage	65/63	66/63	64/64
	Moisture Content: WS/DS (%)		
1. NTR with herbicide	45/58	58/60	61/60
2. NTR without herbicide	44/58	57/60	60/61
3. FTR/conventional tillage	44/56	61/60	62/61

Note: DAP: day after transplanting
Glyphosate used rate: 0.48-1.08 kg a.i/ha

*From Taman Bogo.*Table 4 showed the sandy clay soil physical and chemical properties measured at harvesting time of each season. The bulk density of NTR was declining from previous figure of 1.28 to 1.15 g/cm3 as a result of the increasing porosity from 57.2% to 62.4% after the dry season. While in the FTR, the soil responded the other way. The important implication was that the secondary crops like legumes or maize which normally grown after the second rice will then have a better soil to grow. The chemical soil properties in NTR showed that N content, available P and cation exchange capacity (C.E.C) increased when compared to the FTR. This condition illustrated that with no-tillage, at least the nutrient usually leached during the land preparation was now still in the field. While from time to time the residue from the crop or weeds may enrich the soil. Thus, soil nutrient status will slowly enhanced. CT in upland condition has proven that. CT long-term study (15 consecutive seasons) in Lampung showed that CT can self subsidize fertilizer from its own system by saving nitrogen as much as 53 kg N, and 25 kg P per hectare per year (5). It is a big help for farmers.

Table 4. Soil Physical and Chemical Properties of Lowland Rice Under No-till and Conventional Tillage Systems, Taman Bogo, Lampung, WS 1993/94 and DS 1994.

Soil Properties	Time of Analysis				
	Prev.	End of Wet Season		End of Dry Season	
		FTR	NTR	FTR	NTR
1. Bulk density (g/cm3)	1.28	1.34	1.17	1.38	1.15
2. Porosity (%)	57.2	54.1	60.3	52.6	62.4
3. pH (H2O; 1:2.5)	5.2	5.2	5.3	5.0	5.5
4. Organic matter (%):					
N	0.149	0.145	0.159	0.097	0.185
C	1.336	1.290	1.322	1.168	1.239
C/N ratio	8.966	8.897	8.314	12.041	6.697
5. Av. P2O5 (mg/100 g)	6.994	7.488	19.929	7.689	20.845
6. C.E.C. (me/100 g)	11.439	12.072	13.705	12.592	13.798
7. Cations amount (me/100 g):					
Ca	1.785	1.956	2.052	0.360	0.723
Mg	0.245	0.216	0.221	0.164	0.342
K	0.174	2.279	0.386	0.176	0.113
Na	0.546	0.451	0.489	0.430	0.458
8. Al - saturated	0.798	1.697	1.404	1.750	0.669
9. Fe (ppm)	64.775	88.479	71.671	90.459	78.826

Other aspect studied indicated that the longer period from herbicide application upto transplanting gave the more weed coverage. The period of 12-17 days gave better weed control than the period of 19-24 day, as shown in Table 5.

This because the glyphosate herbicide is a non-selective, post emergence a foliar-applied and translocated herbicide. It has no soil activity, thus can be applied preplant. Shortening that period will give less opportunity for lying seed to grow. The mulch and the earlier crop coverage followed by the flooding seem to have activity on reducing seed growth. So, the use of herbicide is then critical to NTR success.

Table 5. Weed Coverage in Lowland Rice Under No-till and Conventional Tillage Systems, Taman Bogo, Lampung, WS 1993/94.

Treatment	Rate kg a.i./ha	Weed Coverage (%) A	B	C	D
		At 7 DAT (day after treatment)			
1. FTR		< 5	< 5	< 5	< 5
2. NTR+G-12%	0.60	< 5	7.5	10	15
3. NTR+G-12%	0.84	< 5	5	7.5	12.5
4. NTR+G-18%	0.72	< 5	10	15	17.5
5. NTR+G-18%	1.08	< 5	7.5	10	15
			At 21 DAT		
1. FTR		35	35	40	35
2. NTR+G-12%	0.60	15	20	30	45
3. NTR+G-12%	0.84	10	10	35	35
4. NTR+G-18%	0.72	10	15	25	50
5. NTR+G-18%	1.08	15	10	35	35
			At 42 DAT		
1. FTR		30	30	40	40
2. NTR+G-12%	0.60	25	25	15	15
3. NTR+G-12%	0.84	25	20	10	10
4. NTR+G-18%	0.72	25	25	10	15
5. NTR+G-18%	1.08	17.5	20	15	15

Note: A: 7 days after spraying (DAS) + 5 days flooding (DF)
B: 7 DAS + 10 DF; C: 14 DAS + 5 DF; D: 14 DAS + 10 DF;
< 5: below 5%
NTR+G: NTR with glyphosate herbicide 12% or 18%

Table 6. Yield, Value and Net Income of Lowland Rice Under No-till and Conventional Tillage Systems, Taman Bogo, Lampung, WS 1993/94 and DS 1994.

		Tillage System FTR	NTR
Yield (t/ha)	WS 1993/94	5.569	5.636
	DS 1994	4.898	5.446
	TOTAL	10.467	11.082
Gross return (US$/ha)		1,712.94	1,813.42
Labor cost (US$/ha)		550	315.45
Chemical inputs cost (US$/ha)		331.82	400.01
Net return (US$/ha)		831.12	1,097.96
B/C ratio		1.94	2.53

From the 2-season farming cost analysis shown in Table 6, NTR provided 32% higher net return than that of FTR. This extra income was coming from the labor saving (30%) and additional yield (6%), and those made the B/C ratio of NTR farming is 2.53 higher than that 1.94 of FTR.

Conclusion

The no-till transplanted rice (NTR) researchs in lowland ecosystem is just the begining, however, it indicated to have a good prospect. NTR could reduce water requirement, reduce time for land preparation, and maintain soil properties and grain yield the same as conventional did, while increasing farmer's income. As a new system, an integrated and comprehensive researchs are needed to improve the system.

References

1. Ardjasa, W.S., Widyantoro, G.E. Maliawan, W. Hermawan and S. Asmono. 1994. No-till System Using IPA-glyphosate 12% and 18% Herbicides, and the Effect of Fertilization on the Weed Control. Proc. 12th Weed Science Society of Indonesia Conf., Padang. 209-216.
2. Baver. 1972. Soils Physics. 3rd John Wiley and Sons Inc., New York.
3. IRRI Reporter. 1994. IRRI Redesigns Rice Plant to Yield More Grain. IRRI, Philippines. 1-2.
4. Sanchez, P.A. 1976. Soil Management in Rice Cultivation Systems. Philippines Graphic Arts. Ins. 415-448.
5. Utomo, M. 1994. The Role of Conservation Tillage in Soil Improvement in Upland. University of Lampung.

Weed Control by Glyphosate for a Clean Start of No-till Direct Seeded Rice

Matsuo Itoh and Toshikazu Miyamoto
Agricultural Research Station, Monsanto Japan, Ltd.,Ibaraki 300-13, Japan

Abstract. The application system of nonselective herbicides, glyphosate, glufosinate and a paraquat+diquat mixture was studied for a weed-free initiation of no-till direct seeded rice. Glyphosate had a significantly wide application window to control predominant species. Rice safety of all tested chemicals was excellent when rice seeds were adequately covered with soil. *Echinochloa crus-galli* emerged in late April and grew to more than the three leaf stage by planting time. A pre-emergence herbicide, thiobencarb applied before planting did not control the species completely. Thus, for the start of no-till rice, a glyphosate treatment after planting and before emergence of rice seedlings is best to control *E. crus-galli* and other weed species. The amount and size of weeds at seeding affected the stand of rice seedlings and the planting efficiency. A large amount of weed residue killed by glyphosate delayed the emergence of rice seedlings resulting in reduced weight and loss of rice seedlings compared to non-residue plots. Large-sized dead weeds reduced the planting efficiency by twining around the shaft of the seeder. In a no-till field heavily infested with large weeds, therefore, sequential application of glyphosate in March to early April followed by a second application before emergence of rice seedlings is necessary to keep weeds small until planting and then to control those weeds and *E. crus-galli*.
Key words: glyphosate, no-till, direct seeded rice, weed control

Introduction

No-till system of direct dry-seeded rice has been paid attention for saving cost and labor in rice cultivation in Japan[1) 2)]. Direct dry-seeding method enables us to produce rice on a large scale through its less workload for planting than transplant rice. No-till system adds another technical advantage to direct dry-seeded rice. The compact soil of no-till field sustains a heavy seeder in wet conditions, resulting in successful planting on schedule even in rainy weather. However, one of the most important technical hurdles to reach successful no-till cultivation is considered to be weed control from planting time to irrigation at the four to five leaf stage of rice. Established winter weeds and newly emerged summer weeds have to be controlled by herbicides to obtain the good stand of rice seedlings before irrigation. The present paper studied the application system of herbicides to control emerged weeds before, at, or after planting for a clean start of no-till rice cultivation.

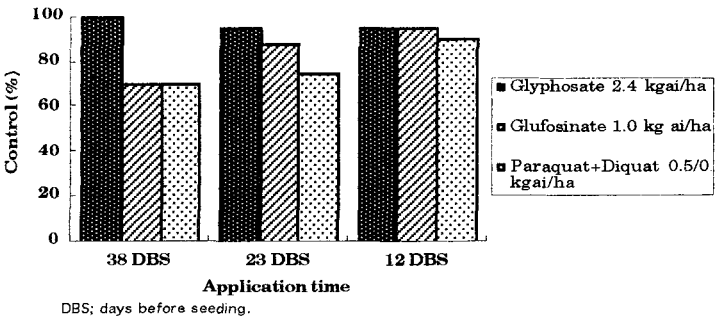
Materials and Methods

Three field tests were carried out in Kurashiki and Sanyo, Okayama, Japan, in 1994. Common weeds in all sites were *Alopecurus aequalis* Sobol. var. *amurensis* (Komar.) Ohwi emerging in fall and barnyardgrass (*Echinochloa crus-galli* (L.) Beauv. var. *crus-galli*) coming out in late April. Isopropyl amine salt of glyphosate (Roundup, 41 % a.i.) at 2.4 kg a.i./ha, glufosinate (Basta, 18.5 % a.i.) at 1.0 kg a.i./ha and a paraquat+diquat mixture (Preglox L, 5%+7% a.i.) at 0.5+0.7 kg a.i./ha were separately applied at six different times before, at, or after seeding to determine their optimum application timing. Sequential application of glyphosate at 2.4 kg a.i./ha on March 3 and May 12 was compared to single applications of glyphosate mentioned above. Water carrier at 500, 1,000 and 1,000 l/ha was used for application of glyphosate, glufosinate and a paraquat+diquat mixture respectively. A mixture of glyphosate at 2.4 kg a.i./ha and thiobencarb (Saturn, 50% a.i.) at 5 kg a.i./ha was also applied with 1,000 l/ha of water carrier before and after seeding to determine the soil residual activity of thiobencarb on barnyardgrass in no-till conditions. Seeds of rice cv. Akebono at 30 kg/ha were drilled at 30 cm row spacing with the no-till seeder by Minoru Sangyo on May 7 (one site in Sanyo) and on May 13 (two sites in Kurashiki). Rice seeds were planted at about two to four cm depth. Slow release fertilizer, LPS fertilizer, at 60 kg/ha of nitrogen was dressed over the whole test field. Plots were randomized complete block design with three replications. Plot size was 10 m^2 (5 m x 2 m). Visual assessments were made for herbicidal activity and rice safety. Rice growth was measured for evaluating the stand of rice seedlings. Barnyardgrass was also measured in size.

Results and discussion

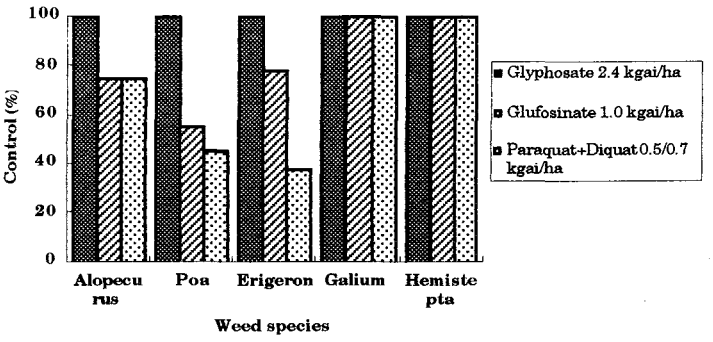
Activity and rice safety

Figs. 1 and 2 show the activity of glyphosate, glufosinate and a paraquat+diquat mixture applied before seeding. As would be expected, weed control by glyphosate lasted longer than glufosinate and a paraquat+diquat mixture. This was true on *Alopecurus aequalis*, *Poa annua* L. and *Erigeron* sp. There was no significant difference in activity on other broadleaf weed species among tested chemicals. Fig. 3 illustrates the activity of glyphosate applied before, at or after rice planting. Glyphosate had a significantly wide application window on predominant species, *A. aequalis*. Rice applied with glyphosate, glufosinate and a paraquat+diquat mixture at five days after planting was uninjured by them (Table 1). Excellent rice safety appeared to be attributed to the adequate cover soil over rice seeds. Therefore, all tested chemicals are able to be applied unless rice seedlings emerge or rice seeds are exposed to them.



DBS; days before seeding.

Fig. 1. Effect of application timing on the activity of herbicides on Alopecurus aequalis.



Data were combined of 3 tested sites at seeding time.

Fig. 2. Weed activity of herbicides at 38 days before seeding.

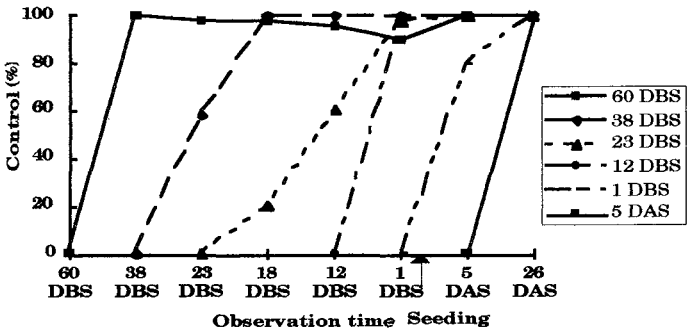


Fig. 3. Activity of glyphosate applied at various application times on Alopecurus aequalis.

Emergence of barnyardgrass

Barnyardgrass started emerging from late April in Okayama. The weed species grew up to more than the three leaf stage by seeding time in the middle of May (Fig. 4). As shown in Table 2, a pre-emergence herbicide, thiobencarb, did not work completely to control the species. Therefore, barnyardgrass has to be controlled by a nonselective herbicide to obtain a clean start of no-till direct seeded rice. Considered the rice safety of glyphosate, a glyphosate treatment after planting and before emergence of rice seedlings appears to be best to control barnyardgrass

Table 1. Rice safety of 3 tested herbicides applied to the soil surface.

Application timing	Rice injury (%) glyphosate (2.4 kgai/ha)	glufosinate (1.0 kgai/ha)	paraquat+diquat (0.5+0.7 kgai/ha)
38 DBS*	0	0	0
23 DBS	0	0	0
12 DBS	0	0	0
0 DAS**	0	0	0
5 DAS	0	0	0

Rice injury shown here is average of 3 sites 26 days after seeding.
*DBS; days before seeding, **DAS; days after seeding.

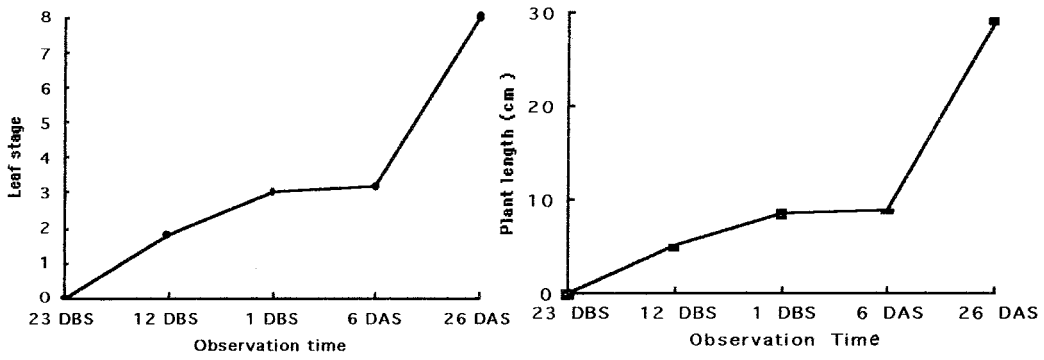


Fig. 4. Changes in growth of barnyardgrass emerged in late April.

and other summer weed species.

Effect of weed residue on rice growth and seeding operation

As shown in Table 3, rice seedlings were significantly reduced in size and weight when the residue of *Alopecurus aequalis* killed by glyphosate covered over the seeding rows. Large amount of weed residue delayed the emergence of rice seedlings resulting in reduced weight and loss of rice seedlings compared to a clean field. Table 4 shows the plant size of weeds controlled by glyphosate at seeding time. *Erigeron* sp. and *Hemistepta lyrata* Bunge had grown over 80 cm tall. Those large-sized dead weeds reduced the planting efficiency by twining around the shaft of the seeder. The poor planting efficiency from weed residue was observed at two of three test sites. Therefore, it is necessary to keep weeds small by herbicides until planting time. Early application of herbicides from March to early April is required to reduce the weed residue in fertile field where weeds grow large and tall.

Table 2. Activity of glyphosate+thiobencarb on barnyardgrass.

Treatment	Rate (kgai/ha)	Control (%)	
		7 DBS*	5 DAS**
glyphosate	2.4	55	35
glyphosate+thiobencarb	2.4+5.0	80	58
untreated		0	0

*DBS; days before seeding, **DAS; days after seeding.

Table 3. Growth of rice seedlings 27 days after seeding at Fujito site.

Treatment	No. of plants/m	Leaf stage	Dry weight/plant (mg)
sequential treatment*	30.3 ± 1.2***	5.0 ± 0.7	106 ± 22
single treatment**	28.3 ± 2.9	3.7 ± 0.7	52 ± 18
untreated	28.7 ± 6.4	4.6 ± 0.6	65 ± 5

*; glyphosate was applied sequentially 60 and 1 days before seeding.

**; glyphosate was applied only 1 day before seeding.

***; Mean ± S.D.

Table 4. Plant size of weeds at seeding time at Fujito site

Species	Plant size (cm)
Alopecurus aequalis	45.3 ± 7.3*
Erigeron sp.	84.5 ± 25.8
Galium spurium	32.7 ± 4.0
Hemistepta lyrata	113.5 ± 35.7
Poa annua	35.4 ± 10.8

*; Mean ± S.D.

The results obtained in the present study suggest that no-till direct seeded rice is able to start with a single treatment of a nonselective herbicide after planting and before emergence of rice seedlings, when small weeds infest the field. Sequential application in March to early April followed by a second application before emergence of rice seedlings is necessary for a clean start of no-till rice. Glyphosate appeared to fit both application systems because of its long term control of weeds and its excellent rice safety.

References

1. Research team on long-term non-tilled rice culture 1994. Investigation into the soil properties and characteristics of rice cultivation in the long-term non-tilled direct seeding rice culture. J. Agri. Sci. 49 (6): 251-256.
2. Tomihisa, Y. 1994. Current status of development on the no-till technology in direct seeded rice. Jpn. J. Crop Sci. 63 (1): 164-168.

Bioherbicidal Control of Annual Bluegrass (*Poa annua* L.).

S. IMAIZUMI, T. NISHINO, M. YAMADA and T. FUJIMORI

JT, Applied Plant Research Laboratory, Yokohama Center

Abstract. Annual bluegrass is well known as the most troublesome weed occurring in golf course and park turfs. Bentgrass greens of golf courses are especially hard hit, because chemical herbicides are not used for fear of their injuring the greens. We have looked toward developing the use of host-specific plant pathogens which can target annual bluegrass without damaging beneficial turf grasses. Exploration in the field for pathogens active against annual bluegrass has resulted in the discovery of the pathogen, *Xanthomonas campestris*. Eighty-nine strains of *Xanthomonas campestris* exhibiting strong pathogenicity against annual bluegrass were collected from 19 prefectures throughout Japan, and of these strains the strongest one, P-482, was selected. Host range testing on main turf grasses, useful agricultural plants and weeds resulted in the findings that P-482 showed no pathogenicity in any plants except for annual bluegrass, in which pathogenicity was vigorous. European turf grasses such as creeping bentgrass (*Agrostis palustris* Huds, - - Penncross, Seaside and Penneagle) and Kentucky bluegrass (*Poa pratensis*) were tolerant to P-482. Optimal cell concentration is 10^8- 10^{10} cfu/ml, while optimal spraying volume is 100-400 ml/m^2. Under these conditions over 75% control was achieved, this value reflecting contributions of both damaged and undamaged plant portions to measured average fresh weight. In pathogenicity comparisons under different temperature conditions, temperatures higher than 25°C in the day and 20°C at night enable P-482 to effectively produce heavy wilting symptoms of annual bluegrass in 7- 10 days. However, at temperatures under 15°C in the day and 10°C at night, the period required for plants to show complete die off was over 4 weeks.
In conclusion, P-482 exhibited high levels of control on annual bluegrass while leaving such essential turf grasses as bentgrass, etc., wholly unaffected.
Key words: bioherbicide, annual bluegrass, *Xanthomonas campestris*

Introduction

Annual bluegrass is well known as the most troublesome weed occurring in golf course and park turfs. Bentgrass greens of golf courses are especially hard hit, because chemical herbicides are not used for fear of herbicide injury to the greens. Golf courses thus resort to weeding by hand or, if damage is severe enough, to returfing, both of which result in untold expenses. We have looked toward developing the use of host-specific plant pathogens which can target annual bluegrass without damaging beneficial turf grasses. Exploration in the field for pathogens active against annual bluegrass has resulted in the discovery of pathogen, *Xanthomonas campestris*. Eighty-nine strains exhibiting strong pathogenicity in annual bluegrass were collected from asymptomatic annual bluegrass of 19 prefectures and 42 locations throughout Japan, and of these strains the strongest one, P-482 was selected.[1,4] Annual bluegrass artificially inoculated with the isolated P-482 developed heavy vascular wilt symptoms. This bacterium produced yellow, mucoid colonies on NA (nutrient agar 23 g/L).

This organism was identified as *Xanthomonas campestris* based on host range, chemotaxonomic tests, fatty acid methyl ester analysis and xanthomonadin production. [2,3,4] Here we report on the herbicidal activity of P-482 and the results of host range experiments.

Materials and Methods

Inoculum production

A cryovial from the refrigerator containing the inoculum of *Xanthomonas campestris* P-482 is thawed. Fifty µl of P-482 suspension is added to 2 ml of YNB medium (yeast extract 5 g/L, nutrient broth 8 g/L). This tube is placed on a rotary shaker at a speed of 200 rpm and shaken for 24-36 hrs at 28°C until a high cell density is apparent. After this pre-culture treatment, 1 ml of bacterium suspension is mixed into 100 ml of YNB and incubated for 22 hrs under 28°C temperature condition. Then bacterium suspensions are spun in centrifuge for 15 minutes at 5000 rpm. Supernatant of bacterium suspension is removed by pouring off excess, and suitable amounts of sterile distilled water are added. Bacterial suspensions are adjusted to an optical density of 0.1 at 575 nm contained 10^8 viable cells per ml for obtaining final concentration of 10^6, 10^7, 10^8, 10^9 and 10^{10} cfu/ml. Cfu means colony forming units. Accurate concentration of viable cells in a bacterial suspension is determined by dilution plate counting method. That is, for determining the number of viable cells in a preparation of *Xanthomonas campestris* cells, a dilution series is readied and small aliquots (100 µl) of diluted bacterium suspension are deposited on a

suitable medium (YNA or NA) and the number of individual colonies formed are counted after 3 days of incubation.

Methods common to herbicidal activity tests

All tests were conducted under the same condition as described below.

Annual bluegrass seeds collected at Utsunomiya were used for tests conducted in the greenhouse. Approximately 25 mg of annual bluegrass seeds were sown in a 5cm x 5cm x 5cm Jiffy pot with Akadama-Magic Soil mixtures, then about 50 seedings were selected out and subsequently placed in a greenhouse at day/night temperature of 20°C/15°C and relative humidity of 35% or above, for 3-4 weeks. Three-4 leaf plants were then selected for experimental use. Nutrient solution ('Monthly'; liquid fertilizer for turf use) was applied once a week throughout the testing period. Watering was done as needed. Five days before inoculation, all plants were moved to their respective greenhouse for temperature acclimation as shown in Table 1 (experimental design). Plants were cut with electric clippers to a uniform height of 2 cm above the pot rim prior to inoculation treatments. Inoculum tests were carried out as described in Table 1 with an aerosol sprayer, then plants were moved into greenhouses. At the time of the application volume experiment, P-482 cells were resuspended in distilled water at concentration of 10^6, 10^7, 10^8, 10^9 and 10^{10} cfu /ml. These cell concentrations were applied to experimental plots at a volume delivery rate of 50, 100, 200 and 400 ml/m^2 (Table 1).

Evaluation was rated using % control at 3 and 4 weeks after inoculation by comparison of fresh weight of entire top growth within each experiment. % control value was calculated by % reduction in fresh weight relative to uninoculated control. Mean values of six pot plants of each replication were used for rating. Acceptable or complete control of annual bluegrass is over 75% data showing plants necrosis or dead.

Table 1 Experimental design

	Temperature condition (°C day/°C night)	Bacterial concentration (cfu/ml)	Application volume (ml/m^2)
Herbicidal activity Temperature / Bacterial concentration	15 / 10 20 / 15 25 / 20	10^6 10^7 10^8 10^9 10^{10}	400
Herbicidal activity Bacterial concentration / Application volume	20 / 15	10^6 10^7 10^8 10^9 10^{10}	50 100 200 400
Host range test	25 / 20	10^9	not sprayed

Host range experiments

Ten major crop plants, 9 turf grasses and 5 strains of annual bluegrass were tested for host range experiments. Scientific and common names of plants tested were shown in Table 4 & 5. In the case of turf grasses, approximately 20- 50 seeds were planted in 5cm x 5cm x 5cm Jiffy pots containing Akadama-Magic Soil mixtures for leaf-clip inoculation. In other grasses, two to three seeds were planted in the same pots as described above. Twelve pots were used for each host range experiment. The pots were placed in the greenhouse (25°C in day / 20°C at night) and watered as needed. They were grown for approximately 2 weeks or until they were of a suitable size for inoculation. Plant test species were inoculated using two methods.

1) Turf grasses: The grass leaves were cut with scissors dipped in bacterial suspension P-482 at 10^9 cfu/ml just before the treatment. (clip inoculation) The plants were placed into dew chamber (100% RH) overnight, and then placed in the greenhouse (25°C in day/20°C in night).

2) Other plants: One drop of bacterial suspension of P-482 at 10^9 cfu/ml was spotted on leaves, after that the leaves were punctured with a bunch of sterilized needles (7-10). (needle inoculation) Dew chamber conditions were the same as above.

In each experiment six annual bluegrass pots were employed as positive control in combination with six negative control application of sterile distilled water. Plants inoculated with P-482 were rated for symptom expression 14 or 21 days after inoculation.

Results and discussion

Herbicidal activity --- Temperature and bacterial concentration

Annual bluegrass seedlings under different temperatures conditions and cell concentrations at 10^6, 10^7, 10^8, 10^9 and 10^{10} cfu/ml were used for pathogenicity comparison. Infection occurred at all temperature and all cell concentration tested. There was a distinct dose-response effect of application cell density. However, % control values increased with increasing temperature conditions over the cell range (Fig. 1 & Table 2). Annual bluegrass inoculated with P-482 showed a significant difference in control depending on the time of evaluation. Before significant disease appeared, wilting symptoms occurred at 5 to 7 days with 25°C/20°C temperature regime and over 10^8 cfu/ml cell concentration, though still kept green color. At 7-10 days after treatment, temperature higher than 25°C in the day and 20°C at night enable P-482 to effectively produce heavy necrosis of annual bluegrass under cell concentration higher than 10^8 cfu/ml. (data not shown)

Table 2 The influence of inoculation temperatures on the development of disease in annual bluegrass seedlings inoculated with different cell concentration of P-482 at 3 weeks after treatment

	Cell conc. (cfu/ml)					
Temp.	0	10^6	10^7	10^8	10^9	10^{10}
15 / 10	0	-35.1 *	-3.3	18.8	49.4	65.4
20 / 15	0	-36.4	9.3	58.8	69.4	73.2
25 / 20	0	-9.8	27.9	84.1	89.0	89.0

* : % Control

At 3 weeks after treatment, effective control values (over 75 % control) were obtained at 25°C/20°C temperature condition with cell concentration higher than 10^8 cfu/ml (Table 2).

Table 3 The influence of inoculation temperatures on the development of disease in annual bluegrass seedlings inoculated with different cell concentration of P-482 at 4 weeks after treatment

	Cell conc. (cfu / ml)					
Temp.	0	10^6	10^7	10^8	10^9	10^{10}
15 / 10	0	22.7	39.4	83.2	87.0	84.0
20 / 15	0	17.7	60.0	85.5	93.8	95.8
25 / 20	0	5.6	56.1	88.0	91.2	93.3

* : % Control

Table 3 indicated at all temperature regime and cell concentration higher than 10^8 cfu/ml, the period required for plants to show dieoff was over 4 weeks. Thus significantly progressed and increased disease expression was observed when inoculated plants were held in a 25°C / 20°C regime compared to those held at 15°C/10°C. As shown in the Table 2, 3 and Fig.1, the effects of P-482 at low temperature (15°C/10°C) conditions application can overtake them at middle (20°C/15°C) and high (25°C/20°C) temperature conditions after approximately one month. However, this sort of slow decline of the target weed is probably ideal for many turf settings since the desired grass will have time to fill in without generating obvious bare zones controlled by P-482 application.

Herbicidal activity----Bacterial concentration and application volume

At low cell concentration as 10^6 cfu/ml, application volume affect to % control values directly (Fig.2). A combination of 10^7-10^9 cfu/ml cell concentration and 50 -100 ml/m^2 application volume reduces P-482 effectiveness; however, 200-400 ml showed increasing symptom developments. The plants inoculated with 10^{10} cfu/ml demonstrated the same control data at all application volume regimes. Acceptable control values over 75% were obtained at combinations of cell concentration and application volume; 10^8 cfu/ml -400 ml/m^2 and 10^9 cfu /ml-100 ml/m^2 at 4 weeks after the treatment. Therefore optimal cell concentration is 10^8-10^{10} cfu/ml, while optimal application volume is 100-400 ml/m^2. This level of application volume of 100-400 ml/m^2 is similar to chemical herbicide use.

Host range experiments

Host range tests were conducted on 11 turf grass species, 9 cultivated crops and 5 annual bluegrass (Table 4 & 5). All 5 populations of annual bluegrass were severely infected (Table 4). Of the species evaluated, the host range of this bacterium, as determined by clip or needle inoculations, was limited to annual bluegrass and rough bluegrass (*Poa trivialis*). These data showed that only annual bluegrass was

severely infected; however, rough bluegrass infection was light, and there was no spread to emerging uninoculated leaves. The marginal susceptibility of *Poa trivialis* would probably preclude significant infection, even within a mowed area. Rough bluegrass is not important for use on Japanese turf. Application to European turf grasses such as creeping bentgrass (*Agrostis palustris* Huds, cultivars of Penncross, Seaside and Penneagle) and Kentucky bluegrass (*Poa pratensis*) were tolerant to P-482.
P-482 exhibited high levels of control on annual bluegrass while leaving such essential turf grasses as bentgrass, etc., wholly unaffected. No symptoms were observed on any of the major crop species by inoculation with needles dipped in bacterial suspension (Table 5). In conclusion, this organism is indigenous and widely distributed throughout Japan. It has been isolated in major geographic regions of this country. This organism is highly specific to annual bluegrass. Host range studies on 19 cultivars of 17 grass species and major crop plants have shown no susceptibility. The host specificity of *Xanthomonas campestris* P-482 makes it an excellent candidate as a biocontrol agent for annual bluegrass, especially in the area bentgrass, Kentucky bluegrass and other cool season grasses are the desired turf.

This biocontrol agent can provide a tool which is needed to the turf manager for control of annual bluegrass.

Table 4. Results of host range testings -- turf grass and annual bluegrass.

common name	cultivar	species scientific name	susceptibility P-482
annual bluegrass	Utsunomiya	*Poa annua*	++
	Atsugi		++
	America- annual		++
	America-perennial		++
	England		++
rough bluegrass	Colt	*Poa trivialis*	+
Kentucky bluegrass	Merit	*Poa pratensis*	-
Canadian bluegrass	Ruebens	*Poa compressa*	-
creeping bentgrass	penncross	*Agrostis palustris*	-
	penneagle		-
	seaside		-
bermuda grass		*Cynodon dactylan*	-
tall fescue	ERA	*Festica elatior*	-
perennial ryegrass		*Lolium perenne*	-
timothy grass	Senpoku	*Phleum pratense*	-
zoysia grass	Kourai	*Zoysia japonica*	-

++: highly susceptible, most plants wilting or dead.
+ : slightly susceptible, a few plants wilting.
- : non-susceptible, no evidence of disease.

Table 5. Results of host range testings -- major crop plants

common name	cultivar	species scientific name	susceptibility P-482
rice	koshihikari	*Oryza sativa*	-
wheat	Chihoku	*Tritucum aestivum*	-
barley	Kashimamugi	*Hordeum vulgare*	-
bean	Turunashisujinashi	*Phaseolus vulgaris*	-
soybean	sapporomidori	*Glycine max*	-
cabbage	Teruterumaru	*Brassica oleracea*	-
tomato	Houkin-2	*Lycopersicum esculentum*	-
sweet corn	Peter corn	*Zea mays*	-
pepper	Togenashi	*Capsicum annuum*	-

++: highly susceptible, most plants wilting or dead.
+ : slightly susceptible, a few plants wilting.
- : non-susceptible, no evidence of disease.

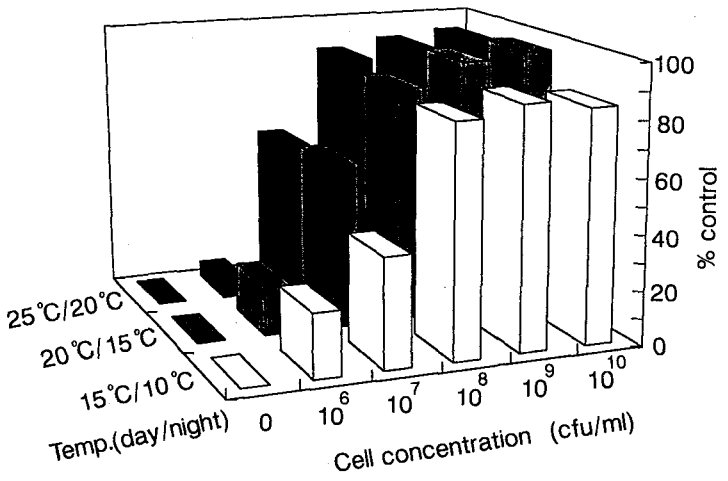


Fig.1 Relation of cell concentration and temperature at 4 weeks after treatment.

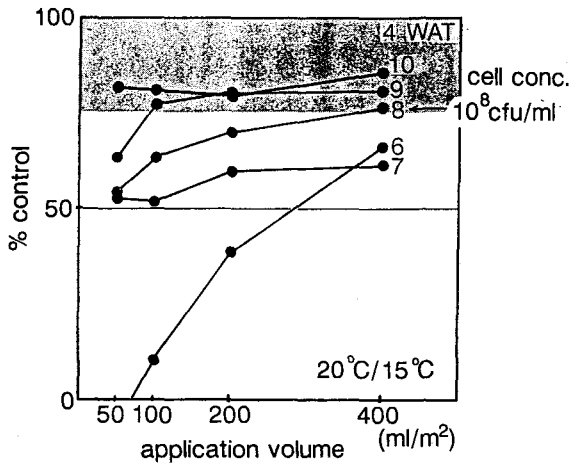


Fig.2 The influence of cell concentration and application volume on the extent of control (%) at 4 weeks after treatment.

References

1) Imaizumi S., R. Houkabe, T. Nishino, Y. Omigawa and M. Yamada 1994. Control of annual bluegrass with bioherbicide. 1) Efficacy testing on weed control of annual bluegrass with a bacterium, *Xanthomonas campestris*. Weed Research, Japan 36, 40-41. (Abstract)
2) Miyabe K., S. Imaizumi, M. Goto and Y. Takigawa 1993. Bacterial wilt of *Poa annua* caused by a pathovar of *Xanthomonas campestris* in Japan. Ann. Phytopath. Soc. Japan 59, 309. (Abstract)
3) Nishino T., S. Imaizumi and M. Yamada 1994. *Xanthomonas campestris* pv. "*poannua*" isolated from symptomless annual bluegrass (*Poa annua*). Ann. Phytopath. Soc. Japan 60, 366. (Abstract)
4) Nishino T. et. al (in press)

STUDY OF ECOLOGICAL MANAGEMENT OF WEEDS IN A WHEAT (BARLEY) FIELD

Tang Hong Yuan, Wang pei Zhong, Wang Xue E, Sheng Guo Hua

Shanghai Academy of Agricultural Sciences, Shanghai, 201106 China,

A field plot test resulted: a) High yielding barley varieties 89-218, 93-1758 and 93 - 1695 effectively controlled *Malachium aquaticum* with an efficacy of 77.8%, 89% and 97.4% compared with wheat 92-01 as check; b) Increasing seed quantity in sowing makes higher crop density, and hastens the germination of seeds, thus enhances crop competition ability to inhibit weeds with efficacy up to 50.5%-66%; c. Ploughing and cultivating before sowing and planting crop kills emerging weeds with an efficacy up to 54%;. d) Adopting a rice-wheat (barley) -cotton-rape cropping system controls weeds with an efficacy of 50-90% compared with the wheat-rice system. Weeds were managded effectively by adopting good crop varieties, cultural practices and crop systems.
Key Words: weed, Ecological management

INTRODUCTION

The farmer have accumulated a lot of experence of cultural practice for weed control in long history of agricultural practice. It was opening the new page that herbicide is using for weed control. That is a high effcacy and modernization measure. But some problem was founded in practce recently, such as weed population shifing, herbicide resistence and pollution problem. So weed integrated management are developed by weed scientist. This paper reports a no-chemical method weed control.

MATHODS AND MACTERIALS

Small plot test: 1, Iligh yielding, more tillages and broad-leafed varities barley 89-218, 93-1736, 93-1695 and Shanghai 10, compared with wheat 92-01 as a check were adopted. Seeds were sowed in same time and seed rate. Weed competition ability was surveied among the different varities. 2, Wheat 92-01 varity was adopted in the tests sowed in different time, seed rate and cultural practice. All tests were made in same field. Weed

distribution in field is even.. The major weeds are *Alopecurus aequalis and Malachium aquaticum*. The experiment was laidout in a complete randomized block design, each plot is 20 sqare meter, replicated three times. Weed and crop were sampled in 11. Fab. 21. Mar. and havest before. Each plot is 0.11 sqare meter.

RESULTS AND DISCUSSION

The test showed that, The high yielding barley varities 93-1696, 89-218, 93-1768 and Shanghai 10 control weeds with an efficay of 55.6%, 60.6%, 49.2% and 40.7% respectively, particular controlling *Malachium aquaticum* with an efficacy of 87.6%, 77.8%, 82.7% and70.6% , respectively (fig 2) . The data showed that barley varities are stronger weed competition than wheat, but while little difference was found between them at early stage until begain of March (fig 1). The results can be explained that, the barley makes more tillage of 68.3%, leaf-broaded 5.8- 26. 6% and shaded rate of 21-32% than wheat (fig 2).

Increasing seed rate makes higher crop density and hastens the seed germination, thus enhances the crop competition ability to inhibit weeds. The data showed that It gave 41% and 65% control of weeds in 3 Millions/ha, and 4..5 Millions/ha compare with I.5 Millions whaet plants in field. particular controlling dwarf weed, such as *Poa annual*, *Lapsana apogonoides* and creeping weeds like *Malachium aquaticum* with efficacy up to 51-81% (fig 3, table 1, 2).

Tabe 1 Comparision of weed control under different crop density

weeds density	wheat density plant/0.11m²	control g/o.11m²	efficacy%	yield kg/ha
Alopecurus	50	31.7	-	2100
aequalis	75	18.7	41.0	3618
100/0.11m²	100	13.3	58.4	4239
handing weed	77	2.4	92.5	4845

compare with 50 plant weeds/0.11m²

table 2 Comparision of weed control in different seed rate

weeds	wheat seed rate 150kg/ha g/0.44m²	wheat seed rate 300kg/ha g/0.44m²	efficacy %
Poa annual	301.3	111.7	62.9
Alopecurus aequalis	71.1	58.6	17.58
Malachium aquaticum	28	5.3	81.0
Lapsana apogonoides	35	17	51.85
total	435.4	240.3	55.1

compare with 150kg/ha

Ploughing and cultivating before sowing and planting crop kill emerged weeds with an efficacy up to 54%(fig 4).

Adopting a rice -wheat(barley)-cotton-rape cropping system controls weeds with efficacy up to 50-90% , compared with rice -wheat continually. Weeds were managed effectively by adopting good crop varieties ,cultural practices and crop systems.

REFERENCE

1, Tang II.Y. 1987
field weed control

2, J.F. Ahrens 1989
The role of govement in economic weed management 12th PAWSS conf.

3, E.M.castin and K. Moody 1989
Effect of different seedling rate, moisture regimes and weed control treatment on weed growth and yield of wet-seeded rice 12th PAWSS conf.

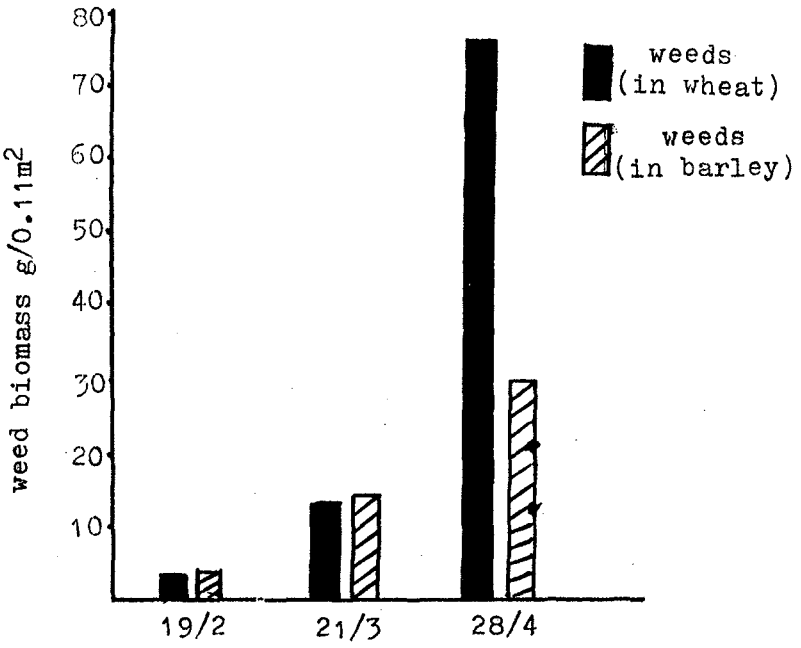


fig 1 comparision of weed growth in different stage

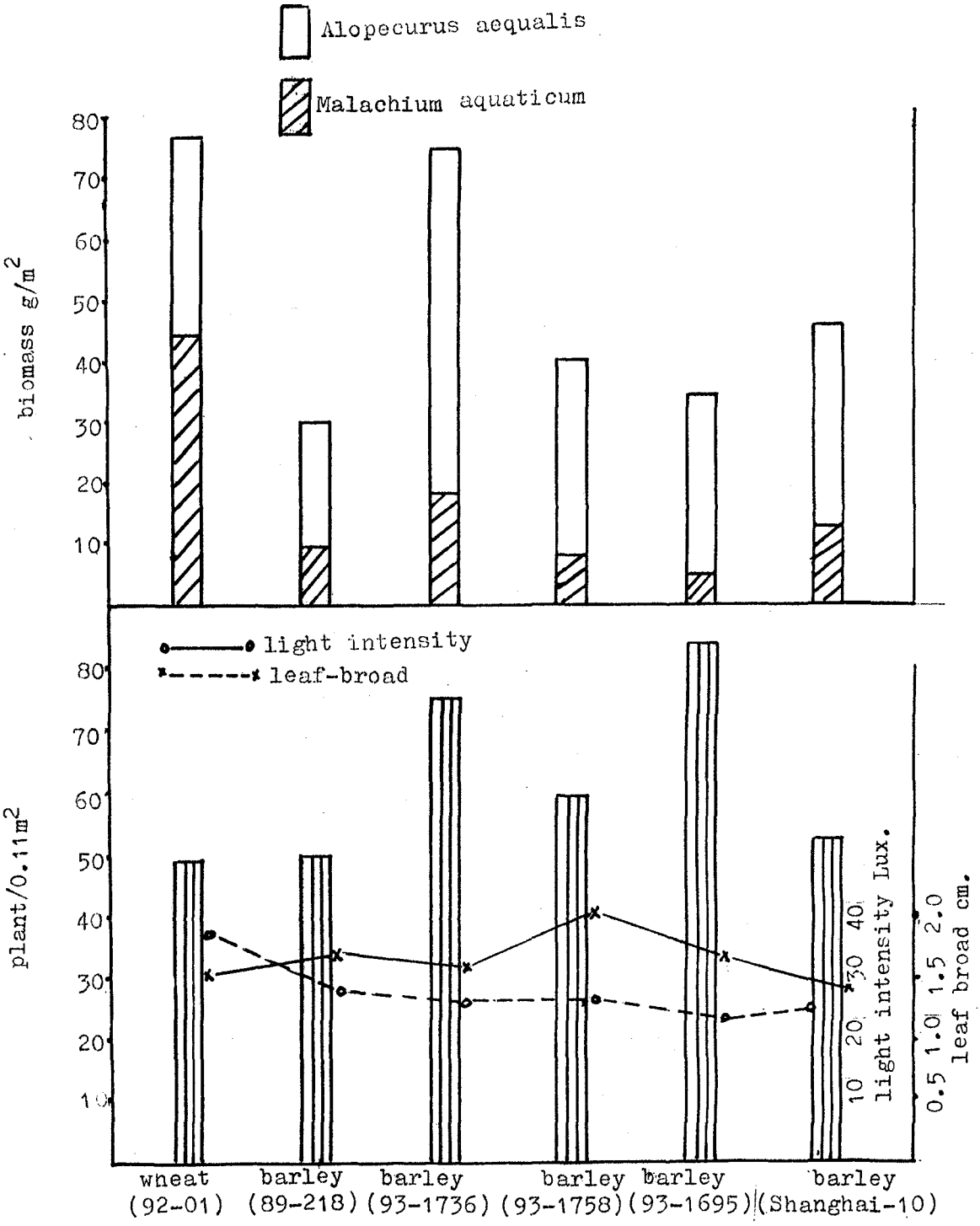


fig 2 Comparision of weed infestation in different varities

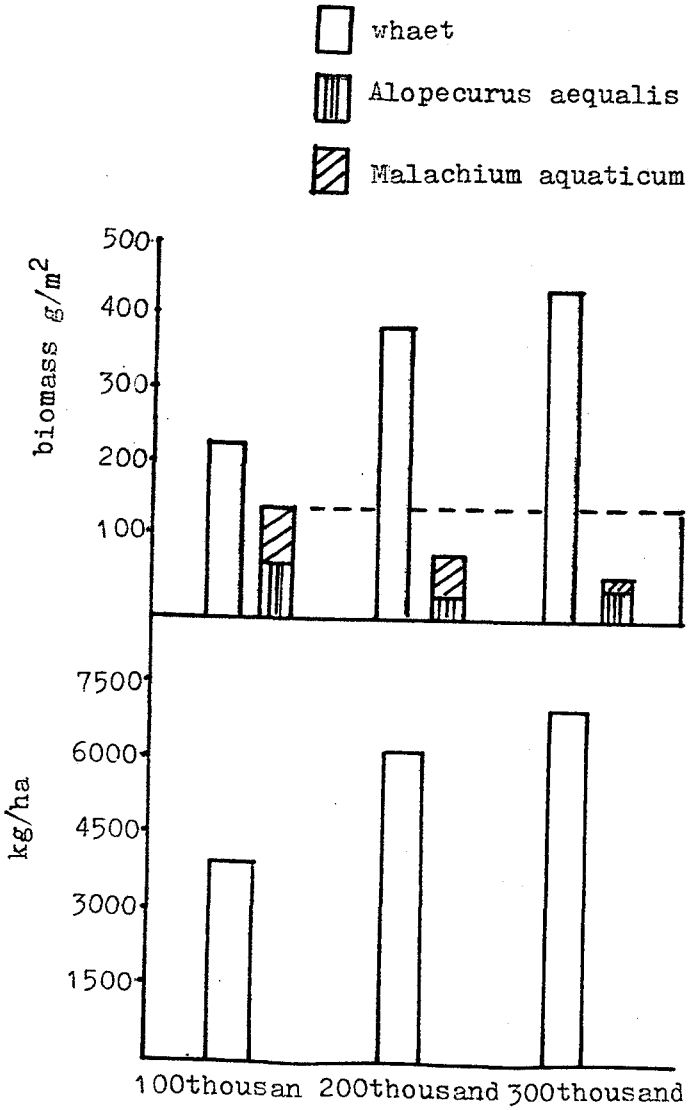


fig 3 Comparision of weed control in wheat under different cultural practices

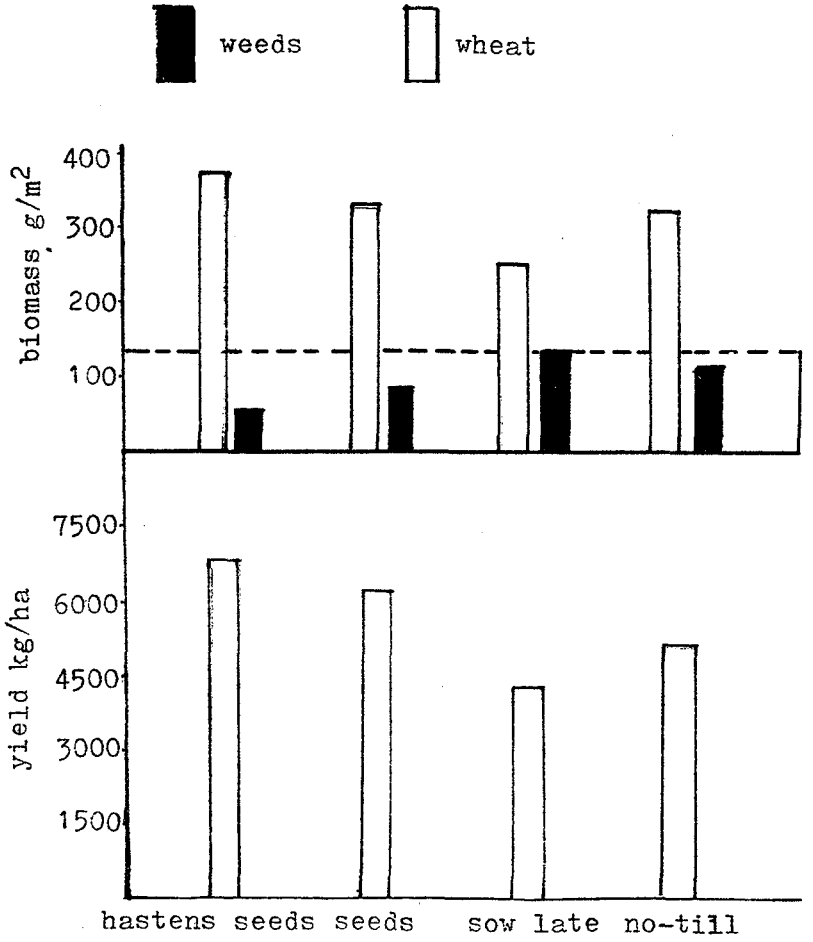


fig 4 Comparision of weed control and crop yield in different agricultural practices

INTEGRATED WEED MANAGEMENT IN GROUNDNUT (Arachis hypogaea L.) INTERCROPPED WITH PIGEONPEA (Cajanus cajan L.) UNDER RAINFED CONDITION

A.N.Tewari* and K.S.Rathi
Chandra Shekhar Azad University of Agriculture and Technology
Kanpur 208002,U.P.,(India)

Abstract:Investigations were carried out to develop integrated weed management technology in groundnut + pigeonpea intercropping during two consecutive years (1993 & 1994) at Kanpur in Gangetic plains of Northern India. Pendimethalin (1.0 kg/ha), metolachlor (1.5 kg/ha), alachlor (1.5 kg/ha), and oxyfluorfen (0.10 kg/ha) alone as well as supplemented with a single hand weeding were evaluated and compared with weedy check and two manual weedings. Weedy condition prevailing throughout the crop period caused reduction in groundnut, pigeonpea and groundnut equivalent yields to the tune of 56.44%, 41.98% and 52.53%, respectively. Herbicides prevented weed emergence till 30-35 days after sowing and thereafter weeds began emerging. Pendimethalin manifested excellent control of associated weeds including Trianthema monogyna, but was ineffective against Commelina benghalensis. Integration of a single hand weeding with pendimethalin (1.0 kg/ha) realized 544 kg/ha (28.30%) more groundnut equivalent yield than its sole application and was at par with two manual weedings. Oxyfluorfen and metolachlor could be considered as next to pendimethalin.

Key words:Integrated weed management, groundnut, pigeonpea, intercropping

Introduction

Groundnut (Arachis hypogaea L.) being a predominant rainfed rainy season crop is traditionally intercropped with pigeonpea (Cajanus cajan L.) in large area of semi-arid tract of India. The slow growing nature of both the crops in the intercropping system coupled with congenial climatic conditions prevailing during rainy season favours the rank and profuse growth of weeds particularly during early stage of the crop. Seasonlong weed competition caused 43.60% and 30.91% reduction in the pod yield of groundnut and grain yield of pigeonpea in groundnut + pigeonpea intercropping system in central tract of Uttar Pradesh at Kanpur (Tewari,1987).

The advent of new broadspectrum herbicides has made it possible to use them for weed control in intercropping.However, there is hardly any chemical which could control all types of associated weeds effectively. Further, available soil applied herbicides like pendimethalin, alachlor etc. have short term control especially during rainy season and late emerging weeds are often escaped. It is thus imperative to use herbicides supplemented with manual weeding as per need. Since groundnut + pigeon

pea intercropping system is gaining great importance in Indian semi arid under rainfed condition and weeds are considered as one of the important factors limiting the productivity of component crops, the present investigation was undertaken.

Materials and Methods

A field experiment was conducted at the Students' Instructional Farm of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (India) during rainy season of 1993 and 1994 under rainfed condition. The soil of the experimental field was sandy loam in texture, low in organic carbon and medium in phosphorus and potassium. The soil reaction is slightly alkaline. The crops received 604 mm and 814 mm rainfall during 1993 and 1994, respectively. Two irrigations were given during the period of dry spell when required. The efficacy of pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine), metolachlor (X-chloro-6- ethyl-N-(2-methoxy-1-methylethyl)-o- acetotoluidide), alachlor(x-chloro 2,6-diethyl-N-(methoxymethyl) acetanilide) and oxyfluorfen (2-chloro-4-trifluoromethylphenyl 3-ethoxy-4-nitrophenyl ether) was evaluated with and without one hand weeding and was compared with weedy check and manual weeding twice (20 and 40 days stage) in a three replicated randomized block design. All the herbicides were applied as pre-emergence spray using knapsack sprayer fitted with flood jet nozzle delevering about 500 L/ha. Nutritional requirement was met by supplying 15 kg N/ha, 30 kg P_2O_5/ha and 45 kg K_2O/ha to groundnut and 18 kg N/ha and 46 kg P_2O_5 / ha to pigeonpea. The sowing of groundnut and pigeonpea was done at row proportion of 5:1 during first week of July and harvested on respective maturity. Weed control efficiency, groundnut equivalent, net profit and return/rupee invested were calculated with the help of following formulae:

$$\text{Weed control efficiency} = \frac{\text{Dry weight of weeds in weedy check} - \text{Dry weight of weeds in treated plot}}{\text{Dry weight of weeds in weedy check}} \times 100$$

$$\text{Groundnut equivalent} = \frac{\text{Grain yield of pigeonpea (q/ha)} \times \text{Market rate of Pigeonpea (Rs/ q)}}{\text{Market rate of groundnut(Rs/q)}}$$

Net profit= Additional income due to weed control-Cost of weed control

$$\text{Return/rupee invested} = \frac{\text{Net profit (Rs/ha)}}{\text{Cost of weed control(Rs/ha)}}$$

Results and Discussion

Weed flora:

Weed composition consisted of Trianthema monogyna L., Commelina benghalensis L., Digera arvensis Forsk, Phyllanthus niruri L.; Echnochloa colona (L.); Link; Dactyloctenium aegyptium (L.) Beauv., Cyperus rotundus L. The overall intensity of weeds was58.88%, 11.20% and 29.90% during 1993 and 40.32%,7.76% and 51.90% during 1994 under broadleaves,grassy & sedges,respectively

Effect on weed control:

Weed control treatments showed significant reduction in weed population (Table 1). Pendimethalin(1.0 kg/ha) prevented the emergence of most of the broadleaved and grassy weeds significantly including T. monogyna which was an aggressive weed to the extent of 62.65 per cent. These results are in conformity with those of Vijay Kumar (1994) who reported 40-50 per cent control of this weed with the application of pendimethalin (1.0 kg/ha). However, this herbicide failed to control C. benghalensis. Metolachlor (1.5 kg/ha) , alachlor (1.5 kg/ha) and oxyfluorfen (0.10 kg/ha) proved effective in reducing the associated grassy and broadleaved weeds significantly. Removal of weeds manually in treated fields eliminated weed competition considerably. The herbicidal application did not show consistent results in reducing C. rotundus.

The effect of weed control treatments on dry matter of weeds was found significant on pooled basis (Table 1). Highest dry matter accumulation was estimated in unweeded (2083 kg/ha)plot. Manual weeding done at 20 and 40 days after planting minimised weed production (287 kg/ha) resulting in maximum weed control efficiency (86.22%). Herbicides brought down dry matter of weeds significantly. Pendimethalin (1.0 kg/ha), alachlor (1.5 kg/ha) and oxyfluorfen (0.10 kg/ha) were at par in weed production on dry matter basis. Differences between metolachlor (1.5 kg/ha) and pendimethalin were found non significant. The overall weed control efficiency ranged from 55.11-68.41 per cent due to application of various herbicides. The efficiency of herbicidal application was further enhanced when single hand weeding was employed which brought down the dry matter production substantially.

Yield of component crops

Groundnut, pigeonpea and groundnut equivalent yield are presented in Table 2.

Groundnut:

The pod yield of groundnut was significantly influenced due to application of weed control treatments on pooled basis. Unchecked weed growth caused 56.44 per cent reduction in pod

Table 1: Density of weeds/m2 and dry matter of weeds (kg/ha) recorded at 75 days after sowing (Pooled mean of 1993 and 1994)

Weed control	Weed density/m2							Dry matter (kg/ha)	Weed control efficiency (%)
	Broadleaved				Grassy		Sedges		
	T. monogyna	C. benghalensis	D. arvensis	P. niruri	D. aegyptium	E. colona	C. rotundus		
Weedy check	8.46 (73.34)	4.45 (20.36)	4.60 (23.14)	6.45 (47.21)	3.16 (9.25)	5.78 (26.84)	12.08 (165..72)	2083	---
Manual weeding twice	3.67 (17.85)	1.67 (2.77)	1.67 (2.77)	2.30 (5.55)	1.00 (0.00)	1.00 (0.00)	9.13 (93.50)	287	86.22
Pendimethalin (1.0 kg/ha)	2.51 (8.33)	4.60 (21.29)	2.80 (9.25)	3.05 (12.03)	1.82 (3.70)	1.00 (0.00)	12.05 (187.94)	778	62.65
Pendimethalin (1.0 kg/ha) Fb One hand weeding	1.82 (3.70)	1.76 (2.77)	1.25 (0.92)	1.51 (1.85)	1.00 (0.00)	1.00 (0.00)	9.75 (107.39)	361	82.66
Metolachlor (1.5 kg/ha)	4.05 (24.99)	1.00 (0.00)	2.73 (8.32)	2.46 (6.47)	2.00 (1.85)	3.45 (13.88)	10.02 (106.47)	658	68.41
Metolachlor (1.5 kg/ha) Fb One hand weeding	3.08 (12.96)	1.00 (0.00)	1.77 (2.77)	1.79 (3.70)	1.51 (1.85)	1.00 (0.00)	8.01 (75.91)	352	83.10
Alachlor (1.5 kg/ha)	5.71 (37.95)	2.84 (9.25)	2.30 (5.55)	3.01 (11.11)	1.00 (0.00)	2.18 (4.62)	11.66 (151.83)	935	55.11
Alachlor(1.5 kg/ha)Fb One hand weeding	3.67 (16.64)	1.53 (2.77)	1.25 (0.92)	2.15 (5.55)	1.00 (0.00)	1.00 (0.00)	10.14 (115.72)	454	78.20
Oxyfluorfen (0.10 kg/ha)	2.90 (12.03)	3.74 (15.73)	2.61 (7.40)	1.94 (4.62)	1.51 (1.85)	2.59 (10.18)	11.30 (174.97)	935	55.11
Oxyfluorfen (0.10 kg/ha) Fb One hand weeding	2.55 (8.33)	2.08 (4.62)	1.25 (0.92)	1.92 (3.70)	1.00 (0.00)	1.82 (3.70)	9.69 (128.68)	398	80.89
C D at 5%	1.45	1.26	1.39	1.71	NS	0.86	1.96	268	--

Original values in parenthesis;Transformed value $\sqrt{X+1}$; Fb indicates Followed by

yield. Herbicides brought about significant increase in groundnut pod yield over unweeded (control) plot. Integration of single hand weeding with pendimethalin (1.0 kg/ha) and metolachlor (1.5 kg/ha) resulted significant increase over sole application of herbicides. Pendimethalin (1.0 kg/ha) followed by single manual weeding recorded similar pod yield to that obtained under manual weeding twice done at 20 and 40 days stage. The need of one hand weeding after 30-35 days of sowing alongwith the herbicides in groundnut was also reported by Vijay Kumar(1994).

Pigeonpea:

Pooled data revealed significant effect on grain yield of pigeonpea dueto application of weed control treatments. Weed competition reduced the grain yield to the tune of 41.98 per cent. Pendimethalin (1.0 kg/ha) and metolachlor (1.5 kg/ha) recorded significantly higher grain yield than that obtained under weedy check. Integration of single hand weeding with these herbicides produced similar grain yield to that recorded in hand weeded twice. However, the quantum of increase due to addition of single hand weeding with pendimethalin (1.0 kg/ha) and metolachlor (1.0 kg/ha) was insignificant compared to sole application of herbicides. The effect of single hand weeding with alachlor (1.5 kg/ha) and oxyfluorfen (0.10 kg/ha) was found promising.

Groundnut equivalent:

Weed control treatments placed significant effect on groundnut equivalent yield. Reduction in grain yield to the tune of 52.53 per cent was noted when weeds were allowed to grow as such. Pendimethalin (1.0 kg/ha), metolachlor (1.5 kg/ha),alachlor (1.5 kg/ha) and oxyfluorfen (0.10 kg/ha) increased the groundnut equivalent significantly over weedy check and the magnitude of increase was 57.01, 50.32, 30.09 and 39.96 per cent, respectively. Each herbicides followed by single hand weeding recorded perceptible increase in grain yield compared to sole application of herbicides. Pendimethalin (1.0 kg/ha) followed by single hand weeding produced groundnut equivalent yield at par to that obtained under hand weeded twice.

Economic return:

All the herbicides fetched handsome net monetary returns due to weed control (Table 2). The highest net profit was realised with manual weeding twice (Rs. 16855 per hectare) followed by pendimethalin (1.0 kg/ha) + one hand weeding (Rupees 15771 per hectare). However, return per rupee spent was more (5.57) with pendimethalin + one hand weeding than manual weeding twice (4.81). The alternative herbicide was considered to metolachlor (1.5 kg/ha) + one hand weeding which registered a net profit of Rs. 11515 per hectare with return per rupee invested of 3.87. This indicated that although manual weeding was effective in

Table 2: Groundnut, pigeonpea and groundnut equivalent yield recorded under different weed control treatments (Pooled mean of 1993 and 1994)

Weed control	Yield of component crops(kg/ha)		Groundnut equivalent (kg/ha)	Cost of weed control (Rs/ha)	Income due to weed control (Rs/ha)	Net profit (Rs/ha)	Return per rupee invested	Labour required/ ha
	Groundnut	Pigeonpea						
Weedy check	821	579	1226	--	--	--	--	--
Manual weeding twice	1884	998	2583	3500	20355	16855	4.81	100
Pendimethalin (1.0 kg/ha)	1300	888	1925	1534	10485	8951	5.83	3
Pendimethalin(1.0 kg/ha) Fb One hand weeding	1766	1000	2466	2829	18600	15771	5.57	23
Metolachlor(1.0 kg/ha)	1267	823	1843	1680	9255	7575	4.50	3
Metolachlor(1.0 kg/ha)Fb One hand weeding	1536	936	2192	2975	14490	11515	3.87	25
Alachlor(1.5 kg/ha)	1129	666	1595	1155	5535	4380	3.79	3
Alachlor(1.5 kg/ha) Fb One hand weeding	1313	830	1894	2450	10020	7570	3.08	31
Oxyfluorfen(0.10 kg/ha)	1242	677	1716	650	7350	6700	10.30	3
Oxyfluorfen (0.10 kg/ha) Fb One hand weeding	1434	844	2025	1944	11985	10041	5.16	27
CD at 5%	254	135	272	-	-	-	-	-

Fb indicates followed by

controlling weeds and gave a little higher net monetary return but return per rupee spent was comparatively low. The use c pendimethalin(1.0 kg/ha) integrated with single hand weeding a reduced number of labour i.e., 23 per ha proved labour savin effective, economical and stable proposition to manual weedin twice especially where labours are costlier and scarce. The othe herbicides though effective but fetched comparatively low income In case of oxyfluorfen, return per rupee invested was quite hig (10.30) but net income was low (Rs. 6700 per hectare). Highe profit per rupee invested on weed control (Rs. 10.7-26.6/Re) a compared to manual weeding (Rs. 6.8-9.6/Re) was also reported b Subbaiah and Nanjappa (1994) in groundnut based cropping syste under rainfed condition.

References

Subbaiah,H. and Nanjappa,H.V.(1994). Economics of chemical wee control under different cropping systems in rainfed groun nut (Arachis hypogaea L.), World Weeds 1(1): 33-39.

Tewari,A.N.(1987). Development of low cost weed management tech nology in legumes/oilseeds based intercropping system Final Technical Report (1985-87) submitted to India Council of Agricultural Research, Deptt of Agronomy C.S.Azad Univ. of Agric and Tech., Kanpur ,India

Vijay Kumar,B.; Anand Reddy,K.& Reddy Nageswara,M. (1994). Chemi cal control of horse purslane (Trianthema portulacastrum) and barn yard grass(Echinochloa crusgalli) in groundnut (Arachis hypogaea L.) ,Indian J. Agron.39(3):491-93.

Germination Characteristics of Horehound (*Marrubium vulgare*) Seed

F. Dastgheib* and R.J. Field

Department of Plant Science, P.O.Box 84, Lincoln University, Canterbury, New Zealand

Abstract. Seed germination of horehound (*Marrubium vulgare* L.) was studied using fresh or 8 year-old seeds. Young seeds had higher germination than old seeds and germinated equally well under dark or light conditions at 25° C, while old seeds showed a positive response to light. Freshly harvested seeds showed a strong response to nitrate, but germinated well without nitrate after 110 days of dry storage. The effect of soil moisture, nitrogen and seed placement depth on seed germination and emergence was studied. Emergence of current year horehound seeds was less than 5% at 25% field capacity of soil regardless of nitrogen level or seed depth. At field capacity, germination of seeds placed on the soil surface was better with nitrogen. Seeds placed at 5 mm depth had lower emergence initially regardless of nitrogen level, but showed a positive response to nitrogen with time. The results are discussed in relation to the opportunities for management of the horehound seed bank and its implication for control of the weed.

Keywords. dormancy, nitrate, seed germination and emergence, seed longevity, weed seed.

INTRODUCTION

Horehound (*Marrubium vulgare* L.) is a pubescent, herbaceous perennial from the *Lamiaceae* family. It is a native of Europe, Asia and North Africa and is widely spread in North America (Young and Evans 1986). Horehound is declared a noxious weed in several states of Australia where it invades farmland and pastoral areas (Carter, 1990; Parsons and Cuthbertson, 1992). In New Zealand, horehound grows initially on sheep camps, along fence lines and in waste areas and encroaches lucerne (*Medicago sativa* L.) pastures. It's reproduction is by seed enclosed in a tubular, persistent calyx which attaches to sheep's wool and reduces it's quality. An understanding of seed biology helps the design of effective weed management strategies. Therefore, a study of horehound seed was undertaken and aspects of seed germination and seedling emergence are reportede.

MATERIALS AND METHODS

Germination experiments

Two seed lots were used; 8 year-old seeds harvested in 1986 and current season seeds harvested in April 1994. Seeds were stored dry at room temperature. All experiments were conducted in 1994. Seed germination tests were carried out in incubators with light, at 25°C constant temperature as described previously (Dastgheib and Field, 1994). For the dark treatments, petri dishes containing seeds were wrapped in black plastic bags and were inspected for germination weekly under a green light. Seeds were placed in petri dishes on filter paper moistened with either de-ionised water or 10 mM potassium nitrate solution. Chilling treatment was performed by keeping the petri dishes at 4°C for a period of four weeks.

Emergence experiment

Germination and emergence of horehound seeds were studied by placing 25 current-season seeds in 40 mm diameter pots filled with a low nitrogen (0.2% N) silt loam soil at depths of 0 or 5 mm. Nitrogen was added at a rate of 400 kg N/ha by adding a solution of urea to half of the pots at the start of the experiment. Pots were maintained in a controlled environment cabinet at 12 h daylength, 300 μ mol/m^2/s light intensity and day/night temperature of 20/10°C. Watering treatment was performed by daily weighing of the pots and adding water to either field capacity or 25% field capacity.

Experimental design and data analysis

All experiments were laid out in completely randomised designs with three to five replicates and were repeated at least once. Analysis of variance was performed on percentage values and on square root transformed data. As the results were similar, the means of the original data are presented.

RESULTS AND DISCUSSION

Table 1 shows the effect of chilling, nitrate and storage on germination percentage of old and young seeds. Chilling had no significant effect on germination percentage of young seeds, but reduced the germination percentage of old seeds. Other reports show the positive effect of chilling on germination of horehound seed which may be a result of variation among sources of seed or seed age (Stritzke, 1975; Young and Evans, 1986; Dastgheib and Field, 1994).

Addition of potassium nitrate resulted in a slight but significant increase in germination of old seeds irrespective of chilling treatment (Table 1). Freshly harvested seeds showed a much greater response to nitrate compared to the old seeds. There was a decrease in the magnitude of the response to nitrogen with storage time for fresh seeds and after 110 days of storage seed germination was equally high with or without nitrate. The increase in germination percentage with storage observed here is similar to the results reported by Stritzke (1975) who found that three months of dry storage at 26°C increased the germination of horehound seed to 80%.

Table 1. Percentage germination of old and young horehound seeds affected by storage, chilling and potassium nitrate, 24 days after incubation.

		Nitrate (mM)	
Seed age	Chilling	0	10
8 years	-	46.4	56.8
	+	30.4	40.8
$LSD_{.05}$		9.45	
16 days	-	8.0	77.6
	+	2.4	70.8
50 days	-	60.8	84.0
80 days	-	36.8	85.6
110 days	-	80.8	74.4
$LSD_{.05}$		15.60	

Irrespective of light treatment, young seeds germinated faster than old seeds and had a greater germination percentage at the final count (Figure 1). Old seeds showed some response to light and the magnitude of the response was greater with incubation time. Germination of young seeds was high in the dark and there was no increase in germination percentage with light (Figure 1).

Emergence of young horehound seeds was affected by soil nitrogen, soil moisture and seed placement depth (Table 2). Emergence did not exceed 5.3% at 25% field capacity. At field capacity there was a significant increase in emergence of seeds placed on the soil surface with additional nitrogen. Seeds placed at 5 mm depth had a much lower emergence irrespective of nitrogen treatment. As seed germination was very low at 25% field capacity, pots from the two watering regimes were paired and were watered to 75% field capacity. Plant count three months after sowing showed that seeds placed at 5 mm depth were able to emerge when they received adequate moisture (Table 2). However, the percent emergence of seeds placed on the soil surface was greater at both nitrogen levels. Addition of nitrogen resulted in an increase in emergence at both seed depths.

Table 2. Percentage emergence of horehound seeds placed at different soil depths under varying nitrogen and watering treatments one and three months after sowing.

Nitrogen	Seed depth	One Month.		Three months
(kg N/ha)	(mm)	F.C.[1]	25% F.C.	75% F.C.
0	0	49.3	4	44
	5	17.3	5.3	22
400	0	77.3	0	78
	5	24	4	48
$LSD_{.05}$		14.60		15.75

[1] Field capacity

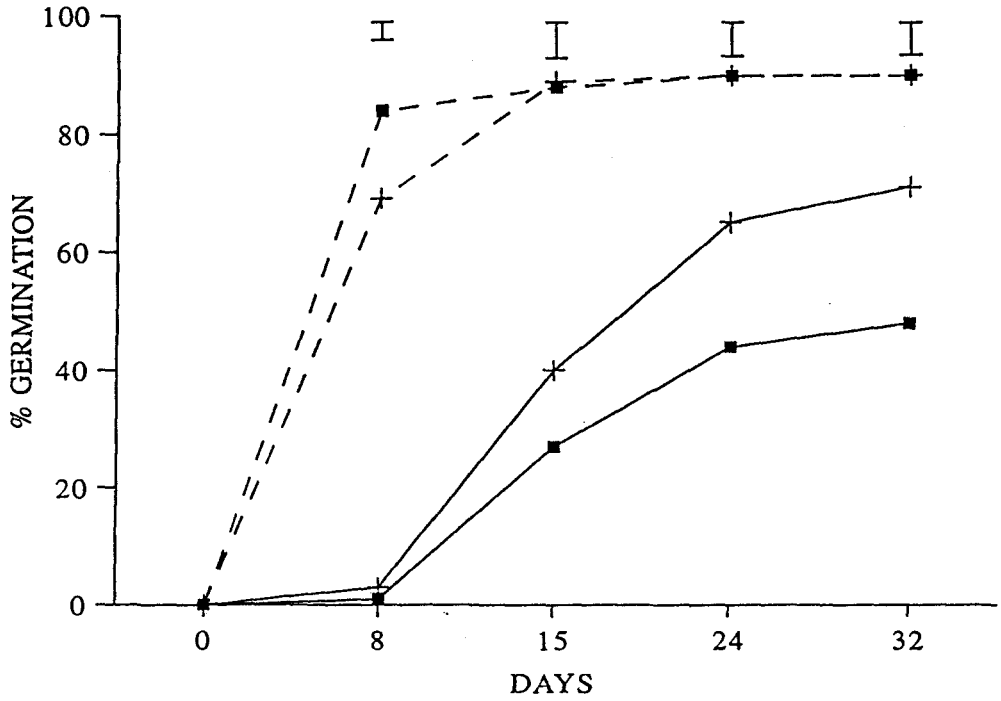


Figure 1. Germination of old (solid lines) and young (broken lines) horehound seeds kept under dark, (■) or light (+) conditions. Error bars are $LSD_{.05}$.

Positive effect of nitrogen on seed germination and seedling emergence of horehound (Tables 1 & 2) is similar to some other weed seeds (Egley, 1986) and is consistent with its occurrence along fence lines and on sheep camps. Although young seeds did not show a requirement for light *in vitro* for gemination (Figure 1), their germination and emergence was higher when placed on the soil surface compared to 5 mm depth (Table 2). It seems that germination was delayed or its rate was decreased at this soil depth as more seedlings emerged three months after sowing. The positive effect of light on seed germination of old seeds (Figure 1) is probably an induced survival mechanism. In all the observations old seeds were less viable and produced less vigorous seedlings than young seeds. Such seedlings might not be able to establish if buried too deeply down the soil profile.

In conclusion, it was found that fresh horehound seeds have a long dormancy which can easily be broken by storage or nitrate treatment. It was also shown that horehound seeds have a longevity of more than eight years during which they might fall into a secondary, induced dormancy and require light for germination. Therefore, deep ploughing plus subsequent cultivations prior to establishment of pasture seems to be a practical strategy for control.

REFERENCES

1. Carter, R.J. 1990. Biology and control of horehound, *Marrubium vulgare. Proc. 9th Aust. Weeds Conf.:* 382-386.
2. Dastgheib, F., R.J. Field. 1994. Seed germination of horehound. *Proc. 47th N.Z. Plant Protection Conf.:* 93-94.
3. Egley, G.H. 1986. Stimulation of weed seed germination in soil. *Review of Weed Science 2:* 67-89.
4. Parsons, W.T., E.G. Cuthbertson. 1992. Noxious Weeds of Australia, Inkata Press, Melbourne, 696 pp.
5. Stritzke, J.F. 1975. Germination characteristics and chemical control of horehound. *J. Range Manag. 28:* 225-226.
6. Young, J.A., R.A. Evans. 1986. Germination of white horehound (*Marrubium vulgare*) seed. *Weed Sci. 34:* 266-270

DIFFERENCE OF WEED OCCURRENCE AND COMPETITION IN TWO CULTIVATION TYPES OF RICE (*Oryza sativa* L.)

I. B. Im* and J. O. Guh**

* National Honam Agricultural Experiment Station, RDA, Iri ,Korea
** Agronomy Department Chonnam National University, Kwangju, Korea

Abstract : This study was conducted to compare the critical period of weed competition among two types of rice(*Oryza sativa* L.) cultivation(machine transplanting with 30-day-old seedlings and flood direct seeding). The species, number of plants and dry weight of weeds increased more in flood direct seeding than machine transplanting with 30-day-old seedlings. When an allowable limit of loss in rice growth was considered as 20%, the allowable period of weed competition was 6 and 9 weeks in flood direct seeding and machine transplanting, respectively. The critical periods of weed competition was either for 5 weeks or the rest period after 8 weeks after transplanting in machine transplanting with 30-day-old seedlings but was from 5 to 7 weeks after seeding in flood direct seeding.
Key words : rice, weed, competition, cultivation type

Introduction

In rice cultivation types the competition of various weeds and rice has been known (1, 4, 5, 6, 7, 8, 9). In technically developed rice cultivation The weed control needs proceeding that can rationally control weeds on various cultivation types. The emergence period of weeds in paddy rice field is more long to early machine transplanting and direct seeding cultivation than conventional hand transplanting (2). The appearance number of weeds in paddy rice field transplanted increase up to 50days after transplanting and thereafter they decrease, but the dry matter weight of weed increased gradually up to heading date of rice (3). It was the objective of this study to establish the critical period of weed control in transplanting and direct seeding cultivation of paddy rice.

Materials and Methods

Variety : Rice cultivar used in this trial was Donjinbyeo of typical japonica type. This among Korea cultivars was acknowledged as the most cultivating cultivar in Korea.
Cultivation : The seeds were sown in the tray box and grown for 30days and then the seedlings were transplanted by machine transplanter at 6th June. Crop density chose at 30cm x 13cm for transplanted rice with 4-5 seedlings/hill. The seeds of 40kg/ha for flood direct seeding were broadcasted in the field by hand at 10th May. Crop protection and other cultural practices were followed by the standard methodology for rice crop in Honam Agricultural Experiment station in 1992.
Determination Method of Weed Competition : The experimental design was used to determine the critical period for weed removal. In one set of treatments, plots are allowed to be weedy for different lengths of time and then kept weed-free until harvest. In another set of treatments, plots are kept weed-free for various periods and then left to become weedy until harvest.

Results and Discussion

1. Weeds Distribution and Difference of Dominance

In the machine transplanting of 30-day-old seedlings the weeds were occurred ten species *Echinochloa crus-galli* (L.)P. Beauv. and so on which amounted to 295 plants/m^2 and 58.8g/m^2 as number of plants and dry weight and, dominance index was 0.313. The species, number of plants and dry weight of the weeds occurred in flood direct seeding increased more than machine transplanting field. Fifteen species *Alopecurus aequalis* var *amurensis*(Kom.) Ohwi and so on were detectable in the flood direct seeding which reached 851 plants/m^2 and 753g/m^2 as number of weeds and dry

weight, respectively. The dominant weed species also varied with cultivation types. They were three species of *Scirpus juncoides* Roxb., *Echinochloa crus-galli*(L.) P. Beauv., and *Eleocharis kuroguwai* Ohwi in machine transplanting with 30-day-old seedlings, whereas occurrence of annual weeds such as *Cyperus difformis* L. and *Monochoria vaginalis* Presl. together the three weed species increased in flood direct seeding cultivation(Table 1).

2. Difference in The Structure of Population

Differences in population structure of rice and weed ; the space ratio occupied by rice was greatly increased at the lower space than 80cm in the machine transplanting of 30-day-old seedling(Fig. 1). But, in flood direct seeding cultivation, the space ratio occupied by weeds was greater than by rice at the overall space(Fig. 2). It was considered because of that the space occupation of weeds was late in machine transplanting but, in flood direct seeding the space was occupied from early growth by weeds.

3. Establishment of Weed Competition Period

Rice growth : When an allowable limit of loss in rice growth considered as 20%, the period of weed-free should be longer than 3 weeks after transplanting in the machine transplanting cultivation and 5 weeks after seeding in the flood direct seeding(Fig. 3). Meanwhile, the allowable period of weed competition was shorter than 6 weeks after seeding in the flood direct seeding. It was 9 weeks after transplanting in machine transplanting of 30-day-old seedlings (Fig. 4).

Heading : When the period of weed free was extended, the heading was delayed by 1day in the machine transplanting of 30-day-old seedlings, meanwhile, the delaying of heading was not observed by the extension of no weeding period. In the flood direct seeding the heading of rice was delayed about 1 week by weed competition of 7 to 8 weeks after seeding, meanwhile it became late 1 day by further elongation of competition period with weeds(Table 2).

Establishment of critical period for weed removal : The critical period of weed competition was searched based on the duration that rice yield was most sensitive to weed presence and during which weeds were kept out. As Fig. 5, in machine transplanting of 30-day-old seedlings the critical period for weed removal was divided to two periods as the field state of weed-free was maintained either for 5 weeks or all the period of growth after 8 weeks from transplanting. On the other hand, in the flood direct seeding the critical period for weed removal was established 1 period as the weed-free must be kept between 5 and 7 weeks after seeding(Fig. 6).

REFERENCE

1. Cho, H. Y., H. S. Lee and Y. W. Kwon. 1983. The growth and yield of paddy rice as affected by competitive duration and density of Flatsedge, *Cyperus serotinus* Rottb.. KJWS. 3(1) : 156-165.
2. Guh, J. O. and S. L. Kwon. 1981. Emergence and growth of weeds in paddy fields as affected by cropping pattern. KJWS. 1(1) : 30-42.
3. Kim. J. K., D. S. Kim, J. H. Lee and B. H. Kang. 1979. Competition between weeds and rice in the machine transplanted paddy the effect of time and weed removal. The research Reports of the office of rural development. Korea 21(Crop) : 131-144.
4. Mcgegor J. T. JR., R. J.Smith, JR., and R. E. Talbert. 1988. Broadleaf signalgrass (*Bracbiaria platypbylla*) duration of interference in rice(*Oryza sativa*). Weed Science 36 : 747-750.
5. Sharma H. C., H.B. Singh and G. H. Friesen. 1977. Competition from weeds and their control in direct-seeded rice. Weed Research 17 : 103-108.
6. Smith R. J., JR. 1974. Competition of barnyardgrass with rice Cultivars. Weed Science 22 : 423-426.
7. Smith R. J., JR. 1983. Competition of bearded sprangletop(*Leptochloa fascicularis*) with rice (*Oryza sativa*). Weed Science. 31 : 120-123
8. Smith R. J., JR. 1984. Competition of spreading dayflower(*Commelina diffusa*) with rice (*Oryza sativa*). Weed Science 30 : 116-119.
9. Yeun, K. B., K. U. Kim, D. H. Shin, I. J. Lee, J. W. Jung and H. K. Kim, 1991. Competition between crop and weed and weed control in dry direct seeded rice. KJWS. 11(3) : 178-186.

Table 1. Weeds appeared in machine transplanted rice field with 30-day-old seedlings and flood direct-seeded rice field

Weed species	Number per m^2		Dry weight per m^2 (g)	
	30MT	FDS	30MT	FDS
Alopecurus aequalis Ohwi	-	11	-	1.4
Echinochloa curs-galli L.	32	200	22.4	597.0
Scirpus juncoides Roxb.	81	206	1.8	20.0
Cyperus difformis L.	6	144	0.1	27.5
Monochoria vaginalis Presl.	6	6	0.1	0.3
Lindernia procumbens Borbas	-	7	-	0.1
Aneilema japonica Kunth	6	6	0.1	0.2
Rotala indica Koehne	6	6	0.1	0.1
Polygonum hydropiper L.	6	6	0.2	0.7
Ludwigia prostrata Roxb.	6	20	0.2	1.6
Aeschynomene indica L.	-	6	-	0.3
Eragrostis multicaulis Steud.	-	6	-	0.1
Centipeda minima L.	-	6	-	0.1
Eleocharis kuroguwai Ohwi	140	210	32.3	94.0
Cyperus serotinus Rottb.	6	11	1.5	9.6
Total	295	851	58.8	753.0
Simpson dominance	0.313	0.270	-	-

* Number and dry weight were measured on 15th July
30MT = Machine transplanting of 30-day-old seedlings
FDS = Flood direct seeding

Table 2. Comparison in heading dates of rice as affected by different duration of initial weed-free or weed competition maintenance under different types of rice planting

(Unit :Dates in August)

Duration	Heading date			
(Weeks)	30MT [a]	FDS [b]	30MT	FDS
	— Weed-free —		— Weed-competition —	
2	-	15	-	16
3	17	15	17	16
4	17	15	17	17
5	17	15	17	19
6	18	15	17	21
7	18	15	17	21
8	18	15	17	23
9	18	15	17	22
10	18	16	17	19
11	18	16	17	17
12	18	16	17	17
13	18	16	17	17
14	18	16	17	17
15	18	16	17	17
16	18	16	17	17
17	18	16	17	17
18	-	16	-	17
19	-	16	-	17
20	-	16	-	17
21	-	-	-	-

[a] 30 MT = Machine transplanting of 30-day-old seedlings
[b] FDS = Flood direct seeding

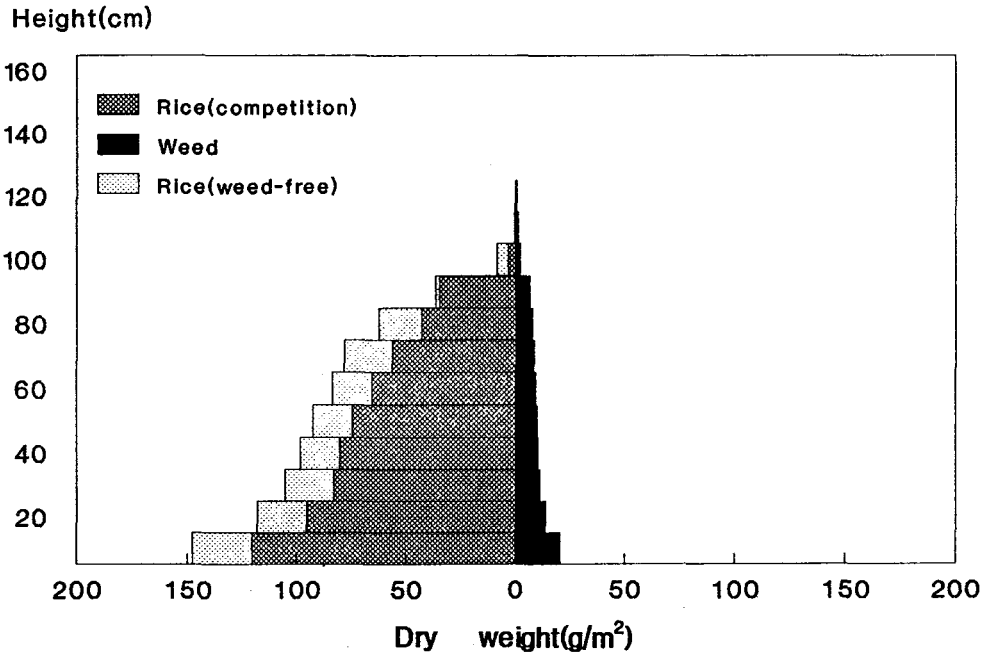


Fig. 1. Canopy structure of rice and weed competed each other and rice grown under weed free condition in machine transplanted rice field with 30-day-old seedlings.

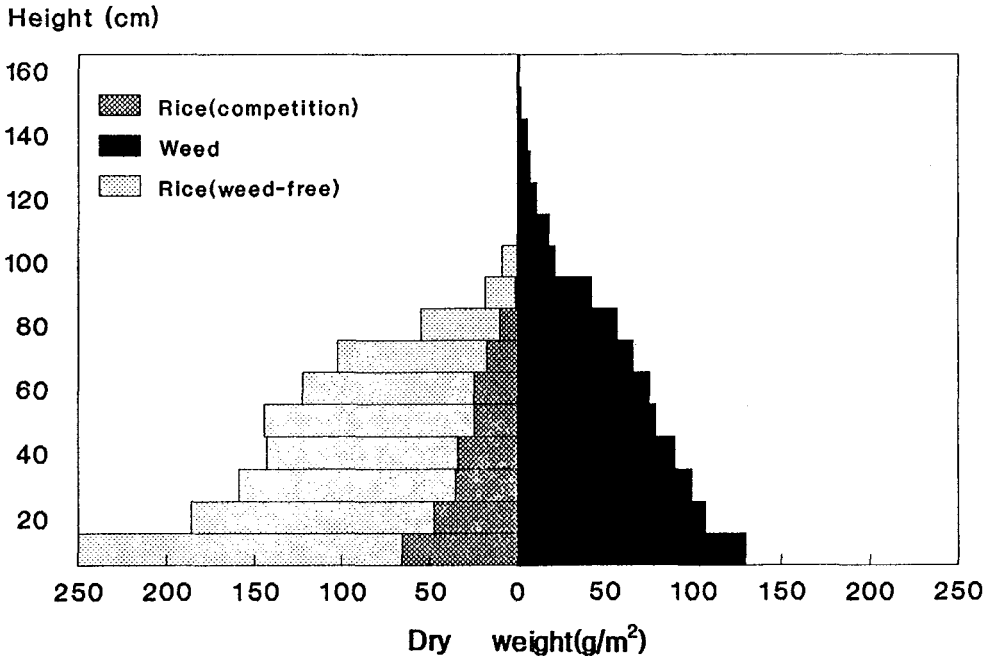


Fig. 2. Canopy structure of rice and weed competed each other and rice grown under weed free condition in flood direct-seeded rice field.

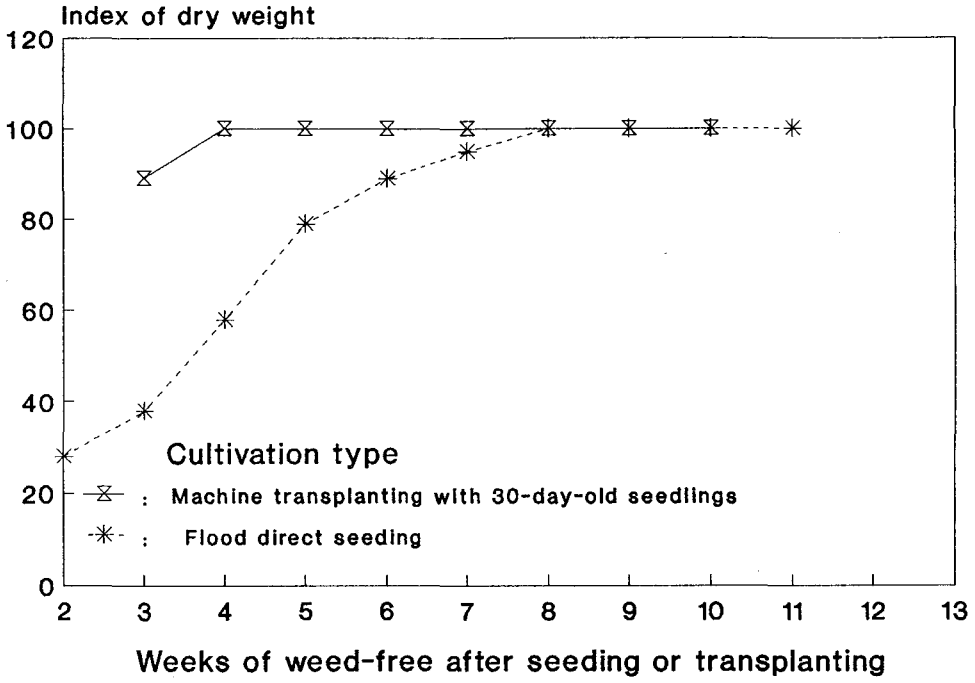


Fig. 3. Dry weight (% of whole period weed-free) of rice as affected by period of weed-free at different cultivation types.

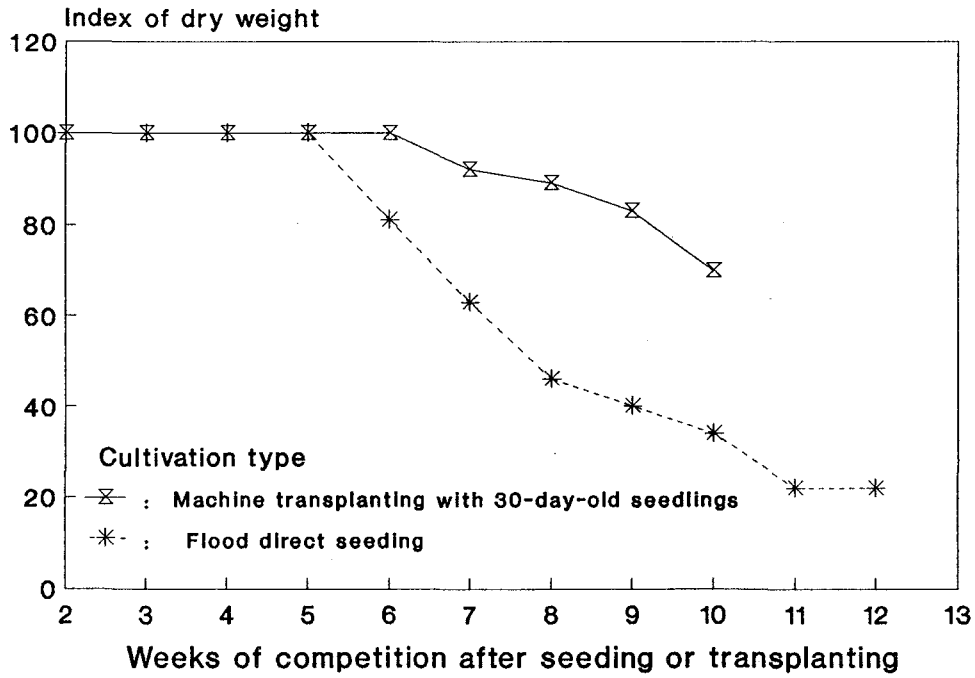


Fig. 4. Dry weight(% of whole period weed-free) of rice as affected by period of weed competition at different cultivation types.

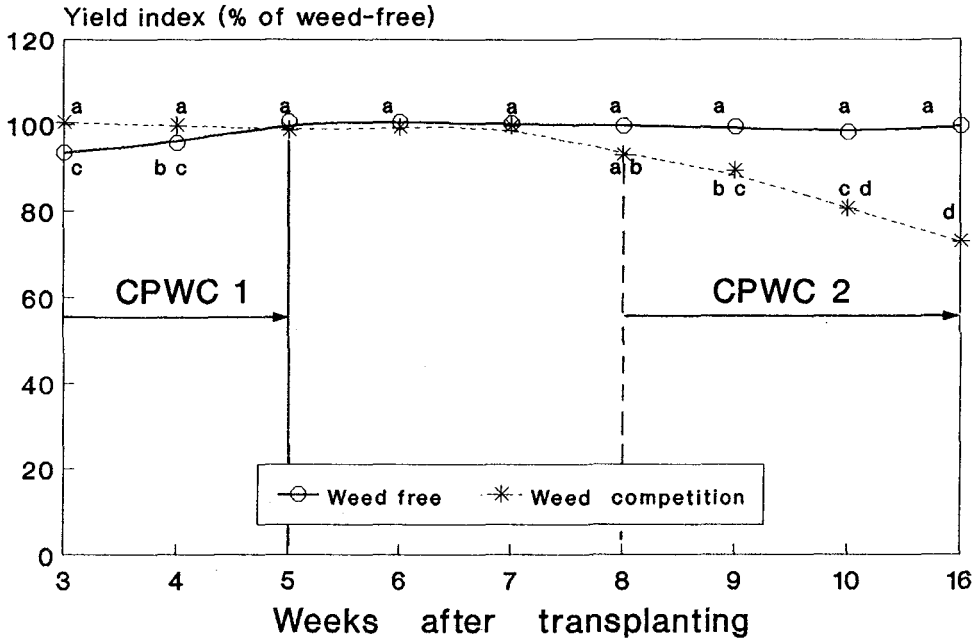


Fig. 5. Critical period of weed competition in machine transplanting with 30-day-old seedlings.
a : Average of three replication, Mean separation by DMRT at 5% level.

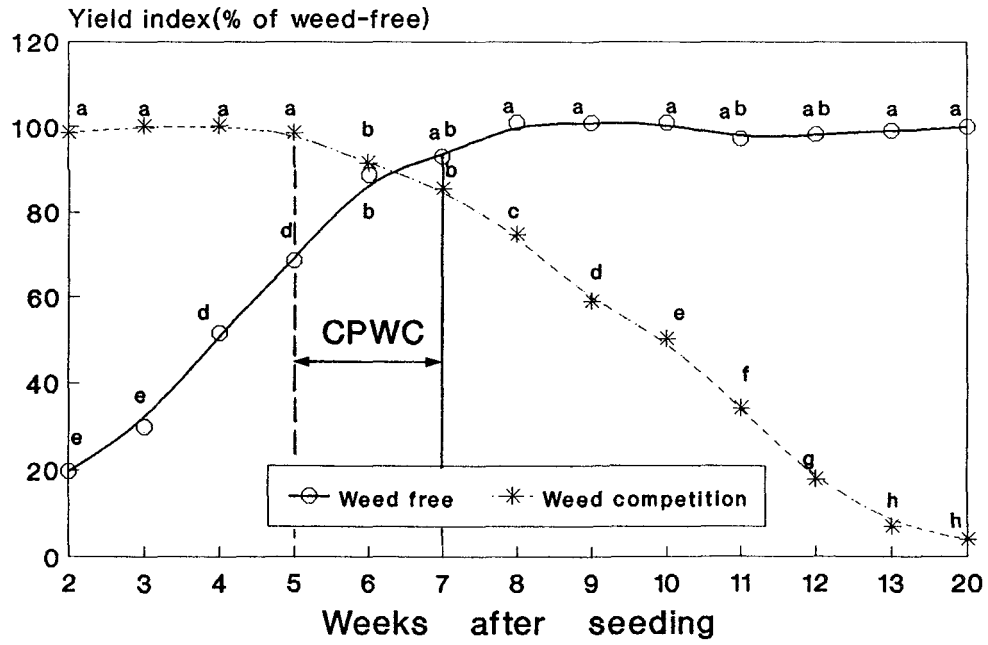


Fig. 6. Critical period of weed competition in flood direct-seeded rice.
a : Average of three replication, Mean seperation by DMRT at 5% level.

Seed Germination Patterns of Capsella and its Adaptive Implication in the Orchard

S.Ishikawa, M.Ikuta and H.Hori
Yoichi Orchard, Experimetal Farms, Faculty of Agriculture,
Hokkaido University, Yoichi, Hokkaido, 046, Japan

Abstruct. To clarify the adaptive strategy of Capsella to the orchard conditon, the influence of weed managements (tillage, mowing and cotrol) on seedling emergence was investigated. After tillage treatment, most seeds germinated and disturbance on the soil surface by a sprayer also resulted in a flush of germinations. These results indicated that disturbance was the most important factor regulating timing in the germination of Capsella. Seeds of plants collected in the orchard showed intermittent germination regulated by dormancy and environmental factors. Such a pattern of seed germination would appear to be a great advantage in an unpredictable environmental situation, because it can permit temporal extention of germination and also ensure replacement seedlings.
The present investigations show that the germination characteristics of Capsella provide a useful adaptive strategy to promot existence in the orchard.

Key words : Capsella, orchard, tillage, intermittent germination, disturbance

Introduction

Capsella bursa-pastris (L.) Medik. is the representative winter annual in Japan. Generally, emergence of Capsella seedlings occurs in fall and these seedlings overwinter as vegetative rosettes that bolt and flower the following spring. However, in the apple orchard of Hokkaido University, Capsella exhibit a very wide distribution of germination time and particularly many seeds germinate after disturbance on the soil surface. It was assumed that Capsella existed in the orchard had mechanisms of adaptation in order to colonize disturbed ground.

To clarify the adaptive strategy of Capsella to the orchard condition, we described the field emergence in relation to different weed managements and also examined the germination behavior under controlled conditions.

Materials and Methods

Experiment 1. Field study

The experiment was carried out in Yoichi Orchard, Experiment Farms of Hokkaido University, located 60 km west of Sapporo. In the study site, young apple trees are planted at intervals of seven meters. Generally, ground managements in the orchard are rotay cultivation after fertilization in spring and mowing in summer. Weeds under trees were controlled by hand hoeing, because cultivation close to the trees may cause mechanical damege to the trunks and root systems.

In 1994, differrent weed managements (tillage, mowing and control) were conducted in three areas (28m x 56m).

Plot 1 : Tillage in spring, mowing in summer
Plot 2 : Tillage in spring, tillage in summer
Plot 3 : No weed control in spring , mowing in summer.

Under different conditions in each plot (under the tree, traces of a sprayer and control), 90 quadrats in spring and 60 quadrats in summer were established. The size of quadrat was 20 cm x 20 cm. After 3 weeks from weed managements, see-

dlings of Capsella in the quadrats were recorded.

Experiment 2. Germination behavior under the contlled conditions

On June 27, seeds of two Capsella plants (plant 1 and plant 2) were collected in the orchard. 1200 seeds of plant 1 were sown immediately on a pan filled with sterilized soil. At the same time, 600 seeds of plant 2 were separately sown on the two pans and the surface of a pan was disturbed on September 11. Also, on July 19, seeds of Capsella plant (plant 3) were collected and 1000 seeds of this plant were sown on a pan. These pans were placed outdoors and watered except rainy day. The seedlings which emerged were counted and removed every day throughout a year except when the pot were under snow cover (from Novenver to March).

Results and Discussion

Field study was carried out in order to examining the seedling emergence in relation to weed managements.

Table 1 presents mean number of seedlings which emerged in a quadrat after weed managements. After tillage treatment, most seeds germinated in spring and also in summer. Although mowing after spring tillage resulted in germinations of a few seeds, not a seedling was found after no weed control and mowing following that cotrol. Roberts and Stokes[3] have shown that rotary cultivation leaves many weed seeds in the top 6 inc. where they germinate best. In the orchard, it is clear that tillage can stimulate germination of Capsella seeds and produce area of bare ground that permit seedling establishment.

Trampling by an air sprayer resulted in a flush of germinations both in spring and summer. It is wellknown that a fine, firm seed-bet, usually results in greater weed emergence that from a rough seed-bed. Traces of sprayer may produce best condition for germination of Capsella seeds.

These results show that disturbance caused by cultivations is key element in field emergence of Capsella in the orchard.

The present investigation show that in spring, many seeds germinated under the apple tree. Popay and Roberts[1,2] sugestted influence soil nitrate on germination Capsella. Since fertilizer applied around the trees before hand hoeing, fertility may affects germination of Capsella seeds.

The second experiment was designed to explor the relationship between germination behavir and field emergence in Capsella.

Germination patterns of Capsella seeds from single plant harvested differnt time shown in Fig 1. From the first germinations on July 28 to last on the following July 2, 86.6 % of sown seeds in plant 1 germinated. Also, 91.8 % of sown seeds in plant 2 germinated during the period from July 28 to May 21. Although two major aggregations of daily germinatins were seen on September and August, the great majority of the germination flushes occurred throughout a year. These germination patterns are consistent with the results obtained by Salisbury[4]. He named such a germination pattern intermittent germination.

The germination patterns were changed under the influence of disturbance(Fig.2). After disturbance, a big germination flush occurred and total germination incresed in nealy 100 %.

From the results of these experiments, it was shown that Capsella seeds indicated intermittent germination regulated by dormancy and environmental factors. Intermittent germination would have a great advantage in an unpredictable enviro nmental situation, because it can permit temporal extension of germination and also ensure replacement seedlings.

References
1) Popay,A.I. and E.H.Roberts 1970. Factors involved in the dormancy and germination of Capsella bursa-pastris (L.) Medik. and Senecio vulgaris L. J.Ecol. 58, 103 - 22.

2) Popay,A.I. and E.H.Roberts 1970. Ecology of Capsella bursa-pastris (L.) Medik. and Senecio vulgaris L. in relation to germination behaviour. J.Ecol.58, 123-139

3) Roberts,H.A. and F.G.Stokes 1965. V.Final observations on an experiment with different primary cultivations. J.Appl.Ecol.2, 307 - 315

4) Salisbury,E.J. 1963. Intermittent germination in Capsella. Nature 199, 1303-1304

Table 1. Mean number of seedlings which emerged in a quadrat (20cm x 20cm) after weed managements.

Weed management	Spring Tillage			Summer Mowing			Spring No weed control			Summer Mowing			Spring Tillage			Summer Tillage		
Date	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
6/17	14.8	87.6	81.9	0	0	0	-	-	-									
9/20	2.9	7.3	2.5	0	0	0	58.5	67.3	21.6									

A : Control, B : Trace of a splayer, C : Under the tree

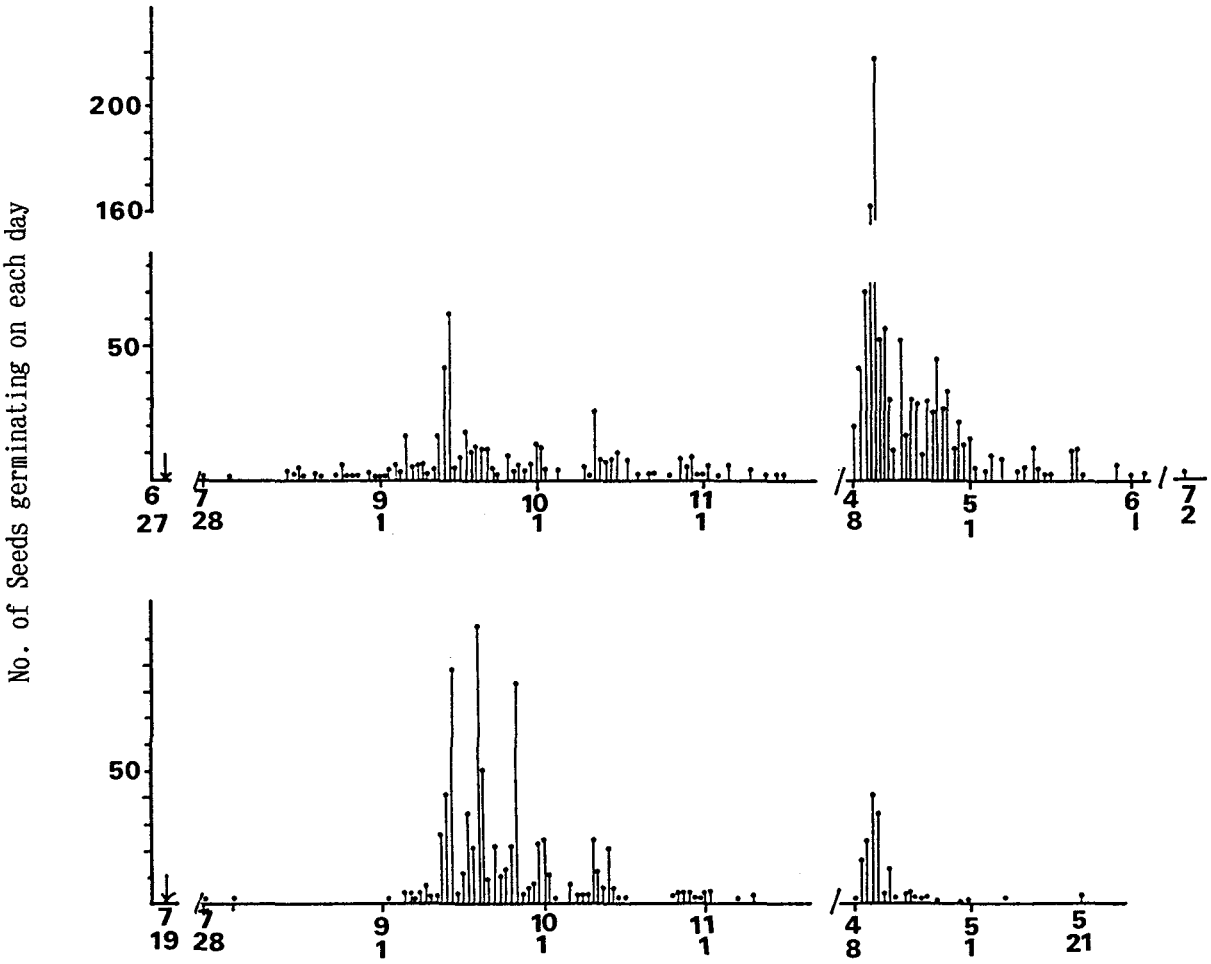


Fig. 1. Daily Germinations of Capsella seeds collected from single plant harvested different time (June 27 and July 20).
A: 1200 seeds from Plant 1 were sown on June 27.
Total germination 86.8 %.
B: 1000 seeds from Plant 3 were sown on July 20.
Total germination 91.8 %.

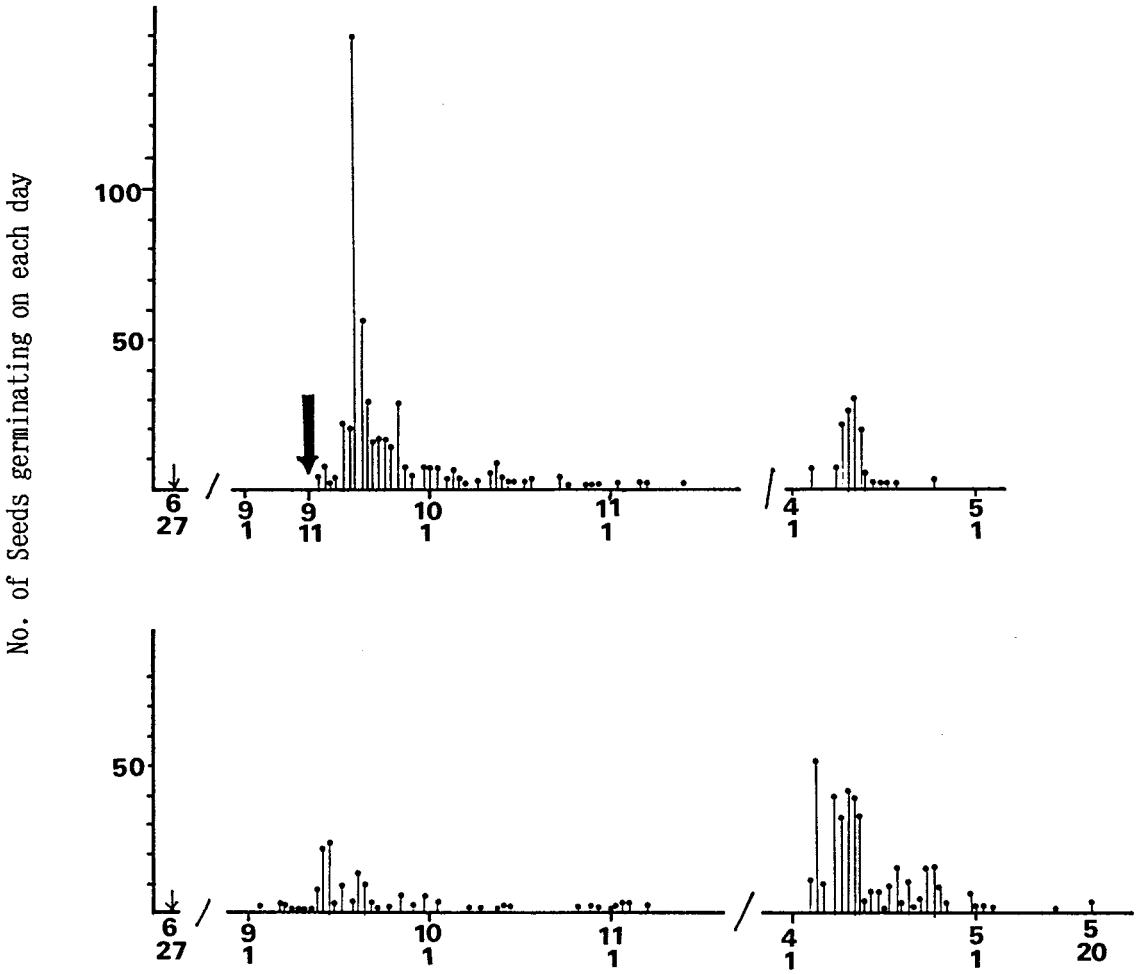


Fig. 2. Effect of Disturbance on Germination patterms of Capsella. 1200 seeds were collected from sigle plant (Plant 2) and 600 seeds were sown separately.
A : No distubance, Total germination 89.5 %.
B : Distubed on September 11, Total germination 99.3 %.
→, indicate disturbance

Effects of Rice (*Oryza sativa* L.) on the Seasonal Variation in Emergence and Growth of *Monochoria vaginalis* var. *plantaginea*

S. Kawaguchi[1], Y. Takeuchi[1], M. Ogasawara[1], K. Yoneyama[1], T. Maeda[2], and M. Konnai[1]

[1]Weed Science Center and [2]Department of Agriculture, Utsunomiya University, Utsunomiya 321, Japan

Abstract. Effects of seeds and seedlings of rice (*Oryza sativa* L.) on seed emergence and growth of *Monochoria vaginalis* var. *plantaginea*, one of the troublesome annual weeds in Japan, were studied in a greenhouse and a paddy field. Rice seeds and seedlings at 2 to 3 leaf-stage promoted the emergence and growth of the weed. By contrast, seedlings at 4 to 5 leaf-stage did not promote the germination of the weed and in fact, inhibited its growth.

Key words: *Monochoria vaginalis* var. *plantaginea*, *Oryza sativa* L., rice seed, rice seedling, seasonal variation in emergence

Introduction

The introduction of labor-saving technologies to rice cultivation has become very important in Japan. One of the labor-saving technologies is direct seeding rice cultivation. However, if it is introduced, the community of weeds will be different from those in transplanting rice cultivation which is the major practice in Japan. *Monochoria vaginalis* is native to tropical Asia and Africa [1]. It is a very serious aquatic annual weed in paddy fields in Asia, growing vigorously and causing a reduction of rice yield because of its heavy competition with the crop for nutrients, light and space. Its seasonal variation in emergence is so long that it emerges continually through the warm season in paddy fields and after disappearance of residual toxicity of applied herbicides. In recent years, it becomes more serious problem in Japan that the number of the weed seems to increase by the introduction of direct seeding rice cultivation. It is known that development and seasonal variation in emergence of weeds are related to the characteristics of dormancy and germination of the weed seeds in a paddy field. This study was conducted to determine the effects of rice seeds and seedlings at different growth stages on seasonal variation in emergence and growth of *M. vaginalis*.

Materials and Methods

Weed seeds: Seeds were collected from the dead plants in November, 1994 in a experimental field of Utsunomiya University and stored dry at 8°C for 7 months. These seeds germinated in the light under flooded conditions at 19°C to 35°C (data not shown).

Greenhouse experiments: Experiments using plastic trays (35 x 28.5 x 25 cm) were conducted in a greenhouse to examine the effects of rice seeds and seedlings on emergence of *M. vaginalis* seeds at Utsunomiya University from June to August, 1994. Plastic trays were filled with sieved volcanic ash-paddy field soil, and 200 seeds/tray of *M. vaginalis* were sown at a 1 cm depth. Rice seeds (*Oryza sativa* cv. Tsukinohikari), incubated in distilled water for 3 days at 27°C (pre-germinated seed), and seedlings, grown for 10 days (2.0-2.2 leaf-stage), 20 days (3.8-4.0 leaf-stage) and 30 days (4.2-4.5 leaf-stage) in the greenhouse, were planted at the density of 5, 10 and 20 plants per tray. Depth of irrigation water in the plastic trays was maintained at 5 cm during incubation.

These trays were placed in the greenhouse and the number of emerged weed were counted 1, 2, 3, 4, 5 and 7 weeks after planting. Each experiment was replicated 4 times.

Field experiments: The field tests were conducted at the Experimental Farm of Utsunomiya University to evaluate the effects of rice plants at different growth stages on emergence and early-growth of *M. vaginalis*, May to July, 1994. In each plot, 1 m by 1 m, *M. vaginalis* seeds were thoroughly mixed into shallow portion of the soil. Rice seeds (*Oryza sativa* cv. Koshihikari), dried or pre-incubated in water for 3 days at 30°C (pre-germinated seed), were sown at a density of 2.5 and 5.0 g per plot. Infant seedling (2.0 leaf-stage) and young seedling (3.2 leaf-stage) of rice plants were transplanted at a density of 80 plants per plot. During the experiments, depth of irrigation water in the field plots was maintained at 5 cm. Total number of emerged weeds and their dry weight were measured 6 weeks after planting. Each experiment was replicated 3 times.

Results

Greenhouse experiments

In the absence of rice plants, *Monochoria vaginalis* emerged 4.3, 15.4, 17.3, 21.1, 21.6 and 21.9% after incubation for 1, 2, 3, 4, 5 and 7 weeks, respectively. By contrast, as shown in Figs. 1 and 2, emerged number of the weed increased in the presence of rice seeds and 10 day-old (infant) rice seedlings, in particular during 1 to 3 weeks of the incubation period. After incubation for 7 weeks, the number of the emerged weed increased in the presence of rice seeds and 10 day-old rice seedlings by 22-47% and by 17-23%, respectively. These results clearly demonstrated that rice seeds and 10 day-old seedlings promoted the emergence of *M. vaginalis*.

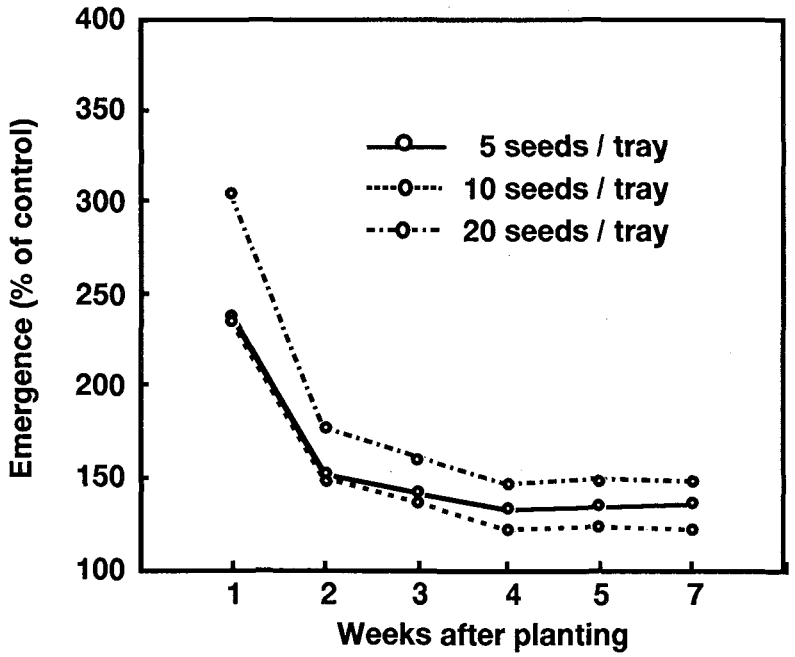


Fig. 1. Effect of pregerminated rice seeds on emergence of *M. vaginalis*.

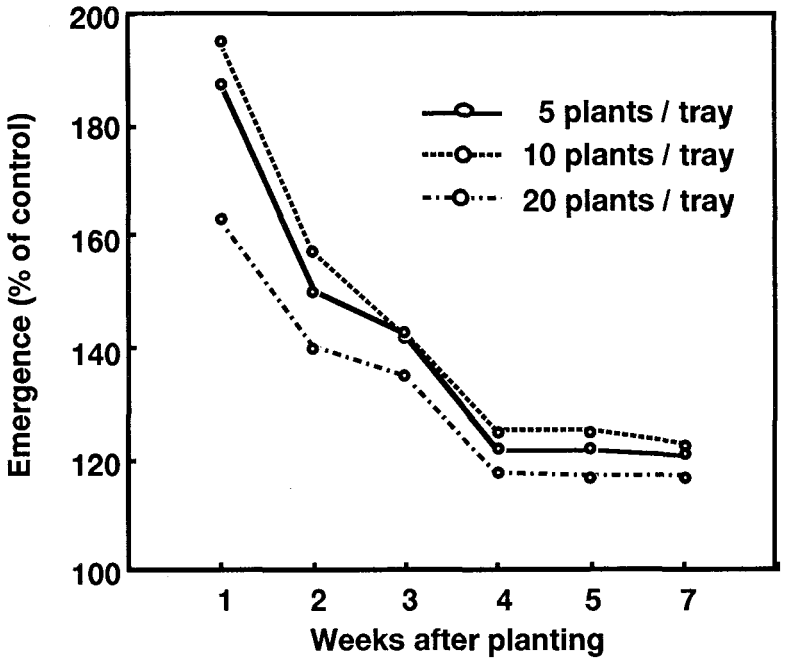


Fig. 2. Effect of 10 day-old rice seedlings (2.0-2.2 leaf stage) on emergence of *M. vaginalis*.

Fig. 3 shows the effects of 20 day-old rice seedlings on the emergence of the weed. Emerged number of the weed increased in the presence of 20 day-old rice seedlings at the density of 5 and 10 plants per plot, while decreased at 20 plants per plot. Finally, the number of the emerged weed decreased slightly or was almost equal to that of control.

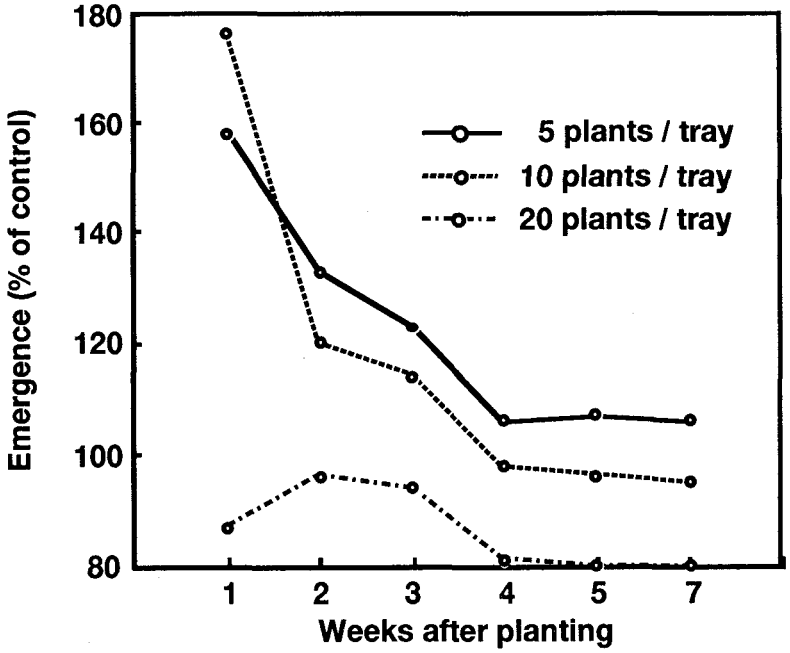


Fig. 3. Effect of 20 day-old rice seedlings (3.8-4.0 leaf stage) on emergence of *M. vaginalis*.

The effects of 30 day-old rice seedlings on the emergence of the weed was shown in Fig. 4. During 1 to 3 weeks of the incubation period, the emergence of the weed was promoted in the presence of 30 day-old rice seedlings, whereas after 4 weeks the emergence of the weed was inhibited. Therefore, the number of the emerged weed decreased by 4-24% after 7 weeks of incubation.

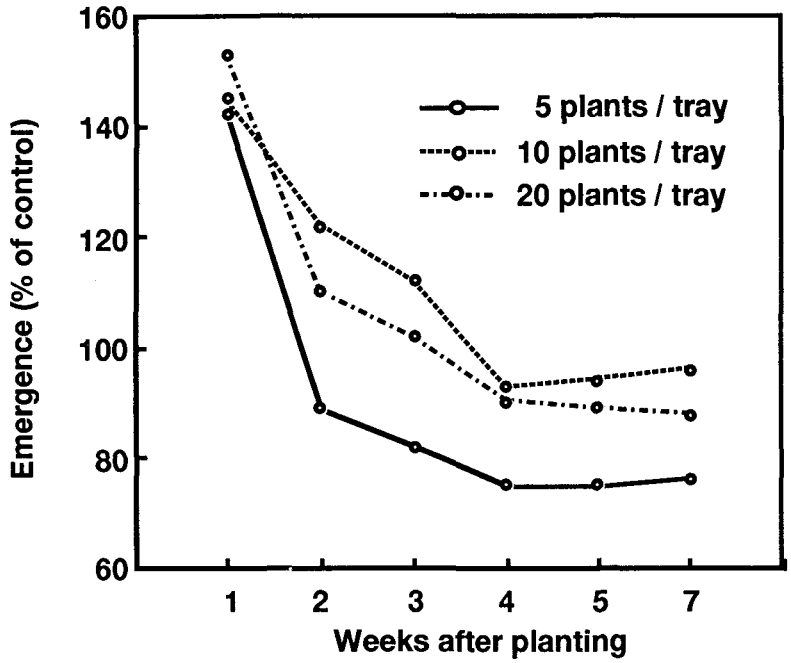


Fig. 4. Effect of 30 day-old rice seedlings (4.2-4.5 leaf stage) on emergence of *M. vaginalis*

Field experiments

Table 1 shows that effects of rice plants on the emergence and growth of *M. vaginalis*. The emergence of the weed was promoted in all the plots where rice plants were growing. These results are very similar to that obtained with the greenhouse experiments. In addition, dry weight of the weeds per 1 m^2 increased in the presence of rice plants, except for the plots where young seedling (3.2 leaf-stage) had been transplanted. The growth of the weed in terms of dry weight per plant was stimulated only in the plots where dry or pre-germinated rice seeds had been sown. By contrast, in the plots where infant seedlings (2.0 leaf-stage) had been transplanted, the dry weight per plant was almost equal to that of the control plots. Furthermore, the growth of the weed was inhibited in the plots where young seedlings (3.2 leaf-stage) had been transplanted.

Table 1. Effects of rice plants on emergence and growth of *Monochoria vaginalis.*

Rice planting system	Number of weed (per m^2)	Dry weight of weed (g/m^2)	Dry weight of weed (mg/plant)
No rice plant (control)	116.0±16.6	10.1±4.7	86
Dry seed			
2.5 g/m^2	204.7±37.5	21.0±6.2	102
5.0 g/m^2	166.7±15.5	17.3±3.8	104
Pre-germinated seed			
2.5 g/m^2	155.7±19.7	12.9±2.6	83
5.0 g/m^2	187.0±25.8	17.1±2.0	91
Infant seedling*			
80 plants/m^2	215.3±27.7	19.3±2.1	90
Young seedling**			
80 plants/m^2	152.3±19.3	10.2±1.4	67

* 2.0 leaf-stage, ** 3.2 leaf-stage

Discussion

From the results obtained in both greenhouse and field experiments, it can be concluded that rice seeds and infant seedlings at 2 leaf-stage promote the germination and growth of *Monochoria vaginalis* as shown in Figs. 1 and 2, and Table 1.

It has been reported that germination of M. vaginalis was stimulated by ethylene and carbon dioxide, and under low oxygen conditions [3]. Furthermore, we found some water-soluble substances released from rice seeds and young seedlings induced the germination of *M. vaginalis* [2].

In paddy rice fields, rice seeds and seedlings release ethylene and carbon dioxide, and consume oxygen in the irrigation water and soil. In particular, more ethylene was released from germinating rice seeds and younger seedlings than from older rice plants [4,5]. Therefore all of these may play an important role in inducing germination of the weed seeds.

Rice seedlings at later leaf-stages, however, did not induce the seed germination of *M. vaginalis.* and, on the contrary, inhibited the growth of the weed, presumably by shading. Consequently, rice seeds and young seedlings, in particular infant seedlings, stimulate but older seedlings inhibit the germination and growth of *M. vaginalis.* Therefore, in direct seeded paddy rice fields, *M. vaginalis* may propagate more easily than in transplanted rice fields.

Acknowledgment

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 06806005).

References

1) Holm, L. G., D. L. Plucknett, J. V. Pancho, and J. P. Herberger 1977. *Monochoria vaginalis* (Burm. f.) Presl and *Monochoria hastata* (L.) Solms (=*M. hastifolia* Presl), *In* "The World's Worst Weeds, Distribution and Biology", The University Press of Hawaii, Hawaii, pp. 338-341.
2) Kawaguchi, S., T. Yokota, Y. Takeuchi, K. Yoneyama, M. Ogasawara, T. Maeda and M. Konnai 1995. *Monochoria vaginalis* seed-germination stimulants from rice seeds,. Weed Res. Japan **40** (Suppl.), 192-193.
3) Momonoki, S. Y. 1992. Effect of ethylene and carbon dioxide on seed germination of *Monochoria vaginalis* var. *plantaginea*, Weed Res. Japan **37** (2), 121-128.
4) Nakayama, M. and Y. Ota 1985. Role of ethylene in the soil atmosphere upon the growth, especially root growth, of crop, Rep. Natl. Inst. Agric. Sci. Jp. **36**, 333-376.
5) Satler, S. and H. Kende 1985. Ethylene and growth of rice seedling, Plant Physiol. **79**, 194-198.

Germination ecology of *Sagina japonica* and *S. maxima*

Shigeru Mariko

Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano, 386-22 Japan

Abstract. *Sagina japonica* is an annual or winter annual species appearing in gardens and crop fields and along roadsides, and *S. maxima* is an annual or perennial species appearing in cliffs and hind sand-dunes along the coastline. These species are similar in life form, but inhabit different enviroments. The present study compared seed ecology in relation to salt environment. Seed weight was heavier in *S. maxima* (27.6 μg) than in *S. japonica* (21.6 μg), and it follows that seed size of coastal plants is larger than that of inland plants. Seeds of both species germinated between 10 and 20°C, indicating that *Sagina* seeds, dispersed mainly in early summer, germinate not in summer but after autumn or the next spring. Higher salt tolerance of germination was found in *S. maxima* than in *S. japonica*. This explains the difference in habitats between both species.

Key words. Germination, *Sagina japonica*, *Sagina maxima*, seed size

Introduction

Sagina japonica Ohwi (Caryophyllaceae) is a annual or winter annual plant appearing in roadsides, gardens and crop fields. *Sagina maxima* A. Gray are a annual or perennial weed existing on cliff rocks and in stable sand dunes along a coastline. Both species have similar life form and reproductive performance, but have some morphological differences, e.g. leaf size and thickness. Physiological differences may be between both species, but not examined. These differences seem to be related to salinity and water environment in their natural habitats[5].

The aim of the present study is to compare the germination responses of *S. japonica and S. maxima* seeds to temperature and salinity in relation to their distributional difference. Seed size, one factor determining the success of seedling establishment, is also compared.

Materials and Methods

Sagina japonica and *S. maxima* seeds were collected from a roadside and a coast in the city of Joetsu (37°11' N, 138°16' E) in June 1994,. The seeds were stored at room temperature in a desiccator for 2 months until germination tests. Fresh weight of 100 seeds was determined for 4 replicates. All germination tests were performed on moist quartz sand in 9-cm petri dishes placed in an incubator in which temperature and photoperiod were controlled. The light regime was a photon irradiance of 150 μmol m^{-2} s^{-1} at the seed level with fluorescent tubes during a 12-h photoperiod, unless specified otherwise. There were 100 seeds per petri dish. Seeds were considered to be germinated when the radicles emerged. Germination was counted every day.

Thermal effects on seed germination were examined at 10, 15, 20, 25, 30 and 35°C. At the same time, sensitivity to salinity at each temperature was determined at five concentrations of sodium chloride solution (0, 0.05, 0.10, 0.20, 0.30 mmol NaCl L^{-1}).

Light requirement for germination was tested by placing seeds in darkness for 0, 21, or 28 days and thereafter by exposing the seeds to a constant temperature of 20°C in the light. The effects of light quality on germination were examined using two film filters cutting a specific wavelength range of radiation, an AG67 filter between 600 and 700 nm and an AG68 filter between 600 and 800 nm[9]. The light level on the top of petri dishes covered with the film filters was maintained at 100 μmol m^{-2} s^{-1}. The two film filters can transmit approximately 50% of the light at 500 nm, which causes slight differences among temperatures in petri dishes, but not appreciable differences in germination performance[8]. The germination count was recorded after 2 weeks.

Statistical differences between mean values were analyzed using the *t*-test. Significance level was $P = 0.05$.

Results and Discussion

Sagina species produce a number of small-sized seeds for a few months, but had different-sized seeds. The mean seed weight of *Sagina maxima* (27.6 μg) was significantly heavier than that of *S. japonica* (21.6 μg). Seed size affects the initial growth of seedlings[2]. *Sagina maxima* seedlings would have more developed root system than *S. japonica* ones after emergence. The sufficient development of root system

Table 1. Effects of constant temperature and salinity on seed germination of *Sagina japonica* and *S. maxima*. Results are mean±sd of four replicates.

Species	Temperature	Salinity level (mmol NaCl L^{-1})				
	(°C)	0	0.05	0.10	0.20	0.30
S. japonica	10	55±1.6	42±8.5	53±2.7	48±6.3	39±2.1
	15	54±3.6	57±6.0	62±8.0	25±4.4	0
	20	58±2.3	46±8.1	29±5.8	14±8.7	0
	25	14±6.7	7±1.8	6±1.8	0	0
	30	0	0	0	0	0
	35	0	0	0	0	0
S. maxima	10	79±4.1	73±2.6	73±8.2	46±2.8	36±6.6
	15	87±0	89±1.1	91±2.9	72±7.7	37±2.5
	20	77±5.4	73±1.5	64±7.1	74±1.0	5±0.4
	25	0	0	0	0	0
	30	0	0	0	0	0
	35	0	0	0	0	0

is important for the survival of pine seedlings in coastal regions where drought in root zone causes seedling death[7]. Shallow soils of cliffs, deposited in crevices and hollows on rock outcrops, are liable to dry up[9], and dune soils are low in moisture and move with the wind[1]. Desiccation and sand movement seriously damage the seedlings with small body and shallow root system. Henceforth, *S. maxima* seedlings would be more successfully established in the cosastal regions.

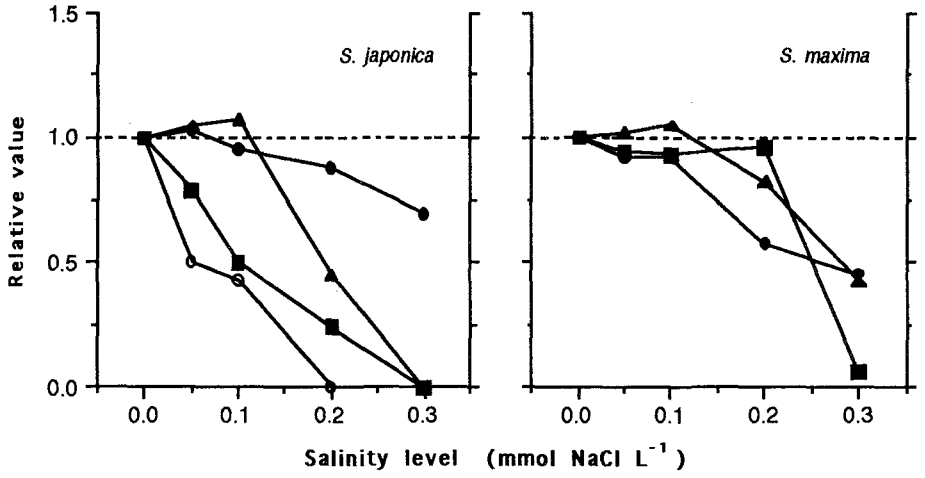


Fig. 1. Interactive effects of salinity and temperature on seed germination in *Sagina japonica* and *S. maxima*. Values are relative to those at 0 mmol NaCl L^{-1}. Symbols: (●) 10°C, (▲) 15°C, (■) 20°C, (○) 25°C.

Table 2. Effects of two film filters excluding radiation spectra between 600 and 700 nm (AG67) and between 600 and 800 nm (AG68) on germination percentage of *Sagina japonica* and *S. maxima* seeds, 14 days after imbibition, at 15°C. Light source was a bank of fluorescent lamps.

Species	Filter	Germination percentage		Statistical
		Mean[1)]	SD	difference[2)]
S. japonica	No filter	57.8	2.2	a
	AG67	0	0.0	b
	AG68	54.0	11.9	a
S. maxima	No filter	73.0	6.1	a
	AG67	0	0.0	b
	AG68	63.5	6.6	a

[1)] Mean of four replicates.
[2)] The same letters show no significant difference at $P = 0.05$ (t test).

Both *Sagina japonica* and *S. maxima* showed high germination percentage between 10 and 20°C (Table 1), indicating that the seeds, dispersed mainly in early summer, germinate not in summer but after autumn or the next spring. This thermal trait of germination is suited for the seedling emergence time of the two *Sagina* species in their natural habitats.

Higher germination percentage was found in *S. maxima* than in *S. japonica* (Table 1). This suggests that a half of *S. japonica* seed population stored for 2 months remained innate dormancy[4)]. The dormancy level of *Stellaria media*, one species of Caryophyllaceae, is intermediate[9)]. In general, winter annuals like *S. japonica* have less dormancy level than summer annuals[10)]. The 2-month-stored seeds of *Sagina maxima*, whose plants often survives for more than 2 years, showed no dormancy, since they germinated at about 90% at the optimum temperature (Talbe 1).

Germination percentage tended to decrease with increasing salinity level (Table 1). This tendency was more marked in *S. japonica* than *S. maxima*, and therefore the latter seeds are able to germinate more successfully in coastal regions where the high level of soil salinity is occasionally found[9)]. The depressive effect of salinity in *S. japonica* was stronger at higher temperatures within a germinable temperature range of 10–25°C (Fig. 1). Such a thermal effect was not found in *S. maxima* whose seeds are exposed to rather high temperature in the daytime.

No germination occurred during any dark period of 0, 21 and 28 days, which was found in *S. japonica* and *S. maxima*. Light including no 600-700 nm radiation significantly inhibited seed germination of both species, while light including no 600-800 nm radiation had no significant effect on germination (Table 2). These results indicate that *Sagina* seeds have the positive photoblastism controlled by phytochrome. When the photoblastic seeds are scattered beneath a dense canopy and buried in soils, enforced dormancy[4)] may occur because of the inhibitory effects of leaf-filtered light and darkness[8)]. Photoblastism is useful to detect gaps in the vegetation and depth in the soil, and therefore is an advantageous trait for the survival of small seedlings[3)].

Acknowledgment: The author thanks Mr. Nakano, Tokyo Metropolitan University, for his kind help.

References

1) Barbour, M.G., T.M. de Jong and B.M. Pablik. 1985. Marine beach and dune plant communities. In "Physiological Ecology of North American Plant Communities" ed. by B.F. Chabot and H.A. Mooney, Chapman and Hall, New York, pp. 296-322.
2) Black, J.N. 1959. Seed size in herbage legumes. Herbage Abst. **29**: 235-241.
3) Fenner, M. 1985. Seed Ecology. Chapman and Hall, London, pp. 87-102.

4) Harper, J.L. 1977. Population Biology of Plants. Academic Press, London, pp. 61-82.
5) Mariko, S., N. Kachi, S. Ishikawa and A. Furukawa. 1992. Germination ecology of coastal plants in relation to salt environment. Ecol. Res. **7**: 225-233.
6) Roberts, H.A. and M.L. Patricia. 1975. Germination of buried and dry-stored seeds of *Stellaria media*. Weed Res. **15**: 199-204.
7) Tazaki, T. 1960. On the growth of pine yearlings in coastal dune regions with special to their drought resistance. Jap. J. Bot. **17**: 239-277.
8) Washitani, I. and T. Saeki. 1984. Leaf-canopy inhibition of germination as a mechanism for the disappearance of *Amaranthus patulus* Bertol. Jpn. J. Ecol. **34**: 55-61.
9) Watanabe, H. 1979. Various light-wavelength selecting filters. In "Research Reports on the Developments of Techniques of Selective Utilization of Light for Plants" Technical Research Association for the Selective Use of Light in Agriculture, Japan, pp. 156-166 (in Japanese).
10) Watanabe, Y. 1978. Physiological and ecological studies on upland weeds in Hokkaido. Res. Bull. Hokkaido Natl. Agric. Exp. Stn. **123**: 17-77 (in Japanese with English summary).

Detection of DNA Fingerprints of *Sagittaria trifolia*

R. Miura[1], C. An[1], T. Kusanagi[1] and R. Terauchi[2]
[1]Faculty of Agriculture and [2]Faculty of Science, Kyoto University,
Sakyo, Kyoto 606, Japan

Abstract. *Sagittaria trifolia* L., a noxious weed in paddy rice, has ability to propagate by sexual (through seeds) and vegetative (through tubers) means. In order to survey the extent of vegetative propagation in this species, we employed DNA fingerprinting technique for the identification of clones. As a preliminary survey, nine individuals collected in paddy fields within a 200 × 300 m area at Mikata, Fukui Prefecture, and five individuals collected from far distant places were used for the DNA fingerprinting study. Genomic DNA was extracted from leaf tissue, digested with restriction enzymes, electrophoresed, and in-gel hybridized with ^{32}P-labeled simple sequence repeat oligonucleotide probes. Individual-specific "DNA fingerprints" were obtained by use of the enzyme *Taq* I and the probe $(GATA)_4$. The probability that all fragments present in an individual are also present in another unrelated individual by chance was estimated to be 1.4×10^{-4}, indicating the discriminating power of the method. The similarity index averaged over pairwise comparisons was 0.46 within the Mikata population and 0.30 among individuals collected from distant places. These results suggest that the local population of *S. trifolia* consists of many genetically distinct clones, and sexual reproduction occurs frequently.

Key words: DNA fingerprinting, microsatellite, clone identification, *Sagittaria trifolia*

Introduction

Many perennial weeds have ability to propagate by both sexual and vegetative means. It is critically important for ecological and genetic study of clonal plants to discriminate among different clones and distinguish between sexual and vegetative offsprings. In several studies, multiclonal population structures were revealed by isozyme analysis (Parks and Werth, 1993; Berg and Hamrick, 1994). However, the number of genotypes detected by isozyme and other conventional genetic markers is usually an underestimate of the real number of clones (Ellstrand and Roose, 1987).

Recently, DNA fingerprinting technique has been applied to natural populations of plants, and have proved to be quite powerful for resolving genotypes (Nybom and Schaal, 1990; Rogstad et al., 1991). DNA fingerprinting is a kind of RFLP analysis, but differs from ordinary one in using multilocus probes specific to hypervariable repetitive sequences in genomes, which are known as "minisatellites" or "microsatellites" (Jeffreys, 1987; Litt and Luty, 1989). DNA fingerprinting with microsatellite-specific oligonucleotide probes has wide applicability to diverse organisms, and can be carried out by in-gel hybridization, which does not require conventional blotting procedures (Epplen et al., 1991; Weising et al. 1991b). We conducted an experiment to examine the potential of oligonucleotide DNA fingerprinting in discriminating among clones of an arrowhead species, *Sagittaria trifolia* L. (Alismataceae).

S. trifolia is a noxious perennial weed occurring in paddy fields throughout Japan. Because seedlings are seldom observed in natural populations, this species has been thought to propagate principally by tubers. Accordingly, previous studies have concentrated on propagation by tubers (Yamakawa, 1986; Itoh, 1989). However, potential propagation by seeds should not be neglected, since it may have significant effects on genetic structure of the population (Yamakawa, 1991). Outcrossing rate may be high due to monoecy and entomophily (Tanaka, 1985).

Materials and Methods

In 1993, a test trial was conducted to determine an appropriate combination of restriction enzymes and probes. Several individuals of *S. trifolia* collected from various

locations in Kinki District, Japan, were used. Genomic DNA was extracted according to Saghai-Maroof et al. (1984) with some modifications. Two to three grams of leaf tissue were ground with liquid nitrogen by an electric mill, and extracted with 20 ml of 2 × CTAB buffer (2% CTAB; 1.4 M NaCl; 100 mM Tris-HCl; 20 mM EDTA; 0.2% mercaptoethanol) at 55°C for 20 min. After extracting twice with 20 ml of chloroform, crude DNA was precipitated by adding 0.6 volume of isopropanol to the aqueous phase. The DNA was redissolved in TE (10 mM Tris-HCl; 1 mM EDTA) and further purified by CsCl density gradient centrifugation using an ultracentrifuge (Beckman L-70 with a 50.4Ti rotor). Alternatively, the crude DNA was purified by repeated phenol extraction. DNA concentration was checked by optical density measurement and gel electrophoresis, and finally adjusted to 250 ng/μl with distilled water.

DNA fingerprinting procedures followed Weising et al. (1991a). DNA sample was digested with three restriction enzymes, *Alu* I, *Hinf* I and *Taq* I. Digested DNA, 4 μg per lane, was run in an 25 cm long, 1% agarose gel (SeaKem GTG Agarose, FMC) in TBE buffer, at 60 V for 36 hours at 4°C, using a submarine electrophoresis apparatus (Nippon Eido NB-1012). After alkali denaturation, the gel was dried by a gel drier and stored until hybridization. Four synthetic oligonucleotide probes, $(CA)_8$, $(CT)_8$, $(GACA)_4$ and $(GATA)_4$, were used for hybridization. $(CA)_8$ is, for example, an abbreviation for the 16 mer, CACACACACACACACA. The probes were ^{32}P-labeled by T4-kinase and purified through a DEAE cellulose column (Whatman). Hybridization was made in 10 ml of hybridization solution (5 × SSPE; 5 × Denhaldt's solution; 10 μg/ml sonicated *E. coli* DNA; 0.1% SDS) at (T_m-5)°C for three hours. The T_m was calculated as the sum of the number of A+T multiplied by 2°C and the number of G+C multiplied by 4°C (Itakura et al., 1984). Washing was made at (T_m-5)°C. Autoradiography was made on an X-ray film (Amersham Hyperfilm) with an intensifying screen for 16 to 48 hours at ambient temperature.

In 1994, samples were collected to determine variability of the DNA fingerprint at two geographical scales, i.e., within a local population and among far distant individuals. Nine individuals were collected in cultivated and abandoned paddy fields within a 200 × 300 m area at Mikata, Fukui Prefecture. Five other individuals were collected from distantly located places in Fukui and Shiga Prefectures. DNA extraction and fingerprinting were performed as above except that only endonuclease *Taq* I and probe $(GATA)_4$ were used, since only this combination produced a clear band pattern in the test trial.

Results and Discussion

Extraction of DNA and detection of DNA fingerprints

Extraction of DNA from *S. trifolia* leaves was relatively difficult due to a high concentration of gelatinous substance. To obtain DNA of sufficient purity, at least twice of chloroform extraction and further purification by a CsCl density gradient centrifugation were necessary. In place of the density gradient centrifugation, three to four times of phenol extraction also gave a satisfactory purity to the DNA. Final DNA yield by these methods was typically less than 30 μg/g leaf fresh weight. Even with these difficulties, a sufficient amount of DNA for several experiments could be obtained from one leaf lamina of the average size.

Hybridization with $(CA)_8$ resulted in a smear pattern. No signal was detected by the probes $(CT)_8$ and $(GACA)_4$. A clear, bar code-like patterns were obtained by the probe $(GATA)_4$. When $(GATA)_4$ was used, digestion of DNA with *Taq* I gave higher resolution of bands than *Alu* I or *Hinf* I.

Variation in DNA fingerprints detected by $(GATA)_4$

Individual-specific DNA "fingerprints" were obtained by hybridization with $(GATA)_4$ following digestion by *Taq* I (Fig. 1). Even within the local population, each individual had a unique combination of bands, and was assigned to a distinct clone. Each individual was scored for presence or absence of 40 distinct bands in a clearly readable size range, approximately 4-25 kbp. For each pair of individuals, a similarity coefficient (D) was calculated according to Wetton et al. (1987) as:

$$D=2N_{AB}/(N_A+N_B)$$

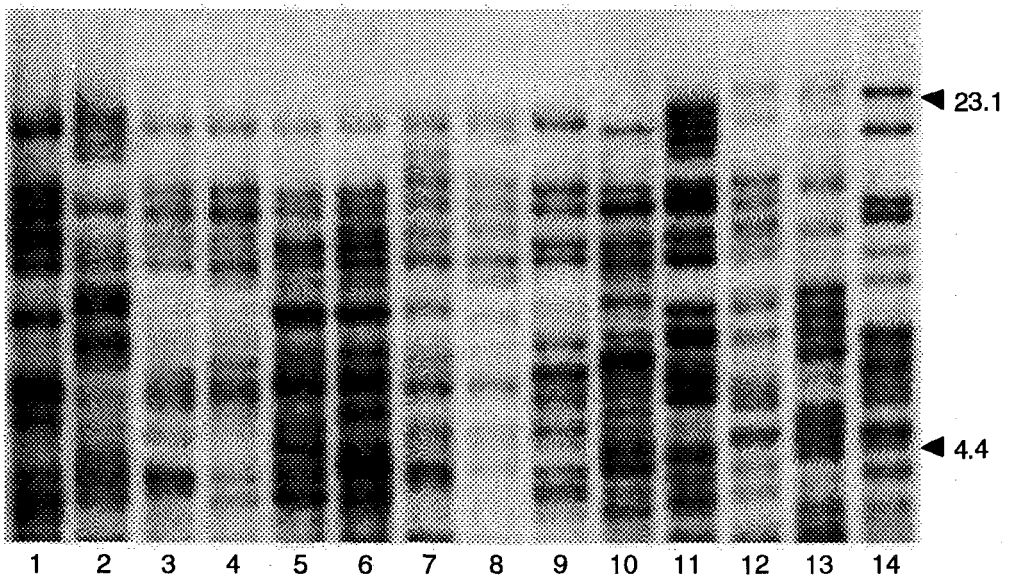


Fig. 1. Autoradiograph of *Taq* I digested DNA of *S. trifolia* probed with $(GATA)_4$. Samples 1-9 were collected within the Mikata population, and 10-14 from distant locations in Kinki District. Fragment sizes are shown in kbp.

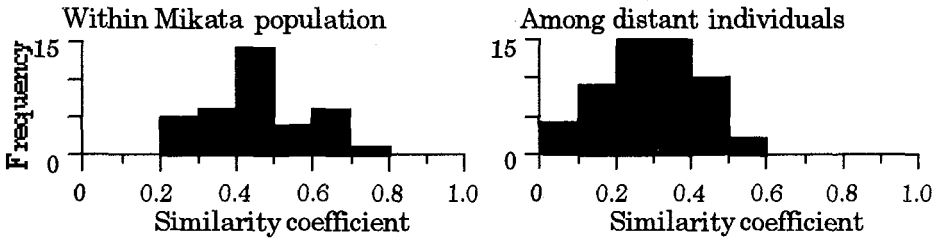


Fig. 2. Frequency distributions of the similarity coefficient within Mikata population and among individuals collected from distant locations

where N_A and N_B are the number of bands in individual A and B, respectively, and N_{AB} is the number of bands shared by individuals A and B. The similarity coefficient was 0.20 to 0.70 (mean = 0.46) within the Mikata population, and 0.00 to 0.49 (mean = 0.30) among the distant individuals (Fig. 2).

Population genetic parameters were calculated for the Mikata population, based on a data set of 33 bands for nine individuals, according to Stephens et al. (1992). Average number of bands per individual was 11.33. The number of loci was estimated to be 7.38 and average heterozygosity, 0.57, assuming the Hardy-Weinberg equilibrium. However, adequacy of the assumption is further to be examined by progeny analysis or by use of single-locus markers. The probability that all fragments present in an individual are also present in another unrelated individual by chance (Jeffreys, 1985) was smaller than 1.4×10^{-4}. This means that, if two individuals having an identical "fingerprint" are encountered in this population, they can fairly safely be judged as ramets of a single clone.

Overall, these results indicate that DNA fingerprinting is a quite powerful tool for analyzing *S. trifolia* population structure. It was revealed that the local population consisted of a number of clones, suggesting frequent sexual reproduction. We are now conducting an extensive analysis of the Mikata population to obtain information on spatial configurations of the clones, dispersal processes and reproductive modes of *S. trifolia*.

Acknowledgements: One of the authors (Terauchi) is greatly indebted to Prof. Dr. G. Kahl, Johann Wolfgang Goethe University, Germany, for his instruction in DNA fingerprinting procedures. We wish to thank Prof. Dr. S. Kawano, Prof. Dr. K. Izui and Dr. T. Aoki of Kyoto University for their helpful advice. A part of this work was conducted at the Radioisotope Center, Kyoto University. This work was partly supported by a grant-in-aid (No. 05760045) from the Ministry of Education, Science and Culture, Japan.

References

Berg, E. and J.L. Hamrick, 1994. Spatial and genetic structure of two sandhills oaks: *Quercus laevis* and *Quercus margaretta* (Fagaceae). *Amer. J. Bot.* 81(1), 7-14.

Ellstrand, N.C. and M.L. Roose, 1987. Patterns of genotypic diversity in clonal plant species. *Amer. J. Bot.* 74, 123-131.

Epplen, J.T., H. Ammer, C. Epplen, C. Kammerbauer, R. Mitreiter, L. Roewer, W. Schwaiger, V. Steimle, H. Zischler, E. Albert, A. Andreas, B. Beyermann, W. Meyer, J. Buitkamp, I. Nanda, M. Schmid, P. Nürnberg, S.D.J. Pena, H. Pöche, W. Sprecher, M. Schartl, K. Weising and A. Yassouridis 1991. Ologonucleotide fingerprinting using simple repeat motifs: a convenient, ubiquitously applicable method to detect hypervariability for multiple purposes. In: *DNA fingerprinting: Approaches and Applications* (eds.: T. Burke, G. Dolf, A.J. Jeffreys and R. Wolff), pp. 50-69. Birkhäuser, Basel.

Itakura, K., J.J. Rossi and R.B. Wallace 1984. Synthesis and use of synthetic oligonucleotides. *Annu. Rev. Biochem.* 53, 323.

Itoh, K. 1989. Ecology and control of an arrowhead, *Sagittaria trifolia* L., a perennial paddy weed. *Weed Res., Japan* 34(2), 101-106. (in Japanese)

Jeffreys, A.J., V. Wilson and S.L. Thein 1985. Individual-specific 'fingerprints' of human DNA. *Nature* 316, 76-79.

Jeffreys, A.J. 1987. Highly variable minisatellites and DNA fingerprints. *Biochem. Soc. Transact.* 15, 309-317.

Litt, M. and J.A. Luty 1989. A hypervariable microsatellite revealed by in vitro amplification of dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Human Genet.* 44, 388-396.

Nybom, H. and B.A. Schaal 1990. DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (*Rubus*, Rosaceae). *Amer. J. Bot.* 77(7), 883-888.

Parks, J.C. and C.R. Werth 1993. A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *Amer. J. Bot.* 80(5), 537-544.

Rogstad, S.H., K. Wolff and B.A. Schaal 1991. Geographical variation in *Asimina triloba* Dunal (Annonaceae) revealed by the M13 "DNA fingerprinting" probe. *Amer. J. Bot.* 78(10), 1391-1396.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen and R.W. Allard 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81, 8014-8018.

Stephens, J.C., D.A. Gilbert, N. Yuhki and S.J. O'Brien 1992. Estimation of heterozygosity for single-probe multilocus DNA fingerprints. *Mol. Biol. Evol.* 9(4), 729-743.

Tanaka, H. 1985. Insect pollination of Sagittaria trifolia L. *Weed Res., Japan* 30, 63-156. (in Japanese)

Weising, K., B. Betermann, J. Ramser and G. Kahl, 1991a. Plant DNA fingerprinting with radioactive and digoxiganated oligonucleotide probes complementary to simple repetitive DNA sequences. *Electrophoresis* 12, 159-169.

Weising, K., J. Ramser, D. Kaemmer, G. Kahl and J.T. Epplen 1991b. Oligonucleotide fingerprinting in plants and fungi. In: *DNA fingerprinting: Approaches and Applications* (eds.: T. Burke, G. Dolf, A.J. Jeffreys and R. Wolff), pp. 312-329. Birkhäuser, Basel.

Wetton, J.H., R.E. Carter, D.T. Parkin and D. Walters 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327, 147-149.

Yamakawa, S. 1986. Genecological study of an arrowhead, *Sagittaria trifolia* L., a perennial paddy weed. Dr. Agric. Sci. thesis, Kyoto University.

Yamakawa, S. 1991. Intraspecific variation of *Sagittaria trifolia* L., a perennial paddy weed. *Weed Res., Japan* 36(1), 8-16. (in Japanese)

Role of Seed Coat on Seed Dormancy in *Persicaria vulgaris* Weeb et Moq.

K. Nakatani *[1] and K. Noguchi [2]

[1] National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki 305, Japan

[2] National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan

Abstract. Experiments were carried out to determine the role(s) of seed coat in seed dormancy in *P.vulgaris*. The material seeds for these experiments were harvested from plants grown in a controlled environment at 25°C, 14 hour day-length condition. After harvest, they were stored at 22°C under dry condition or at 5°C under wet condition. Without any treatments, the seeds did not germinate at all. The seed dormancy was broken by treatment such as the peeling of seed coat or cutting off a part of seed coat. The germination of the seeds was promoted markedly by these treatments during after-ripening. This effect was substituted by gibberellic acid treatment. Partial removal of the seed coat advanced water absorption of the seed. The ethanolic extract of seed coat or that of embryo and albumen did not decrease the germination rate, however, each suppressed the elongation of radicles. These results suggested that the physiological function of seed coat on seed dormancy changed during the ripening process. The seed coat prevents the water absorption and may supply growth inhibitor(s) before and/or during after-ripening stage, and it plays only a role as a water barrier after after-ripening stage.

Key wards *Persicaria vulgaris,* seed dormancy, seed coat.

Introduction

Many weed species have dormancy on their seeds, concerning with an irregular emergence of weeds in the field. The seed of *Persicaria vulgaris* shows strong dormancy (2,3). The strength of the seed dormancy, the vigor of seed germination or light demand for germination was influenced by the photoperiod and/or temperature condition during growing season (5). The dormancy is broken by a storage at 5°C under wet condition or the scarification of seed coat with H_2SO_4 (4). These phenomena suggest that the seed coat participates in seed dormancy in *P.vulgaris*. However, it remains to be clarified that the physiological role of seed coat on seed dormancy in detail. Therefore, in the present study, we carried out the experiments to determine the role of seed coat on seed dormancy in *P.vulgaris*.

Materials and Methods

The material seeds for all experiments were harvested from the plants grown in a phytotron under controlled environmental condition such as 25°C in temperature, 14 hours in photoperiod and 70% in relative humidity. The seeds were harvested at 30 days after the flowering. The seeds were stored at 22°C under dry condition and 5°C under wet condition.

Experiment 1. Effects of removal of seed coat on germination during after-ripening of seeds

Seed coat of the materials was peeled completely or cut partially immediately after the harvest, and 20, 40, 60, 80 and 100 days after the harvest. After the seed coat removal, germination tests were done using 4.5 cm petri dishes with filter paper (Toyo No 2) and 2 ml of distilled water or 100 ppm GA_3 was put in the each dishes. The dishes were placed in an incubator at 30/20°C (daytime/nighttime temperature, each 12 hour) and 12 hours' lighting during 10 days. This test was done with two replications.

Experiment 2. Effects of seed coat removal on the water absorption of seed

The rates of water absorption of seeds were compared between two kinds of materials such as seeds stored at 5°C under wet condition and seeds stored at 22°C under dry condition. The seed coats of the seeds stored at 22°C under dry condition were partially cut as shown in Fig. 1. The rates of the water absorption of these seeds were measured for 10 days at 28°C in the dark. Thirty seeds were placed on each dish and this test was done with three replications. The water absorption rate was calculated as the weight of water in the seed per the dry weight of the seed and shown in percentage.

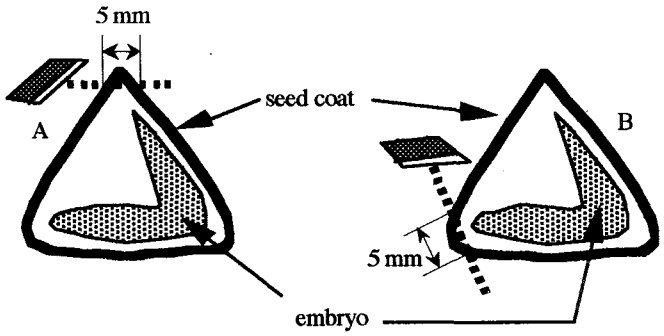


Fig. 1 Cutting position of seed coat.
A: top of seed, B: bottom edge of seed.

Experiment 3. Effect of extract from seed organs of *P.vulgaris* on germination

Dormant seeds that stored at 22°C under dry condition were separated into seed coat and embryo-albumen. Each parts of seeds were homogenized and extracted by ethanol or distilled water. The extracts were adsorbed to filter paper in the dishes. In the case of the ethanolic extract, ethanol was evaporated before injection of water. The seeds stored at 5°C under wet condition were subjected for the germination test as a bioassay of activity. The germination test was done under the same conditions as those in the experiment 1.

Results and Discussion

Experiment 1. Effects of removal of seed coat on germination during after-ripening of seeds

Intact seeds stored at 22°C under dry condition did not germinate at all during storage. The dormancy of intact seeds was not broken by GA_3 treatment (Fig. 2:○,●). The removal or partial cut of seed coat increased germination percentages of seed. After 20 days after storage, the germination percentages of peeled seeds were always high (Fig. 2:△,▲). On the other hand, the germination percentages of partially cut seeds increased in progress with after-ripening, and GA_3 treatment increased the germination percentage (Fig. 2:□,■). Therefore, it was thought that the treatment of GA_3 acted as substitution for after-ripening.

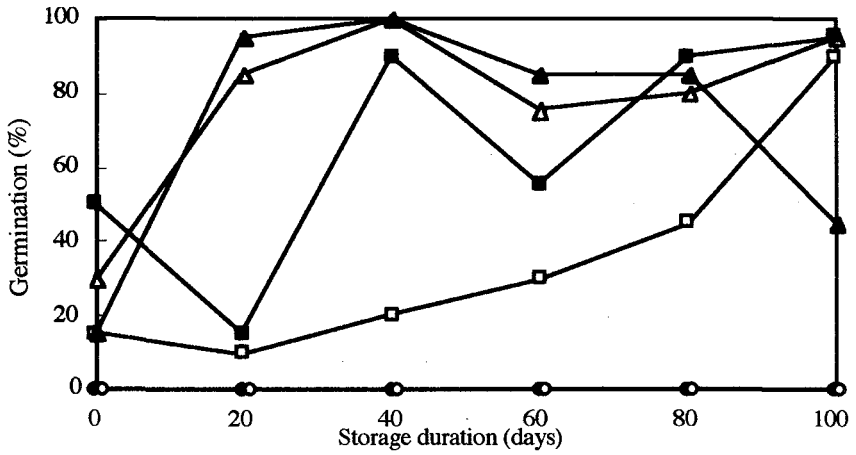


Fig. 2 Effect of removal of seed coat and GA3 treatment on the germination of *P.vulgaris* seeds stored under dry conditions.

○,●: intact seeds, □,■:seed coat patially cut, △,▲:seed coat removed. Open symboles show the data from water medium, and closed symboles show that from the medium containning100ppm of GA3.

On the other hand, the dormancy of seeds stored under cool and wet condition was broken gradually after 60 day's storage and was broken almost perfectly without any seed coat treatments during storage for 100 days (Fig. 3:○,●). The partial cut of seed coat increased the germination percentage remarkably after 20 days of storage (Fig. 3:□,■). GA_3 treatment did not increased germination percentage of these seeds remarkably as compared with the seeds stored under dry condition. Although the removal of seed coat increased the germination percentage on the 20th day of storage, the germination percentages were decreased after 40 days of storage (Fig. 3:Δ,▲). The reason for decline of the germination percentage was considered that the embryo of the seeds stored at 5°C under wet condition were damaged easily at cutting off seed coat.

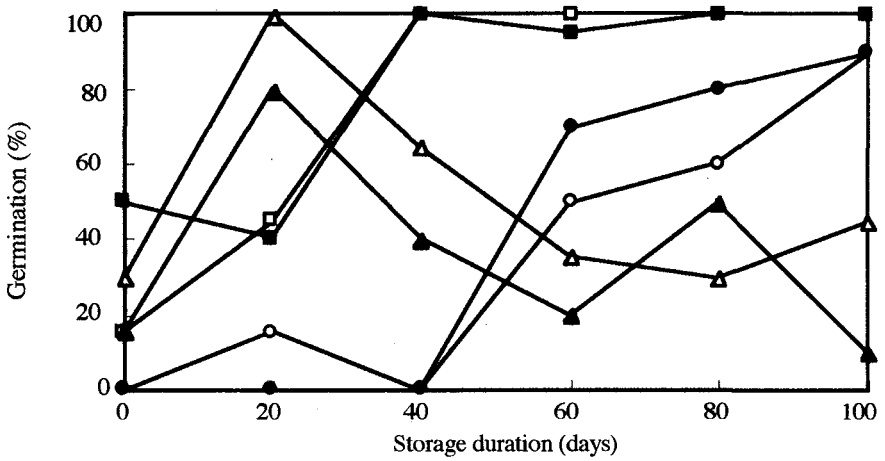


Fig. 3 Effect of removal of seed coat and GA3 treatment on the germination of *P.vulgaris* seeds stored under wet and cool conditions.

Symboles in the figure are same as those in Fig. 2.

These results suggested that the seed coat prevented the germination of dormancy seeds and GA_3 was substituted the after-ripening of seeds in *P.vulgaris*.

Experiment 2. Effects of seed coat removal on the water absorption of seed

Ordinary under optimal condition of water supply, the kinetics of water uptake by seed is triphasic, *i.e.*, first, second and third phase (1). The cutting of seed coat promoted the water uptake of first phase (Fig. 4). The water uptake of third phase of the cut seeds started after 48 hours from imbibition, while the water uptake of intact seeds was stopped at second phase. The cut seeds at the top of seed (Fig. 1 A) showed higher water absorption than those at the edge (Fig. 1 B, Fig.4). In the case of seeds stored under wet and cool condition, the water uptake of first phase was finished already during storage and water uptake of third phase was increased rapidly (Fig. 5). The cutting of seed coat substituted the storage under wet and cool condition for of breaking of dormancy on water absorption. The effect of the treatment was different as cutting position of seed coat (Fig. 6).

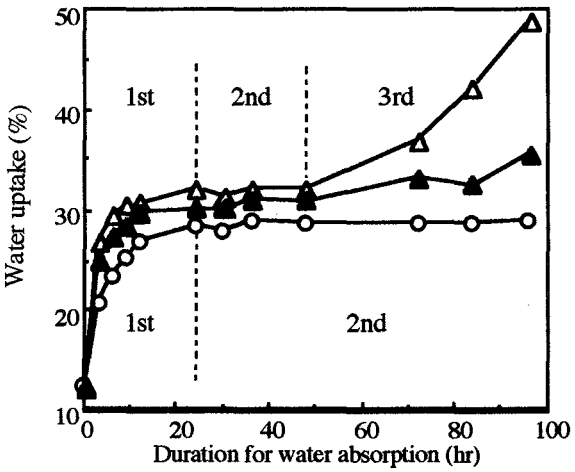


Fig. 4 Effects of the cutting of seed coat on the water uptake during germination of seeds stored under dry condition.
○ :intact seeds (stored under dry condtions)
△:seed coat was cut at the top of seeds
▲:seed coat was cut at the bottom edge of seeds

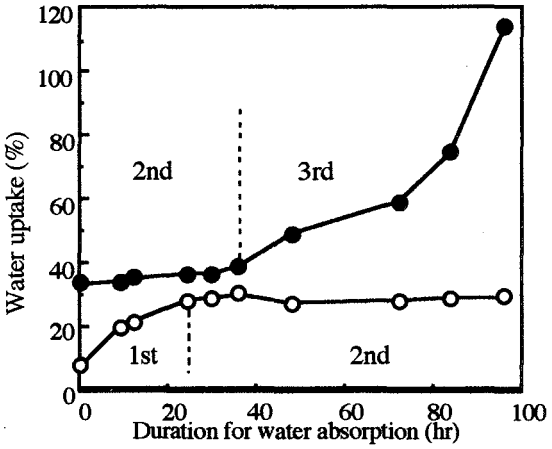


Fig. 5 Effects of the storage condition of seed on the water uptake during germination.
○:seeds stored under dry condtions
●:seeds stored under wet and cool condtions for 78 days

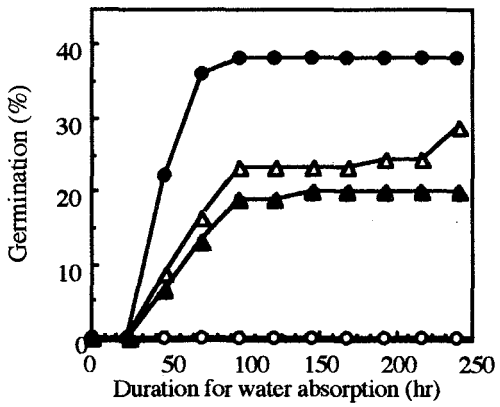


Fig. 6 Effects of the storage condition and the cutting of seed coat on germination of *P.vulgaris*.
○:intact seeds stored under dry condtions
●:intact seeds stored under wet and cool condtions
△:cut seed at the top of seeds stored under dry condtions
▲:cut seed at the bottom edge of seeds stored under dry condtions

Experiment 3. Effect of seed the extract from seed organs of *P.vulgaris* on germination

The ethanolic and water extract from seed coat under dormant stage did not decrease the germination percentage of dormant broken seeds, and the extract from embryo and albumen also did not prevent the germination of seeds (Table 1). However, the ethanolic extract from seed coat or that from embryo and albumen suppressed the elongation of radicles of *P.vulgaris* (Table 1).

Table 1 Effects of the extracts from the seed organs on the germination and radicle elongation of P. vulgaris

Extracting solvent	Organs extracted	Concentration for the assay (g fr.wt. equivalent /ml)	Germination (%)	Radicle length (mm) (ratio to the blank)
100%Ethanol	Blank	-	100	12.8 (100)
	Seed coat	1.17	100	1.5 (12)
	Embryo & Albumen	1.50	100	2.0 (16)
	Blank	-	100	21.8 (100)
	Seed coat	0.47	100	13.0 (60)
	Embryo & Albumen	0.47	100	5.3 (24)
80%Ethanol	Blank	-	100	
	Seed coat	0.32	100	
	Seed coat	0.48	85	
	Embryo & Albumen	0.32	100	
	Embryo & Albumen	0.48	100	
Water	Blank	-	100	
	Seed coat	0.25	100	
	Seed coat	0.13	100	
	Embryo & Albumen	0.25	100	
	Embryo & Albumen	0.13	100	

It was reported that 5,7-dihydroxychromone, a flavanoid decomposition product, isolated from seeds of *Polygonum lapathifolium* inhibited the germination of velvet leaf seeds (7). That compound was also isolated from *Polygonum persicaria* (6). In this experiment, we did not detect the activity of germination inhibitor(s) from dormant seed organs of *P.vulgaris*. However, it is still not clear whether the seed dormancy of *P.vulgaris* may be controlled rather physically in the term of water barrier.

From the results obtained in the present study, it is suggested that the physiological function of seed coat on seed dormancy changed during the ripening process. The seed coat prevents the water absorption. It plays only a role as a water barrier at least after after-ripening stage. The role of growth inhibitors on the seed dormancy before and/or after after-ripening is still not clear. More investigations are needed to clarify the contribution of growth substance(s) to seed dormancy.

References

1. Bewley,J.D. and M.Black 1985. Seeds. Plenum Press,New York, pp.115-124.
2. Hammerton,J.I. 1967. Studies on weed species of the genus *Polygonum* L. Variations in seed weight, germination behaviour and seed polymorphism in *P.persicaria* L. Weed Res. 7, 331-348.
3. Justice,O.L. 1941. A study of dormancy in seeds of *polygonum*. Cornell Agric.Exp. Sta. Mem. 235, 1-43.

4. Nakatani,K. and K.Noguchi 1989. Breaking dormancy and germination in *Persicaria vulgaris*. Weed Res.Japan 34(suppl.), 73-74.
5. Nakatani,K. K.Noguchi and T.Kusanagi 1992. Effects of photoperiod and temperaturre during the growing period of mother plants on seed dormancy and germination in *Persicaria vulgaris* Webb et Moq. Weed Res.Japan 37, 226-231.
6. Romussi,G. and G. Ciarallo 1974. 5,7-Dihydroxychromone from *Polygonum persicaria* seeds. Phytochem. 13:2890-2891.
7. Spencer,G.F. and L.w.Tjarks 1985. Germination inhibition by 5,7-dihydroxychromone, a flavanoid decomposition product. J.Plant Growth Regul. 4, 177-180.

In vitro Induction of Salt Tolerance in Vetiver Grass

M. Na Nakorn* [1], S. Surawattananon [1], C. Wongwattana [2] and K. Namwongprom [1].
[1] Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand
[2] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.

Abstract Vetiver grass (*Vetiveria zizanioides* Nash) ecotype Srilanka was induced for salt tolerance by culturing calli from young inflorescence on MS medium supplemented with 5 uM 2,4- D and 0.5 - 3.0 % NaCl , at 0.5 % intervals for 45 days. At 0.5 - 1.5 % NaCl 100% of calli survived. At 2, 2.5, and 3.0 % NaCl, their survival percentage dropped to 82.5, 25, and 0%, respectively. The survived calli regenerated to plantlets when transferred to hormone and NaCl free MS medium for 30 days. However, the regeneration percentage declined with the increased concentration of NaCl. The 1.0 - 2.0 % NaCl treated calli regenerated only 10 - 20 %, while there was no regeneration in 2.5 % NaCl although the cultures were observed over 60 days. All obtained plantlets were retested for their salt tolerance by culturing on MS medium with NaCl at the same level as their calli were treated, in comparision with the untreated plantlets. The result clearly showed that the survival percentage of tolerant plantlets was higher.

Key words : salt tolerance, vetiver grass, *Vetiveria zizanioides*, *in vitro* induction, NaCl tolerance

Introduction

At present, tissue culture techniques are commonly employed to improve the salt tolerance plants i.e. in tobacco (Nabors et al., 1980), rice (Yoshida et al., 1983) and alfalfa (Winicov, 1991).

Vetiver (*Vetiveria zizanioides* Nash) is a grass that has a high tendency to prevent soil erosion, soil surface runoff, and increase the underground moisture. This grass can survive on many soil types, regardless of fertility and can grow in a wide range of climates.Their deep penetrating root system can effectively fixed the soil. (National Research Council, 1993). Truong (1995) reported that the mature plants could tolerate mild salt stress but there are no resulting from the *in vitro* study. This work is therefore conducted aiming at inducing the vetiver to be able to tolerate the higher salty degree. Thereby it can grow and improve the soil quality of salt affected area of Thailand particularly in Northeastern part which occupied about 2.8 million hectares of inland saline soil or about 82 % of total salt affected area (Land Development Department, 1992).

Materials and Methods

Callus induction

Young inflorescence of vetiver grass ecotype Srilanka were surface sterilized by spraying with 95 % alcohal and flamed, then were cut into 1 cm. length pieces. These explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 15 μM 2,4-D to activate callus induction. After 15 days, calli formed were transfered to cultured on proliferation medium, MS with 10 μM 2,4-D, and kept for furthur 60 days without any subculturing. Two kinds of calli were developed. One was translucent and watery nonembryogenic callus (NC) and the other was compact nodular and creamy white colour embryogenic callus (EC). EC was selected and cut into small pieces of 0.2 cm. in diameter for salt tolerance induction.

Salt tolerance induction

The divided embryogenic calli were culture on MS medium supplemented with 5 μM 2,4-D and 0-3 % NaCl at 0.5 % interval for 45 days. Total of 50 calli were observed in each treatment. Their survival percentage and callus characters were throughly recorded. Later the salt treated calli were transfered to cultured on regeneration medium or hormone and NaCl free MS medium, for 30 days. Regeneration percentage was recorded. The plantlets obtained were tested for their salt tolerance degree by culturing for 45 days on MS medium supplemented with 5 μM BAP and NaCl at the same concentration as their calli had formerly received. The survival percentage of treated plantlets was observed.

The culture conditions used for those experiment were 16/8 hours light/dark, 38 μmol m^{-2} s^{-1} cool white light and at 27± 2 °C.

Results and Discussion

Calli of vetiver grass ecotype Srilanka were treated with NaCl supplemented in culture medium at various concentration from 0-3.0 %, each at 0.5 % interval for 45 days. The survival percentages were shown in Fig.1. The results showed that 100 % of calli survived at 0-1.5 % NaCl, and when the salinity increased to 2, 2.5 and 3.0 % the survival percentage declined to 82.5, 25 and 0 % respectively. The results were clearly shown that maximum level of NaCl that vetiver calli could tolerate was 2.5 %.

When transferred the survived calli to cultured on NaCl-free regeneration medium, the regeneration percentage (Fig.1) indicated that salt stress directly affected the regeneration capacity of vetiver calli. At 0.5 % NaCl, calli regenerated at 60 % which was 30 % less than the control, although calli were still embryogenic. Eventhough the results in some experiments showed that mild salt stress effected as increasing plant regeneration (Yoshida et al.,1983; Heszky et al., 1991; Unnikrishnan et al.,1991) which possibly related to osmotic pressure of medium and the requirements for the early stages of ontogenesis (Heszky et al., 1991).

At concentration higher than 1 % NaCl, calli proliferated with non-embryogenic calli (NC), watery and translucent in appearance.The NC spread over the callus mass and increased their volume correspond to the salt concentration . At 1.0-2.0 % NaCl the regeneration percentage of treated calli were markedly dropped to 10-20 %. At 25 % NaCl no plantlets able to regenerate although the cultures were observed over 60 days. Similar results obtained in some plant species such as in sorghum (Bhas-Karan et al., 1983), *Pennisetum* (Chandler and Vasil, 1984) and rice (Heszky et al.,1991) indicated that cells or calli had loss their regeneration capacity when exposed to high salt concentration. In addition McCoy (cf. Tal, 1990) found that chromosomal abberrations were involved in the loss of plant regenerability.

Survived plantlets were tested for their salt tolerance by culturing on MS medium added with NaCl at the same level as their calli had formerly received. It was found that at 2.0 % NaCl of which calli could survived and regenerated, the plantlets could not survived (Fig. 2). This result indicated that plantlets tolerated to lower level of NaCl than their calli. Heszky et al. (1991) reported that salt tolerance at various ***in vitro*** developmental stages; callus induction, proliferation and regeneration, were all different. The tolerant calli may develop plantlets which tolerate to lower or higher level of salt stress than their calli. Tal (1990) listed both positive and negative correlations of tolerance response of many plants between whole plant and their cultured tissue or cells.

When determined salt tolerance at each concentration of NaCl, the result showed that survival percentage of treated plantlets was higher than the untreated plantlets. The highest concentration which plantlets from both sources able to tolerate were the same, 1.5 % NaCl. However, at this level treated plantlets performed better than untreated plantlets (Fig. 3). They had green leaves while the untreated plantlets showed necrosis symptom. When the treatment has extended to 60-70 days, these untreated plantlets died while treated plantlets increased in their growth. This means that the testing time of 45 days for this experiment is not long enough to justify the tolerate plants.

In conclusion, this experiment has induced vetiver grass *in vitro* that can tolerate up to 1.5 % NaCl and their survival rate was higher than the normal plants that had a tendecy to tolerate not higher than 1.0 % NaCl.

References

Bhas-Karan, S., Smith R.H., Schertz K.F. 1983. Sodium chloride tolerant callus of *Sorghum bicolor* (L.) Moench.. J. Plant Physiol. 112:459-463.

Chandler, S.F., Vasil K. 1984. Selection and characterization of NaCl tolerant cells from embryonic cultures of *Pennisetum purpureum* Schum (Napier grass). Plant Sci. Lett. 37:157-164.

Heszky, L.E., L.S. Nam, I.K. Simon, E.Kiss, K.Lögös, D.Q. Binth. 1991. *In vitro* studies on rice in Hungary. pp.619-641. *In* Y.P.S. Bajaj.(ed.). Biotechnology in Agriculture and Forestry. vol 14 Springer-Verlag, Berin.

Land Development Department. 1992. Annual Report. Ministry of Agriculture and Co-operatives, Bangkok, Thailand. pp. 70-75.

Murashige, T., F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nabors, M.W. Gibbs, S.E., Bernstein, C.S., Meis, M.E. 1980 NaCl-tolerant tobacco plants from cultured cells. Z. Pflanzenphysiol. 97:13-17.

National Research Council. 1993. Vetiver Grass : A Thin Green Line Against Erosion. National Academy Press, Washington, D.C. pp. 49-58.

Tal, M. 1990. Somaclonal variation for salt resistance. pp.236-257. *In* Y.P.S. Bajaj (ed.). Biotechnology in Agriculture and Forestry. vol. II. Somaclonal Variation in Crop Improvement. Springer-Verlag, Berlin.

Truong, P.N.V. 1995. Vetiver grass, its potential in stabilization and rehabilitation of degraded saline land. Vetiver Newsletter 13:27-28.

Unnikrishnan, S.K., Prakash, L., Josekutty, P.C., Bhatt, P.N., Mehta, A.R. 1991. Effect of NaCl salinity on somatic embryo development in *Sapindus trifoliatus* L. J. Exp. Bot. 42:401-406.

Winicov, I. 1991. Characterization of salt tolerant alfalfa (*Medicago sativa* L.) plants regenerated from salt tolerant cell lines. Plant Cell Reports 10:561-564.

Yoshida,S., M. Ogawa, K. Suenaga, H.C. Ye. 1983. Induction and selection of salt-tolerance mutant rices by tissue culture-recent progress at IRRI. pp.237-254. *In* Proc. Worksh. Cell and Tissue culture Techniques for Cereal Crop Improvement. Science Press, Beijing.

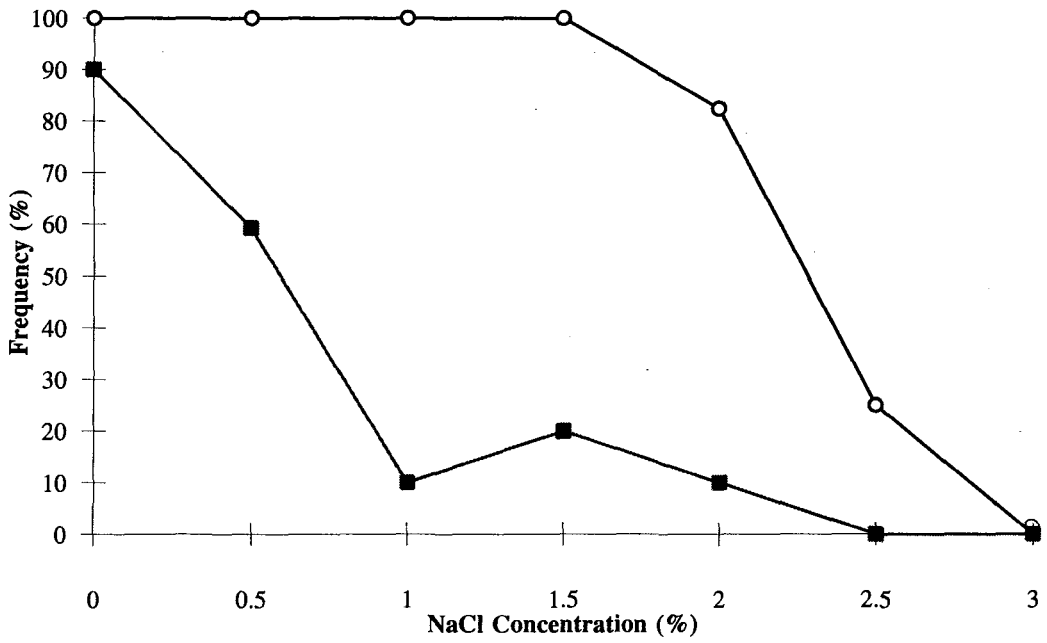


Fig. 1 Survival (—■—) and regeneration (—○—) percentage of calli after treated with various concentration of NaCl.

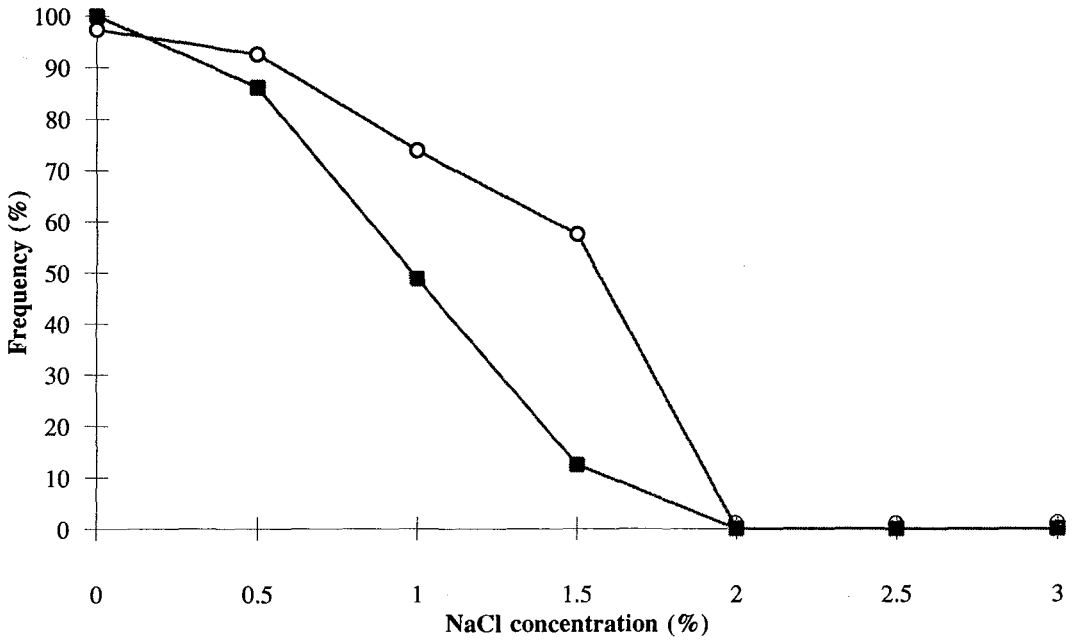


Fig. 2 Survival percentage of treated (■) and untreated calli (○) after 45 days cultured on MS medium supplemented with various concentration of NaCl.

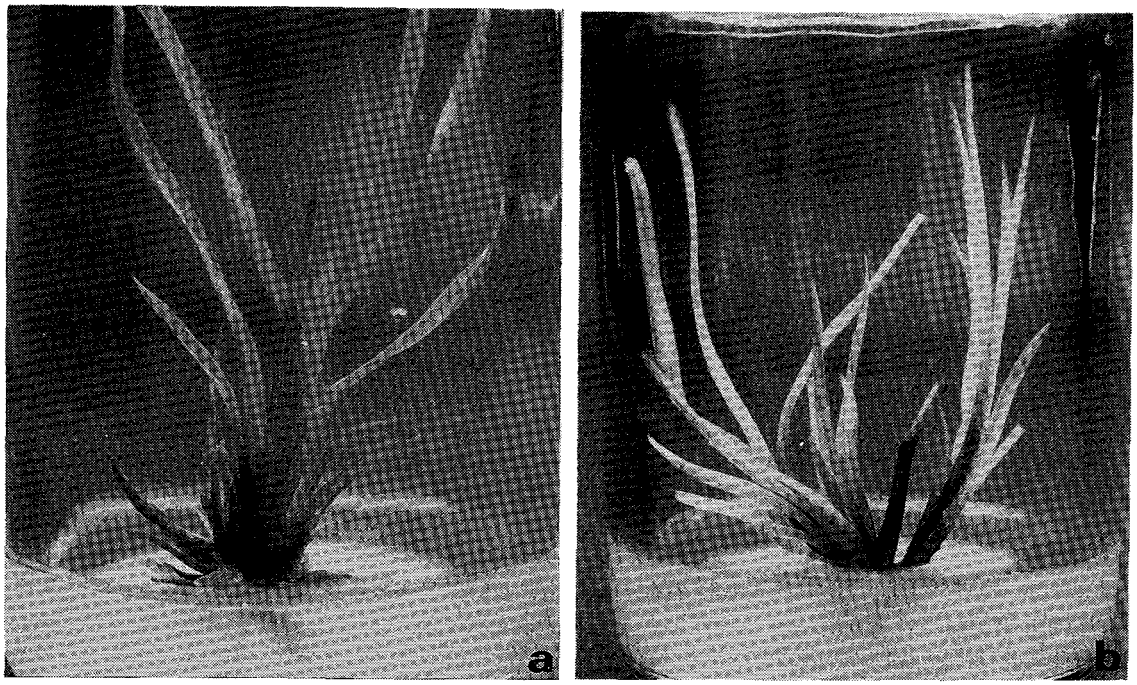


Fig. 3 Responses of plantlets regenerated from treated (a) and untreated calli (b) after 45 days culturing on MS medium supplemented with 5 μM BAP and 1.5 % NaCl.

POPULATION DYNAMICS OF HORSENETTLE (*Solanum carolinense* L.) SHOOTS IN PASTURES

T. Nishida * and N. Harashima
National Grassland Research Institute, Tochigi, Japan

Abstract. A study was conducted in 1994 at the National Grassland Research Institute to follow the population dynamics of horsenettle shoots in pastures. Five quadrates (1 m×1 m) were set up in a pasture. Each pre- and post-grazing period (rotational grazing, one week grazing and three weeks rest), shoot number and growth stage was recorded from May to October (the end of the grazing season). The number of damaged shoots was also counted as a post-grazing measurement. The numbers and diameters of berries were recorded in each quadrate in November when most berries were mature. At the same time, the diameters of berries located outside the quadrates were measured and the number of seeds per berry was counted to obtain a regression curve between the diameter and the number of seeds. The number of horsenettle shoots reached a maximum (31 shoots/m^2) in late August, and slightly decreased thereafter. Shoots continued sprouting throughout the experimental period and half or more of them, except those which sprouted in August, persisted until the end of the grazing season, though shoots were damaged by cattle. Shoots, which sprouted by July, set berries, while those, which sprouted in August and September, reached the flowering and budding stage, respectively. Horsenettle set berries by August. Budding, flowering and setting berries continued throughout the remainder of the grazing season and only 7.4 berries/m^2 were counted in November because of damage by grazing cattle. The number of seeds as calculated from the regression curve was 111/m^2.

Key words: Horsenettle, *Solanum carolinense* L., Population dynamics, Shoot, Pasture

Introduction

Horsenettle (*Solanum carolinense* L.) is a persistent perennial weed that reproduces from seeds, root cuttings, and creeping roots. This species, which is native to the southern part of the United States (5), is a noxious weed in Ontario (Canada) and the north eastern part of the United States (3). In Japan, Tsuji recorded the presence of horsenettle in 1906 (1), however, generally, it was not noticed as a very troublesome weed in pastures until the 1970's (10, 11). In recent years, it is also becoming a problem in fodder corn fields (8).

Horsenettle is difficult to control because of its extensive root system. Several studies have indicated that control of this species may result from clipping or herbicidal application made during the period of low root reserve (5,11). However, the most effective period for herbicidal application has not been clarified (2,4, 7).

The knowledge of the life cycle of weeds is needed to achieve acceptable control. Nichols et al. (7) have reported the biology of this species in bermudagrass pastures in Georgia. In Japan, however, information on the life cycle of horsenettle in pastures is lacking. Therefore, a field study was conducted to describe the changes in population and growth stage, and seed reproduction in a temperate grass pasture.

Materials and methods

Experimental site. The experiment was conducted in 1994 at the National Grassland Research Institute, Tochigi, Japan. The experimental site was located at 36° N and 139° E. The soil was brown lowland soil and slightly covered with volcanic ash. It also has a layer of rounded gravel appearing about 20 cm below the soil surface and is well drained (Entic Haplumbrepts, fragmental, mixed, mesic) (6). It was a hot, dry summer in 1994 (Fig. 1).

Site management. The pasture was dominated by cooksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundenacea* Schreb.), red top (*Agrostis alba* L.), sweet vernal grass (*Anthoxanthum odratum* L.) and white clover (*Trifolium repens* L.). The pasture was 1.2 ha and divided into four paddocks. Four cows with 6 calves grazed one week on each paddock in rotation, i.e., each paddock was grazed every four weeks. Grazing began in early April and ended in late October. The pasture received 24 - 36, 24 - 48 and 24 - 36 kg/ha N, P_2O_5 and K_2O, respectively on each of the five following dates: November 29, 1993, March 3, June 6, July 29 and September 26, 1994. The total amount of fertilizer was 152, 180 and 152

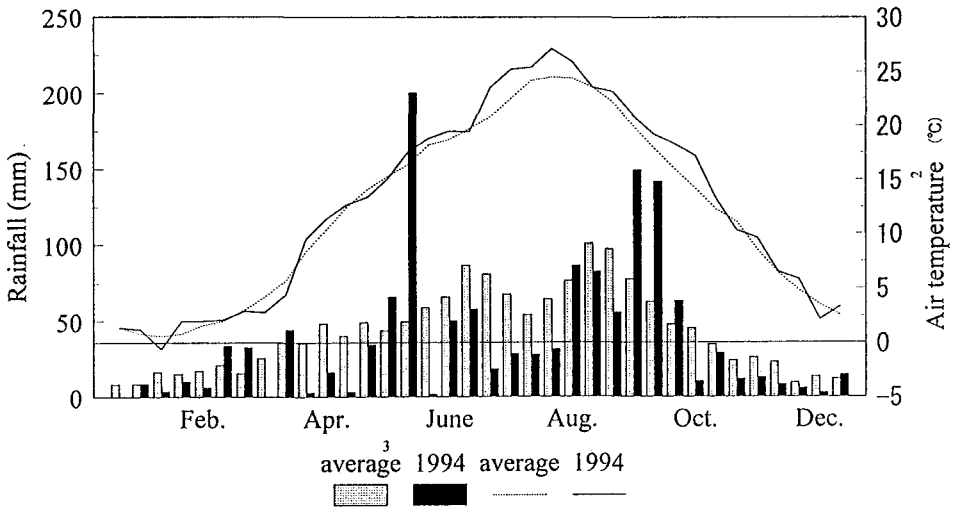


Fig. 1. Meteorological data at the experimental site.
1: Total rainfall in ten days. 2: Average temperature over ten days. 3: Average over 1961 - 1990.

kg/ha of N, P_2O_5 and K_2O, respectively. Dry matter yield (not including horsenettle) was 6.8 t/ha and the consumption rate was 97 % in 1994.

Measurements. Horsenettle covered approximately one fifth of a paddock. Five quadrates (1 m × 1 m) were set up at the site. Each pre- and post-grazing period, all shoots (emerged erect branches), shoots with buds, shoots with flowers, and shoots with berries were counted from May 27 to October 21. Each shoot was marked with a color ring which indicated the sprouting date of the shoot as a pre-grazing measurement. The number of damaged shoots was recorded as a post-grazing measurement. The numbers and diameters of berries were recorded in each quadrate on November 21 when most berries were mature. At the same time, the diameters of berries located outside of the quadrates were measured and the number of seeds per berry was counted in order to obtain a regression curve between the diameter and the number of seeds.

Result and discussion

Population dynamics. The number of horsenettle shoots reached a maximum (31 shoots/m^2) in late August, and slightly decreased thereafter (Fig. 2). Shoots continued sprouting throughout the experimental period (Fig. 3). The initial shoot numbers in the cohorts were larger in the spring than those in the summer, and the latter were larger than those in the autumn. Cattle damaged about 83 % of the shoots on the average over the grazing periods (Table 1). The percentage of damaged shoots was lower in the summer, though they were not significantly different from the others. This concave curve may imply that it was difficult for cattle to touch the inside shoots of the stands because horsenettle was thick in the summer. The percentage of dead shoot numbers during a grazing period to those in the pre-grazing period tended to be higher in the first half of the experimental period (Table 1). However, the population still increased in this period because new shoots sprouted rapidly (Figs. 2, 3). In spite of being damaged by cattle, approximately half or more of the horsenettle shoots, except for those which sprouted in August, persisted until the end of the grazing season (Table 2). The reason why the percentage of persisted shoots was low in the cohort of August was speculated as follows. They were not as strong as those which sprouted by July because they did not grow in a favorable period for vegetative growth in terms of both environmental and physiological conditions and were influenced by well-established shoots. In addition, they were exposed to grazing longer than those which sprouted in September or October.

Only one seedling was recorded in this study and it persisted for one month (Fig. 2). Grazing cattle did not damage it but it withered one month after it emerged.

Growth stage. Several horsenettle shoots already had buds in late May and began flowering by June (Table 3). Horsenettle set berries by August, and budding, flowering and setting berries continued throughout the remainder of the grazing season. Maximum flowering occurred in the late summer, but the number of shoots with berries remained constant to when they were first counted.

Shoots, which sprouted by July, set berries, while those, which sprouted in August and September,

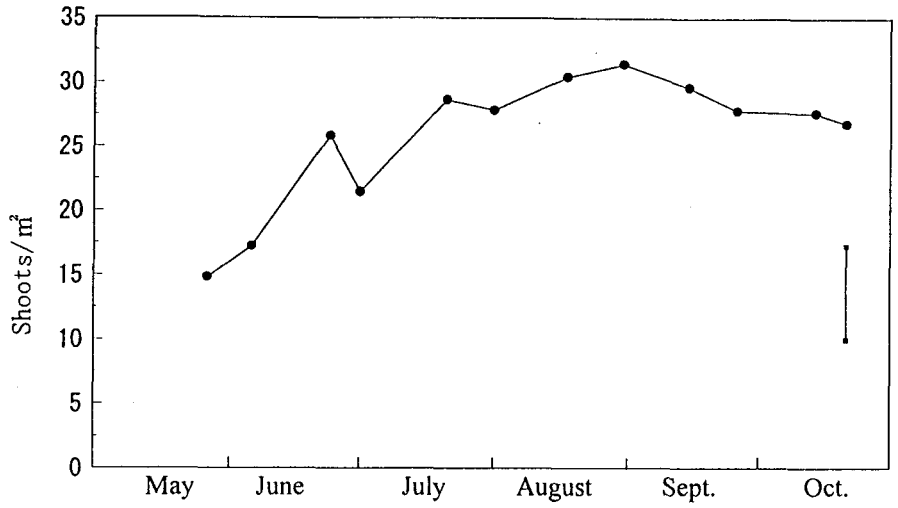


Fig. 2. Horsenettle shoot population in a temperate grass pasture.
A vertical bar represents common standard deviation.

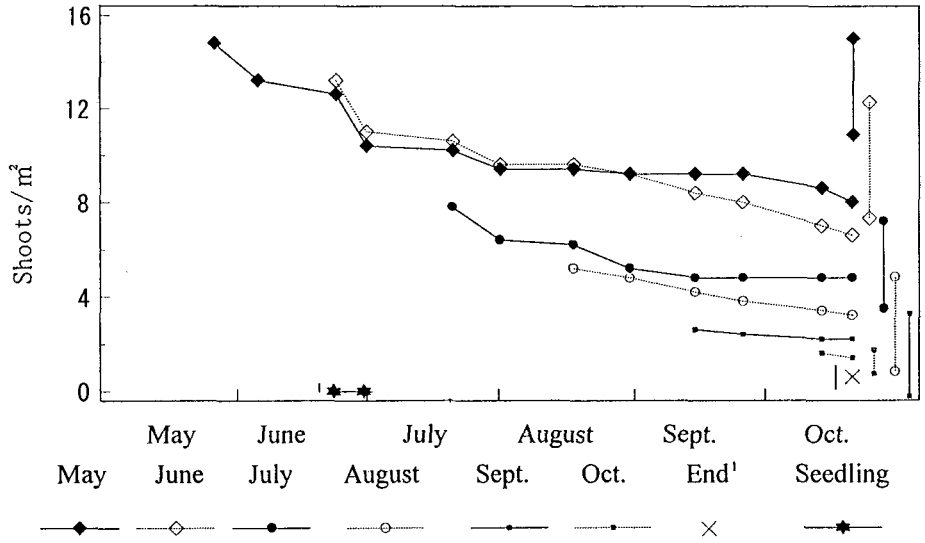


Fig. 3. Horsenettle shoot population in each cohort.
1: The end of the grazing season. Vertical bars represent common standard deviation in each cohort.

Table 1. The percentage of damaged and dead horsenettle shoots in a grazing period.

	Gazing					
	May	June	July	Aug.	Sept.	Oct.
Damaged shoots (%)	93	91	69	73	79	93
Dead shoots (%)	12	17	12	6	4	5

Figures are averages over the five plots. Averages within a row are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 2. The percentage of persisted horsenettle shoots until the end of the grazing season in each cohort.

	Cohort					
	May	June	July	Aug.	Sept.	Oct.
Persisted shoots (%)	56ab	49ab	59ab	37b	89a	88a

Figures are average over the plots in which the cohort sprouted. Averages within a row followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 3. Number of horsenettle shoots with buds, shoots with flowers and shoots with berries per m^2 in pre-grazing measurements.

	Total	Cohort				
		May	June	July	Aug.	Sept
— Date —	Shoots with buds (shoots/m^2)					
May 27	1.6b	1.6bc				
June 24	10.4a	7.0a A	3.4a A			
July 21	9.2a	4.2b A	4.4a A	0.6a A		
Aug. 18	9.4a	3.0bcA	2.2a A	2.4a A	1.8a A	
Sept. 15	9.0a	2.8bcA	2.2a A	1.2a A	1.4a A	1.4 A
Oct. 14	0.6b	0.4c A	-	-	0.2a A	-
	Shoots with flowers (shoots/m^2)					
June 24	0.4b	0.4b	-			
July 21	1.0b	0.6b A	0.4b A	-		
Aug. 18	10.4a	3.4a AB	4.4a A	2.2a AB	0.4a B	
Sept. 15	7.8a	2.4abA	3.2a A	1.2a A	1.0a A	-
Oct. 14	2.2b	1.2abA	0.4b A	-	0.6a A	-
	Shoots with berries (shoots/m^2)					
Aug. 18	2.4a	1.4a A	0.8a A	0.2a A	-	
Sept. 15	3.4a	1.6a A	1.2a A	0.6a A	-	-
Oct. 14	3.4a	1.6a A	1.0a A	0.8a A	-	-

A shoot with buds, flowers and berries was counted as a shoot with berries. The cohort of October stayed in the vegetative growth stage. Figures are averages over the five plots. Averages within a column followed by the same lower-case letter or those within a row followed by the same capital letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

reached the flowering and budding stage, respectively. Shoots, which sprouted in October, stayed in the vegetative growth stage. There were several shoots which already had buds when they were initially counted, in cohorts except the cohort of October. The number of shoots with flowers tended to peak in the late summer in each cohort, though it was clear only in the cohorts of May and June. In addition, there were no significant differences between cohorts in the number of shoots with flowers on the same date, except for the cohort of August on August 18th. Thus, it was considered that the later sprouting shoots reached the reproductive stage more quickly than the earlier sprouting shoots and they flowered at the same time.

Seed production. Although many flowers and immature berries were observed, only 7.4 berries/m^2 was counted on November 21 (Table 4) because of damage by cattle. The regression curve between the diameter and the number of seeds had good correlation (Fig. 4). The number of seeds as calculated from the regression curve was 111/m^2.

Table 4. Number of berries per m^2 and seed number per m^2.

Berries/m^2	Seeds/m^2
7.4 (7.5)	111(116)

Figures are averages over the five plots. Figures in parentheses are standard deviation over the five quadrates.

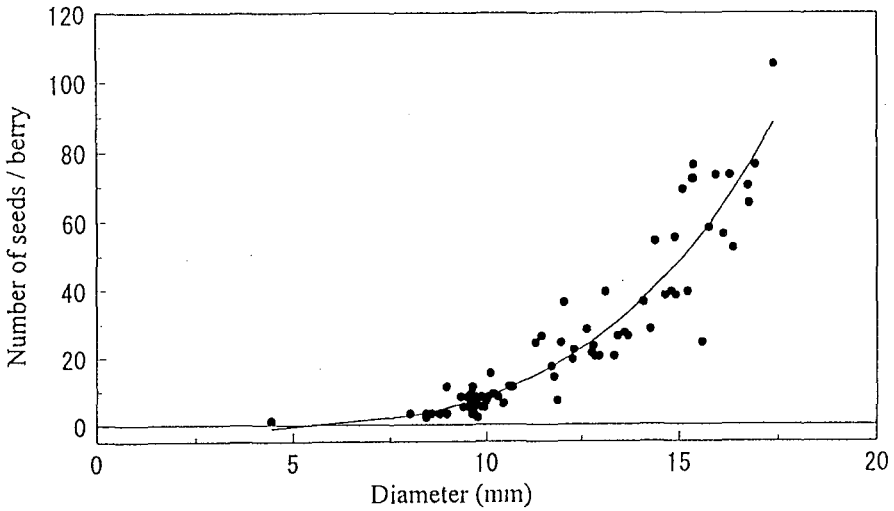


Fig. 4. The regression curve between the diameter of horsenettle berries and the number of seeds.
Number of seeds = 9.862 x 10^(-4) x (diameter)^4 - 1.563 r2 = 0.87 ($p < 0.01$)

Discussion. Only one seedling was observed and it did not establish in this study. Germination of horsenettle seed was inhibited by horsenettle plants incorporated into the soil and this process can result in a density-dependent regulation of population size (9). Therefore, it is suggested that horsenettle seedlings to recruit into an established stand seldom happens in a pasture. It was observed that grazing reduced the seed production of horsenettle. However, it can be considered that grazing contributes to the control of horsenettle by defoliation and trampling of young shoots in the early summer rather than reduction of the seed production from this respect. The large size of the cohort in June to compensate for the damage by cattle suggests that severe grazing (or frequent cutting) at this time may effectively reduce carbohydrates in the root system. Several studies (5, 7) suggest that the principal translocation from spring to early summer is acropetal. This indirectly supports our idea.

In order for herbicides to be effective, they must be applied during the period in which the principal translocation of this weed is basipetal. The study of Nichols et al. (7) suggests that accumulation of root carbohydrates begins less than one month after maximum flowering. This implies that treatment with herbicides in the late summer or early autumn may be effective under this experimental condition. Further experiments are necessary to determine the most effective period to control horsenettle with herbicides under grazing conditions.

Acknowledgments: The authors are grateful to Dr. K. Okada, Director of Department of Grazing Animal Production, National Grassland Research Institute for the critical reading of the manuscript. We also wish to thank Ms. Aoki who investigated grazing cattle in the same pasture and agreeably allowed us to do this investigation.

References

1. Asai, Y. 1993. Green invaders. 30-31. Asahi shinbunsya. Japan.
2. Banks, P. A., M. A. Kirby, and P. W. Santelman. 1977. Influence of postemergence and subsurface layered herbicides on horsenettle and peanuts. Weed Sci. 25:5-8.

3. Bassett, I. J. and D. B. Munro. 1986. The biology of Canadian weeds. 78. Solanum carolinense L. and Solanum rostratum Dunal. Can. J. Plant Sci. 66:977-991.
4. Grorrell, R. M., S. W. Bingham, and C. L. Foy. 1988. Translocation and fate of Dicamba, Picloram, and Triclopyr in Horsenettle, Solanum carolinense. Weed Sci. 36:447-452.
5. Ilnicki, R. D., T. F. Tisdell, S. N. Fertig, and A. H. Furrer, Jr. 1962. Life history studies as related to weed control in the northeast. 3－Horsenettle. Agric. Exp. Stn., Univ. Rhode Island, Bull. 368. 54 pp.
6. Kurashima, K., K. Oota, T. Kusaba, H. Amano, K. Yamamoto, T. Kimura, H. Kondo, and G. Saito. 1993. Classification and characteristics of soils in National Grassland Research Institute. Misc. Publ. Natl. Grassl. Res. Inst. 3. 47 pp.
7. Nichols, R. L., J. Cardina, and T. P. Gaines. 1991. Growth, reproduction and chemical composition of horsenettle (Solanum carolinense). Weed Tech. 5:513-520.
8. Shimizu, N., S. Uozumi, T. Nishida, N. Harashima, K. Matoba, Y. Tamura, M. Tachibana, K. Ito, K. Hagino, S. Sato, R. Kobayashi, and H. Tateno. 1994. Recent situation of emergence of alien weeds invading grasslands and forage crop fields. Weed Res., Japan. 39 Supplement Ⅰ:228-229.
9. Solomon, B. P. 1983. Autoallelopathy in Solanum carolinense － reversible delayed germination. Am. Midland Nat. 110:412-418.
10. Suzuki, Kanae. 1975. Ecology and control of horsenettle. Shokutyo. 9(6):10-17.
11. Takematsu, T., M. Konnai, Y. Takeuchi, and N. Ichizen. 1979. Study on the ecological properties and control of perennial weed, horsenettle. Utsunomiya Daigaku Nogakubu Gakujutsu Hokoku. 10:93-102.

Systematical Classification of *Echinochloa* Species

Sang-jo Park, Kil-Ung Kim and Dong-Hyun Shin
Dept. of Agronomy, Coll. of Agri., Kyungpook Nat'l Univ. Taegu 702-701, Korea

Abstract. Echinochloa accessions collected in Korea varied in height, standing type, heading time, seed weight and tiller number. On the basis of cluster analysis of 16 morphological descriptors, accessions were divided into two groups. Group I is spreading (decumbent) and open tillering type, producing small seeds at two months after seeding. Group II is erect and compact tillering type. Group II is segregated into 4 subgroups; subgroup IIa has early heading, small seed size, open and nodding panicle, subgroup IIc having short height, great number of tillers and late heading, subgroup IId having long stem length, wide flag leaf and small seed size. Subgroup IIb has middle size and specific polypeptides 3 and 10.

Introduction

It was reported by Yabuno (1975) that *E. crus-galli* var. *crus-galli, E. crus-galli* var. *praticloa, E. crus-galli* var. *formosensis* and *E. oryzicola* are present in Korea. Identification of *Echinochloa* species is not simple due to intraspecific morphological variation. This study was conducted to find out the useful markers in identifying *Ecinochloa* species with systematical analysis.

Material and Methods

Thirsty-eight accessions collected in Korea and two accessions obtained from Yabuno were used in this study. Main stem length, tiller number, primary rachis-branch number, longest primary rachis-branch length, flag leaf length and width, panicle length, awn length, 4th indternode diameter and length, plant growing type, internode number of main stem, and days to the first heading were measured from heading to maturing stage. These morphological descriptors were subjected to cluster analysis using the average linkage clustering strategy and associated dendrogram.

For the seed protein analysis by two dimentional electrophoretic system, protein was extracted from decoated seeds with 9 M urea solution. The isoelectric focusing and the sodium dodecyl sulphate polyacrylamide gell electrophoresis were followed by the mothod of Kim et al. (1992). The gels were stained with silver soluton. For the comparisons of seed protein variability, protein aliquots of individuals were combined in one pools. This combined-protein gel was used as templates to detect polymorphisms. The presence of spot was encoded as 1 and its absence as 0. The correspondence analysis was made in order to describe the data structure.

Results and Discussion

Morphological descriptors. The result of cluster analysis is expressed as dendrogram for morphological descriptors (Fig. 1). Thirsty-eight Korean accessions were easily segregated into two groups with plant standing type, spreading (Group I) and erect type (Group II). Group I grows at the slant or creep before heading stage. It was erect to decumbent and have open tillering at the maturity. Group I was distinguished by side panicles (1 to 2 per stem) produced from side tillers. First panicle headed at 69.5 ± 8.9 days after seeding. One thousand seeds weight ranged from 1.33 ± 0.3 g. Group I varied in plant height, awn length, tiller number, stem colour, heading, and seed size.

Group II having erect type and compact tillering was divided into 4 subgroups on the base of heading time, seed size, tiller number, height, panicle shape and primary rachis-branch number. Subgroup IIa (accessions EC03 and EC20) produced the first panicle early as accessions of Group I. Its panicle shape was open, long and nodding at maturity. It produced small seed without awn (1000 seeds weight; 1.2 g). This subgroup was classified into *E. crus-galli* by the mean of Yabuno's seed morphological method. Subgroup IIb (accessions EC23, Ec32, EC33 and EC34), 1.4 to 1.5 m tall at maturity, produced the first panicle about one month later than Group I. The panicle shape was closed and not nodding at maturity. Its seed weight was two times greater than that of Group I and subgroup IIa. Subgroup IIc (accessions EC02, EC15 and EC24) had a middle height at maturity and made many tillers (87.1 ± 9.9). Heading time was the latest among groups, the first panicle at 105 days after seeding. One thousand seed weight of this subgroup (2.9 ± 0.9 g) was heavier than those of another subgroups. Some plant of EC24 had ligules and auricles as rice. Accession number EC38 was divided into subgroup IId, which have been cultivated for eating. Height became over 1.7 m at maturity and flag leaf width was two times wider than those of anothers. Subgroup IId produced awnless small seed (1.2 g/1000 seds).

Protein variability. Protein polymorphism of each accession was not variable. Eleven among intense polypeptides were differently stained (Fig. 2). It is clear that polypeptides 3 and 10 have a close relationship with erect and large seed accessions of Group II (Fig. 3). In case of EC03, EC20 and EC38, erect type by cluster analysis with morphological descriptors, they have a similar protein polymorphisms as the spreading type.

Based on the results of cluster analysis with morphological descriptors and correspondence analysis with polypeptides, *E. oryzicola* species were well segregated from *E. crus-galli* species with morphological descriptors, erect, compact tillering heading time and large seed, and polypeptides 3 and 10.

References

Kim, K.U., K. Oono, D.H. Shin, S. Virigool, K. Y. Shinozaki, and S.Y. Hong. 1992. Isolation and characterization of gene(s) related to preservation of plant cells and its transfer to economic plant. I. Survival mechanism of dried calli and isolation of gene(s) related to preservation of rice cells. Korean J. Plant Tissue Culture 19:93-100

Yabuno, T. 1975. The classification of geographical distribution of genus *Echinochloa.* Weed Research (Japan) 20:97-104

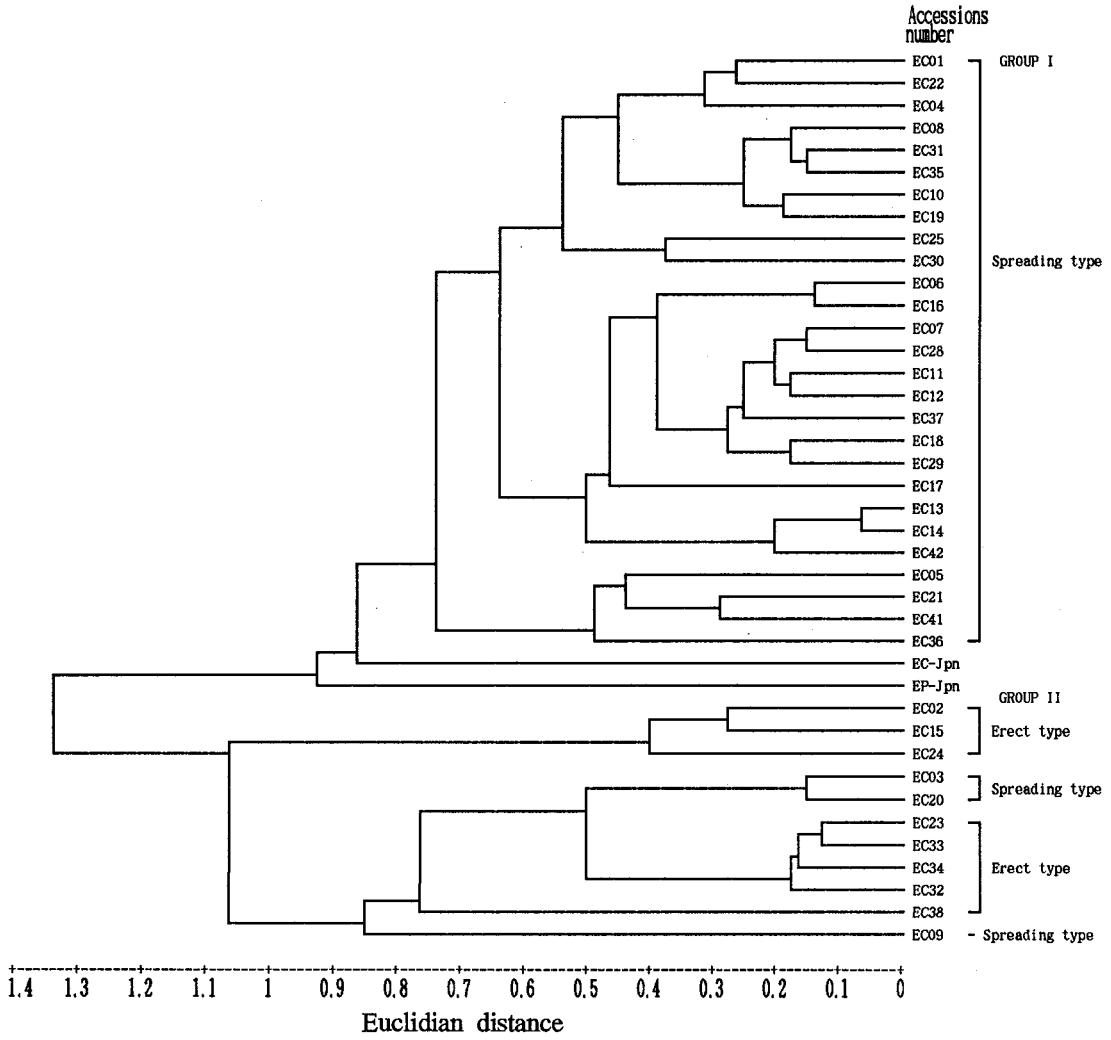


Fig. 1. Dendrogram made by average linkage cluster analysis of 16 morphological descriptors.

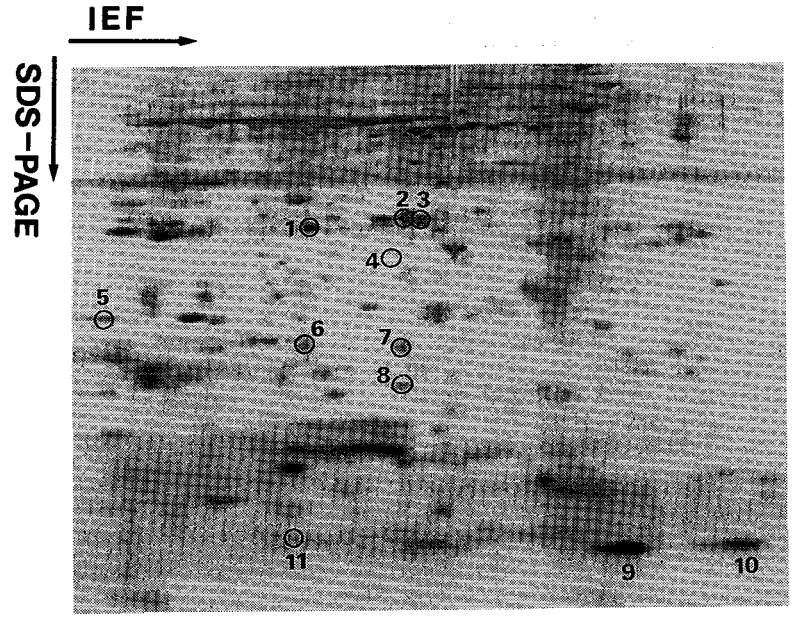


Fig. 2. Two-dimentional protein pattern obtained from the bulked protein pools of *Echinochloa* accessions. Polypeptides marked with numerical numbers are used in correspondence analysis.

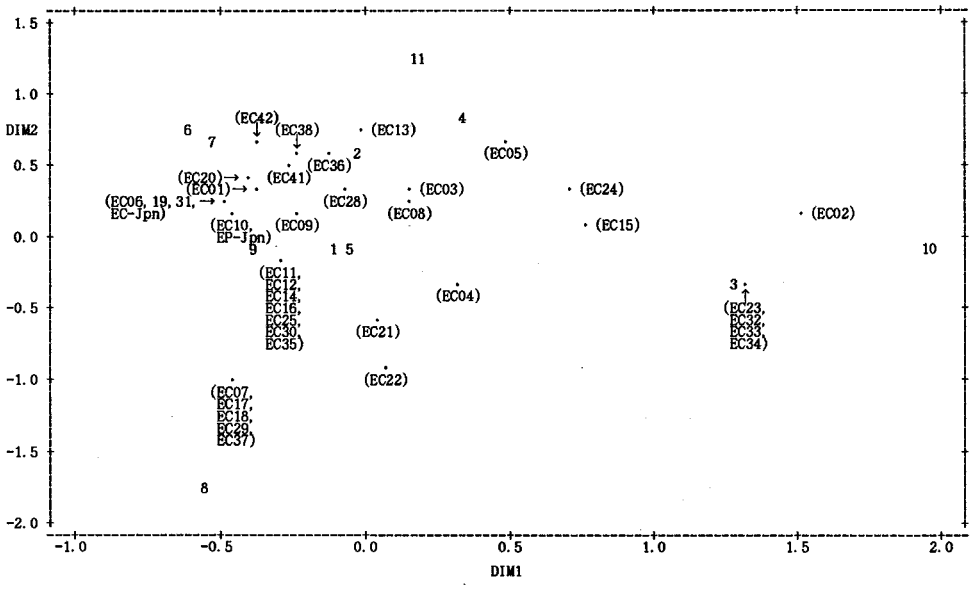


Fig. 3. Distribution of the individuals and polypeptides (numerical numbers) in the first plane of the correspondence analysis.

Allelopathic Potential of Gooseweed (*Sphenoclea zeylanica* Gaertn.) in Submerged Soil.

C.* Premasthira and S. Zungsonthiporn

Weed Science Group, Botany and Weed Science Division , Department of Agriculture , Chatuchak, Bangkok 10900, Thailand.

Abstract : The effects of gooseweed incorporation in submerged soil on the growth of rice and paddy weed seeds was conducted in a greenhouse. The results showed that the decomposed gooseweed in submerged soil can inhibit growth and delay flowering stage of white head (*Eclipta prostrata* Linn.) red springletop (*Leptochloa chinensis* (L.) Ness) and barnyard grass (*Echinochloa crusgalli* (l.) Beauv.)

" ***Key Words*** " : gooseweed (*Sphenoclea zeylanica* Gaertn.) , Submerged soil, Paddy weed, incorporated.

Introduction.

Gooseweed is a troublesome weed in paddy fields. Especially in submerged area in the central region of Thailand. Gooseweed grows well during dry season in submerged soil. They are incorporated in soil before broadcasting rice seeds. According to Chou and Lin (1976), the residues of rice plant in submerged soil affected the growth of rice plant growing in the next season. Moreover, although some weed can not release toxic substances through root exudation, when they decompose in soil the toxic substance will come out (Johnson III and Coble, 1986). Gooseweed is an allelopathic weed of which substances inhibited the growth of weed seeds (Premasthira , 1985 and Premasthira , 1990a).Thus when gooseweed is incorporated in soil before rice growing, the plant growth inhibiting substances from gooseweed will affect weed seeds in that soil. Besides, 3 - 4 weeks after gooseweed is incorporated in the soil, rice plant receivers the least effect from gooseweed residues (Premasthira, 1990b) . Therefore, the effect of plant growth inhibiting substances of gooseweed in soil on the growth of weeds was investigated.

Materials and Methods

Five seedlings of gooseweed were grown in a square pot (67 cmW x 80 cmL x 45 cmH.) Sixty days after planting, gooseweed at the mature stage was cut into 1 inch pieces and mixed into that soil. The water level in these pots was kept about 1 inch above the soil surface. After three weeks of incubating gooseweed in the soil, rice and 4 kinds of paddy weed seeds were broadcasted in the pots. The weed seeds were barnyard grass (*Echinochloa crusgalli* (L.) Beauv.) , red springletop (*Leptochloa chinensis* (Linn.) Nees) , white head (*Eclipta prostrata* Linn.) and small flower umbrella plant (*Cyperus difformis* Linn.) . Rice and weed seeds were grown in the untreated soil as a control group. Visual observation of growth was conducted and height of plant was measured 30 and 45 days after planting. Yield component of rice plant was checked at harvesting time. The experimental design is randomized complete block , with four replication.

Results and Discussions.

The growth of rice and weeds growing in the treated soil was observed. The result showed that 30 days after planting, red springletop and white head weed were evidently inhibited as compared with those in the untreated soil. All kinds of experimental weed plant appeared light green, while weed plant in the untreated soil was dark green. The flowering stage of weeds growing in the treated soil was delayed. Forty-five days after planting , rice plant growing in the untreated soil was darker green than that growing in the treated soil. However, the experimented weed plant was lighter green than that in the untreated soil. The height of rice and weeds was measured 30 and 45 days after planting, the results are in Figure 1. Thirty days after planting , the rice plant in the treated soil was shorter than that in the untreated soil, but it was not significantly different. The red springletop , white head

and barnyard grass growing in the treated soil were significantly shorter than those in the untreated soil. Anyhow, the small flower umbrella plant in the treated soil was taller than that in the untreated soil. Forty-five days after planting, the white head growing in the treated soil was still significantly shorter than that in the untreated soil. While the red springletop and barnyard grass was still shorter than those in the untreated soil, they were not significantly different. Figure 2 shows the number of tiller of rice plant and grassy weed. It shows that the rice plant in the treated soil has fewer tiller than that in the untreated soil, but the number of tiller of red springletop and barnyard grass were not significantly different in both types of soil. The yield component of rice plant at harvest is in Table 1. It shows that the weight of filled grain and 1000 grains of the rice plant growing in the treated soil was more than that growing in the untreated soil, although the number of kernel of rice plant in the treated soil are less than those in the untreated soil. From the results, gooseweed was incorporated in submerged soil, decomposed and released the phytotoxic substances into the soil. Especially in the submerged soil , the decomposition of plant is very active (Welbank, 1963). In addition, the phytotoxic substances of gooseweed in the soil affected the growth of white head weed more than red springletop and barnyard grass, respectively. Yet, the occurrence increases the effect on the small flower umbrella plant. The effect of substances of gooseweed in the soil was similar to that of the extracted substances of this plant on the weeds in laboratory. (Premasthira, 1990a)

It can be concluded that gooseweed which was incorporated in submerged soil has allelopathic potential to control weed in paddy field , especially white head , red springletop and barnyard, etc. Since gooseweed, a troublesome weed in paddy fields, provides substances inhibiting as well as promoting the growth of plant, it will be a great benefit for weed control if a good management is emphasized.

References.

Chou , C.H. and H.J. Lin. 1976. Autointoxication mechanism of *Oryza sativa*. L. 1. Phytotoxic effect of decomposing rice residues in soil.J. Chem. Ecol. 2: 353-376

Johnson III , W.C. and H.D. Coble. 1986. Effects of Three weed Residues on Weed and Crop Growth. Weed Sci. 34 : 403-408

Premasthira , C. and S. Zungsonthiporn. 1985. Plant Growth Inhibiting Effects of Weed Species with Reference to Allelopathy. Proc. 2. The Tenth Conference of Asian - Pacific Weed Science Society p. 458-462

Premasthira, C. and S. Zungsonthiporn. 1990a. Influence of Allelopathic Substances from *Sphenoclea zeylanica* Gaertn. on Growth of Weeds. Thai Agricultural Research Journal vol 8 : 29-34

Premasthira, C. and S. Zungsonthiporn. 1990b. Effects of gooseweed (*Sphenoclea zeylanica* Gaertn.) Incorporated in Soil on Rice Growth. Annual Research Report. Weed Science Group, Botany and Weed Science Division . Department of Agriculture. 10 pp.

Welbank, P.J. 1963. Toxin production during decay of *Agropyron repens* (Couch grass) and other species Weed Res. 3 : 205-214

Table1: Yield component of rice plant growing in gooseweed incorporated soil (treated soil) and untreated soil (control)

Treatment	No.of kernel	filled grain. (gm)	unfilled grain (gm)	weight of 1000 seed(gm)	Straw weight (gm)
Untreated soil.	59.00	82.57	14.67	25.74	75.76
Treated soil.	41.5	85.02	9.78	26.87	108.33

Note: Harvesting area = 1 ft^2 / pot

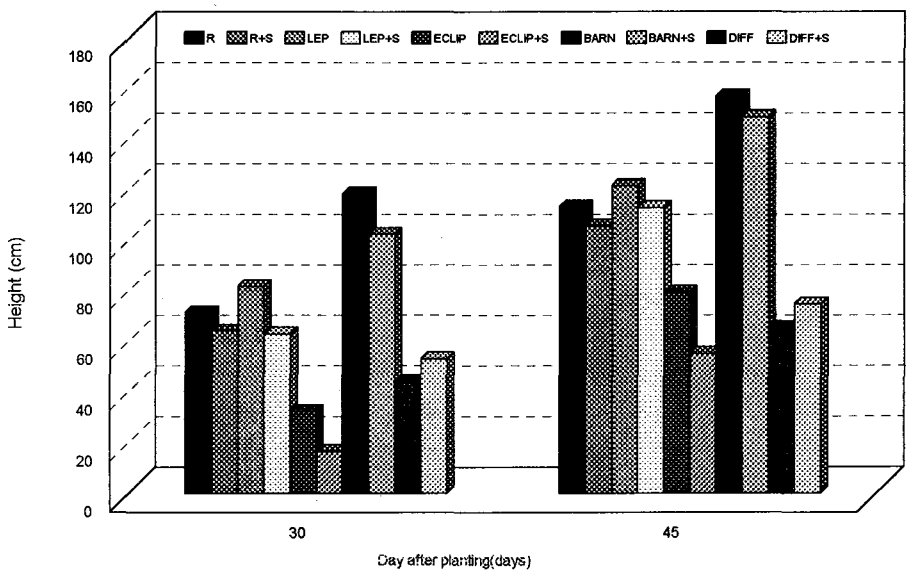


Fig. 1 Height of rice and weeds growing in treated soil (soil incorporated with gooseweed) compared with untreated soil.

R = rice grown in untreated soil, R+S = rice grown in treated soil
LEP = red springletop grown in untreated soil, LEP+S = red springletop grown in treated soil
ECLIP = white head grown in untreated soil, ECLIP+S = white head grown in treated soil
BARN = barnyard grass grown in untreated soil, BARN+ S = barnyard grass grown in treated soil
DIFF = small flower umbrella plant grown in untreated soil,
DIFF+S = small flower umbrella plant grown in treated soil

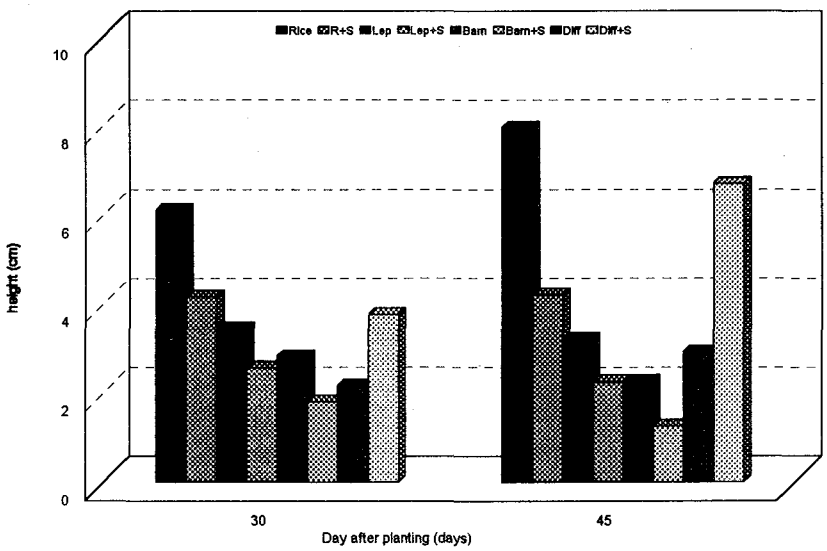


Fig.2 Number of tiller of rice and weeds growing in treated soil (soil incorporated with gooseweed) compared with untreated soil.

EFFECTS OF (Z)-3-HEXENOL AND RELATED VOLATILE COMPOUNDS ON LETTUCE GERMINATION AND GROWTH

T. Shibuya[1], Y. Fujii[2] and K. Noguchi[1]
[1] National Agriculture Research Center, Tsukuba 305, Japan
[2] National Shikoku Agricultural Experiment Station, Zentsuji 765, Japan

Abstract. (Z)-3-Hexenol and related volatile compounds were tested for lettuce germination and initial growth responses. These compounds were naturally detected from many kinds of plant leaves. Germination and initial growth tests were conducted using seeds and seedlings with vapor of the test compounds in sealed containers. Fifty percent reduction in germination after 48 h incubation was caused by 0.05μmol/l of (Z)-3-hexenol, 0.1μmol/l of (E)-2-hexenal, 0.2μmol/l of hexanol, 0.6μmol/l of hexanal, and 1.5μmol/l of (Z)-3-hexenyl acetate, respectively. Fifty percent reduction in radicle and hypocotyl elongation of seedlings after 48 h incubation resulted from 0.7 μmol/l of (Z)-3-hexenol, and 0.8 μmol/l of (E)-2-hexenal, respectively. In this test, alcohols were more toxic than aldehydes or ester, and unsaturated compounds were more toxic than saturated ones. The germination of seeds exposed to (Z)-3-hexenol in wet condition were strongly inhibited, but any inhibitory effect were not observed when the seeds were exposed to (Z)-3-hexenol in dry condition.

Key words. (Z)-3-hexenol, volatile compound, germination inhibition, growth inhibition,

Introduction

A number of plant species produce volatile compounds that inhibit or stimulate growth of other plants(1,2,6,9-11), micro organisms(3,10) and insects(8,12) in laboratory bioassays. There is some evidence that terpenes may influence vegetation patterning in certain plant communities(10,11). (Z)-3-Hexenol and related compounds were volatiled from many kinds of plant leaves and the amount of flax were increased when plant leaves were injured(4). One of the roles of (Z)-3-hexenol for insects was reported. (Z)-3-Hexenol was one of phagostimulants for *Epilachna fulvosignata*(12). But the roles for plants were not clear. In this report, authors examined the effects of (Z)-3-hexenol and related compounds on germination and growth of plants. For this purpose lettuce seed was chosen as the test plant because of its rapid response.

Materials and Methods

EXP. 1. Effects of (Z)-3-hexenol and related compounds on germination.

(Z)-3-Hexenol and related compounds were obtained from commercial sources. Lettuce seeds, variety Grate Lakes 366 were stored in dark desiccator at 5 °C before using. Germination tests were conducted in sealed containers, volume 200 ml. Seeds were germinated on wet filter paper with vapor of the test compounds for 48 h at 25 °C in dark. Test compounds were diluted to each concentration in ethanol and added 1 μl by pippetting into small glass cups placing in the center of sealed container. Control was added 1 μl of ethanol. The test compounds volatiled in container according to their vapor pressures. Concentrations of compounds in air were checked by gas chromatography. After 48 h incubation, germinated seeds were counted. Bioassay was performed on 20 seeds of each container with 3 replications and averaged data were used.

EXP. 2. Effects of (Z)-3-hexenol and (E)-2-hexenal on growth.

Germinated seeds, radicle 2-3 mm, were used for growth tests. So, germinated seeds were incubated on wet filter paper with or without vapor of the test compounds for 48 h at 25 °C

in dark. After 48 h incubation, radicle and hypocotyl length were measured. The others were the same as EXP. 1.

EXP. 3. Germination of seeds exposed to (Z)-3-hexenol.

Seeds were exposed to (Z)-3-hexenol in wet and dry conditions. In wet condition, seeds were absorbed distilled water for 24 h at 5 °C before exposure. Then, the outside water of seeds was removed by filter paper, and the seeds were exposed to (Z)-3-hexenol in sealed container for 1-6 days. The concentration of (Z)-3-hexenol in container was 1 μmol/l. After exposure, the seeds were germinated on a wet fitter paper in a new petri dish. After 5 days, germinated seeds were counted. In dry condition, dry seeds were exposed to 1 μmol/l of (Z)-3-hexenol for 1-6 days. The others were the same as in wet condition.

Results and Discussion

EXP. 1. Effects of (Z)-3-hexenol and related compounds on germination.

Fig. 1 shows the relation between test compounds concentration and germination percentage after 48 h incubation. (Z)-3-Hexenol was most toxic. Fifty percent reduction in germination after 48 h incubation was caused by 0.05μmol/l of (Z)-3-hexenol, 0.1μmol/l of (E)-2-hexenal, 0.2μmol/l of hexanol, 0.6μmol/l of hexanal, and 1.5μmol/l of (Z)-3-hexenyl acetate, respectively. Alcohols were more toxic than aldehydes or ester, and unsaturated compounds were more toxic than saturated ones. It had been reported that (Z)-3-hexenol and (E)-2-hexenal were synthesized from linolenic acid(5,7). It is thought that (Z)-3-hexenol and (E)-2-hexenal had high toxicity because of high affinity for membranes.

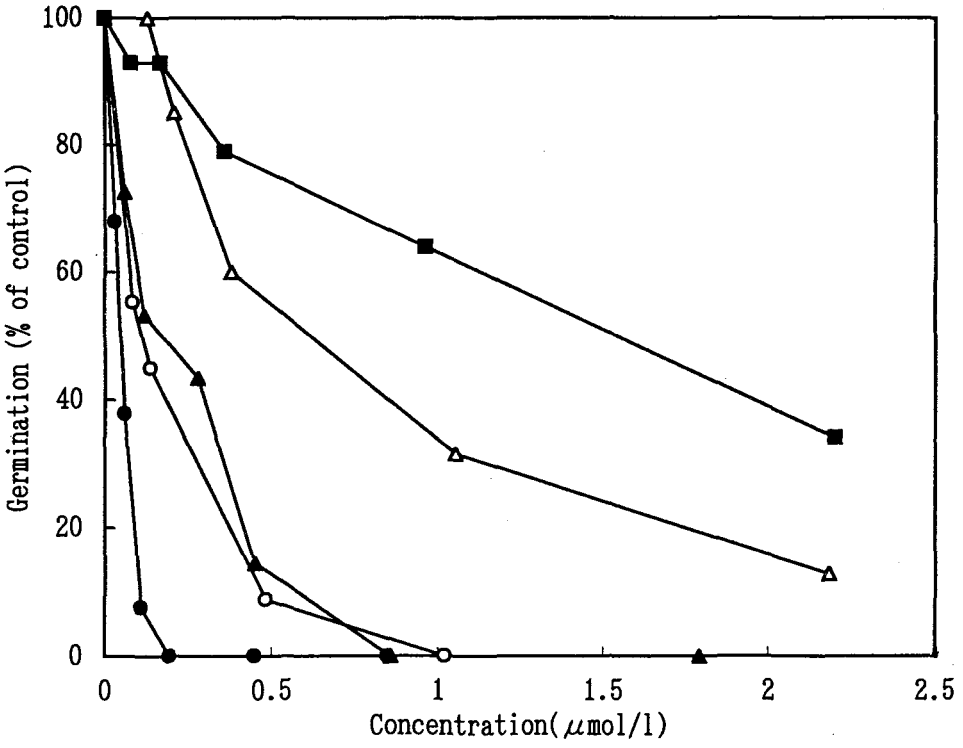


Fig. 1. Relation between test compounds concentration and germination percentage after 48 h incubation.
● (Z)-3-hexenol, ○ (E)-2-hexenal, ▲ hexanol, △ hexanal, ■ (Z)-3-hexenyl acetate

EXP. 2. Effects of (Z)-3-hexenol and (E)-2-hexenal on growth.

Fig. 2 shows the relation between concentration of (Z)-3-hexenol or (E)-2-hexenal and lettuce radicle length. The length of radicle was reduced by presence of (Z)-3-hexenol or (E)-2-hexenal. The trend of length of hypocotyl was the same as radicle. Fifty percent reduction in radicle and hypocotyl elongation of seedlings after 48 h incubation resulted from 0.7 μmol/l of (Z)-3-hexenol, and 0.8 μmol/l of (E)-2-hexenal, respectively. It is thought that these concentrations would be changed according to incubation period.

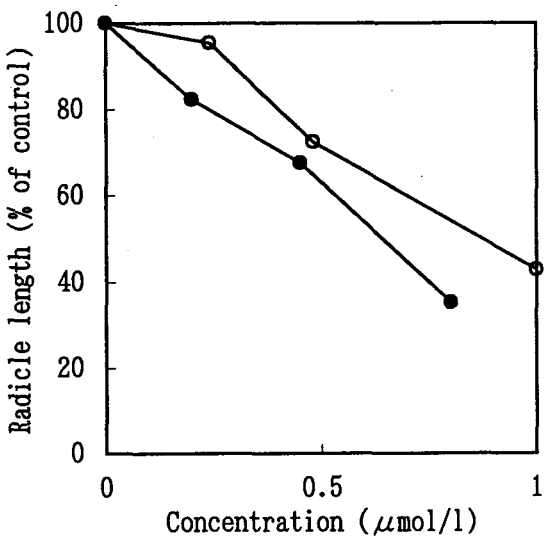


Fig. 2. Relation between test compounds concentration and radicle length after 48 h incubation.
● (Z)-3-hexenol, ○ (E)-2-hexenal

EXP. 3. Germination of seeds exposed to (Z)-3-hexenol.

The germination of seeds exposed (Z)-3-hexenol in wet condition was strongly inhibited (Fig. 3). The seeds exposed to (Z)-3-hexenol for 1 day germinated about 40 %. The seeds exposed for more 2 days in wet condition were germinated about less than 10 percentage of control. But, in dry condition, any inhibitory effect were not observed(Fig. 3).

It had been reported that exposure of dry *Bromus* seeds for 1 day to volatiles from *Trichostema* foliage inhibited growth when the seeds were later moistened and the major volatile inhibitor from *Trichostema* was terpinen-4-ol(6). It suggests that the inhibition mechanism is different according to characteristics of compounds.

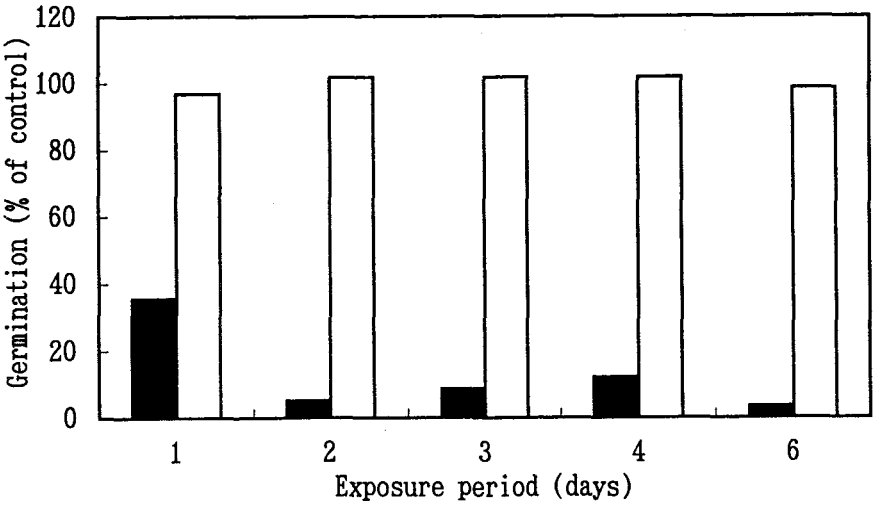


Fig. 3. Germination of seeds moistened after 1,2,3,4 and 6 days of exposure to (Z)-3-hexenol.
■ seeds absorbed water before exposure, □ dry seeds

(Z)-3-Hexenol did not effect on dry seeds. The surface of dry seeds may not absorb (Z)-3-hexenol. Water was necessary when (Z)-3-hexenol inhibits germination and elongation. So, it is suggested that (Z)-3-hexenol inhibits metabolism of moistened seeds. The strong toxicity of (Z)-3-hexenol may be used for weed management. (Z)-3-Hexenol and related compounds are natural ones, so they may impact less for environment. Further investigation of their inhibition mechanism is necessary.

Acknowledgment: the authors acknowledge Dr. T. Yasuda for his critical and useful suggestion.

References

1. Asplund, R.O. 1968. Monoterpenes: relationship between structure and inhibition of germination. Phytochemistry 7,1995-1997.
2. French, R.C. and G.R. Leather 1979. Screening of nonanal and related volatile flavor compounds on the germination of 18 species of weed seed. J. Agric. Food Chem. 27,828-832.
3. French, R.C., R.K. Long, F.M. Latterell, C.L. Graham, J.J. Smoot, and P.E. Shaw 1978. Effect of nonanal, citral, and citrus oils on germination of Conidia of *Penicillium digitatum* and *Penicillium italicum*. Phytopathology 68, 877-882.
4. Fujii, Y., T. Shibuya, and T. Yasuda 1986. Volatile substances emitted from plants. Bulletin of the National Institute of Agro-Environmental Sciences 1, 69-82.
5. Hatanaka, A. and T. Harada 1973. Formation of *cis*-3-hexenal, *trans*-2-hexenal and *cis*-3-hexenol in macerated *Thea sinensis* leaves. Phytochemistry 12, 2341-2346.
6. Heisey, R.M. and C.C. Delwiche 1984. Phytotoxic volatiles from *Trichostema lanceolatum*(Labiatae) . Amer. J. Bot. 71, 821-828.
7. Kajiara, T. T. Harada, and A. Hatanaka 1975. Isolation of Z-3-hexenal in tea leaves, *Thea sinensis*, and synthesis thereof. Agri. Biol. Chem. 39, 243-247.
8. Klocke, J.A. M.F. Balandrin, R.P. Adams, and E. Kingsford 1985. Insecticidal chromenes from the volatile oil of *Hemizonia fitchii*. J. Chem. Ecol. 11, 701-712.
9. Lorber, P. and W.H. Muller 1976. Volatile growth inhibitors produced by *Salvia leucophylla*: effects on seedling root tip ultrastructure. 63,196-200.
10. Muller, W.H. 1965. Volatile materials produced by *Salvia leucophylla*: effects on seedling growth and soil bacteria. Botan. Gaz. 126, 195-200.
11. Muller, C.H., W.H. Muller, and B.L. Hanes 1964. Volatile growth inhibitors produced by aromatic shrubs. Science 143,471-173.
12. Murray, R.D.H., A. Martin, and G.O. Stride 1972. Identification of the volatile phagostimulants in *Solanum campylacanthum* for *Epilachna fulvosignata*.

DORMANCY , GERMINATION AND EMERGENCE OF *SCIRPUS NIPPONICUS* SEEDS

T. Sumiyoshi[1] and K. Itoh[2]
1 Kyushu Natl. Agric. Exp. Stn. ,Chikugo,Fukuoka 833, Japan
2 Tohoku Natl. Agric. Exp. Stn. ,Omagari,Akita 014-01, Japan

Abstract. A study was conducted to investigate the effect of storage conditions on seed dormancy, germination and emergence of *Scirpus nipponicus* Makino, a perennial paddy weed. The seeds were stored in submerged soil in test tubes, or buried 5 to 10 cm deep in the field. Matured seeds were kept in deep dormancy. Air dried seeds and the seeds stored for 31 to 60 days in submerged soil didn't germinate at all. The dormancy was broken after storage of seeds in submerged soil at 5°C for three and one half years, and a germination percentage of 35.4% was achieved. Seeds buried 5 to 10cm deep in the field in October did not germinate the next spring, while the seeds of other *Scirpus* species germinated 91.5% to 16.2 %. However, 50.8% of *S. nipponicus* seeds germinated the second spring. When the same seeds were sown in the submerged soil of pots, emergence percentages were very low.

Key words: *Scirpus nipponicus*, dormancy, germination, emergence, paddy weed.

INTRODUCTION

Scirpus nipponicus Makino is a Cyperaceae weed, and is distributed in Hokkaido, Honsyu, Sikoku, Kyusyu, Korea and Ussuri[7]. In Japan, this weed has become troublesome recently in the paddy fields of northern part, especially Tohoku district. Although several effective herbicides are available now, it is difficult to eliminate this weed because of its ecological characteristics such as long duration of emergence. Infested area is slightly increasing (Table 1).

After the discovery of paddy fields infested by *S. nipponicus* in Aomori prefecture in 1977[8], many researches with this weed were conducted. They included distribution[8,15], weed damage[6,10], control with herbicides[4,6,9,10,11,18], and so on.

Although *S. nipponicus* produces tubers and seeds, almost all seedlings in the paddy fields were from tubers, and only a few were from seeds[12]. Fresh weight of a tuber ranged widely from 6 to 193 mg[6]. One plant usually produces 15 to 20 tubers[8], but there was an example that 1916 tubers were produced by one plant under pot condition[20]. The seed is 2.5mm long and 1.5mm wide[8], and the weight is 1.19mg/one seed[17].

There were many other researches[8,12,20] with *S. nipponicus* tubers, such as tuber formation, sprouting characteristics, and so on. But little is known about the seeds of this weed. We think that the seeds of *S. nipponicus* play an important role when this weed invades new fields, because the seeds are much smaller than the smallest tubers and easy to be carried by water, animals, machines, and so on. So the research with the seeds is important as well as the tubers.

The objectives of this research were to investigate the effect of storage conditions on seed dormancy, germination and emergence of *S. nipponicus*, and to compare with other *Scirpus* species about seed dormancy

and germination.

Table 1. Paddy fields* infested with *S. nipponicus* in Tohoku district**.

Year	Aomori	Iwate	Akita	Miyagi	Yamagata	Fukusima
1981	2	-	-	-	-	-
1985	20	2	3	0	-	0
1990	28	6	3	1	-	1
1994	27	8	2	1	-	1

* Percentages of infested aera by cultivated aera.
- Uninvestigated.

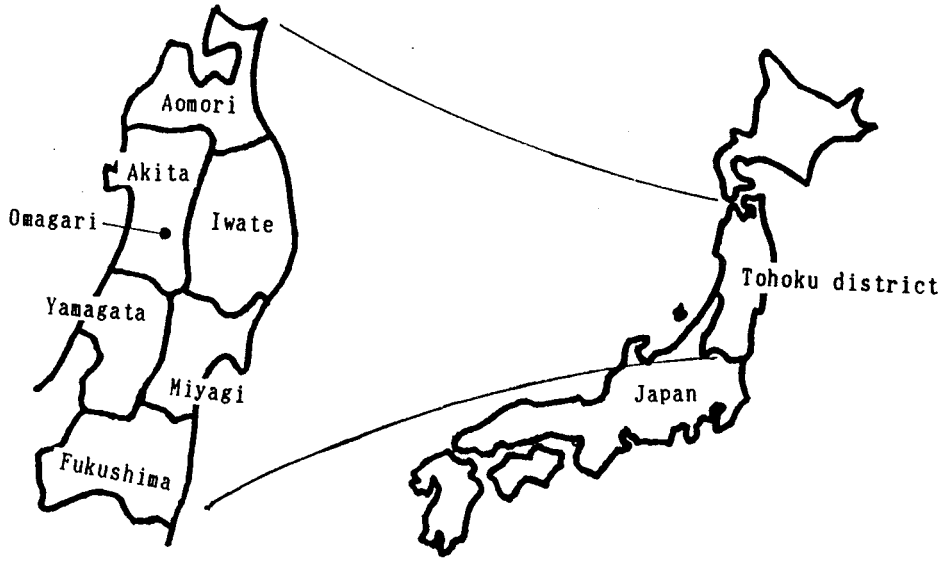


Fig. 1. Tohoku district.

MATERIALS AND METHODS

Storage of seeds in a test tube condition.

S. nipponicus and *S. juncoides* Roxb. subsp. *juncoides* Roxb. were tested in this study. The seeds of these weeds were collected at paddy fields in Tohoku National Agricultural Experiment Station in 1987 and 1994. After air dried, about 300 seeds were mixed with 5ml sifted soil by 0.5mm mesh in test tubes 16.5mm in diameter, and watered to make submerged condition. The test tubes were incubated 5°C and 15°C for 30 to 60 days. With *S. nipponicus* seeds, storage at 5°C for three and one half years also tested. After storage the seeds were washed, and germination tests were conducted.

Storage of seeds under field condition.

First, 5 *Scirpus* species, *S. nipponicus*, *S. juncoides* subsp. *juncoides*, *S. planiculmis* Fr. Schm., *S. yagara* Ohwi and *S. triqueter* L. were tested in this study. The seeds of these weeds were collected at paddy fields or from plants grown in sample pots in abovementioned station in 1987. After air dried, the seeds were buried 5 to 10 cm deep in pots with sifted soil by 0.5mm mesh,

** Data from Bull. of Tohoku branch, The Japan Association for Advancement of Phyto-Regulators vol.17,21,26,30(1982,1986,1991,1995).

and the pots were buried in the field in October in 1987. The seeds were recovered in May the next year, and germination tests were conducted.

Second, only *S. nipponicus* seeds collected in 1991 and 1992 were tested, and were buried similarly in November in the same year. The seeds were recovered the second spring, respectively. Germination and emergence tests were conducted.

Germination and emergence tests.

Germination tests were conducted with two types of seed-bed. One was wet filter paper on petri-dish (WFP), and the other was sample tube filled with water (ST). 50 seeds were placed on each seed-beds in three replicates immediately after recovered and washed, and then incubated at constant temperature; 30, 25, 20, 15°C and/or alternating temperature; 30/20, 25/15°C, under light(12hr) condition. Germination percentages were measured after 20 to 60 days.

Emergence tests were conducted with wagner pots 16cm in diameter. 100 seeds were sown on submerged soil in the pots in two replicates immediately after recovered and washed, and then pots were placed under film roofed house and in the phytotorons of constant temperature; 30, 25, 20, 15°C. Emergence percentages were measured after 60 days.

RESULTS AND DISCUSSION

Storage of seeds in a test tube condition.

Air dried seeds of *S. nipponicus* didn't germinate, and also the seeds stored for 31 to 60 days didn't (Table 2). Air dried seeds of *S. juncoides* subsp. *juncoides* germinated 27.3% in sample tubes, and higher germination percentages were achieved after storage of seeds for 31 to 60 days (Table 3).

The seeds of *S. nipponicus* stored in submerged soil at 5°C for three and one half years germinated 35.4%. Germination of the seeds began from 15 days after placed on seed-bed, and ended by 30 days (Fig. 2).

These results showed that *S. nipponicus* seeds were kept in deeper dormancy after rippened than *S. juncoides* subsp. *juncoides* seeds. In other reports[12,14], germination percentages of seeds after storage for about one to two months in submerged soil at 5 to 15°C were 93-100% of *S. juncoides* R_{0X6}.

Table 2. Effect of storage* conditions on germination percentages** of *S. nipponicus* seeds.

Storage		WFP			ST		
Temp.	Days	30/20	25/15	25	30/20	25/15	25
—	—	0.0	0.0	0.0	0.0	0.0	0.0
5°C	31d	0.0	0.0	0.0	0.0	0.0	0.0
	60d	0.0	0.0	0.0	0.0	0.0	0.0
15°C	31d	0.0	0.0	0.0	0.0	0.0	0.0
	60d	0.0	0.0	0.0	0.0	0.0	0.0

* Seeds were collected in 1994, and stored in submerged soil in test tubes in February 1995.

** Germination percentages were measured 30 days after the seeds were placed on each seed-beds under light condition.

subsp. *hotarui* T.KOYAMA, 21-83% of *S. wallichii* NEES, and 93-100% of *S. smithii* A.GRAY subsp. *leiocarpus* T.KOYAMA on wet filter paper at 25/15°C under light condition. So, it's thought that primary dormancy of *S. nipponicus* seeds is the deepest among these species. And it's also thought that long duration of storage in submerged soil is needed to break the dormancy of *S. nipponicus* seeds.

Table 3. Effect of storage conditions* on germination percentages** of *S. juncoides* subsp. *juncoides* seeds.

Storage		WFP			ST		
Temp.	Days	30/20	25/15	25	30/20	25/15	25
—	—	0.2	0.0	0.0	25.4	27.3	0.0
5°C	31d	2.3	3.1	6.8	70.9	83.6	3.0
	60d	18.7	25.6	15.2	81.6	76.1	12.9
15°C	31d	39.4	12.1	17.0	89.5	89.2	11.7
	60d	46.3	1.7	13.8	89.5	81.9	1.3

* Seeds were collected in 1994, and stored in submerged soil in test tubes in February 1995.
** Germination percentages were measured 14 days after the seeds were placed on each seed-beds under light condition.

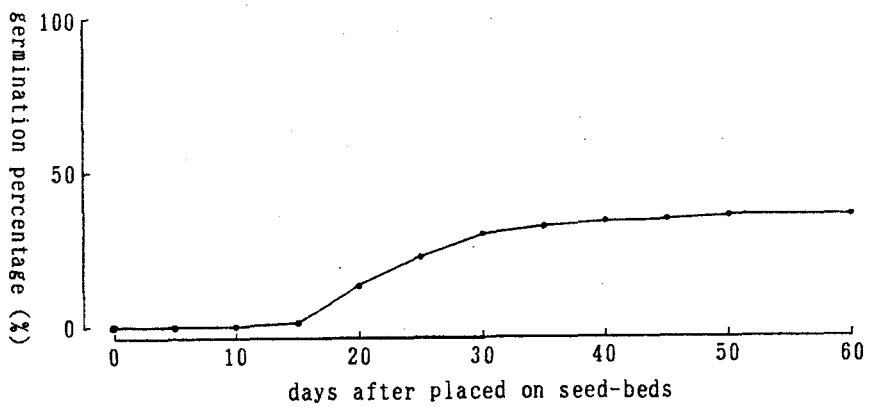


Fig. 2. Germination patern of *S. nipponicus* seeds on WFP seed-bed at 25/15°C under light condition.
* The seeds were collected in 1987, and stored in submerged soil at 5°C for three and one falf years.

Storage of seeds under field condition.

The seeds of all *Scirpus* species tested germinated the next year of burying, but *S. nipponicus*. The germination percentages ranged from 91.5% of *S. juncoides* seeds to 16.2% of *S. yagara* seeds (Table 4).

The seeds of *S. nipponicus* recovered from fields the second year of burying germinated, and the highest germination percentages were 50.8% in 1994 and 6.1% in 1993 (Table 5).

These results showed that *S. nipponicus* seeds were kept in deeper dormancy after storage for a winter, compared with other *Scirpus* species. In other reports[13,14], the germination percentages of the seeds after storage

for a winter under field condition were 100% of *S.juncoides* subsp. *hotarui*, 95-100% of *S. wallichii*, and 100% of *S. smithii* subsp. *leiocarpus* on wet filter paper at 25/15°C under light condition. And the germination percentages of the seeds after storage for two winter were 91% of *S.juncoides* subsp. *juncoides* and 99% of *S. wallichii* [17], both of them were higher than that of *S. nipponicus*. So, it's thought that breaking dormancy of *S. nipponicus* seeds under field condition is the slowest among these species. And it's also thought that at least two winter of storage is needed to break the dormancy of *S. nipponicus* seeds under field condition.

The germination percentages of the seeds recovered in 1994 were higher at 25°C than any other constant temperature, so it seemed that optimum temperature for germination of *S. nipponicus* seeds was 25°C. And the germination percentages at alternating temperature (25/15°C) were always higher than those at constant temperature, so alternating temperature seemed to be suited for germination of *S. nipponicus* seeds (Table 5).

Table 4. Germination percentages* of *Scirpus* species seeds** after storage for a winter under field condition.

species	germination percentage
S. nipponicus	0.0
S. juncoides	91.5
S. planiculmis	42.2
S. triqueter	88.2
S. yagara	16.2

* Germination percentages were measured 20 days after the seeds were placed on WFP seed-beds at 25/15°C under light condition.
** Seeds were buried in October 1987, and recovered in May 1988.

Table 5. Germination percentages* of *S. nipponicus* seeds** after storage for two winter under field condition.

Buried Year	Recovered Year	Recovered Date	Seed-bed	Incubation temp.(°C) 25/15	30	25	20	15
1991	1993	1,June	WFP	5.3	0.1	-	-	-
			ST	6.1	0.0	-	-	-
1992	1994	6,May	WFP	33.4	1.1	20.8	0.2	0.0
			ST	33.4	0.2	17.2	0.0	0.0
		7,June	WFP	50.8	1.6	32.4	0.3	0.0
			ST	37.7	1.2	8.4	9.2	0.0

* Germination percentages were measured 60 days after the seeds were placed on each seed-beds under light condition.
** Seeds were buried in November each year.
- Not researched.

Emergence from submerged soil.

The seeds of *S. nipponicus* which germinated 50.8% and 6.1% emerged only 3.5% and didn't from submerged soil of pots in 1994 and 1993,

respectively (Table 6). It means that *S. nipponicus* seeds couldn't emerge from submerged soil, even if the seeds could germinate under experimental conditions.

In other reports[13], the emergence percentages of the seeds after storage for one or two winter under field condition were 90-100% of *S.juncoides* subsp. *juncoides* and *S. wallichii*. The seeds of *S. planiculmis* emerged when placed on the soil in the paddy field, but they didn't emerge if covered with soils[2].

Shading and decrease in oxygen supply were considered to be one of the reasons for low emergence percentages of *S. nipponicus* seeds. About shading, as *S. nipponicus* seeds germinated under light condition, they are thought to be light needed seeds. About oxygen supply, dormant seeds of *S. juncoides* subsp. *juncoides* germinated more in low oxygen partial pressure of atmosphere than in high oxygen partial pressure[5]. In our research, *S. juncoides* seeds germinated more in sample tubes filled with water than on wet filter paper (Table 3), this acknowleged the report. But germination percentage of *S. nipponicus* seeds in sample tubes were not different with or slightly lower than those on wet filter paper (Table 5). So, it's thought that low oxygen partial pressure isn't suited for germination of *S. nipponicus* seeds.

Table 6. Emergence percentages* of *S. nipponicus* seeds** after storage for two winter under field condition.

Buried	Recovered		Temp. condition (°C)				
Year	Year	Date	House	30	25	20	15
1991	1993	1,June	0.0	0.0	0.0	0.0	0.0
1992	1994	6,May	1.5	1.5	1.0	0.0	0.0
		7,June	3.5	0.0	2.5	0.3	0.0

* Emergence percentages were measured 60 days after the seeds were sown in the submerged soil of pots. Each pots were placed under film roofed house or in the phytotorons.

** The seeds were buried in November each year.

Conclusion.

Our researches showed that not only deep dormancy of the seeds, but also environmental conditions of paddy fields such as shading, oxygen supply, or others may prevent the seeds of *S. nipponicus* from emerging from soil.

About the reproductive strategy among major perennial *Scirpus* weeds in Japan, *S. juncoides* subsp. *juncoides* and *S. wallichii* depend mainly on seeds[2], whereas *S. nipponicus* and *S. planiculmis* depend mainly on tubers[1,6]. In this study, the primary dormancy of *S. juncoides* subsp. *juncoides* seeds were not so deep and the seeds were easy to germinate, that is necessary for this weed to reproduce in paddy fields. On the other hand, the primary dormancy of *S. nipponicus* seeds were the deepest among *Scirpus* species and long duration of storage was needed for the seeds to germinate. It means that the seeds of *S. nipponicus* play an important role in survival rather than reproduction in paddy fields. And also the seeds must play an

important role when this weed invades new fields.

References

1) Chiba, K. 1992. Studies on the ecology and control of *Scirpus planiculmis* Fr. Schm., a perennial paddy weed. Bull. Akita Pref. Coll. Agr. **18**, 1-54.(in Japanese with English summary)
2) Dakeishi, S. 1988. Ecology and control of *Scirpus planiculmis*. Syokucho **22**(7),21-25.(in Japanese)
3) Iwasaki, K., T. Watajima and H. Hagimoto 1981. Wintering abilities of three *Scirpus* taxa, and their control. Weed Res., Japan **26**, 104-110. (in Japanese with English summary)
4) Kanagawa, M., R. Tanaka and M. Ino 1992. Effects of several herbicides on the growth of *Scirpus nipponicus*. Weed Res., Japan **37** (suppl.),196-197.(in Japanese)
5) Kataoka, T. and Kim S. Y. 1978. Oxygen requirement for seed germination of several weeds. Weed Res., Japan **23**,9-12.(in Japanese with English summary)
6) Kinoda N. 1988. Ecology and control of *Scirpus nipponicus*. Syokucho **22**(7), 14-19.(in Japanese)
7) Kitamura S., G. Murata and T. Koyama 1964. Coloured illustrations of herbaceous plants of Japan. (Monocotyledoneae). Hoiku-sya, Osaka, 219 pp.(in Japanese)
8) Kudo T., N. Kinoda and M. Namioka 1981. Ecology and control of *Scirpus nipponicus*. 1. Distribution and morphology. Weed Res., Japan **26**(suppl.), 87-88.(in Japanese)
9) Kudo T., N. Kinoda and M. Namioka 1981. Ecology and control of *Scirpus nipponicus*. 2. Control with Herbicides. Weed Res., Japan **26**(suppl.), 89-90.(in Japanese)
10) Kudo T., N. Kinoda and M. Namioka 1982. Ecology and control of *Scirpus nipponicus*. 3. Influences of infestation of S. nipponicus on the rice yield. Weed Res., Japan **27**(suppl.),7-8.(in Japanese)
11) Seito F. 1995. Control of *Scripus nipponicus* with several sulfonylurea herbicides. Weed Res., Japan **40**(suppl.),12-13.(in Japanese)
12) Sumiyoshi, T.,Y. Sato and J. Harada 1991. Distribution of weeds of *Scirpus* species on paddy fields in Tohoku district, and, dormancy and germination of seeds. Weed Res., Japan **36**(suppl.),110-111. (in Japanese)
13) Sumiyoshi, T. and K. Itoh 1994. Studies of emergence characteristics of *Scirpus* weeds. 2. Variations of germination and emergence rates of *S. juncoides* and *S. wallichii* among years. Weed Res., Japan **39**(suppl.), 192-193.(in Japanese)
14) Sumiyoshi T. 1994. Studies on the asynchronous emergence of *Scirpus* weeds in cool region. 6. Seed germination characteristics of *S. juncoides* subsp. *hotarui* and *S. smithii* subsp. *leiocarpus*. Tohoku Agric. Res. **47**,35-36.(in Japanese)
15) Sumiyoshi, T., K. Itoh and K. Suzuki 1995. Studies of emergence characteristics of *Scirpus* weeds. 3. Distribution of *Scirpus* species on paddy fields around the Omono river in Akita. Weed Res., Japan **40** (suppl.),70-71.(in Japanese)
16) Sumiyoshi, T. and K. Itoh 1995. Studies of emergence characteristics

of *Scirpus* weeds. 4. Seasonal variation in emergence of *S juncoides* and germination characteristics of *S. nipponicus*. Weed Res., Japan **40** (suppl.),72-73.(in Japanese)
17) Sumiyoshi, T.; unpublished.
18) Takahashi H. and M. Takahashi 1995. Effects of several sulfonylurea herbicides on *Scripus nipponicus*. Weed Res., Japan **40**(suppl.),14-15. (in Japanese)
19) Uetake, T., S. Yamauchi and K. Iwasaki 1982. Ecology and control of *Scirpus nipponicus*. 2. Emergence of tubers by cutting of bud and tuber formation depth. Weed Res., Japan **27**(suppl.),9-10.(in Japanese)
20) Yamauchi S. and T. Uetake 1981. Ecology and control of *Scirpus nipponicus*. 1. Characteristics of sprouting and emergence of tubers, and tuber formation time. Weed Res., Japan **26**(suppl.),85-86.(in Japanese)

Impact of Decayed Weeds on Growth of Sugarcane and Certain Crops

Kleopan Suwanarak[1], Samaporn Supasilapa[1]
Sermsiri Kongsaengdao[1] and Misako Ito[2]

1) Botany and Weed Science Division, Department of Agriculture, Bangkok 10900, Thailand
2) Weed Science Laboratory, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Abstract : Impact of decayed weeds on growth of sugarcane *(Saccharum officinarum* L.) and certain crops i.e. peanut *(Arachis hypogeae)*, soybean *(Glycine max)*, mungbean *(Phaseolus aureus)* and chinese cabbage *(Brassica pekinensis)* were investigated in the greenhouse of Botany and Weed Science Division, Department of Agriculture, Bangkok, from September, 1992 to July, 1993. Decayed *Trianthema portulacastrum* at the compositions of 2.5, 5.0, 7.5 and 10.0% w/w enhanced growth of sugarcane whereas these compositions inhibited the germination and growth of peanut, soybean, mungbean and chinese cabbage. Although decayed *Pennisetum setosum* and *Digitaria ciliaris* severely reduced on growth of cane plants, decayed *P. setosum* tened to have less effect than *D. ciliaris*. Fresh weight of sugarcane grown in 10.0% decayed *P. setosum* was reduced by 53.1% compared with a 73.2% reduction of its fresh weight grown in 10.0% decayed *D. ciliaris*.

Key words : decayed weeds, allelopathy, competition, *T. portulacastrum, D. ciliaris, P. setosum*

Introduction

The subject of interaction between plants is a very complex one. The phases include the concept of competition and that of biochemical inhibition or allelopathy. The allelopathy is distinct from competition in that it implies the suppression of some factor which is required by some other species sharing the same habitat (Datta *et al.*, 1974 and Szczepanski, 1977). Allelopathic substance can be produced from leaves and other plant parts falling on the ground and decomposing by weathering and soil microorganisms released into various phytochemicals which may affect nearby species directly or indirectly (Rice, 1964).

Some main weed species seem to have allelopathic potential which causes effects on crop growth as indicated by Singh (1968), showing that phytotoxic substances in *Cyperus rotundus* inhibited the germination of seeds and growth of ten crop species and this was confirmed by Das & Pal (1970) on rice seedlings. Sen (1981) reported the yield losses of 50.0 and 39.6% due to burnt weed biomass of *C. rotundus* in crops of bajra and til, respectively. substances, passing out from the leaves of a plant after they have been shed, are known to inhibit the germination, growth or occurrence of other plants. An alkaloid absinthin present in the leaves of *Artemisia absinthium* may be washed out, which severely inhibits the growth of all adjoining plant species.

Some main weed species in sugarcane field i.e. *T. portulacastrum, D. ciliaris* and *P. setosum* may have allelpathic potential on the crops. Therefore, the impact of decayed weeds was investigated in order to study the allelopathic effects on cane growth and certain other crops.

Materials and Methods

The studies consisted of 3 experiments as follows.-

1. Impact of decayed *T. portulacastrum* on growth of sugarcane. The study was carried out from September to December, 1992. Whole plants of weed and samples of soil were collected from the cane field. Collected weed plants were air-dried for 48 hours, then oven-dried at 58°C for 72 hours and then ground. Soil was oven-dried at 85°C for 72 hours. Ground weed and soil were mixed at the composition of 0.0, 2.5, 5.0, 7.0 and 10.0% w/w, then filled in a plastic box (0.24 x 0.30 m). All plastic boxes were put in the greenhouse where average temperature of 28-30°C was maintained for seven days, incubation. Two cane-setts variety U-Thong 1 each of two buds were planted in the plastic boxes, water was supplied as needed. Randomized complete blocks were designed with five treatments and three replications. Length, number and fresh weight of cane stalks were measured 16 weeks after planting.

2. Impact of *T. portulacastrum* on growth of certain crops. The experiment was carried out from September to October, 1992. Testing materials were prepared the same as experiment 1. Seeds of peanut, soybean, mungbean and chinese cabbage were seeded in the plastic boxes. Water was supplied daily. Percentage germination of seeds, shoot and root length and dry matter of the crops were measured three weeks after seeding.

3. Impact of *D. ciliaris* and *P. setosum* on growth of sugarcane. Weed plants were prepared as in the two previous trials. Timing of study was from April to July, 1993. Height and fresh weight of sugarcane were measured six weeks after planting.

All experiments were carried out in the greenhouse of Botany and Weed Science Division, Department of Agriculture, Bangkok.

Results and Discussions

1. Impact of *T. portulacastrum* on growth of sugarcane. As shown in Table 1, decayed *T. portulacastrum* at composition of 2.5, 5.0, 7.5 and 10.0% did not influence growth of sugarcane but they seemed to induce growth of the crop, because length, number and fresh weight of cane stalks were increased in pararell to the increasing of percentage weed composition. The composition of decayed weed at 2.5, 5.0, 7.5 and 10.0% gave cane weight of 84.6, 77.9, 92.4, 151.7 and 163.8 g/stalk, respectively.

Table 1. Effects of decayed *T. portulacastrum* on cane variety U Thong 1 assessed 16 weeks after planting.

Composition (%)	Length (cm)	Number (Stalks/plant)	Fresh weight	
			g/stalk	% 1)
0.0	66.5	3.5	84.6	100.0
2.5	64.0	3.0	77.9	92.1
5.0	68.4	4.2	92.4	109.2
7.5	75.2	4.5	151.7	179.3
10.0	86.1	3.8	163.8	193.6
LSD. 0.05	8.7	0.8	11.0	-
0.01	12.3	-	15.0	-

1) Based on 84.6 g/stalk of treatment without decayed weed = 100%.

2. Impact of *T. portulacastrum* on growth of certain crops. It was found that percentage germination of all crops was decreased in relation to increasing of the decayed weed composition. *T. portulacastrum* seemed to have inhibitory effect on germination of chinese cabbage much more than on other crops. The composition of decayed weed over 5.0% caused germination reduction lower than average (Table 2).

Table 2. Decayed *T. portulacastrum* on germination of crops.

Composition (%)	Germination (%)[1]			
	Peanut	Soybean	Mungbean	Chinese cabbage
0.0	60.0[2]	90.0	55.0	65.0
2.5	80.0	80.0	40.0	35.0
5.0	60.0	70.0	45.0	40.0
7.5	40.0	60.0	40.0	30.0
10.0	40.0	60.0	30.0	20.0

1) Assessed three weeks after seeding.
2) Average of two replications.

Decayed *T. portulacastrum* in every percentage composition caused strong inhibition of root and shoot length of all crops. Decayed weed at 2.5, 5.0, 7.5 and 10.0% gave dry weight of peanut 52.9, 41.2, 29.4 and 23.5% respectively of non-decayed weed treatment. All treatments composed of decayed weed gave less percentage dry weight of chinese cabbage than other crops. It could be explained that decayed *T. portulacastrum* at compositions of 2.5, 5.0, 7.5 and 10.0% respectively caused inhibitory effects on both germination and growth of peanut, soybean, mungbean and chinese cabbage (Table 3, 4, 5 and 6).

Table 3. Effects of decayed *T. portulacastrum* on growth of peanut. Assessed three weeks after seeding.

Composition (%)	Length (cm)		Dry weight	
	Shoot	Root	g/plant	%
0.0	11.3[1]	14.4	0.17	100.0
2.5	10.8	8.3	0.09	52.9
5.0	10.0	7.7	0.07	41.2
7.5	6.8	2.6	0.05	29.4
10.0	4.1	2.5	0.04	23.5

1) Average of two replications.

Table 4. Effects of decayed *T. portulacastrum* on growth of soybean. Assessed three weeks after seeding.

Composition (%)	Length (cm)		Dry weight	
	Shoot	Root	g/plant	%
0.0	15.1[1)]	10.0	0.33	100.0
2.5	10.7	7.7	0.10	30.3
5.0	10.3	6.1	0.09	27.3
7.5	6.8	5.6	0.07	21.3
10.0	3.4	4.8	0.04	12.1

1) Average of two replications.

Table 5. Effects of *T. portulacastrum* on growth of mungbean. Assessed three weeks after seeding.

Composition (%)	Length (cm)		Dry weight	
	Shoot	Root	g/plant	%
0.0	10.8[1)]	7.4	0.18	100.0
2.5	10.2	5.2	0.15	83.3
5.0	9.6	5.4	0.12	66.7
7.5	9.5	4.4	0.11	61.1
10.0	7.7	4.0	0.05	27.8

1) Average of two replications.

Table 6. Effects of decayed *T. portulacastrum* on growth of chinese cabbage. Assessed three weeks after seeding.

Composition (%)	Length (cm)		Dry weight	
	Shoot	Root	g/plant	%
0.0	4.8[1)]	3.3	0.07	100.0
2.5	4.1	2.6	0.02	28.6
5.0	3.1	2.5	0.01	14.3
7.5	2.8	2.1	0.01	14.3
10.0	2.3	1.6	0.009	12.9

1) Average of two replications.

3. Impact of *D. ciliaris* and *P. setosum* on growth of sugarcane. It is shown in Table 7 and 8 that the highest cane plant was found in non-decayed soil. *D. ciliaris* tended to have inhibitory effects on growth and fresh weight of sugarcane much more than *P. setosum*. Average length of cane plant in the decayed *P. setosum* and *D. ciliaris* were 25.8 and 19.4 cm respectively whereas average fresh weight of sugarcane in these treatments were 55.4 and 40.3 g/plant. Composition of both decayed weeds at 2.5% up to 10.0% caused influential effects on growth of sugarcane such as fresh weight of cane plants were 44.7, 49.0, 60.3 and 73.2% respectively in the treatments 2.5, 5.0, 7.5 and 10.0% decayed *D. ciliaris*, respectively. Also, losses were 24.5, 30.1, 53.1 and 40.1% in the same order composition of decayed *P. setosum*, respectively. From this experiment, it may be seen that decayed *D. ciliaris* and *P. setosum* caused inhibition of growth of sugarcane although *D. ciliaris* appeared to be more effective than *P. setosum*.

Table 7. Effects of decayed *D. ciliaris* on length and fresh weight of cane plants. Assessed six weeks after planting.

Composition (%)	Cane plants		
	Length (cm)	Fresh weight (g/plant)	Reduction[1)] (%)
0.0	38.5[2)]	74.0	0.0
2.5	14.5	40.9	44.7
5.0	15.5	37.7	49.0
7.5	16.0	29.3	60.3
10.0	12.5	19.8	73.2

1) % Reduction based on weight of treatment without decayed *D. ciliaris*.
2) Average of two replications.

Table 8. Effects of decayed *P. setosum* on length and fresh weight of cane plants. Assessed six weeks after planting.

Composition (%)	Cane plants		
	Length (cm)	Fresh weight (g/plant)	Reduction[1)] (%)
0.0	39.5[2)]	80.4	0.0
2.5	28.8	60.7	24.5
5.0	26.8	49.8	30.1
7.5	17.0	48.2	40.1
10.0	17.0	37.7	53.1

1) % Reduction based on weight of treatment without decayed *P. setosum*.
2) Average of two replications.

Consideration on these experiments, in particular, weed species such as *T. portulacastrum*, showed adverse results to the tested crops. The growth of sugarcane was induced when it was cultivated in all decayed *T. portulacastrum* treatments. This appearance seemed related to its prominent habitation in the cane fields. Because it is usually found at the early stage of cane growing when it is covered with *T. portulacastrum* of 80% or more, the cane plant does not show any growth reduction compared with the cane plant free from weed.

Conclusions

Decayed *T. portulacastrum* at 2.5, 5.0, 7.5 and 10.0% w/w caused enhancement of cane growing. In contrast, decayed weed inhibited germination and growth of peanut, mungbean, soybean and chinese cabbage. Even though decayed *P. setosum* and *D. ciliaris* at the same composition also showed strongly inhibiting growth of cane plants, *P. setosum* tended to have less effect on the crop than *D. ciliaris*.

References

Das, T. M. and S. K. Pal. 1970. Effects of volatile substances of aromatic weeds on germination and subsequent growth of rice embryos. Bull. Bot. Soc. Bengal. **24**: 101-103.

Datta, S. C. and S. P. Singha Roy. 1974. Allelopathy and inhibitors. Sci. & Cult., **40**: 47-59.

Rice, E. L. 1964. Inhibition of nitrogen fixing and nitrifying bacteria by seed plants. Ecology **45**: 824-837.

Sen, D. N. 1981. Crop weed interactions. *In* Ecological Approaches to Indian Weed. pp. 166-195.

Singh, S. P. 1968. Presence of growth inhibitor in the tubers of nutgrass *(Cyperus rotundus* L.*)*. Proc. Indian Acad. Sci. **67**: 18-23.

Szczepanski, A. J. 1977. Allelopathy as a means of biological control of water weeds. Aquatic Botany **3**: 193-197.

Effect of Brassinosteroids on Seed Germination of Weeds

Y. Takeuchi[1], K. Yoneyama[1], M. Ogasawara[1], Y. Omigawa[1], M. Konnai[1], T. Takematsu[1] and A. D. Worsham[2]

[1]Weed Science Center, Utsunomiya University, Utsunomiya, 321, Japan
[2] Crop Science Department, North Carolina State University, Raleigh, NC, USA

Abstract. Brassinosteroids applied at early stage of conditioning shortened the conditioning period required before seeds of a parasitic weed, *Orobanche minor*, would germinate after exposure to a germination stimulant. Brassinosteroids eliminated the inhibitory effects on conditioning and subsequent germination of the seeds caused by germination stimulants applied during early stages of the conditioning period. Brassinosteroids applied after conditioning increased the rate of the seed germination induced by stimulants in the light and in the dark. Brassinosteroids also promoted the seed germination of *Aeginetia indica*, a parasite of upland rice and increased the rate of the seed germination induced by *dl*-strigol. Brassinosteroids at low concentrations such as 10^{-17}~10^{-7} *M* promoted seed germination of the weeds, *Digitaria sanguinalis, Amaranthus retroflexus, Vernonica persica* and *Rumex obtusifolius.*

Key words : seed dormancy, germination stimulation, weed seeds, brassinosteroids.

Introduction

Because weed seeds in the soil may be in different stages of dormancy, germination within a seed population may be nonsynchronus, thus, spreading seedling emergence over a period of time and causing the farmer to repeatedly face similar weed problems and apply control measures. Chemical manipulation of weed seeds may be made more susceptible to weed control methods and control practices made more efficient [2].

Brassinosteroids, derivatives of brassinolide, have recently been recognized by some as a new class of plant hormones [1]. Brassinosteroids applied at early stage of conditioning promoted the process in witchweed (*Striga asiatica*) seeds [5]. It also increased the rate of the seed germination induced by stimulants, such as natural stimulant of root exudate of host plants and strigol isolated from cotton (*Gossypium hirsutum* L.), a non-host plant. This paper describes the effect of brassinosteroid on seed germination of two parasitic weeds: clover broomrape (*Orobanche minor)* and *Aeginetia indica*, and five non-parasitic weeds: *Amaranthus retroflexus, Digitaria sanguinalis, Vernonica persica, Echinochloa crus-galli* and *Rumex obtusifolious*. Clover broomrape and *Aeginetia indica* are both root haloparasites which attack, in particular, red clover and tomato, and upland rice and sugarcane respectively.

Materials and methods

Chemicals.

dl-Strigol was synthesized in the chemical laboratory at Weed Science Center in Utsunomiya University. Brassinolide was purchased from Fuziyakuhin Company, Toyama, Japan. 24-Epibrassinolide and compound A [22*R*, 23*R*-epoxy-28-homobrassinolide 2,3-acetonide] were generous gifts of Drs. Takatsuto and Kamuro. GA_3 was purchased from Aldrich Company, Milwaukee, USA.

Seed germination

(1) *Orobanche minor* Seeds were collected from the plants in a red clover field in Tochigi prefecture in Japan in June and stored at room temperature. Five hundred clover broomrape seeds were placed in a 9-cm petri dish and immersed in 5 ml of conditioning solution at 27°C for 7 to 16 days. After the conditioning, the seeds were rinsed with distilled water and blotted. Groups of 50 seeds each were transferred to 6-cm petri dishes containing a sheet of filter paper wetted with 2 ml of the terminal treatment solution. After the terminal treatment, germination of the seeds was determined under a 20x binocular microscope. Standard errors of the means were computed for all the experiments as the means of three separate experiments.

(2) *Aeginetia indica* Seeds were collected from the plants parasitized rice plants in a upland-rice fields in Tochigi prefecture in Japan in October and stored dry at room temperature for 7 month. Thirty seeds were placed in a 2-cm petri dish and immersed in 5 ml water or test solutions at 27°C for 15 days in the light and dark. After the incubation, germination of the seeds were determined under a 20x binocular microscope.

(3) *Amaranthus retroflexus* (A), *Digitaria sanguinalis* (D), *Vernonica persica* (V), *Echinochloa crus-galli* (E) and *Rumex obtusifolius* (R) Seeds of A and D were collected in the research fields of NC State University in USA in October and stored at 5°C for 5 months. Seeds of V and E, were collected in October and R were collected in May in the fields of Utsunomiya University, and they were stored at room temperature for 5 months.

The procedures of the experiments of the seed germination were the same as described in the experiment of *Aeginetia indica* seeds.

Results and discussion

(1) *Orobanche minor* Brassinolide and 24-epibrassinolide at 10^{-15}~10^{-9} *M* did not induce germination of 3 month- and 10 month-old seeds conditioned in water. However, when brassinolide or GA_3 was used with *dl*-strigol, the rate of germination was greatly increased (data not shown). Ten month-old seeds conditioned in water for 7, 10, 13 and 16 days germinated 5±3%, 40±5%, 26±2% and 5±3% respectively, in the terminal treatment of 10^{-8} *M dl*-strigol solution. While, seeds conditioned in 10^{-8} *M* brassinolide for 7, 10, 13 and 16 days germinated 19±4%, 62±2%, 52±5% and 18±4% in the terminal treatment solution. Seeds conditioned in 10^{-4} *M* GA_3 for 7, 10, 13 and 16 days germinated 28±2% 82±3%, 62±5% and 25±4% respectively, in terminal treatment solution (Table 1).

Table 1. Percent of germination of 10 month-old clover broomrape seeds condition in water, 10^{-4} *M* gibberellin (GA_3), 10^{-8} *M* brassinolide (BR), 10^{-8} *M* 24-epibrassinolide (EBR), 10^{-8} *M* compound A (A) for 7-16 days before 2 day-terminal treatment with 10^{-8} *M dl*-strigol at 27°C.

Conditioning media	Days of conditioning			
	7	10	13	16
water	5±3	40±5	26±2	5±3
10^{-4} *M* GA_3	28±2	82±3	62±5	25±4
10^{-8} *M* BR	19±4	62±2	52±2	18±4
10^{-8} *M* EBR	30±3	71±4	57±2	21±3
10^{-8} *M* A	35±6	78±5	63±4	20±2

In this experiment, germination percentage seemed to reach a maximum after 10 days of conditioning and then dropped with further incubation. These results indicated that after prolonged exposure to moist conditions, the seeds might have entered into the stage of "wet dormancy" and became less sensitive to the stimulant. Brassinolide and GA_3 applied at early stage of conditioning promoted the rate of these process and shortened the conditioning period required. In contrast, brassinolide and GA_3 prevented the seeds from entering into the stage of the wet dormancy when applied at later stage of conditioning. All of the other brassinosteroids tested also promoted the rate of conditioning. In particular, compound A was highly active in promoting the conditioning process.

Inhibitory effect of *dl*-strigol was observed in the seed germination of clover broomrape. When twelve month-old seeds were conditioned for 10 days and treated with terminal treatment with *dl*-strigol for 0-8 days, the seeds conditioned in water germinated 0% after conditioning and 66±3% with 8 day-terminal treatment. On the other hand, seeds conditioned in 10^{-8}M *dl*-strigol germinated 32±4% after conditioning and 50±3% with 8-day terminal treatment. These results suggested that a portion of the seeds might be at shallow dormant stage and remaining seeds might be at the middle to deep dormant stage. Seeds conditioned in BR, GA_3 and the combinations of these two chemicals with *dl*-strigol, germinated more than 48±3% after conditioning and more than 65±6% after 2 day-terminal treatment. BR and GA_3 showed synergistic effect on seed germination, and consequently the inhibitory effects of *dl*-strigol addition during conditioning in 12 month-old seeds were largely eliminated by brassinolide and brassinolide plus GA_3 during this period. Light has been found to inhibit the conditioning process and germination of the seeds. Seeds conditioned in water in the light did not germinated with *dl*-strigol terminal treatment in the light. When brassinolide and GA_3 were used as conditioning medium, they greatly increased the rate of seed germination induced by *dl*-strigol, especially in the light. Brassinosteroids may promote the conditioning presumably by increasing permeability of seed coat and/or membranes [4]. It was also suggested that brassinosteroids may reduce the inhibitory effect of light on the seed germination through modulation of phytochrome response.

Table 2. Percent of germination of 12 month-old *O. minor* seeds conditioned in the combination of *dl*-strigol (ST) with gibberellin (GA_3), brassinolide (BR) for 10 days followed by 0-8 days-terminal treatment with *dl*-strigol (10^{-8} *M*). Each value represents the mean of 3 replications ± SE of the means.

Conditioning media	Days of terminal treatment			
	0	2	5	8
water	0	28±6	55±5	66±3
10^{-8} *M* ST	32±4	34±5	41±3	50±3
10^{-8} *M* ST + 10^{-9} *M* BR	28+3	65±3	78±3	85±2
10^{-8} *M* ST + 10^{-4} *M* GA_3	51±4	75±5	85±2	90±2
10^{-8} *M* ST + 10^{-9} *M* BR + 10^{-4} *M* GA_3	65±6	8±3	90±5	93±4

(2) *Aeginetia indica* The seeds germinated continually in the dark without host plants. Seven month-old seeds incubated in water, 10^{-7} *M* brassinolide, 10^{-8} *M dl*-strigol and the combination of brassinolide with 10^{-8} *M dl*-strigol for 15 days in the dark, they germinated 4±2%, 34±4%, 42±5% and 83±4% respectively. The result indicated that brassinolide combined with *dl*-strigol greatly enhanced the seed germination and that brassinolide and *dl*-strigol showed a synergistic relationship in increasing the seed germination. Brassinolide might have served to increase the sensitivity to *dl*-strigol in the germination by lowing the degree of dormancy.

Table 3. Percent of germination of 7 month-old *Aeginetia indica* seeds after 15 days of incubation in water, 10^{-7} *M* brassinolide (BR), 10^{-8} *M dl*-strigol and 10^{-7} *M* brassinolide plus 10^{-8} *M dl*-strigol at 27°C in the dark.

Germination media	Germination (%)
water	4±2
10^{-7} *M* BR	34±4
10^{-8} *M dl*-strigol	42±5
10^{-7} *M* BR + 10^{-8} *M dl*-strigol	83±4

(3) Germination of *Amaranthus retroflexus, Digitaria sanguinalis, Vernonica persica, Echinochloa crus-galli,* and *Rumex obtusifolius* seeds. These seeds were incubated in the dark. *Amaranthus retroflexus* seeds germinated 50±2% in water and 68±2%~80±5% in 10^{-13}~10^{-11} *M* brassinolide, respectively. *Digitaria sanguinalis* seeds germinated 30±5% in water and 42±4%~50±5% in 10^{-19}~10^{-13} *M* brassinolide, respectively. *Vernonica persica* seeds germinated 20±3% in water and 40±1%~55±5% in 10^{-13}~10^{-11} *M* epibrassinolide, respectively. *Echinochloa crus-galli* seeds germinated 15±2% in water, 40±4% in 10^{-14} *M* brassinolide, 28±5% in 10^{-4} *M*, 42±3% in 3X10^{-4} *M* GA_3 and 69±6%~92±2% in 10^{-14} *M* brassinolide plus 10^{-4}~3X10^{-4} *M* GA_3, respectively. The results indicate that brassinolide and epibrassinolide were effective for promoting the seed germination and that brassinolide was highly active in the promotion of germination of gramineous seeds, such as *Digitaria sanguinalis* and *Echinochloa crus-galli* seeds. Brassinolide was more active than GA_3 in promoting the seed germination of *Echinochloa crus-galli* and a synergism was observed between these two substances.

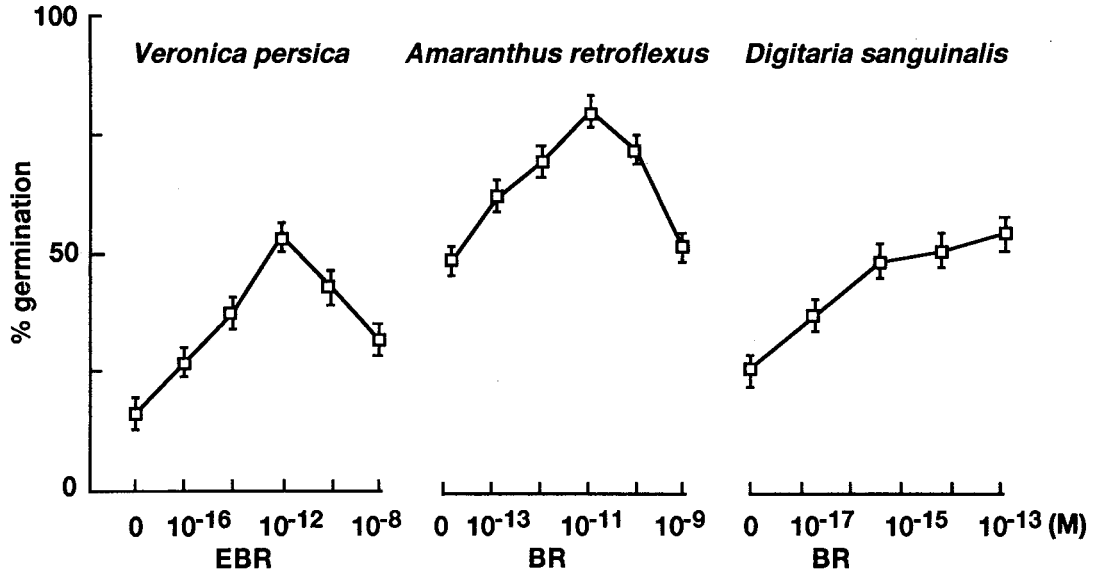


Fig. 1 Germination percentage of *Veronica persica, Amaranthus retroflexus* and *Digitaria sanguinalis* seeds incubated in water, 24-epibrassinolide (EBR) or brassinolide (BR) for 8 days at 25°C in the dark.

Table 4. Percent of germination of 5 month-old *Echinochloa crus-galli* seeds incubated in water, 10^{-14} *M* brassinolide (BR), 10^{-4} *M* gibberellin (GA_3) and combination of 10^{-14} *M* brassinolide plus 10^{-4} *M* GA_3 or $3X10^{-4}$ *M* GA_3 for 7 days at 20°C in the light.

Germination media	Germination (%)
water	15 ± 2
10^{-14} *M* BR	40 ± 4
10^{-4} *M* GA_3	28 ± 5
$3X10^{-4}$ *M* GA_3	42 ± 3
10^{-14} *M* BR + 10^{-4} *M* GA_3	69 ± 6
10^{-14} *M* BR + $3X10^{-4}$ *M* GA_3	92 ± 2

Brassinolide might have promoted the penetration of GA_3 into the seeds and/or the biochemical actions in the seeds. *Rumex obtusifolius* seeds have been reported to require light for germination [3]. In our study, the requirement of light was also evident. The seeds germinated 12±4% and 70±5% in the dark and the light, respectively. However seeds scarified with 70% sulfuric acid for 15 minutes, germinated 85±4% and 100% in the dark and the light, respectively (data not shown). The results indicated that the seed coats physically restrict the growth of the embryo. When seeds were incubated in 10^{-11} ~10^{-9} *M* brassinolide or 10^{-4}~10^{-3} *M* GA_3 in the dark, they germinated 10±2%~13±2% and 12±4%~14±3%, respectively. However they germinated 19±5%~60±4% in the combinations of 10^{-11}~10^{-9} *M* brassinolide with 10^{-4}~10^{-3} *M* GA_3 in the dark. Brassinolide and GA_3 showed a synergistic effect in the seed germination and their combinations greatly increased the rate of the seed germination. They also increased the rate of the germination in the light. Brassinolide and GA_3 might have synergistically increased the growth potential of embryo and/or decrease the mechanical restriction.

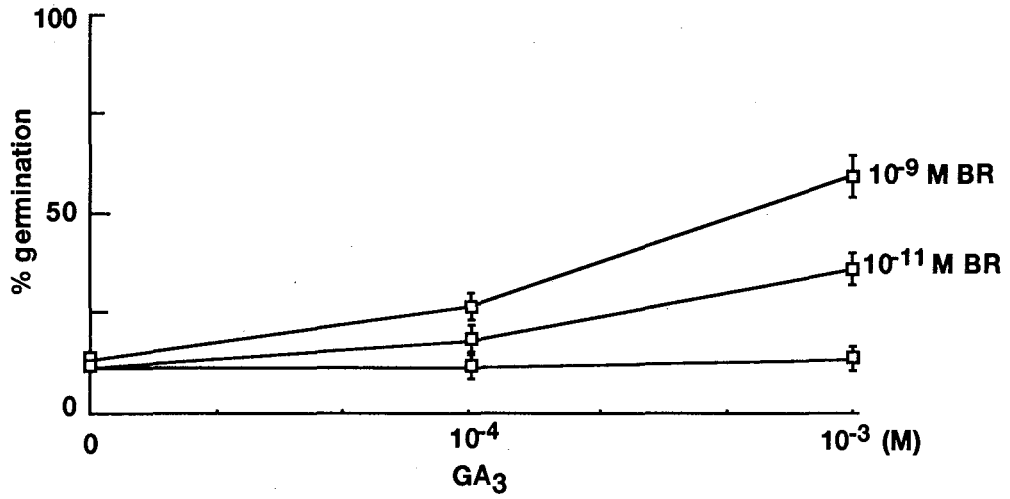


Fig. 2. Germination percentage of *Rumex obtusifolius* seeds incubated in water, 10^{-11} ~10^{-9} *M* brassinolide (BR), 10^{-4}~10^{-3} *M* gibberellin (GA_3) and combinations of 10^{-11} ~10^{-9} *M* BR and 10^{-4}~10^{-3} *M* GA_3 for 6 days at 20°C in the dark.

Conclusion

A significant practical application of the findings reported herein could be that the addition of brassinosteroids or combinations of brassinosteroids and gibberellins to seed germination stimulants such as *dl*-strigol or its analogs applied to soil to cause "suicidal germination" might greatly increase the effectiveness of the parasitic weed management approach. The results also demonstrated that brassinosteroids alone or brassinosteroids with stimulants could significantly enhance and synchronize the germination of the weeds, *Digitaria sanguinalis, Amaranthus retroflexus, Vernonica persica* and *Rumex obtusifolius* seeds.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 0630510).

References

1) Cutler, H. G. 1991. Brassinosteroids through the looking glass: An appraisal. *In* "Brassinosteroids, Chemistry, Bioactivity and Applications", Cutler, H. G., T. Yokota and G. Adam (eds), Washington DC, ACS, pp. 334-345.
2) Egley, G. H. and S. O. Duke 1984. Physiology of weed seed dormancy and germination. *In* "Weed Physiology, Vol. I. Reproduction and ecophysiology", Duke, S. O. (ed), CRC Press, Boca Raton, FC.
3) Staden, V. J. and P. F. Waring 1972. The effect of light on endogenous cytokinin levels in seeds of *Rumex obtusifolius*, Planta **104**, 126-131.
4) Takeuchi, Y., Y. Omigawa, M. Ogasawara, K. Yoneyama, M. Konnai and A. D. Worsham 1994. Effects of brassinosteroids on conditioning and germination of clover broomrape (*Orobanche minor*) seeds. Plant Growth Regul. **16**, 153-160.
5) Takeuchi, Y., A. D. Worsham and A. E. Awad 1991. Effect of brassinolide on conditioning and germination of witchweed (*Striga asiatica*) seeds. *In* "Brassinosteroids, Chemistry, Bioactivity and Applications," Cutler, H. G., T. Yokota, and G. Adam (eds), Washington DC, ACS, pp. 298-306.

Seasonal Change in Reproductive Allocation of *Pinellia ternata*

T. Tominaga and S. Sano
Faculty of Agriculture, Shinshu University, Ina, Nagano 399-45, Japan

Abstract. *Pinellia ternata* is an Araceae perennial weed and reproduces by seeds, bulbils produced on both lamina joints and petioles, and cormlets. This weed expands four different types of leaves and its reproductive mode is largely dependent on the leaf type. For establishing an effective control program, an understanding of its complicated and ingenious reproductive system is very important. Three hundred plants were sampled once a month from March to November 1994 in an orchard garden and each plant was divided into component structures. Dry matter weight of each structure was measured after drying at 80 C for 48 hours. This weed emerged continuously from April to October. Only a few plants produced flowers. On the other hand, 15% of the sampled plants formed bulbils on petioles from May to July, 19% in September and 27% in October. The recruitment of offspring was primarily done by producing bulbils. The dry matter partitioning ratio of corm decreased to 63% in July, but that of the leaf reached its maximum, 34%. That of the bulbil was 2% to 6% from May to October and the ternately compound leaf type had the highest value. The reproductive allocation was 67% to 86% during the growing season. This may explain the persistence of this weed population in fields.

Key words: life cycle, perennial weed, *Pinellia ternata*, reproductive allocation, seasonal change.

Introduction

Pinellia ternata (Thunb.) Breit. is an Araceae perennial weed and widely distributed in orchards and other fields in Japan (Ohwi, 1983). It reproduces by seeds, bulbils produced on both lamina joints and petioles, and cormlets. The recruitment of offsprings is primarily done by bulbil formation on petiole. This weed expands four different types of leaves (Tominaga, 1992) and its reproductive mode is largely dependent on the leaf type that closely related to corm weight (Tominaga and Nakagaki, 1993).

It is difficult to control this perennial weed (Kasahara, 1969). For establishing an effective weed control program, an understanding of its complicated and ingenious reproductive system is very important. Size of reproductive organ greatly influences the emergence, early growth and competition with other plants at early growth stage (Stebbins, 1971) and the number of propagules also influences the persistence of the weed population. In spite of the importance of the study from such a viewpoint, reports on the seasonal change in dry matter allocation were scarcely found out. In this study, we clarified the seasonal change in dry matter allocation to reproductive organ of *P. ternata.*

Materials and Methods

Three hundred plants of *P. ternata* were sampled once a month from March 24 to November 16, 1994 in an orchard garden of the Research Farm, Faculty of Agriculture, Shinshu University. Sampled individuals were classified into four groups on the basis of their leaf type. These were the cordate, auriculate, tripartite and ternately compound leaf types (Tominaga, 1992).

Each plant was divided into component structures such as root, corm, leaf, bulbil and sexual reproductive organ composed of scape, spadix, spadix appendage and spathe. Leaf area of each plant and fresh and dry weight of each structure were measured. Dry weight measurement was done after drying at 80 C for 48 hours. Root could not be collected completely and its biomass seemed to be very low, so it was omitted from total dry matter weight when reproductive allocation (RA) was calculated as follows;

$$\text{RA (\%)} = \frac{\text{Dry weight of corm, bulbil and sexual reproductive organ}}{\text{Total dry matter weight}} \times 100$$

Results and Discussion

This weed emerged in early April and continued to do until early October. Total biomass of this weed was the largest in June and gradually decreased thereafter. The ternately compound leaf type had the largest biomass and the cordate leaf type had the smallest one throughout the growing season (Fig. 1). Specific leaf weight (SLW = leaf weight / leaf area) was the highest in June with the only exception of the tripartite leaf type (Fig. 2). Photosynthesis seems to have been the highest at that time. The percentage dry matter of corm was also the highest in June, 32% and in other months it ranged from 19% to 22% (Fig. 3). Photosynthate may have been stored in corm in June. This weed prefers moderately moist habitat, but in the summer of 1994, it was very hot and dry in the area where the sampling was done. Low biomass in the summer was probably caused by this hot and dry condition.

Only 1% of the sampled plants had a sexual reproductive organ in May to July. These individuals were the ternately compound leaf type without exception. This weed produced sexual reproductive organ when its corm dry weight exceeded 0.4 g in this population (Tominaga and Nakagaki, 1993). The extremely low ratio resulted from the reason that there were few plants with such corm in this population. On the other hand, 15% of the sampled plants formed bulbils on petioles from May to July, 19% in September and 27% in October (Fig. 4). The recruitment of offspring was primarily done by producing bulbils. The individuals that produced bulbils on petioles had the tripartite or ternately compound leaf, but the cordate or auriculate leaf type seldom produced bulbils. The tripartite and ternately compound leaf types

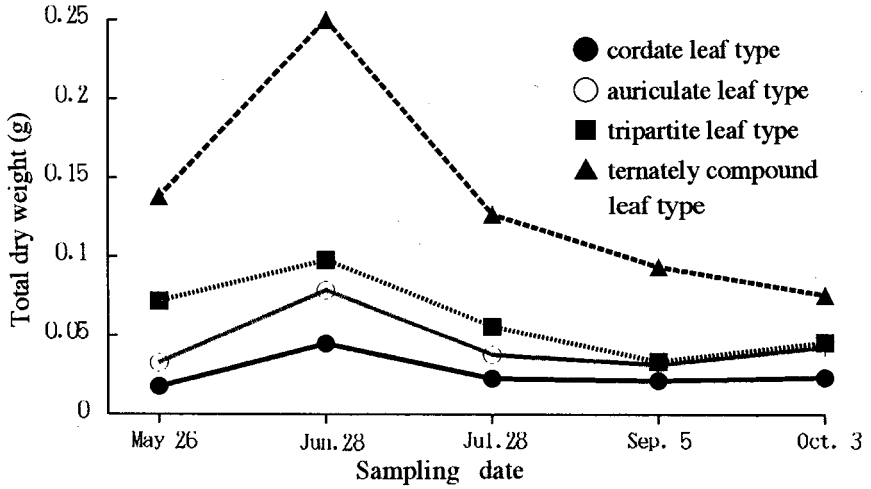


Fig. 1. Seasonal change in biomass of each leaf type

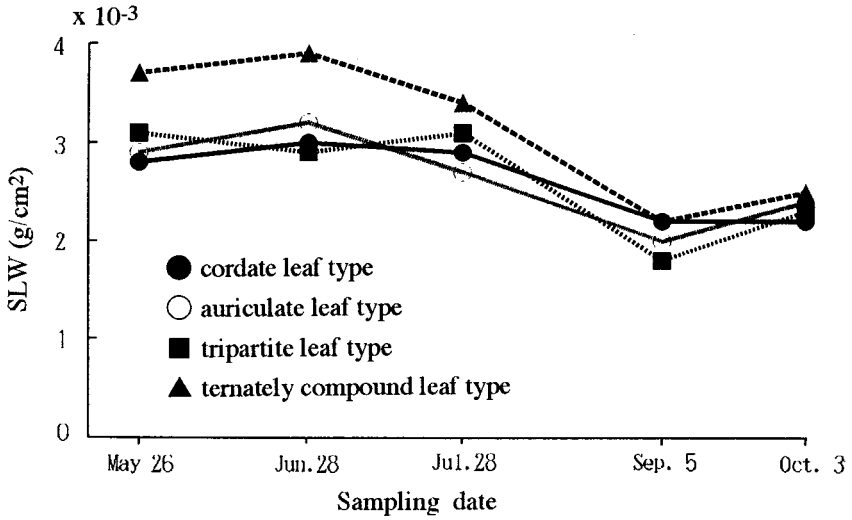


Fig. 2. Seasonal change in specific leaf area of each leaf type

played an important role in the persistence of the population. SLW decreased in fall (Fig. 2), whereas the number of individuals that produced bulbils increased (Fig. 4). Photosynthate may have been transported to bulbils and corms in the tripartite and ternately compound leaf types and to corms in the cordate and auriculate leaf types.

The seasonal change in dry matter allocation of each leaf type was shown in Fig. 5. The cordate and auriculate leaf types showed similar seasonal change in dry matter allocation. Corm occupied 64% to 79% in the cordate leaf type and 66% to 83% in the auriculate leaf type during the growing season. The dry matter partitioning ratio to corm was higher in the cordate and auriculate leaf types than the tripartite and ternately compound leaf types throughout the growing season. The cordate and auriculate leaf types seldom produced bulbils, so photosynthate may have been allocated to corm in these two leaf types. On the other hand, the tripartite and ternately compound leaf types produced bulbils. Bulbil occupied 1% to 8% of the total biomass in the tripartite leaf type and 1% to 6% in the ternately compound leaf type. The ternately compound leaf type produced sexual reproductive organ in May to July and its dry matter allocation was 4% to 15%. In June when the sexual reproductive allocation was the highest, 15%, the dry matter allocation to bulbil was very low, 1% in this leaf type. There seems to be a trade-off relationship between sexual reproduction and bulbil formation.

The seasonal change in reproductive allocation (RA) of each leaf type showed similar pattern (Fig. 6). RA of each type showed its maximum in June, thereafter it decreased in summer and increased again in October. In June, photosynthesis was the highest as described above and its product was transported to

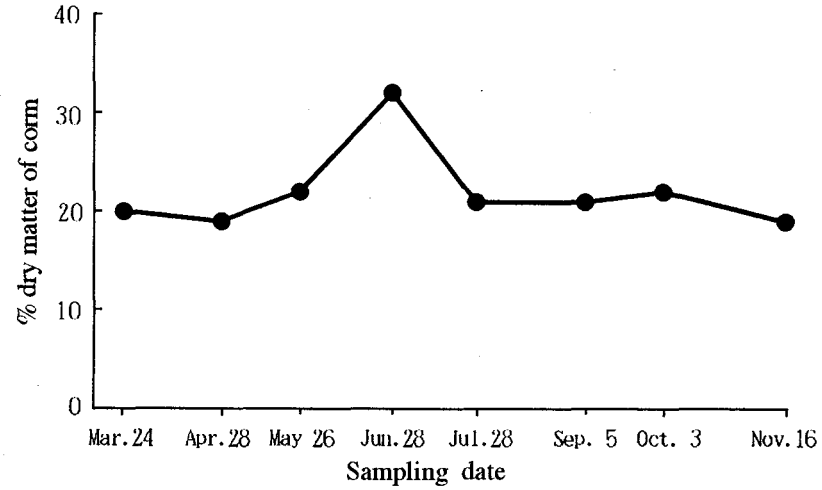


Fig. 3. Seasonal change in percentage dry matter of corm

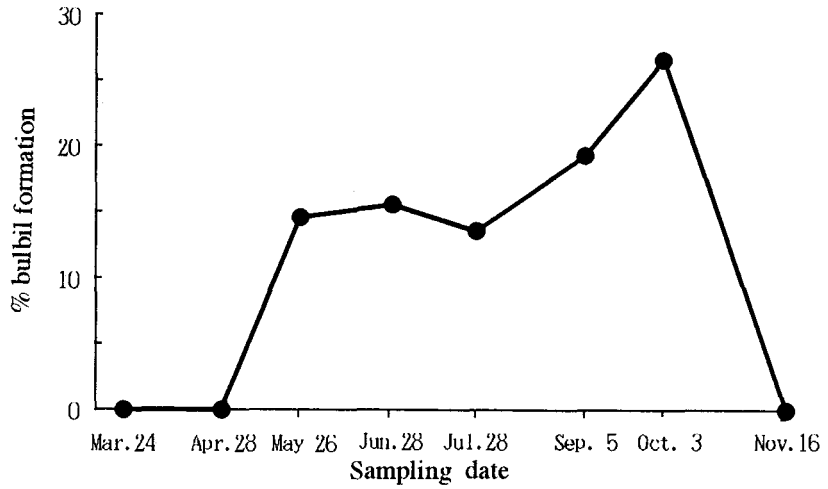


Fig. 4. Seasonal change in bulbil formation rate

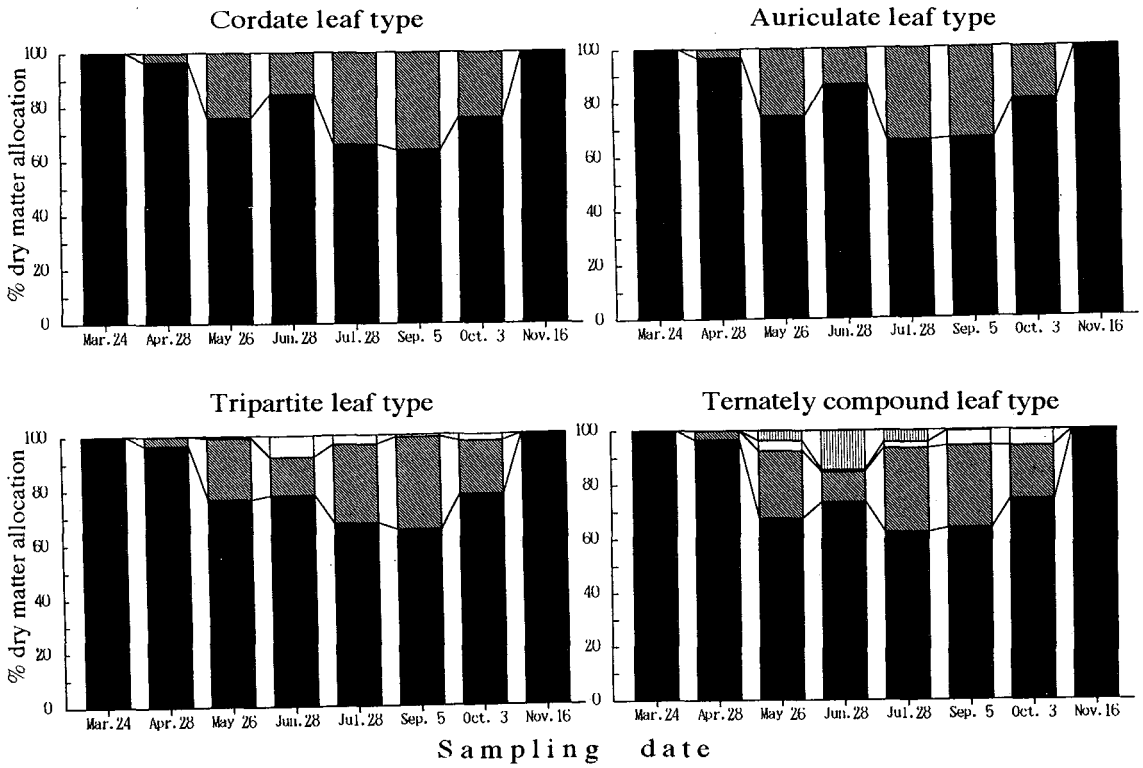


Fig. 5. Seasonal change in dry matter allocation to each component structures

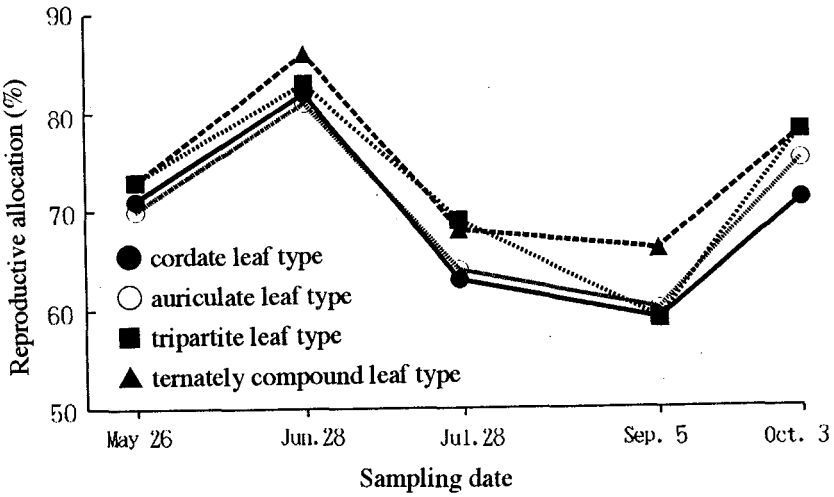


Fig. 6. Seasonal change in reproductive allocation of each leaf type

sexual and asexual reproductive organs. In October, just before the death of aerial parts, the photosynthate was allocated to corm in the cordate and auriculate leaf types and to corm and bulbil in the tripartite and ternately compound leaf types. This was supported by the increase of the bulbil formation rate in October (Fig. 4).

RA was the highest in the ternately compound leaf type throughout the growing season. This leaf type had the largest biomass and produced both sexual and asexual reproductive organ. The frequency of this leaf type was the highest in the population (Tominaga, 1992). The high reproductive allocation during the growing season may be one of the reasons for the persistence of this weed population in fields instead of weeding. The control of the ternately compound leaf type in June and October seems to be effective for decreasing the production of reproductive organ of this weed.

References

1. Kasahara, Y. 1969. Weeds of Japan Illustrated - Seeds, Seedlings and Plants-. 372-373. Yokendo Ltd., Tokyo.
2. Ohwi, J. 1983. Flora of Japan. 296. Shibundo, Tokyo.
3. Stebbins, G. L. 1971. Adaptive radiation of reproductive characteristics in angiosperms. II: Seeds and seedlings. Annu. Rev. Ecol. Syst. 2; 237-260.
4. Tominaga, T. 1992. Leaf types dependent on corm size of *Pinellia ternata*. Weed Res., Japan 37 (suppl.); 132-133.
5. Tominaga, T. and A. Nakagaki 1993. Corm size dependent reproduction of *Pinellia ternata*. Weed Res., Japan 38 (suppl.); 118-119.

Surrvey of Weeds in Salt-Affected Area in Northeast Thailand.

C. Wongwattana[1], S. Surawattananon[2] and M. Na Nakorn[2].

[1] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.
[2] Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand.

Abstract Seven locations in salt-affected areas in Northeast Thailand were selected for a weed survey using data from the reports and the saline soil map of the Department of Land Development, Thailand. Soil samples were also collected at the same locations to determine the soil salinity and pH values. The areas which have high soil salinity problem are in NRM, MSK and KKN. Soil salinity (EC value) in 7 locations were in the range of 0.0 - 7.9 mS/cm. Soil pH of NRM were rather high when compared to those of the other locations. Thirty eight families with 138 species of weeds were found in salt-affected areas in 7 locations. Comparing among different soil salinities, 26 families with 64 species were found in areas which have soil EC value 2.13 - 2.60 mS/cm and 15 families with 32 species were found in the areas with 6.0 - 6.8 mS/cm EC value. The species that often found in saline soil which have EC value more than 2 mS/cm were *Dactyloctenium aegyptium* (L.) P.B., *Chloris barbata* Sw., *Cynodon dactylon* (L.) Pers. and *Synostemon bacciformis* G.L. Webst.

Key words: weed, survey, salt tolerance, saline soil, Northeast Thailand

Introduction

There are two types of saline soil in Thailand. One is the coastal saline soil formed along the coastline and the other is the inland saline soil in Northeast Thailand. Salt-affected area in Northeast is estimated to be 17 % of the total area of the Northeast. The dominant salt in both types of saline soil is sodium chloride (1, 4). Salt-affected area in Northeast distributes in every provinces and the salinity vary from low to high. Some plants and weeds can grow in low saline soil but the areas with high salinity are usually left bare land or bare spot with the white film of salt on soil surface. The inland salt contaminated areas have begun expanding due to human interference; deforestation, reservoirs construction, salt manufacture, etc.

Weeds flourishing in the rainy season and those in the dry season in salt-affected area were different (3). Most of the rainy season weeds seemed to be tolerant to both moderate salinity and reductive state of soil. On the contrary, most of the dry season weeds were tolerant to high salinity but not tolerant to reductive state of soil. Nine families with 28 species of plants were reported to be found in saline soil in Khon Kaen Province (5), both dicot and monocot, including some species of weeds. Many species of plants were reported to be salt-tolerant (2), and some of those were in the same genus of the common weeds in Thailand, e.g. *Leptochloa*, *Paspalum*, *Chloris*, *Sporobolus* and *Echinochloa.*

The purpose of this study is to determine the weeds species in the salt-affected area and to select the proper weeds species to study further on the salt tolerance.

Materials and Methods

Materials

1) Herbariums, knifes and scissors for weeds and plants collection.
2) Soil sampling equipments
3) pH meter
4) EC meter for soil salinity determination.

Methods

1) Seven locations (provinces) in Northeast Thailand which have saline soil problem, Nakhonratchasima (NRM), Mahasarakam (MSK), Khon Kaen (KKN), Skonnakhon (SKN), Udonthani (UDN), Surin (SRN) and Srisaket (SSK) were selected by using the data from the reports and saline soil map of the Department of Land Development, Thailand.

2) Two to three sub-locations in each location (totally 17 sub-locations) were selected for the weed survey and soil salinlty determination, using data from the local offices of the Land Development.

3) Inspected the areas where the survey would be done.

4) Surveyed the weeds in each sub-location and collected the soil samples (0 - 20 cm. depth) at the same places.

5) Identified weeds species in each sub-location and determined the pH and salinity (EC values) of soil samples.

5.1 For soil pH evaluation, soil sample was dissolved in water at the rate of 1:2 (soil : water).

5.2 For soil salinity (EC value) evaluation, soil sample was dissolved in water at the rate of 1:5 (soil : water).

6) Comparing weeds species among the locations and among the different soil salinity.

Results and Discussions

EC values of soils in each location are different. The areas which have high soil salinity problem are in NRM, MSK and KKN, the EC values of soil are in the range of 0.0 - 7.9 mS/cm. (Table 1). In some areas of these locations, the white film of salt could be inspected on soil surface and no plants could grow there. The EC value of soil in bare land were sometime more than 26 mS/cm.

From 7 locations, soils pH in NRM were rather high (9.12 in average) when compared to other locations (4.77 - 6.42 in average) (Table 1).

Thirty eight families with 138 species of weeds were found in salt-affected area in 7 locations (17 sub-locations) (Table 2). Among those, 22species were found at more than 3 sub-locations and 3 species were found at more than 9 sub-locations (*Synostemon bacciformis* G.L. Webst., *Chloris barbata* Sw., and *Dactyloctenium aegyptium* (L.) P.B.).

Comparing among the different soil salinity in each location, 26 families with 52 genus and 64 species were found in the areas which have soil EC value 2.13 - 2.60 mS/cm. and 15 families with 28 genus and 32 species were found in the areas with 6.0 - 6.8 mS/cm. soil EC value.

Among the 81 species (28 families) of the weeds found in areas which have soil salinity (EC value) more than 2 mS/cm. (2.13 - 6.8 mS/cm.) in this study, 13 species (8 families) were found at 2 sub-locations, 8 species (6 families) were found at 3 sub-locations, 2 species (*Synostemon bacciformis* G.L. Webst.and *Cynodon dactylon* (L.) Pers.) were found at 4 sub-locations and 1 species was found at 5 and 6 sub-locations (*Chloris barbata* Sw. and *Dactyloctenium aegyptium* (L.) P.B., respectively) (Table 3).

Leaves of *Portulaca piloca* Linn. grew in saline soil were thicker and more succulent than the normal ones, like egg shape, and had green-red to red color (compared to the green normal ones). The portulaca leaf tasted salty from the salt accumulated in it. *Synostemon bacciformis* G.L. Webst. was also found in many locations of salt-affected areas and its lower leaves were thicker than the normal ones.

Leaves of *Sporobolus* sp. grown in saline soil (at KKN by the Land Development researchers) could excrete salt absorbed from soil, small salt crystals could be seen on its leaves. The other *Sporobolus* sp., found at MSK, grew well in high saline soil until its maturation. At the late stage of growth, the plant was on the land covered with white salt-crystal. But seeds of the *Sporobolus* grew in saline soil had low germinatig ability.

The data shown in this report were collected from salt-affected areas in late rainy season. The study will be done again at the same locations in rainy and dry seasons in order to determine the changes in soil pH and salinity (EC values) and weed species.

From the survey, some weed species (Table 4) which were potentially salt-tolerant and had high distributing ability were selected to study further on salt tolerance and on their propagating ability in saline soil. The proper salt-tolerant weed species will be used to improve the soil in salt-affected areas.

References

1) Mitsuchi, M., P. Wichaidit and S. Jeungnijnirund. 1986. Outline of soils of the Northeast plateau Thailand, Their characterstics and constraints. Technical paper No. 1, Agricultural Development Research Center in Northeast, Khon Kaen, Thailand. 79 p.

2) National Research Council. 1990. Saline Agriculture. Salt-tolerant plants for developing countries. Report of a Panel of the Board on Science and Technology for International Development. National Academy Press, Washington. 143 p.

3) Puengpan, N., T. Subhasaram, K. Shiraishi and H. Wada. 1990. Soil-plant relationship at moderately to strongly salt-affected area in Northeast Thailand. The 29th Kasetsart University Annual Conference. p: 79-85

4) Takai, Y., T. Nagano, M. Kimura, J. Sugi and S. Vacharotayan. 1987. Coastal and inland salt-affected soils in Thailand, Their characteristics and improvement. Nodai Research Institute, Tokyo University of Agriculture. p: 99 - 120.
5) Thammathaworn, S., A. Thammathaworn and P. Chantaranothai. 1984. A survey of vegetation in saline condition of Muang Khon Kaen. Department of Biology, Khon Kaen University.

Table 1 Soil pH and salinity (EC value) in salt-effected areas of 7 locations in Northeast Thailand (the range and mean of sub-locations in each location)

Locations	EC (mS/cm)		pH	
	Range	Mean	Range	Mean
NRM	0.0 - 7.9	2.17	7.9 - 9.9	9.12
SRN	0.0 - 1.2	0.62	4.9 - 7.1	5.58
SSK	0.1 - 2.1	0.75	4.4 - 8.4	6.42
KKN	0.0 - 3.8	1.59	4.3 - 8.1	6.16
MSK	1.3 - 6.0	3.28	4.7 - 8.0	5.65
SKN	0.0 - 3.6	1.69	3.8 - 7.8	5.38
UDN	0.3 - 3.2	1.45	4.3 - 5.8	4.77

1 EC and pH values of the top 0-20 cm. depth soil in the area of weed survey

Table 4 Potentially salt-tolerant weeds selected from salt-affected areas in 7 locations of Northeast Thailand

Family	Scientific Name
Amaranthaceae	*Gomphrena celosioides* Mart.
Euphorbiaceae	*Synostemon bacciformis* G.L. Webst.
Gramineae	*Chloris barbata* Sw.
	Chrysopogon aciculatus (Retz.) Trin.
	Cynodon dactylon (L.) Pers.
	Dactyloctenium aegyptium (L.) P.B.
	Eragrostis spp.
	Panicum repens Linn.
	Paspalum distichum Linn.
	Sporobolus spp.
Papilionaceae	*Aeschynomene* sp.
	Sesbania sp.
	Desmodium triflorum (L.) Df.
Portulacaceae	*Portulaca pilosa* Linn.

Table. 2. List of weed species found in salt-affected areas in 7 locations of Northeast Thailand

Scientific name	Scientific name
Acanthaceae	*F. dichotoma* (L.) Vahl.
Hygrophila sp.	*F. glacicenta*
unknown	*F. globulosa* (Retz.) Kunth
Aizoaceae	*F. littoralia* Gaud. *var. littoralis*
Trianthema triquetra Willd. ex Rettler	*F. miliacea* (L.) Vahl.
T. portulacastrum Linn.	*F. velata*
Amaranthaceae	*Fuirena ciliaris* (L.) Roxb.
Achyranthes aspera Linn	*Scirpus juncoides*
Alternanthera sessilis DC.	*S. purshianus* Fernald
A. pungens H.B.K.	Elatinaceae
Amaranthus hybridus Linn.	*Bergia capensis* Linn.
Gomphrena celosioides Mart.	Eriocaulaceae
Apocynaceae	*Eriocaulon odoratum* Dalz.
Trachelospermum jasminoides Linn.	*Eriocaulon sp.*
Casealpiniaceae	Euphorbiaceae
Cassia occidentalis	*Euphorbia hirta* Linn.
Celastraceae	*Phyllanthus niruri*
Maytenus marcanii Ding Hou	*Synostemon bacciformis* G.L. Webst.
Chenopodiaceae	Gramineae
Suaeda maritima Dum.	*Aristida chinensis* Munro
Commelinaceae	*A. cumingiana* Trin et Rupr
Aneilema herbaceum n (Roxb.) Wall	*Brachiaria distachya* (L.) Stap f.
A. loureirii Hance	*B. reptans* (L.) Gard. & Hubb
A. scaberrimum Kunth	*Chloris barbata* Sw..
Commelina benghalensis Linn.	*Chrysopogon aciculatus* (Retz.) Trin
Cyanotis axillaris Roem.& Schult	*C. orientalis* (Desv.) A. Camus
Murdannia nudiflora (L.) Brenan	*Cynodon dactylon* (L.) Pers.
Compositae	*Dactyloctenium aegyptium* (L.) P.B.
Eclipta prostrata Linn.	*Dichanthium annulatum* (Forsk) Stapf.
Elephantopus scaber Linn.	*Digitaria biformis*
Eupatorium odoratum	*D. ciliaris* (Retz.) Koel
Grangea maderaspatana Poir.	*D. sp.*
Pluchea indica	*Diplachene fusca* P. Beauv.
Sphaerathus africana	*Echinochloa colona*
Vernonia cinerea (L.) Less	*E. crus-galli* (L.) Beauv.
Convolvulaceae	*Eleusine indica*
Impomoea aquatica Forsk.	*Eragrostis atrovirens* (Desf.) Trin.
Merremia sp.	*E. brownii* (Kunth) Nees & Steud
Cyperaceae	*E. elongata*
Bulbostylis barbata (Rottb.) C.B. Clarke	*E. pilosa* (L.) P.Beauv.
Cyperus compressus	*E. tenella* (L.) P.Beauv.
C. difformis	*E. uniloides* Nees.
C. imbricatus Retz.	*Eriochloa procera* (Retz.) C.E. Hubb
C. iria Linn.	*Ischaemum barbatum* Retz.
C. haspan	*I. imbricatum* Stapf.
C. procerus Rottb.	*I. sp.*
C. pulcherrimus Willd. & Kunth	*Oryza rufipogon* Griff
C. rotundus Linn.	*Panicum repens* Linn.
C. tegetifarmis Boeck.	*Paspa lidium* Flavidum
Eleocharis dulcis (Burm.f.) Henschel	*Paspalum comersonii* Lamk.
Fimbristylis aestivaris	*P. distichum* Linn.

Table. 2. (Continued)

Scientific name	Scientific name
Sacciolepis indica (L.) Chan	*Aeschynomene americana* Linn.
Setaria sp.	*A. sp.*
Sporobolus coromandelianus (Retz.) Kunth	*Tephrosia purpurea* Pers.
Vetiveria nemoralis (Balansa) A. Camus	Passifloraceae
Labiatae	*Cocinia cineria*
Anisomales indica	Pontederiaceae
Hydrophyllaceae	*Monochoria vaginalis* Presl. Agfl.
Hydrolea zeylanica Vahl.	Potulacaceae
Liliaceae	*Portulaca oleracea* Linn.
Asparagus cochinchinchinensis	*P. pilosa* Linn.
A. racemosus	Rubiaceae
Lythraceae	*Hedyotis diffusa* Willd.
Ammania baccifera Linn.	Salvadoraceae
Rotala indica (Willd.) Koechne	*Azima sarmentosa* (bl.) Benth.et.Hook
Leucus aspera (Willd.) Link	Scrophulariaceae
Malvaceae	*Bacopa monnieri* Pennell
Abutilon hirtum	*Centranthera siamensis* Yamazaki
Hibiscus sp.	*Limnophila geoffrayi* Bonati
Sida sp.	*Lindernia hyssopioides* (L.) Haines
Sida acuta Burm.	*L. sp.*
Urena lobata Linn.	*L. succosa* (Kerr.ex.Barn.) Philcex
Marsiliaceae	*Mazus sp.*
Marsilia crenata Presl.	*Scoparia dulcis* Linn.
Mimosaceae	Sphenocleaceae
Mimosa invisa Mart.	*Sphenoclea zeylanica* Gaertn.
Mimosa pudica L.	Sterculiaceae
Nyctaginaceae	*Helioteres lanata* Kurz.
Boerhavia erecta	*Melochia corchorifolia* Linn.
Olacaceae	*Waltheria inolica* Linn.
Olax scandens Roxb.	Tiliaceae
Onagraceae	*Corchorus aestuants* Linn.
Jussiaea adscendens L.	*Triumfetta annua* Linn.
J. linifolia Vahl	Typhaceae
J. parviflora Roxb.	*Typha sp.*
Papilioneceae	Xyridaceae
Desmodium triflorum DC.	*Xyris indica* L.
	Zygophyllaceae
	Tribulus terrestris

Table 3. List of weed species that often found in saline soil which have EC value more than 2 mS/cm. (2.13 - 6.80 mS/cm.) in 7 locations of Northeast Thailand.

No. of sub-locations that weed was found	Scientific Name of Weeds	
2	Aizoaceae	*Trianthema portulacastrum* Linn.
	Celastraceae	*Maytenus marcanii* Ding. Hou.
	Commelinaceae	*Aneilema herbaceum* (Roxb.) Wall.
		Murdannia nudiflora (L.) Brenan.
	Cyperaceae	*Cyperus iria* Linn.
		C. rotundus Linn.
	Gramineae	*Ischaemum imbricatum* Stapf.
		Panicum repens Linn.
		Sporobolus coromandelianus (Retz.) Kunth.
	Malvaceae	*Sida* sp.
		Urena lobata Linn.
	Onagraceae	*Jussiaea adscendens* L.
	Typhaceae	*Typha* sp.
3	Amaranthaceae	*Gomphrena celosioides* Mart.
	Cyperaceae	*Bulbostylis barbata* (Rottb.) C.B. Clarke
		Fimbristylis dichotoma (L.) Vahl.
		Fuirena ciliaris (L.) Roxb.
	Gramineae	*Chrysopogon aciculatus* (Retz.) Trin.
	Hydrophyllaceae	*Hydrolea zeylanica* (L.) Vahl.
	Portulacaceae	*Portulaca pilosa* Linn.
	Tiliaceae	*Corchorus aestuans* Linn.
4	Euphorbiaceae	*Synostemon bacciformis* G.L. Webst.
	Gramineae	*Cynodon dactylon* (L.) Pers.
5	Gramineae	*Chloris barbata* Sw.
6	Gramineae	*Dactyloctenium aegyptium* (L.) P.B.

WEED PREVENTION BY INTERCROPPING OF WHEAT AND BARLEY

Hajime Araki*, Tomokazu Hamada, Michiaki Ito,
Toshinori Fukuyama and Kazuhiro Nakano
Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-21, Japan

Abstract Wheat (cv. Mulch-mugi for green manure) and barley (cv. Minori-mugi) were introduced in crop production for weed control, not seed production. For examination of the weed control-effect in autumn sowing and spring sowing in row spacings in a watermelon patch, seeds of wheat and barley were sown by hand in October 1993 with the seeding density of 4 kg/10 a, while wheat seed was sown early in April 1994 with the seeding density of 0 to 10 kg/10 a. In the autumn sowing, elongated culms were cut down early in June 1994 and became straw mulch. Light intensity (photon flux density) on the surface of the ground was suppressed by the straw mulch of wheat and barley. Suppression of light intensity in wheat straw mulch was greater than that of barley 1 month after cutting because of the large volume and toughness of wheat straw in comparison with barley straw. Germination from fruit body (grain) attached in the cut culm occurred late in June, and new leaves developed above ground. Suppression of light intensity by new leaves in barley was more than that in wheat, and photon flux density on the surface of the ground was reduced to 30% of solar radiation. Weed quantity in the interrow space was effectively reduced, especially by wheat straw mulch. In the spring sowing, weed growth was more vigorous than wheat vegetative growth, and weed production was not noticeably reduced.

Key words; Weed prevention, Wheat and barley, Straw mulch, Live mulch, Light intensity

Introduction

In the cultural weeding work, labor saving and efficiency have been expected to establish a sustainable agriculture. Straw mulch is a useful and traditional technique to prevent weed growth over the past years, and used to conserve moisture, lower surface temperature, fertilize the soil and protect the soil from rain (Partiquin, D.G. 1988). In the open field cultivation, it takes a long hours of work to cover the ground with rice straw or wheat and barley straw. Besides, straw laid on mulches is often blown off by a strong wind. In the present study, when barley and wheat with the autumn sowing ability were intercropped, weeding system through shading the ground-surface by straw mulches or vegetative growth was examined.

Materials and Methods

1.Cultivation of watermelon

Watermelon nurseries were planted on a high ridge (10 cm height,t 90 cm width, 2 m m distance between the ridges), 2.5 m distance between the plants, in the open field of University Farm, Niigata University, on May 10, 1994, and watermelons were cultivated till early in August. High ridges planted watermelons were added chemical fertilizers (N: 6.4 kg/10a, P_2O_5: 9.6 kg/10a, K_2O: 8 kg/10a) and covered with black plastic film. Interrow spaces were covered with barley and wheat straw mulch or wheat live mulch. Vines of watermelon grew over the straw mulch or live mulch.

2. Mulching by barley and wheat straw

Wheat (*Triticum aestivum* L. cv. Mulch-mugi for green manure) and barley (*Hordeum vulgare* L. cv. Minori-mugi) were sown with the seeding density of 4 kg/10 a, in the interrow space of watermelon field on October 20, 1993. Next spring, elongated culms were cut down early in June 1994 and became straw mulch. After heading of wheat and barley, light intensity of solar radiation (photon flux density) on the surface of

the ground and sunshine above were measured; shading ability of wheat and barley straw mulches was also estimated. Weed quantities (dry weight) in the interrow space covered with wheat and barley straw mulch were measured early in August. Interrow spaces cultivated wheat and barley were added compound fertilizers (N: 8 kg/10a, P_2O_5: 8 kg/10a, K_2O: 9 kg/10a) for basal dressing on October 20, 1993, and ammonium sulfate (N: 8 kg/10a) for topdressing on April 7, 1994.

3. Live mulch by wheat vegetative growth

Seeds of wheat (*Triticum aestivum* L. cv. Mulch-mugi for green manure) were sown in the interrow space of watermelon field with the seeding density of 0 to 10 kg/10 a on April 7, 1994. Interrow spaces were added compound fertilizers (N: 8 kg/10a, P_2O_5: 8 kg/10a, K_2O: 9 kg/10a) before sowing. Wheat vegetative growth was observed and weed quantities (dry weight) in the interrow space covered with wheat live mulch were measured on June 14, 1994.

Results

1. Weed prevention by barley and wheat straw mulch

Average tillering number per plant was 3.4 in barley and 7.0 in wheat in April 7, 1994 (Table 1). Photon flux density of ground surface under developed wheat culms was less than 10 $M/m^2 \cdot day$ even fine day and light passing ratio to ground surface from sunshine above was 23%. Few weeds grew in the communities of wheat and barley plants.

Just after cutting down developed culms, the photon flux density of ground surface under wheat straw mulch was less than 3 $M/m^2 \cdot day$ on a cloudy day and less than 14 $M/m^2 \cdot day$ on a fine day; light passing ratio to the ground surface was approximately 20 % (Fig. 1). The same results were observed for barley straw mulch.

One month after cutting down, the photon flux density of ground surface under wheat straw mulch was less than 4 $M/m^2 \cdot day$ on cloudy day. It was 14 $M/m^2 \cdot day$ on cloudy day and 20 $M/m^2 \cdot day$ on fine day under barley straw mulch, and the light passing ratio was 40-61% . Though wheat straw was tough, barley straw was easy to break.

Germination from fruit body (grain) attached in the cut culm began late in June, and new leaves developed above ground. Width of new leaf was 8.0 mm in wheat and 12.3 mm in barley (Table 1). Photon flux density under the new leaves was 27 $M/m^2 \cdot day$ in wheat and 15 $M/m^2 \cdot day$ in barley. Shading ability by new leaves in barley was larger than for wheat because of the broad barley leaf.

The quantity of weed (dry weight) was 980 g/m^2 in interrow space without straw mulch (Fig. 2). On the other hand, the weed production decreased to 180 g/m^2 in barley straw mulch and 20 g/m^2 in wheat straw mulch.

Table 1 Comparison of number of tillers and width of leaf grown by germination from fruit body in wheat and barley sown in autumn in the previous year.

	No. of tillers		Leaf width(mm)	
	Ave.	SE	Ave.	SE
Wheat	7.0	0.3	8.0	0.3
Barley	3.4	0.2	12.3	0.2

Observation date: April 7, 1994

2. Weed prevention by wheat live mulch

Wheat sown in April grew vegetatively and covered the ground with its leaves (wheat live mulch). Initial growth of wheat was slower than some weeds, such as green pigweed, water pepper and green panicum. Weed quantity was 111 g/m^2 in the interrow space without wheat sown in April (control; 0 kg/10a) on June 14 and the same level as control in the interrow space sown with the seeding density of 1, 2 and 4 kg/10a (Fig. 3). In the interrow space sown with more than 8 kg/10a wheat, the weed production decreased and 40 g/m^2 was recognized in 10 kg/10a.

Discussion

Barley and wheat straw mulch suppressed the light intensity at ground surface and served to decrease weed growth. They have been called a "smother crop" for weed (Putnam, A.R. and DeFrank J. 1983). The difference in the shading ability of ground surface between wheat and barley sown in autumn was due to their botanical characteristics such as number and toughness of culm and size of new leaves grown by germination from fruit body. Weed suppression in the interrow space covered with straw mulches resulted from the shading at ground surface by those botanical characteristics. From the view point of maintaining the low light-intensity at ground surface for a long period, cv. Mulch-mugi, which is the wheat cultivar for green manure and has a large number of tillers, is promising material for ground cover.

The live mulch of wheat in the present study had a lower weed-reducing effect in comparison with wheat and barley straw mulch, because weed growth was more vigorous than initial growth of wheat in spring and shading ability on the ground surface by wheat leaves was small.

Some cultivars in barley grow more vigorously in the vegetative stage more than wheat used in the present study (Takahashi and Fukuyama 1983). It is necessary to select cultivars which provide ground cover rapidly for weed prevention by wheat and barley live mulch.

References

Partiquin, D.G. 1988 Weed control in organic farming system. p.304-317. In: Miguel A.A. and Matt L.(eds.) Weed management in Agroecosystem: Ecological approaches. CRC Press, Florida.

Putnam, A.R. and J. DeFrank 1983 Use of phytotoxic plant residues for selective weed control. Crop Prot. 2:173

Takahashi, R. and T. Fukuyama 1983 Catalogue of barley germplasm in Okayama University. Okayama University.

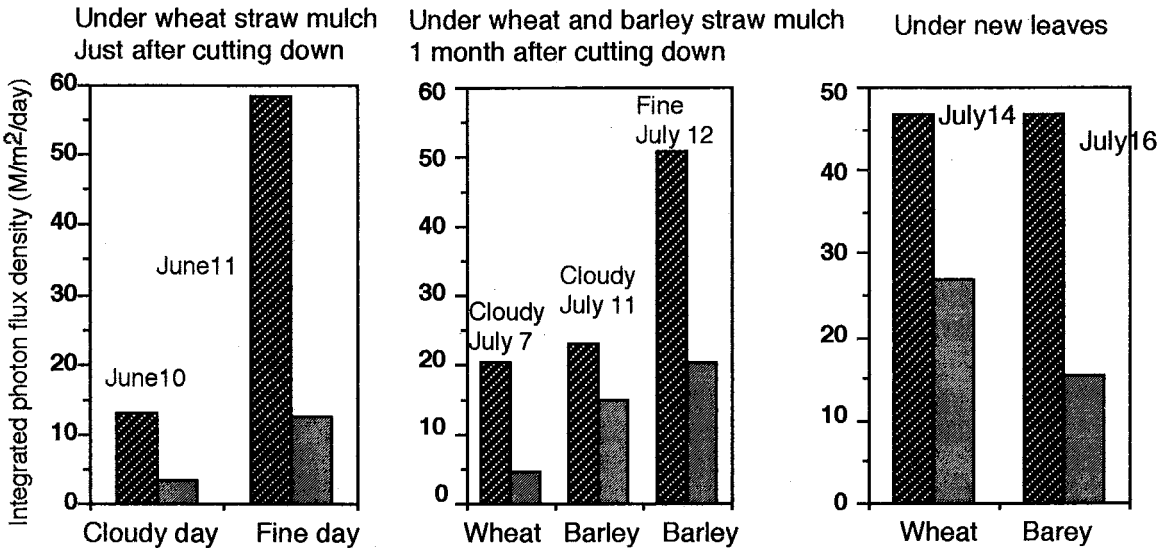


Fig. 1 Decrease of light intensity at ground surface by wheat and barley straw mulch.
Cutting date; June 8, 1994.
Sunshine Ground-surface

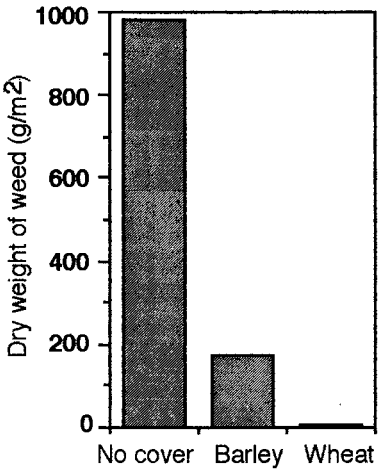


Fig. 2 Effect of ground cover by wheat and barley straw mulch on weed production.

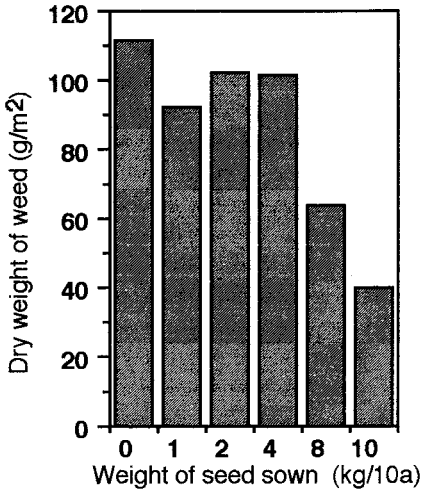


Fig. 3 Effect of seeding density of wheat sown in April on weed production.
Sowing date: April 7
Investigating date: June 14

Weed Flora of Nontillage Sown Soybean(*Glycine max*) on Rotational Paddy Fields of Southern Japan

S. Nakayama , Y. Kawana, M. Takabayashi, S. Eguchi and H. Morita
National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan

Abstract. To develop a proper weed control method in nontillage sown soybean(*Glycine max* L.) in double cropping with wheat(*Triticum aestivum* L.) on rotational paddy fields of southern Japan, weed flora of the soybean was studied in comparison with that of conventional tillage culture. In nontillage culture total biomass of weeds was much larger than in conventional culture, and number of weed species in nontillage culture was also greater. The most remarkable change in weed flora was that tall goldenrod (*Solidago altissima* L.) was predominant in nontillage culture while this species did not infest conventional soybean. Tank-mix combination of thiobencarb + prometryn and foliar applied herbicides, glyphosate and glufosinate, presented excellent control efficacy against weeds including tall goldenrod in nontillage culture. The tank-mix combination is considered a reliable and labor-saving weed control method in nontillage sown soybean.
Key words. Soybean(*Glycine max* Merr.), Nontillage, Weed flora, Tall goldenrod(*Solidago altissima* L.), Tank-mix combination

Introduction

It is rainy season in early to middle July as proper seeding time of late-summer soybean(*Glycine max* Merr.) in double cropping with wheat(*Triticum aestivum* L.) in rotational paddy fields in southern Japan. Retardation in seeding with rainfall was a serious technical problem in soybean culture in the area. Harada *et al.* (1987) examined nontillage sown soybean culture in order to avoid the retardation, therefore they recommended to advance the seeding to middle June out of proper time because of weed infestation.

In the present study, in order to evolve an effective weed control method in nontillage sown soybean with proper seeding time, changes in weed flora was investigated in comparison with that of conventional tillage culture, and control effects of tank-mix combination of a soil applied herbicide and foliar applied herbicides were determined in the soybean.

Materials and Methods

All experiments were conducted on rotational paddies of alluvial clay loam during 1987 to 1988 at Kyushu National Agricultural Experiment Station, Chikugo, Fukuoka. Paddy rice(*Oryza sativa* L.) in single cropping was cultivated till the previous year, and soybean in double cropping with wheat was cultivated throughout the experimental years. Soybean, cultivar Fukuyutaka, was sown at the density of 20 cm apart in hills x 65-70 cm apart in rows on June 23, 56 days after harvest of wheat(DAH) in 1987, June 11(41 DAH) in 1988 and June 17(48 DAH) in 1989. Fertilizer was applied at the rate according to the standard of the Station.

Characteristics of weed flora

In 1987, the first season of the upland culture after rotation from paddy, plots with and without tillage at soybean seeding were arranged. Plots were applied with thiobencarb + prometryn (Saturn-Valor EC, 50.0% + 5.0% a.i.) at the rate of 4.0+0.4 kg a.i./ha. Dry weight of every weed species in a 100 cm x 60 cm quadrat for each plot at harvest of soybean, November 11, was determined.

In 1988 and 1989, each plot in 1987 was divided into two plots, with and without tillage at wheat seeding. Plots were applied with no herbicide. In order to calculate Summed Dominance Ratio(SDR_2), plant height and cover class in PENFOUND & HOWARD's method of every weed species were determined in a 60 cm x 60 cm quadrat for each plot on July 7, August 9 and September 12 in 1988 and August 8 in 1989. August 9 in 1988 and August 8 in 1989 were the daies of molding.

Plot size was 5.6 m^2 in 1987 and 2.8 m^2 in last two years with two replications.

Efficacy of tank-mix combination

In 1988, the first season of upland culture in the field, four herbicide treatments in no-till culture were designed as follows: soil application of thiobencarb + prometryn 4.0 + 0.4 kg a.i./ha (abbreviated to TP in below) and combined application of thiobencarb + prometryn 4.0 + 0.4 kg a.i./ha mixed with paraquat-dichloride + diquat-dibromide(Preeglox L Liq, 5.0% + 7.0% a.i.) 0.5 +0.7 kg a.i./ha(TP + PD), thiobencarb + prometryn 4.0 + 0.4 kg a.i./ha mixed with glyphosate (Roundup Liq, 41.0% a.i.) 4.1 kg a.i. /ha(TP + Gly) and thiobencarb + prometryn 4.0 + 0.4 kg a.i./ha mixed with glufosinate(Basta Liq, 18.5% a.i.) 3.7 kg a.i. /ha(TP + Glu). In 1989, four herbicide treatments in no-till culture were designed as follows: TP, TP + Gly, TP + Glu and unweeded(UW). The dosage of all herbicides were equal to those in the previous year except Glu reduced to 1.85 kg a.i. /ha. And thiobencarb + prometryn 4.0 + 0.4 kg a.i./ha was applied in conventional tilled soybean in both year for control. The herbicides were applied on the day of seeding.

Dry weight of every weed species was determined in two 30 cm x 30 cm quadrates in a plot on August 9, 28 days after treatment(DAT), in 1988 and August 8(22 DAT) and November 13(119 DAT) in 1989. Exceptionally a 300 cm x 180 cm quadrat for each plot was used for tall goldenrod (*Solidago altissima* L.) in the final determination. Plot size was 10.5 m^2 with two replications.

Results and Discussions

Characteristics of weed flora

Dry weight of weeds in no-till and conventional tilled soybean at harvest time in 1987 were shown in Table 1. Total biomass of weeds in no-till culture was approximately 170 times as large as that in tilled culture. Of the total weed biomass in no-till culture, biomass of tall goldenrod was more than 95%. On the other hand this species did not occur in tilled culture.

Table 1 Dry weight of weeds at harvest time of soybean in 1987

	No-till	Tilled
	g/m^2	g/m^2
Tall goldenrod	181.9	0
Chinese sprangletop	7.1	1.0
Others	0.0	0.1

SDR_2 of weeds and the crop in no-till and tilled culture on August 9 in 1988 and August 8 in 1989 were shown in Table 2. Number of weed species occurred in no-till soybean was greater than that of tilled culture. 22 species occurred in no-till culture through the two experimental years while in tilled culture 15 species were observed. Of these weed species, 14 species occurred in the both treatments, and the rest, eight species in no-till culture and one in tilled culture did not occur in the alternatives. Of the eight species only occurred in the no-till culture, three were Composite species which had pappi on achene.

It was the most remarkable difference between no-till and tilled culture in weed flora that

Table 2 Effects of tillage on Summed Dominance Ratio(SDR2) of weeds and soybean at molding time

Year	1988		1989			
Winter cropping	Tilled wheat		Tilled wheat		No-till wheat	
Summer cropping	No-till soybean	Tilled soybean	No-till soybean	Tilled soybean	No-till soybean	Tilled soybean
Acalypha australis			3.4			
Alopecurus aequalis var. *amurensis*					4.0	
Centipeda minima	5.3	3.4	1.3		2.0	
Conyza sumatrensis	8.8					
Cynodon dactylon		10.1		4.8		
Cyperus spp.	44.3	13.2	23.6		13.4	14.9
Digitaria ciliaris	25.0	22.8	40.3	20.4	64.9	
Echinochloa crus-galli var. *formosensis*	49.4	23.9	16.8	25.6	26.1	66.7
Eclipta prostrata	3.8	5.0	4.7	25.8	3.9	7.7
Eleusine indica		2.6			11.6	
Eragrostis multispicula	23.3	10.0		9.1		4.2
Euphorbia supina	8.7	3.7	10.9	7.5	13.0	7.6
Fimbristylis miliacea	11.8	3.2	6.0	6.4	3.1	6.8
Gnaphalium japonicum	11.0					
Leptochloa chinensis	13.7	5.3				
Mazus pumilus	2.6		3.4	9.6		11.0
Mollugo pentaphylla		2.6	2.6		1.8	
Oxalis corniculata					3.1	
Persicaria longiseta	16.0		8.6		9.8	
Portulaca oleracea	17.1	45.7	3.6		4.9	
Rorippa islandica	20.6	1.0		65.6	0.9	22.0
Solidago altissima	100.0		100.0		100.0	
Soybean	80.7	100.0	32.2	55.0	24.5	93.6

tall goldenrod was predominant in no-till culture while the species was not established in tilled culture. In no-till soybean, SDR_2 of the species was greater than the crop. Tillage at wheat seeding did not prevent the infestation of tall goldenrod in no-till soybean.

Seasonal changes in SDR_2 of major weeds, tall goldenrod, *Echinochloa crus-galli* (L.) Beauv. var. *formosensis* Ohwi, common purslane (*Portulaca oleracea* L.), Southern crabgrass (*Digitaria ciliaris*(Retz.) Koeler and *Cyperus* spp., and soybean in 1988 were shown in

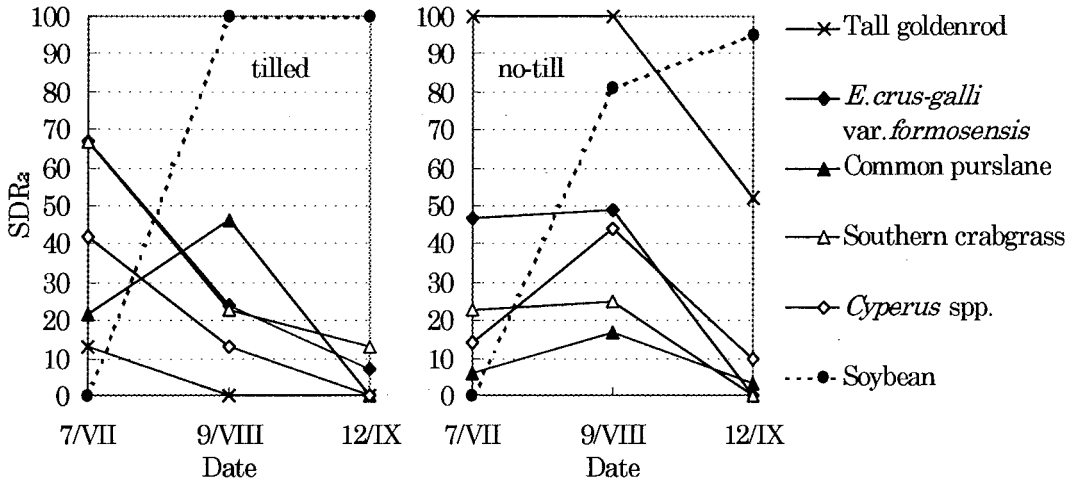


Figure 1. Changes in Summed Dominance Ratio(SDR_2) of major weeds in tilled and no-till soybean in 1988.

Figure 1. After determination in August, molding was done in both no-till and tilled culture. Therefore all major weeds except tall goldenrod, were declining in the interval of determination in August and September. Only tall goldenrod had higher SDR_2 value even in September.

Efficacy of tank-mix combination

Results in 1988 and 1989 were given in Fig. 2 and Fig. 3 respectively. Although the most part of weed biomass was caused by *E.crus-galli* var. *formosensis* and eclipta(*Eclipta prostrata* (L.) L.) in all plots, effects of the foliar applied herbicides were determined excepting eclipta because thio-bencarb + prometryn, the soil applied herbicide mixed in the combined applications, has less efficacy for eclipta in nature. TP + PD, TP + Gly and TP + Glu provided 50.2%, 79.2% and 84.2%

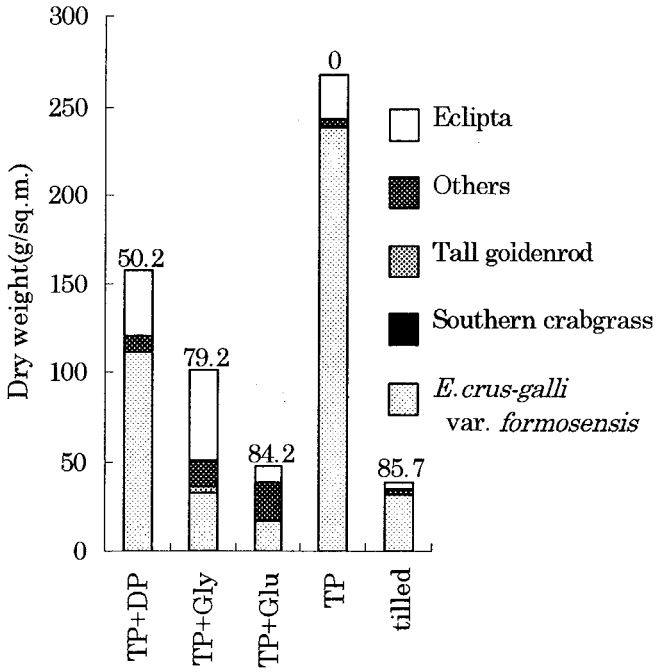


Figure 2. Effect of tank-mix combination of foliar applied herbicides and thiobencarb + prometryn on weed bio-mass, 28 days after treatment in 1988. Numbers on bars mean percentage of control.

control respectively at 28 DAT in 1988. And TP + Gly and TP + Glu provided 99.6% and 99.1% control respectively at 22 DAT in 1989. Although total weed biomass at 119 DAT in 1989 lowered because of increased dead matter of summer annuals including *E.crus-galli* var. *formosensis*, only tall goldenrod had biomass more than 100 g/m^2 in TP and UW. Throughout the experiments in both year, weeds including tall goldenrod were controlled with TP + Gly and TP + Glu in no-till culture as well as in tilled culture applied with thiobencarb + prometryn.

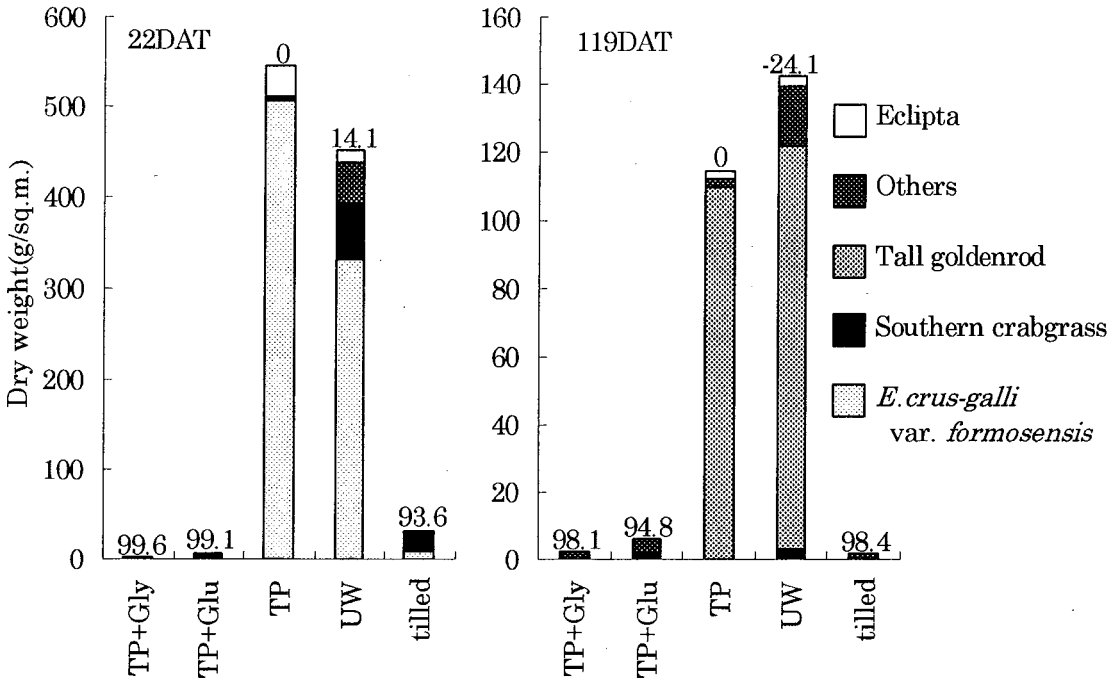


Figure 3. Effect of tank-mix combination of foliar applied herbicides and thiobencarb + prometryn on weed biomass, 22 days and 119 days after treatmen in 1989. Numbers on bars mean parsentage of control.

In soybean it is a considerable factor of weed loss that weed stains grains with sap as soybean is harvested, besides yield reduction caused by competition. Examining the characteristics of weed flora from this view point, tall goldenrod which was taller than soybean and had high SDR_2 even in the latter stage of culture is considered the most troublesome weed in nontillage sown soybean.

Seed dispersal in tall goldenrod occurs in middle November to middle January(Shimano *et. al.* : 1973) or early December to late January(Enomoto & Nakagawa : 1977, Yukinaga *et. al.* : 1975) in western Japan. And elongation in rhizomes of this species begins in early June(Yukinaga *et. al.* : 1975) or early July(Enomoto & Nakagawa : 1977) in the area. In soybean-wheat double cropping, therefore, dispersed seed of tall goldenrod invades fields after seeding of wheat, and then seedlings begin to elongate rhizomes at about seeding time of soybean.

In present experiments, tall goldenrod was predominant in nontillage sown soybean while the species did not infest conventional soybean at all. The results imply that the tillage with seeding prevent establishment of tall goldenrod in the conventional soybean.

Tank-mix combination of thiobencarb + prometryn and foliar applied herbicides, glyphosate and glufosinate, presented excellent control efficacy against weeds, including tall goldenrod, in nontillage sown soybean. The tank-mix combination is considered a reliable and labor-

saving weed control method in nontillage sown soybean, which prevents establishment of tall goldenrod.

References

1) Enomoto, T. and K. Nakagawa 1977 Ecological studies on *Solidago altissima* L. 1. A comparison of the reproductive strategy between the plants grown from seed and those grown from rhizome. Weed Res., Japan 22, 202-208

2) Harada, K. and S. Imabayashi, N. Manabe, Y. Ohga and S. Kojyo 1987 Non tillage culture of soybean in rotational paddy field. Bull. Fukuoka Agric. Res. Cent. A-6:53-58

3) Shimano, I., S. Kuwano and T. Furuya 1973 Reproduction and control of tall goldenrod (*Solidago altissima*). Weeds in Kyushu 3, 9-13

4) Yukinaga, H., K. Ide, K. Ito and M. Shimada 1975 Control of *Solidago altissima* with Asulam. Weed Res., Japan 19, 46-50

Changes in Weed Seed Densities in Soil of Paddy Fields under Consecutive Herbicide Application

Ryuichi Sago and Masakazu Komatsuzaki
Experimental Farm, Faculty of Agriculture, Ibaraki University
Ami-town, Ibaraki,JAPAN 300-03

Abstract: More than 30 years have passed since an effective herbicide for annual weeds became a fundamental tool to control weeds in Japan. However changes in the weed seed densities in the soil of paddy fields have not been clarified under consecutive herbicide applications. We observed seed densities in soil during 12 years in rice fields of Kanagawa prefecture that had been treated with herbicides for at least 30 years. The number of weed seeds was estimated by the number of seedlings that emerged from paddy soil samples collected from the paddy fields. Weed seed densities in soil including the 6 species that emerged ranged from 2,264 to 10,650 per m^2 in 1982. The weed seed densities in soil were reduced from the above numbers in 1982 to 354-1,847 per m^2 in 1994. The number of seeds decreased by 78%.

Key words: seed density in soil, paddy field, consecutuve herbicide applications, single cropping of rice, annual weed

Introduction

More than 30 years have passed since weed control for rice cultivation began to depend on herbicides. During 3 decades, herbicides were used for the control of not only annual but also perennial weeds. The dominant weed species in paddy fields were influenced by the kind of herbicides applied6,8). The application of effective herbicides for three years enabled to eradicate the perennial weeds, but not the annual weeds4). Many studies have been carried out on the analysis of the vegetation in paddy fields treated with herbicides. However changes in the weed seed density in soils of paddy fields have not been clarified under consecutive herbicide applications. It was reported that the seedbank density can not be estimated based on the subsequent weed flora5). However, Wilson10) showed that the seed composition in the upper 15-cm soil horizon was correlated with the number of redroot pigweed, yellow foxtail and barnyardgrass seedlings which were expected to emerge in the following year. Also we7) reported that the density of seeds in soil was correlated with the dominant rank order of weed seedlings that emerged in the following year. We conducted the current study to analyse the changes in the seed density in soil during a period of 12 years in rice fields in Kanagawa prefecture.

Materials and Methods

Survey was conducted during the period 1981 - 1994 in 4 rice fields in Kanagawa prefecture where rice plants had been cultivated for more than 70 years consecutively and treated with herbicides for at least 30 years (Table 1). Sources of water irrigation were different among the 4 rice fields. For three years from 1981, the same herbicides had been applied in subplots, which were prepared by dividing one rice field into 2 or 3 plots with the same size. In the A and B rice fields the same herbicides were applied from 1984 to 1993 dependent on farmer`s selection. In the C and D rice fields the same herbicides were applied from 1984 to 1991. The number of residual weed populations ranged for 0 to 9.1 /m^2 after weeding was completed in 1994.

To determine the number of viable seeds, soil samples were taken from each subplot after tillage in spring. Fifty random samples were taken from each subplot at the level of cultivated soil layers. Each soil

sample was thoroughly mixed and kept in a plastic container 11 cm in diameter and 6cm deep with water at a depth of 1 cm , placed in a greenhouse where the temperature was not controlled allowing viable seeds to germinate. Soil samples containing seeds were watered as needed. Seedlings were identified, counted and removed at the seed-leaf stage to avoid the interaction of weeds periodically throughout about 100 days of the germination period beginning in May and through September. Weed seeds of 6 annual weed species were identified, including Lindernia procumbens (Krock.) Borbas,Elatine triandra Schk. var. pedicellata Krylov, Rotala indica (Wild.) Koehne var. uliginosa (Miq.) Koehne, Cyperus difformis L., Ammannia multiflora Roxb. and Monochoria vaginalis (Burm. f.) Presl var. plantaginea (Roxb.) Solms-Laub.

Table 1. Herbicides application and weed management in 4 rice fields

Field Name	A	B	C	D
Irrigation source	Tamagawa Brook	Tamagawa Brook	Suzukawa River	Hanamizu River
Beginning of Paddyfield culti.	since 1927		since 1926	
Field Area	9.1a	9.1a	9.6a	7.0a
year without cultivation	1971	no	1989	no
barnyard manure application	annually until 1990	annually until 1989	every 2 years	every 2 years
Herbicide Application 1981-1983	A1:chlornitrofen+thiobencarb/simetryn, A2:Pyrazoxyfen+simetryn/MCPB/bentazon, A3:chlornitorofen+thiobencarb/simetryn/MCPB	chlornitrofen+thiobencarb/simetryn	C1:chlornitrofen/daimron+simetryn/MCPB/bentazon,C2:pyrazoxyfen+simetryn/MCPB/bentazon,C3:chlornitrofen/daimron+molinate/simetryn/MCPB	D1:chlometoxyfen+thiobencarb/simetryn,D2:pyrazolate+molinate/simetryn/MCPB
since 1984	chlornitrofen+thiobencarb/simetryn, thiobencarb/simetryn/MCPB (from 1994) Bensulfuron-methyl/thiobencarb/mefenacet+thiobencarb/simetryn hand weeding annually performed		chlornitrofen+thiobencarb/simetryn (from 1992) pretilachlor+thiobencarb/simetryn hand weeding annually performed	
Residual weed density(No./m2)	9.1	3.2	0	1.2

*Manufactured herbicides were used.

**Residual weed density covered an area of 30 m^2

Results

The number of seeds in soil, which were sampled from 9 subplots of rice fields in 1982 and 1983, was counted and the results are shown in.Fig. 1. Lindernia procumbens, Elatine triandra, Rotala indica , Cyperus difformis were dominant. Therefore the number of total annual weeds,Rotala indica and Cyperus difformis seeds are shown in Fig. 1 ,but the number of Lindernia procumbens and Elatine triandra seed were combined due to the difficulty in differentiating Lindernia procumbens from Elatine triandra at the seed-leaf stage. The number of seeds in 9 subplots of soil in 1983 decreased, and the number of Lindernia procumbens , Elatine triandra, Rotala indica, Cyperus difformis and total annual weed seeds in 1982 were significantly correlated with the number in 1983 .These results suggest that the number of weed seeds declined year by year as a result of herbicide application.

Fig. 2 shows the number of seeds in the soil of 4 paddy fields in which herbicides were applied for 12 years.The number of weed seeds in 1994 was smaller than in 1982,1983 except for Rotala indica in the B paddy field in 1983 and Lindernia procumbens and Elatine triandra in the C paddy field in 1994. The weed seed density in 4 paddy fields in 1982 ranged from 10,650~ 2,264/m2, but the weed seed density in 1994 decreased by 1,847- 354 /m^2 .The density of total weed seeds in the A,B,C,D paddy fields declined by

82.7, 84.4, 54.3, 90.7, respectively and the overall decline of the density of weed seeds in 4 paddy fields was 78.0%.

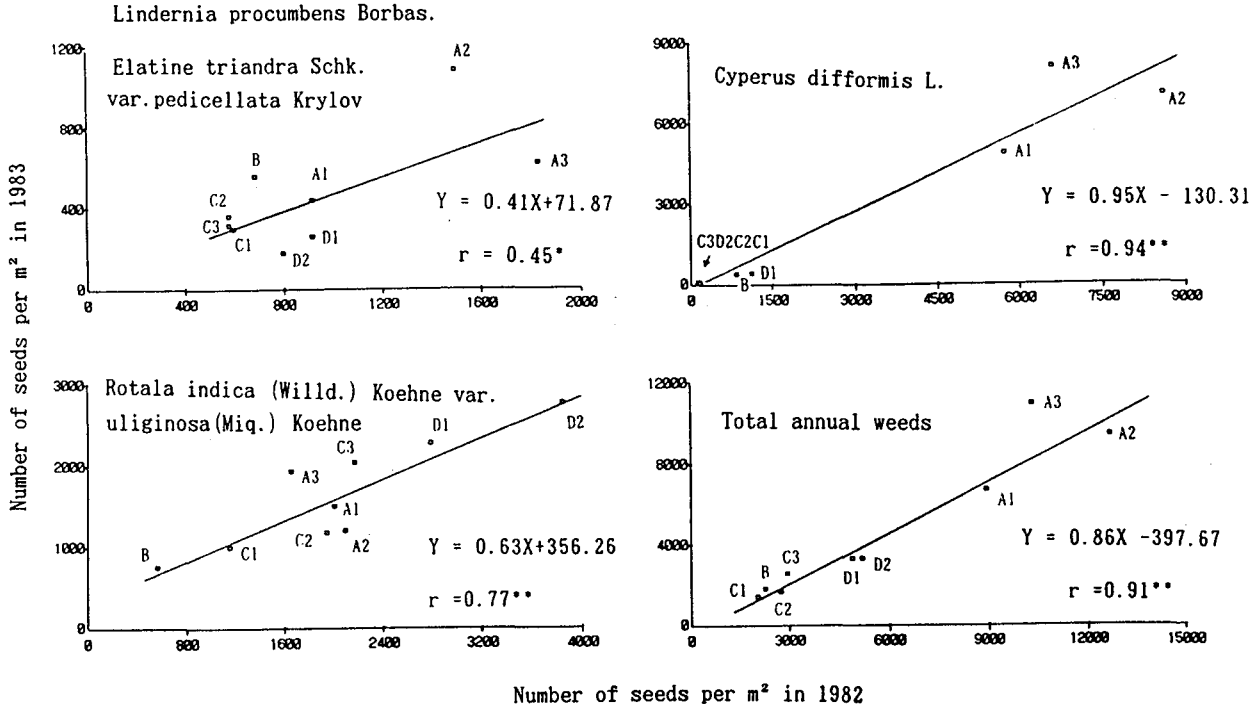


Fig.1 The number of seeds in the soil of 9 subplot of rice fields in 1982 and 1983.

* ** indicate significant difference at 5%, 1% level of probability

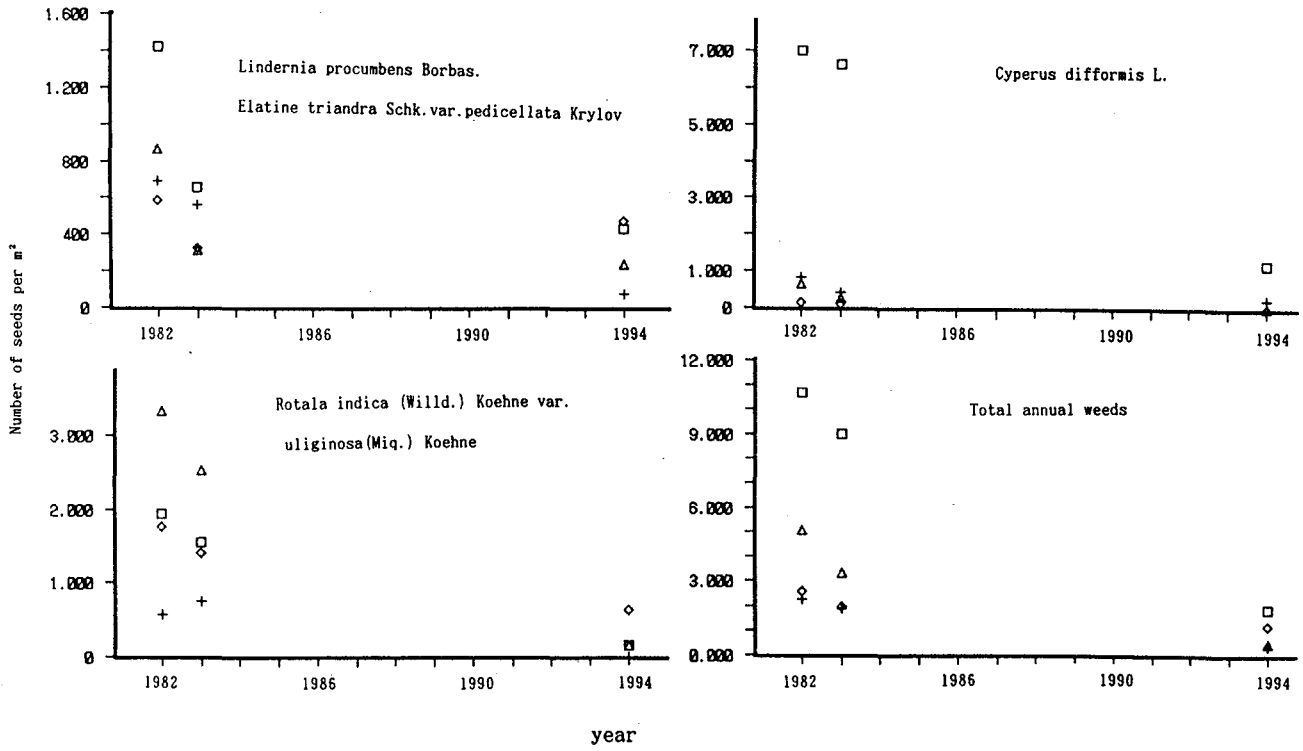


Fig.2 Changes of the number of seeds in the soil of 4 rice fields

Field : □ A, + B, ◇ C, △ D

Discussion

Studies on seedbanks in arable soil have led to the development of many techniques for estimating the seed density from soil samples. There are two basic techniques to measure the seedbank: in one seeds are germinated in a glasshouse tray, and the other is the seed extraction method. Ball & Miller[1] compared the glasshouse and seed extraction techniques and concluded that the two seedbank estimation methods were correlated with one another. Forcella[2,3] concluded that the glasshouse technique appeared to be more reliable than seed extraction for correlations with actual field seedling densities. We[7] reported previously that the glasshouse tray method enabled to estimate a seedbank in paddy fields based on the sampling date, tray size and conditions of emergence or dormancy breaking. We also indicated that the glasshouse tray method enabled to compare the number of seeds in soil that were sampled from paddy fields during the season of seed dormancy breaking.

In many paddy fields the number of residual weeds is very small, because of consecutive herbicide applications in Japan .It was considered that the number of seeds in soil decreased continuously .

We analysed the soil of 4 paddy fields with different sources of irrigation and the number of seeds in soil decreased in all the 4 fields. These result indicate that the seeds carried from a river do not always contribute to the increase of the soil seedbank, due to effective herbicide applications.

Since the rate of decline varies with the weed species or weed populations, a larger number of plots should be used to determine the rate of decline precisely.

Changes in weed seed densities in the soil of paddy fields indicate that the use of herbicides enables to minimize weed problems and that seedbank density estimation is useful for predicting weed infestation[9,10].

References

1. Ball D.A.and S.D.Miller 1989. A comparison of techniques for estimation of arable soil seedbanks and their relationship to weed flora. Weed Research 29, 365-373.
2. Forcella F. 1992. Prediction of weed seedling densities from buried seed reserves Weed Research 32,29-38.
3. Forcella F.,R.G.Wilson,K.A.Renner,J.Dekker,R.G.Harvey,D.A.Alm,D.D.Buhler and J.Cardina 1992. Weed seedbanks of the U.S. corn belt: Magnitude,variation, emergence and application. Weed Science 40:636-644.
4. Fujiwara,S.,R.Sago,T.Uezono,H.Sugawara,S.Nishi,K.Tuchida 1988. The study on estimation ation of lowland weed emergence (2) the analysis of vegetation in paddy fields treated with herbicides. Weed Research,Japan 33(supplement),95-96. (in Japanese)
5. Hayashi I. 1978. Seedbank population -Succession of plant comunity and their mechanism. Asakura-shoten,Tokyo,pp.193-203. (in Japanese)
6. Miyahara M. 1992. Lowland weed ecology and their control. Zenkoku-nouson-kyouikukyoukai,Tokyo,pp.20. (in Japanese)
7. Sago R.,S.Ohnishi,T.Uezono,K.Ikai,K.Hamada 1983.The study on estimation of lowland weed emergence (1). Weed Research,Japan 28(supplement),161-162. (In Jananese)
8. Sago R. 1991.Estimation of lowland weed emergence and rotation of herbicides application. shokuchou 25(5),195-204. (In Japanese)
9. Schweizer E.E. and R. L.Zimdahl 1984. Weed seed decline in irrigated soil after rotation of crops and herbicides. Weed Science 32,84-89.
10. Wilson R.G.,E.D.Kerr and L. A. Nelson 1985. Potential for using weed seed content in the soil to predict future weed problems. Weed Science 33,171-175.

Emergence of Shepherds purse (*Capsella bursa-pastoris* L.) in an Orchardgrass (*Dactylis glomerata* L.) Grassland in Warm Temperate Areas of Japan

K.Sato, T.Imura, T.Kosako and Y.Fujii
Shikoku National Agricultural Experiment Station,
Zentsuji Kagawa 765,Japan

Abstract. Shepherds purse invades grasslands and disturbs the establishment of sown grasses in warm temperate areas of Japan. An experiment by randomized block design was conducted to evaluate the relationship between the emergence of shepherds purse and four cutting times on the first cutting of the orchardgrass grassland sown in early winter. Crown coverage(%) of shepherds purse was about 50% at ealier cuttings on 25 April and 9 May, while it decreased to about 20% at later cuttings on 23 May and 6 June($P < 0.05$). Dry matter (g/ m^2) and botanical composition(%) of shepherds purse was significantly lower at the cutting time on 6 June. It was concluded that making the first cutting after 23 May was an effective method for establishing orchardgrass.
Key words : Emergence, Shepherds purse, Orchardgrass grassland, Warm temperate areas.

Introduction

In warm temperate areas of Japan, winter seeding is one of promising method to renovate the grassland dominated with southern crabgrass (*Digitaris ciliaris*). But in the next spring, shepherds purse invades grasslands (Sato et al.1994) and disturbs the establishment of sown grasses (Sato et al.1995). In the same areas, the establishment of grasses using the winter seeding is required for the beef cattle production system(Imura.1993).

This study was conducted to evaluate the relationship between the emergence of shepherds purse and four cutting times on the first cutting of the orchardgrass grassland sown in early winter.

Materials and Methods

This experiment was carried out by using randomized four block design from 1993 to 1994 at the experimental sloping field of Shikoku National Agricultural Experiment Station in Kagawa Prefecture. Prior to the over seeding, this field dominated with southern crabgrass were grazed by Japanese Cattles.

The experimental plot was 2.5m × 2.5m. The seeding rate of orchardgrass(cv.Natsumidori) was 2,000 germination seeds per m^2 on December 16 in 1993. Application amounts of N, P_2O_5 and K_2O were 6.0g, 3.7g and 3.7g per m^2 on March 16 in 1994. Four cutting treatments on the first cutting of orchardgrass grassland, were on April 25, on May 9, on May 23 and on June 6, respectively. The plant samples were cut at 5cm height after measuring the coverage (%) of main species per a 0.5m × 0.5m quadrat in each plot. Cut samples were devided into each species and their dry matter were weighed after drying at 70 °C . Plant length (cm) and plant height (cm) were measured the six plants in each plot.

Results and Discussion

As shown in Table 1, crown coverage of shepherds purse on April 25 and May 9 were 48.0% and 50.0%, respectively, while it decreased to 24.5% on May 23 and 17.3% on June 6. Coverage of henbit(*Lamium amplexicaule* L.) on April 25 was the largest value of 28.8% among cutting times. Coverage of orchardgrass was highter at later cutting than at earlier cutting. Others coverage were not over the 6.0%.

Table 2. shows the results of main species dry matter (g/ m^2). Dry matter of shepherds purse was significantly lower at the latest cutting time on June 6. Dry matter of henbit was only significantly higher at the earlist cutting time on April 25, while those of orchardgrass was higher

at later cutting time. Dry matter of annual bluegrass (*Poa annua* L.), persian speedwell (*Veronica persica* Poir.) and common chickweed (*Stellaria media* L.) were smaller weight under 11.6g.

Table 1. Crown coverage(%) of main species and others on the orchardgrass grassland at each cutting.

Cutting time	Shepherds purse	Henbit	Orchard -grass	Others(species number)
Apr.25	48.0 a[a)]	28.8 a	12.0 a	5.0 a (6)
May 9	50.0 a	6.3 b	36.3 b	5.7 a (7)
May 23	24.5 b	0.8 c	64.5 c	4.5 a (7)
June 6	17.3 b	0.5 c	76.8 d	2.0 a (4)

a) Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2. Dry matter (g/ m^2) of main six species in the orchardgrass grassland.

Cutting time	Shepherds purse	Henbit	Annual bluegrass	Persian speedwell	Common chickweed	Orchard -grass
Apr.25	74.6 a[a)]	93.2 b	3.7 a	1.9 a	2.0 a	17.8 c
May 9	76.6 a	52.1 a	7.0 a	11.6 a	8.4 a	60.4 b
May 23	69.8 a	42.5 a	9.2 a	10.9 a	3.0 a	82.3 ab
June 6	46.1 b	42.7 a	8.5 a	8.0 a	6.1 a	108.9 a

a) Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 3. shows the results of the botonical composition (%) of main six species. The percentage (21.4%) of shepherds purse on June 6 was significantly lower than the values at the cutting time on April 25 and on May 9. Henbit was significantly the largest value(46.3%) on April 25 among the cutting times, while annual bluegrass,persian speedwell and common chickweed were small values and not significantly at the cutting times,respectively. But the orchardgrass tends to increase at later cutting times.

Table 3. Botanical composition (%) [b)] of main six species in the orchardgrass grassland.

Cutting time	Shepherds purse	Henbit	Annual bluegrass	Persian speedwell	Common chickweed	Orchard -grass
Apr.25	39.8 a[a)]	46.3 b	1.8 a	1.2 a	1.1 a	9.2 a
May 9	34.5 a	24.2 a	3.3 a	5.3 a	3.9 a	27.4 a
May 23	31.2 ab	19.1 a	4.0 a	5.2 a	1.3 a	36.9 a
June 6	21.4 b	19.9 a	3.6 a	4.4 a	3.8 a	46.9 a

a) Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.
b) Based on dry matter (g/ m^2).

As shown in Table 4, plant height(cm) of shepherds purse, henbit and orchardgrass were not

significantly among cutting treatments. But shepherds purse was the highest plant height (cm) among three species. Plant length (cm) of orchardgrass was not significantly about 50 cm at the later cutting times.

Table 4. Plant height (cm) of shepherds purse, henbit, orchardgrass and plant length (cm) of orchardgrass.

Cutting time	Plant height(cm)			Plant length(cm)
	Shepherds purse	Henbit	Orchardgrass	Orchardgrass
Apr.25	34.4 a[a)]	20.2 a	28.6 a	31.5 a
May 9	33.5 a	20.7 a	22.5 a	44.2 b
May 23	37.6 a	21.5 a	30.7 a	49.4 b
June 6	37.8 a	18.5 a	27.3 a	49.3 b

a) Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

From the facts described in Table 1 and Table 4, we consider that competitive ability of shepherds purse over main other species tends to decrease by the deterioration of the growth of shepherds purse under the later cutting times. Especially, dry matter(Table 2) and botanical composition(Table 3) of shepherds purse was significantly lower at the cutting on June 6. We surmise that the deterioration of shepherds purse is occured in the latter part of May. It was concluded that making the first cutting after 23 May was an effective method for establishing orchardgrass. Proper management of shepherds purse is not confirmed yet, and further experiments should be done concerning this point, such as Nashiki et al(1986).

Acknowledgment : The authors are grateful to Shyichi WATANABE, Masahiro MIYATAKE and Masaki AKIYAMA for their help in the field works. We would also like to acknowlege Mrs.Tomoe HOSHINA for her great assistance.

References

1)Imura,T.(1993):Studies on improvement of beef cattle production efficiency by making good use of grazing.,Bull.Shikoku Natl.Agric.Exp.Stn.No.56,1−111.

2)Nashiki,M.,T.Nomoto,R.Meguro and K.Sato(1986):Effect of natural conditions and management of pastures on weed invasion in cooperative livestock farms in Japan., Weed Res., Japan, **31**(3).221−227.

3)Sato,K.,T.Imura, T.Kosako amd Y.Fujii(1994): Relationship between weed biomass and establishment of vetch seeded after early winter grazing., Weed Res., Japan, **39**(Suppl. I), 182−183.

4)Sato,K.,T.Imura and T.Kosako(1995):Effect of grass species seeded in early winter on the emergence of weeds in the grassland of warm temperate areas., Weed Res., Japan, **40**(Suppl. I), 88−89.

Control of Spiny Amaranth(*Amaranthus spinosus* L.) and Velvetleaf(*Abutilon theophrasti* Medic.) with Cover Crop in Forage Corn(*Zea mays* L.).

Seturo Sato, Koji Tateno and Ryoji Kobayashi

Department of Grasslands, Kyushu National Agricultural Experiment Station, Kumamoto, Nishigoshi, 861-11, Japan

Abstract. A field experiment was conducted to determine the effect of cover crop on the control of spiny amaranth(*Amaranthus spinosus*) and velvetleaf(*Abutilon theophrasti*) in forage corn(*Zea mays* L.). Forage corn was sown in a field intentionally infested by spiny amaranth or velvetleaf. In order to control these weeds, Italian ryegrass(*Lolium multiflorum*)was sown as a cover crop at a density of 0.3 and 0.6 kg/a, or atrazine + alachlor was applied pre-emergence at a rate of 7.1g + 10.8ml a.i./a. The cover crop at either sowing density and herbicide application gave complete control of spiny amaranth. In contrast, neither treatment controlled velvetleaf well. Fodder yield of corn was reduced from 12.5 to 18.9% in plots sown with the cover crop although it increased from 4.9 to 10.5 % in plots applied with herbicide compared with untreated check. This decrease in yield was probably due to the competition of soil water from the cover crop under extremely low precipitation during the growing season.

key words : spiny amaranth, velvetleaf, forage corn, cover crop, pre-emergence herbicide

Introduction

Spiny amaranth and velvetleaf are major serious weeds in forage corn sown in spring, especially in early April in Kyushu(4,5,6). These weeds reduce the fodder yield of corn and disturb the harvest mechanically. These species are often difficult to control adequately with pre-emergence herbicides used at present i.e., atrazine and alachlor, due to herbicide tolerance of the weeds and/or emergence and establishment after the herbicides lose their effects(3,6). Therefore it has become necessary to select alternative means to control these weeds and cover crop is thought to be an applicable cultural means for it(7).

The objective of the study was to determine the effect of cover crop both on control of the growth of the two weed species in forage corn and on the corn yield.

Materials and Methods

In 1994 a field experiment was conducted to determine the effect of cover crop both on the control of spiny amaranth and velvetleaf in forage corn and on corn yield at the Kyushu National Agricultural Experiment Station, Nishigoshi, Kumamoto, on thick high humic Andosols (Melanudands). On April 14 forage corn(cv. Pioneer 3352) was sown at a density of 660 seeds/a or one seed per 20cm of row in four plots which have six rows with 3m long and 0.75m width between rows. After sowing of the corn

each of the two weeds was sown to infest the all five interrows of each plot at a density of 17.8g/a in spiny amaranth and of 23.0 g/a in velvetleaf, respectively. In order to control the weeds of the interrows, three of the plots were treated with either cover crop at two different sowing densities or pre-emergence herbicide. Italian ryegrass(cv. Tatiwase) was sown as a cover crop at a density of 0.3 and 0.6 kg/a(CT(0.3) and CT(0.6)) and atrazine(Gesaprim, 47.5% a.i.) + alachlor(Lasso, 43% a.i.) was applied at a rate of 7.1g + 10.8ml a.i./a (HbT). The last plot was not treated for weed control(untreated check). Both sowings of the corn and the weeds and all weed control treatments were conducted on the same date. Plots were fertilized with a compound fertilizer at a rate of 1kg N/a before sowing and were added equal rates 26 days after sowing. Plots were arranged as a randomized complete block design with two replications of four treatments. Growth of the weeds, cover crop and corns were monitored 70 days after sowing and at the harvest time(97 days after sowing).

Results and Discussion

Control percentage of spiny amaranth for each treatment is shown in Fig.1. On 70th day after sowing, cover crop sown at either density of 0.3 or 0.6 kg/a(CT(0.3) and CT(0.6)) gave 95.5 and 96.2 % spiny amaranth control, respectively, and atrazine + alachlor(HbT) gave complete control(100%). At harvest time of forage corn, all treatments gave close to complete control of the weed, at 99.8 to

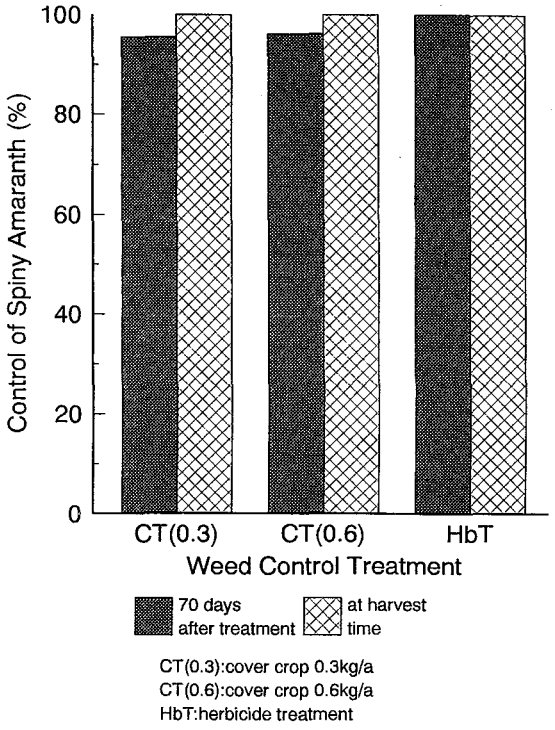


Fig.1. Effect of Cover Crop and Herbicide on Control of Spiny Amaranth.
(Weed control was expressed as a percentage of untreated check).

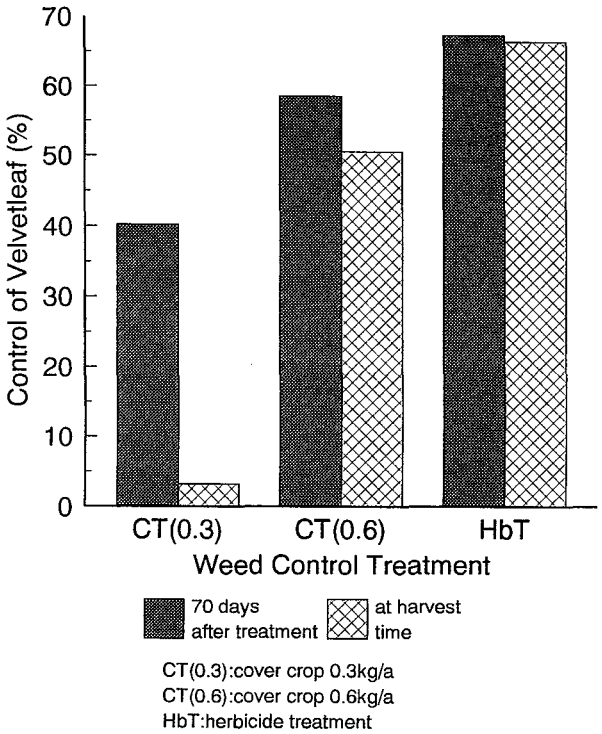


Fig.2. Effect of Cover Crop and Herbicide on Control of Velvetleaf.
(Weed control was expressed as a percentage of untreated check).

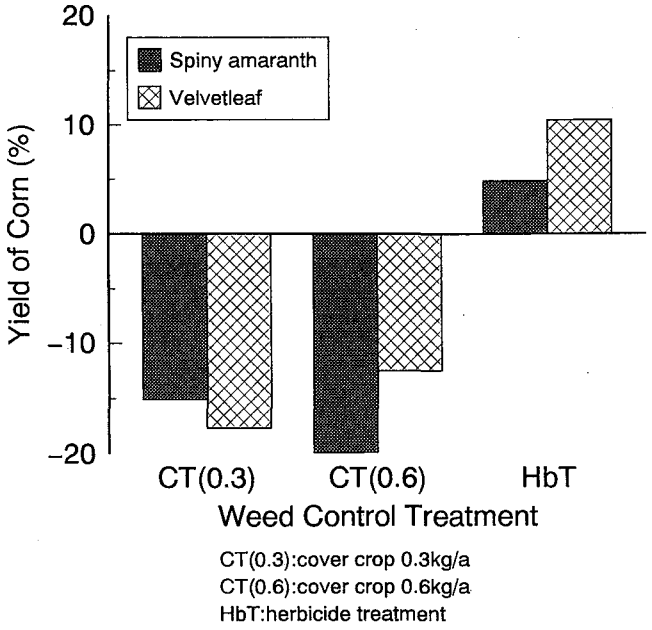


Fig.3. Effect of Cover Crop and Herbicide on Forage Corn Yield.
(Yield was expressed as a percentage of untreated check).

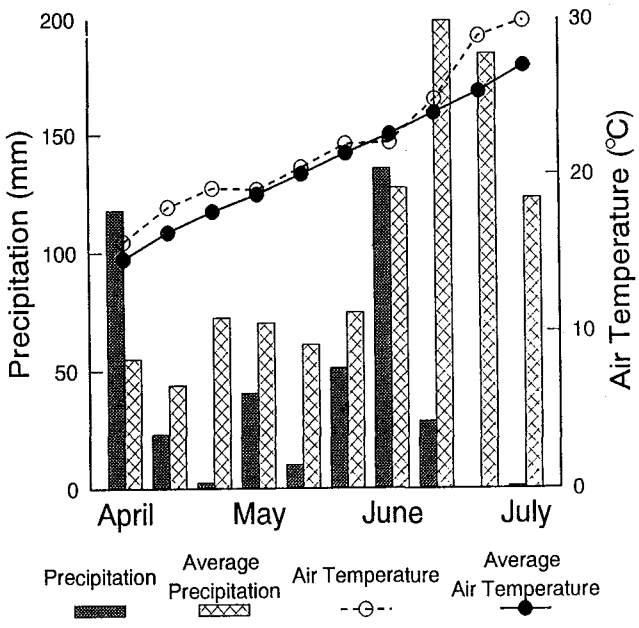


Fig.4. Precipitation and Air Temperature during the Field Experiment.

100 %. Thus, CT(0.3), CT(0.6) and HbT gave excellent control of spiny amaranth both during corn growing season and at harvest time(Fig.1). Control percentage of velvetleaf for each treatment is shown in Fig.2. On 70th day after sowing, CT(0.3) and CT(0.6) gave 40.2 and 58.5 % velvetleaf control, respectively, and HbT gave 67.3 % control. At harvest time, CT(0.3) did not control the weed although CT(0.6) gave control to some extent(50.5%). HbT gave 66.4 % control, which was most excellent in all of the treatments. Thus, control of velvetleaf was most effective with HbT, moderate with CT(0.6) and least effective with CT(0.3) on both investigating dates(Fig.2).

CT(0.3) and CT(0.6) reduced the fodder yield of corn by 15.1 and 19.9 % in the spiny amaranth control experiment and by 17.7 and 12.5 % in velvetleaf control one, respectively, compared with untreated check. In contrast, HbT was increased fodder yield by 10.5 and 4.9 % in the spiny amaranth control experiment and velvetleaf control one, respectively, compared with untreated check. Thus, cover crop reduced the fodder yield, whereas herbicide treatment increased it(Fig.3).

Both cover crop and herbicide gave better control of spiny amaranth than expected on both investigating dates. This excellent control with cover crop was due to competition of little soil water with spiny amaranth under extremely less

Table 1. Biomass in interrows 70 days after sowing. (g/m²)

	spiny amaranth control exp.				velvetleaf control exp.			
	check	CT(0.3)	CT(0.6)	HbT	check	CT(0.3)	CT(0.6)	HbT
cover crop	–	244.3	442.0	–	–	154.6	241.6	–
spiny amaranth	72.8	3.3	2.8	0	–	–	–	–
velvetleaf	–	–	–	–	200.0	119.6	82.9	65.4
other weeds	28.7	109.1	44.8	20.0	107.0	32.7	24.0	4.5
total	101.5.	356.7	489.6	20.0	307.0	306.9	348.5	69.9

precipitation during the experiment than that of an averaged year(Fig.4). Similar excellent control with herbicide was attained by elongation of its efficacy due to extremely low precipitation.

In contrast, neither treatment controlled velvetleaf well. Optimum growth temperature for Italian ryegrass used as a cover crop is lower than that for velvetleaf. Air temperature from mid-April to early May in the experimental year was slightly higher than that of an averaged year, and the high temperature was thought to be more favorable for early growth of velvetleaf than for cover crop. Moreover, air temperature during late June to mid-July was comparatively higher, so that velvetleaf might successfully compete with cover crop. Control percentage of velvetleaf at harvest time revealed that cover crop sown at 0.3 kg/a density was unable to suppress the growth of velvetleaf during late June to mid-July. To obtain effective control of velvetleaf in forage corn with cover crop, it is necessary to sow corn and cover crop at an earlier date so that cover crop would grow more rapidly than velvetleaf in lower temperature conditions.

Spiny amaranth was controlled with cover crop, in contrast, velvetleaf was not. This result suggests that seedlings of velvetleaf are more competitive than those of spiny amaranth under low precipitations and the superior competitive ability of velvetleaf seedlings might be due to viror of their seeds.

Although it was reported that atrazine and atrazine combinations did not control velvetleaf well(3), treatment with the atrazine + alachlor controlled the weed to some extent in our experiment. Some effective control was due to prolonged efficacy caused by extremely low precipitation.

Weeds are often harmful when they suppress the growth of crops with competition, resulting in reduced yields. In our experiments, although spiny amaranth was controlled well during the growing season of corn, fodder yields of corn treated with cover crop were reduced, whereas for those treated with herbicide, they increased. Similar reduction and increase of fodder yield were observed in the velvetleaf control experiment. Biomass in interrows 70 days after sowing for each treatment is shown in Table 1. Biomass in interrows was larger in CT(0.3), CT(0.6) and untreated check than in HbT in both experiments, and biomass including unanticipated other weeds(mainly southern crabgrass (*Digitaria adscendence*)). High negative correlations were observed between the interrows biomass and

fodder yields of corn(r=-0.845 and -0.765 in spiny amaranth and velvetleaf control experiment, respectively). Although herbicide did not control velvetleaf well, it suppressed the other weeds in interrow, resulting in reduction of the interrows biomass. This reduction of the biomass caused avoidance competition of soil water and nutrients between the plants in interrows and forage corn, so that the fodder yield of the corn in HbT was larger compared with those of other treatments for weed control.

The field used in our experiments was infertile because it had been left unplanted for several years. Infertility of the field might be also an important factor causing fodder yield reduction. Accordingly it might be concluded that weed control in forage corn with cover crop is applicable to more fertile fields.

Acknowledgments: The authors thanks Dr. Yoichi Nada and Mr. Yasuharu Sanada, Kyushu National Agricultural Experiment Station, or their comments on the manuscript.

Reference

1. Eaton, B.J., O. G. Russ, and K.C. Feltner. 1976. Competition of velvetleaf, pricky sida, and Venice mallow in soybeans. Weed Sci. **24**(2). 224-228.
2. Harvey,R.G., R.H. Andrew, and A.W. Ruscoe. 1977. Giant foxtail and velvetleaf control in sweet corn. Agron. J. **69**(5). 761-764.
3. Ritter, R.L. 1986. Triazine resistant velvetleaf and giant foxtail control in no-tillage corn. Proc. 40th annual meeting of the Northeastern Weed Sci. Soc. 50-52(abstract).
4. Sato, S., K. Tateno, and R. Kobayashi. 1994. Influence of seeding date on flowering and seed production of velvetleaf(*Abutilon theophrasti* Medic.) Weed Res, Japan 39(4). 243-248.
5. Simize, N. et al. 1994. Recent situation of emergence of alien weeds invading grasslands and forage crop fields. Weed Res., Japan **39**(suppl.). 228-229(in Japanese).
6. Tateno, K. and R. Kobayashi. 1993. Research of naturalized weed occurrence in forage crop fields
in the Kyushu District. J. Japan Grassl. Sci. **39**(suppl.) 65-66(in Japanese).
7. Triplett, B. G. Jr. 1985. Principle of weed control for reduced-tillage corn production. In "Weed Control in Limited Tillage Systems" ed. by A. F. Wiese, Weed Science Society of America, Illinois, pp 26-40.

CURRENT STATUS OF WEED SCIENCE STUDIES IN YUNNAN OF CHINA

Sun, Xizhi Guo, Yiqing You, Chengli

Yunnan Academy of Agricultural Sciences, Yunnan 650205 China

Abstract Based on the description of the present state of studies on weed science in Yunnan, the present paper anlalyzes the trends of weed science research developing from the investigation on weed classification and distribution to the study of biological characters of weed individuals or population; from single control measure to multiple measures; from chemical control to integrated control; and from the applying single herbicide to mixture preparation. The further measures of weed science studies in Yunnan are put forward in this paper: close cooperation with other subjects, focusing research on key points, paying attention to weed integrated control and biological studies in weed population, making compound preparations and popularizing the mixture of herbicides posityvely.

key words: *Weed Biology*, *Herbicide*, *Control technique*, *Allelopatty*

Yunnan has characteristics of stereoscopic agriculture with a varied topography and climate, canning water and temperature distribution in time and space uneven. Under the complex condtions of ecology and cultivation, weeds in farmland are in great variety and weed encroachment is serious. In the whole province the planted area is 4,600,000 ha and the weed encroached area accounts for about 70% of planted area, including about 700,000 ha seriously encroached. Since the 1980s the agricultural production in Yunnan has developed faster than before and at the same time of stressing grain production , the farm structure has been adjusted, to develop tobacco, sugarcane, tea, rubber, fruits, vegetables, medicinal plants and other economic plants with great efforts. With the development of agriculture production, many new problems in weed control have been put forward and have promoted the development of weed science. At the moment the chemical controlled area is up to 25% of the planted area in Yunnan Province.

I. Study course and status

The weed studies and its control in Yunnan have gone through three stages. In the mid—late 1960s introduction, experimentation and demonstration of herbicides were mainly done in paddy fields; in the 1970s demonstration and popularization of herbicides and beginning of the biological study on individuals of main weeds in paddy fields ; and in the 1980s a systematic investigation on farmland weeds, the researches on characteristic effect of herbicides and its application techniques, and integrated weed control were carried out. Since 1980 weed science has developed

faster with a certain depth and its contents related to most fields of weed science.

Ⅰ. Ⅰ Weed studies

Ⅰ. Ⅰ — Ⅰ Weed investigation

From 1981 to 1985 the weed encroachment in farmland has been investigated systemitically. In 1987—1990 the supplementary investigation was conducted mainly in orchards. The results show that the weeds in farmland of Yunnan belong to 102 families, 629 species, including 224 species in paddy, 405 in dryland; the main harmful weed species number 151, quarantine weeds 6 and newly recorded 8. Trend of the weed distribution is cleared from south to north, and also with increase of the latitute and the elevation. The thermophile weeds reduce and cold tolerant weeds increase and the community composition trends to simplicity. We have identitied well the weed types, occurrence, distribution and encroachment, and put forward the grade standards for weed encroachment. According to regional differences of the weed encroachment the province is preliminarily divided into 5 regions: humid—terrid weed region of north tropic belt with three—crop harvest a year, xerothermic weed region of south subtropic belt with three—crop harvest a year including double—crop rice, temperate—humid region of mesothermal subtropic belt with harvest of two crops a year including rice and winter crop, cool—humid weed region of north subtropic belt, warm—temperate belt with harvest two crops a year including rice and winter crop and cold region of moderate belt, cool temperate belt with one—crop harvest a year. The weed community composition, indicative weeds and main weeds in the staple crop fields in each region have been made clear, and a set of weed specimens from farmland prepared.

Ⅰ. Ⅰ — Ⅱ Studies on weed biology

Since 1970 we have studied on weed types, their occurrence conditions and patten of growth in fields of rice, maize, wheat, upland rice, tobacco, sugarcane, fruit tree and other crops. The key points we studied are the occurrence conditions, propagation habit, population dynamics and the position and effect in plant community of Pondweed (*Potamogeton distinctus*), Barnyardgrass (*Echinochloa crusgalli*), Croftonweed (*Eupatorium adenophorum*), Nytgrass Galingale (*Cyperus rotundus*), Lalang Grass (*Imperata cylindrica*), Chinese Pennisetum (*Pennisetum alopecuroides*) and other weeds thus the bases have been provided for control measures and control techniques. For example we have studied Pondweed a vicious weed in paddy, on its occurrence conditions, propagation, creeping habit and other biological characters, and made clear that when its branch stem leaf is tendergreen, its chemical tolerance is weakest and it is the best stage for application of herbicides. Studies on individual biology and population dynamics of Croftonweed showed that Croftonweed is a transitional type of the secondary vegetation formed and developed under special conditions. The population 1—4 years old is in vigorous growth phase, 5—6 years maturity phase, after that getting into veteran stage and gradually becoming succeeded by a ligneous plant community. Through the above researches we have put forward the control measures, namely, fixation of crop land, chemical control of the population of low age before reforesting, or planting crops, controlling the population and

shortening its longevity

Ⅰ. Ⅱ Studies on herbicides

We have mainly studied on introduction, screening, experiment, demonstration of new herbicides, characteristics of their action and herbicide mixing.

Since 1980 we have introduced Goal, Butachlor, Oxadiazon, Paraquat, Glyphosate, Molinate, Benvil, Quizalofop—ethyl and several other kinds of herbicide, made the medicinal efficiency experiment and studied on safeness, the best application time, and application techniques in rice, wheat, maize and various crops. Simultaneously, the herbicide researches have been carried out on weed—killing activity, selectivety, residual effect duration, leaching and percolating property in soil, efficiency, the safe and effective dosage and the best application techniques. The research on activity and selectivity of herbicides shows that the main factors causing harmful effects on rice by Goal are the shoot age and the water depth in the field; studies on the characteristics of Glyphosate show, that application time is the main factor affecting killing effects on Couch Grass, and the best applying time is at the three—leave stage and at this stage Couch Grass absorbs much more amount of the herbicide with the downward translocation in a large quantity and the sugar content being lower in the rhizome. In relation between the activity of herbicides applied in soil and the humidity of the soil the activity in herbicides that is mainly absorbed by roots increases with the increase of soil humidity, while the efficiency of those mainly absorbed by coleoptile reduces with the rise of soil humidity. The trend apparently depends on the vapour pressure and water solubility of the herbicides.

In the mid 1980s we began to study herbicide mixing having screened many formulations with synergized action, such as Alachlor+Simazine, Glyphosate+2,4—D ect. ,and carried out the evaluation of the synergized action of dual mixing in herbicides and the formulation control. The results show that each of the methods already reported abroad, such as Collby's, similar to Collby's, double—parameter, dosage transformation, regression analysis,differntiation, has its own merits. Among them dosage transformation, Collby's and differentiation are far superior. They can be used for herbicide mixing. Differentiation has the functions of correctly expressing the character, size, source of interaction in herbicide and providing the best formulations. In the late 1980s and the early 1990s we studied on the herbicides mixing and produced Kunming No. 1, No. 2 successfully making up for the shortage of single herbicide which is narrow in weed control pedigree, attaining the effect that application for only once can control weed in the season effectively, reducing the dosage and costs greatly. It is the first herbicide in paddy with high activity, low costs and application for only once in our province, enabling our province to get into the "super—high efficiency" era of paddy weed control.

Ⅰ. Ⅲ Studies on weed control techniques

Ⅰ. Ⅲ— Ⅰ Chemical control

Chemical control is indispensable and important technique for realizing agricultural modernization. Since the 1980s on the base of studies on characteristics of herbicide effect, we

have intensified herbicide application techniques, demonstration and popularization. As a result chemical controls on the main crops were widely practised in our province. For example Goal application techniques were practised in transplanting paddy fields amounting to over 75,000 ha in popularization and the development of high efficiency, low dosage chemcal controls in paddy were promoted; studies on techniques of soil incorporated with herbicides in dryland provided a simple application method for dryland crops; the techniques of non—tillage plus chemical control after rice were also studied. At present, the chemical control area is developed from 540,000 ha to 600,000 ha in the mid 1980s up to more than 1,000,000 ha in our province.

Ⅰ. Ⅲ — Ⅱ Weed control with film covering

Cultivation with film covering has been developed in our province since the 1980s, and widely used in many crops. Because of the effect of increase of temperature and humidity the weed encroachment is serious. For resolving the problem, we studied the weed control with colour film and herbicides film in time. The results show that colour and herbicides films have both ideal effect for weed control, and the black film is the best. The technique has already been demonstrated in the production in our province.

Ⅰ. Ⅲ — Ⅲ Biological control

Bio — control is the weed control by using the interaction among biological species. In Yunnan province the research is starting late, but the development is rapid. The main researches we have done are as follows:

Introducing parasitic insects. we introduced *Procecidochares utilis* from Tibet in 1984 and after systematic research began to release the insect in 1985. It reproduced 4—5 generations a year and spread 15 kiometers away. The percentages of seed setting and seed germination of Croftonweed were reduced and the spread and the density of population of Croftonweed were effectively controlled.

Substitution control. Using Armand Pine (*Pinus armandii Franch*), Yunnan Pine (*Pine Yunnanensis Franch*), White clover (*Trifolium repens*) as artificial vegetation could control the occurrence and encroachment of Croftonweed and the area controlled by this method is already 40000 ha. Using low stemmed legumes and herbage as artificial vegetation could control weed encroachment in orchards. Now the technique has been popularized in orchards in mountainous areas of our province.

Weed control with fungus. Researches revealed that *Mycovellosiella eupatorii — odorati* (Yen) has significant inhibiting effect on plant height and numbers of leaf and flower of croftonweed. The research on morbidity of Ustilago robenhorstiana showed that it is safe for gramineous crops but the morbidity on Crabgrass (*Digitaria sanguinalis*) is up to 95%, with its tillering numbers, plant height and seed setting percentage reduced significantly.

Ⅰ. Ⅲ — Ⅳ Integrated weed control.

Since 1984 we have carried out the researches on integrated weed control for Croftonweed and orchard weeds. In orchards after chemical weed control, low stemmed legumes are planted

with notillage or lesstillage, and artificial vegetation to control weed reoccurrence improves the ventilation and light penetrability in orchard, reduces occurrence of disease and insect pests, lessens soil erosion, and increases the comprehensive productivity of the orchard. The researches have been successful and demonstrated and applied in production. After controlling Croftonweed with Glyphosate, reforesting, or planting low stemmed legumes or herbage and releasing *Procecidochares utilis* as a supplementary measure could effectively control the occurrence and encroachment of Croftonweed.

Ⅰ. Ⅳ Researches on other aspects.

Ⅰ. Ⅳ— Ⅰ Research on allelopathy of plant.

The research on extract of Croftonweed revealed that the water extract has apparently inhibiting effect on seed germination of other plants and the extract from leaf is most inhibitive.

Ⅰ. Ⅳ— Ⅱ Screening for herbicide—resistant strains of plants.

We have done resistance screening from somatic cell clone in tobacco with Glyphosate and obtained the Glyphosate —resistant tobacco strain. It grows normally and the main economic characters are the same as the check.

Ⅱ. Developing trends.

The development and popularization of weed science marks the degree and level of agricultural modernization in a country or a region. Summerizing the weed science researches in our province since the 1980s reveals the following characteristics and trends.

Ⅱ. Ⅰ From investigation of weed species and distribution to researches on weed biology.

Along with the accomplishment of investigation of weed species, distribution and encroachment, the researches on the characteristics of weed biology have been stressed with the aim of weed control, and have provided bases for formation of control strategies. The new problems of weed biological research would be further put forward in the studies on weed integrated control and biological research in weed population has started and it is increasingly paid attention to. For this reason the direction weed of researches has changed from the investigation of species, distribution to biological characters, from individual biology to both individual and population of the weed.

Ⅱ. Ⅱ From applying herbicides seperately to using mixed preparations.

At present chemical control is still an important measure for weed control in the world. Since single herbicide has some limitation, the mixed herbicides and mixture preparations have developed rapidly. In our province the weed in farmland mainly has been treated with herbicide mixing to increase weed —killing efficiency, enlarge herbicide pedigree, prolong the effective duration, reduce costs and promote the development of energy—saving cultivation in agriculture.

Ⅱ. Ⅲ From single control measure to multiple measures, from chemical control to integrated control.

Developing agriculture of high yield, high efficiency and superior quality requires weed science research at a high level. For the weed control, only one measure or several measures

combined mechaically could not attain the aim of being economical, safe and high efficiency. So we need proceed from the whole farmland ecological system, take protecting beneficial living things as a prerequisite to increase land productivity, make full use of regulatory mechanism of ecological system itself and manmade factors, set up a set of organic, harmonized integrated control system. This is the aim for every weed scientist to strive jointly. Our successful researches of integrated weed management in Croftonweed and in orchards have marked that the weed control in our province has developed to a new stage, from simple chemical control to integrated weed management.

Ⅲ. The future measures and suggestion

Ⅲ. Ⅰ Close cooperation with other subjects, focusing the research on key points

Weed science is a new borderline subject divided and developed from botany, with its contents wide, theory and practic strong, thus only by close cooperation with other subjects including agronomy, biology, chemistry etc. to carry out integrated research can it be adaptable to and capable of accomplishment of the task of weed science researches. However under the situation of research force being weak in our province for the time being, the weed science research should not only consider the needs of the subject itself. It must focus the research on key points with the fundamental aim of weed control. The key points are researches on weed biology, especially distinguishing weed shoots, the relation between weeds and crops, the economic range of weed control with its control index, characteristics of herbicide effect and control techniques. Under the above studies the research field should be properly extended: doing some gene transfer for herbicide resistance selectively and breeding for weed resistance, developing herbicides and their additives, antagonism, and inductive weed killing.

Ⅲ. Ⅱ Paying attention to integrated control and biological studies in weed population

As related above, only proceeding from the whole farmland ecological system, fully utilizing regulatory mechanism of agricultural ecological system itself, establishing mutual harmonious integrated control system of chemical control, biological control and the control measures in agrculture can be beneficial for living things which would be protected to the maximum possible degree, and the negative effect of harmful living things and some measures can be controlled to increase land productivity. To the weed integrated control research as the frontiers of weed control research, an adequate attention must be paid and it must be greatly strengthened. Besides continuing research on individual biology and population dynamics in the weed biologcal researches, biological studies on weed population should be specially intensified so as to make clear the species, their patten of increase and decline and mutual relation in the farmland ecological system, and the relation of species and cultivation measures with weeds, crops and other plants, and should be definite about the position and action of weed in ecological system in order to provide the bases for developing integration control.

Ⅲ. Ⅲ Intensifying research on compound preparations and popularizing positively the mixing of herbicides

Compound preparation or the mixing of herbicides is far superior to single herbicide, which is generally recognized, so compound preparations are developed positively in many countries and over 40% of the herbicide commodities are compound ones. In recent years, the researches on compound preparations have been carried out in our country, for example, Kunming No. 1, No. 2. have produced notable benefits in their demonstration and popularization. This should be fully affirmed, but it must be also seen that some mixing preparations are short of scientific bases and a few of abuse even existed. For this reason in addition to establishing and perfecting the related statutes of register, production, sale and usage of compound preparations, it is more obligatory to intensify researches on methods for evaluating efficiency of mixing in order to put it on the base of science and set up corresponding system of test and approval

STATUS AND CONTROL OF WEEDY RICE IN THE MUDA AREA MALAYSIA

Md. Zuki, I.; Watanabe, H.; Ho, N.K.
Muda Agricultural Development Authority (MADA)
ALOR SETAR, 05990, MALAYSIA

Abstract

Weedy rice (Oryza sativa) is rice plant with undesirable easy shattering characteristics. It was first detected sporadically in the Muda area in 1990. Severe infestation was subsequently reported in 1993 affected approximately 168 hectares. Morphologically, weedy rice is identical to cultivated rice at the vegetative stages. However, at the reproductive phase, the panicle morphology is widely variable. The spikelets (grains) are sometimes pigmented or have long awns. The grains shatter early and easily, resulting in severe yield losses ranging from 30 - 90%. Segregation in culm length indicated that the fields were infested by heterogeneous rice plants probably resulting from crossing with cultivated rice. Studies on the control measures of weedy rice were conducted in off-season 1993. Five crop establishment methods were tested namely wet seeding, dry seeding, mechanical row seeding, mechanical transplanting and manual transplanting. Results from this study showed that manual transplanting with 100mm of standing water can reduce the infestation of weedy rice up to 100%. Proper land preparation coupled with stale seedbed technique using paraquat and glufosinate ammonium for preplant treatment can reduce the density of weedy rice in the infested field. The degree of control under various crop establishment methods are in the following order: manual transplanting > mechanical transplanting and mechanical row seeding > wet seeding > dry seeding.

Introduction

The Muda Irrigation Scheme is located in the North West of Peninsular Malaysia. It is the biggest rice granary area in the country with a total of 97,000 hectares being planted with rice by approximately 63,000 farming families.

Double cropping of rice in the Muda area was initiated in 1970 after the completion of irrigation infrastructure. With the continuous practices of direct seeding method due to shortage of labour, grassy weed have become competitive weeds. During the off season in the early 1990's due to the acute water shortage problem, farmers were shifted from wet seeded culture to the dry seeded. Under dry conditions field favoured more grasses and weedy rice to gain dominance especially in dry seeding field with good emergence of volunteer seedling.

This paper briefly discusses the important topics related to weedy rice such as morphology, status of infestation and the control measures in the Muda area Malaysia.

Morphology of Weedy Rice

Weedy rice can be defined as rice plants (Oryza sativa) which occur as weeds within the rice fields. It is unwanted plants caused by easy shattering characteristics and resulting in severe yield losses ranging from 30 - 90%. Weedy rice in Malaysia is supposed to have originated from cultivated rice, because its genetic structure of the plant showed close similarity to cultivated rice (Abdullah et. al., 1994).

Observation conducted by MADA revealed that weedy rice has 4 important characteristics namely easy shattering, plant height (long culm), pigmented grain and long awn (refer to Table 1).

Morphologically, weedy rice is identical to cultivated rice at the vegetative stages. However, at the reproductive phase, the panicle morphology is widely variable.

Watanabe, et. al, 1994, also reported that weedy rice in the Muda area could be characterized by easy shattering trait, tall plant height, pigmented grain and grain with long awn. Wide variation also occurs in their morphologies in the population. Segregation in culm length and heading period of progenies of weedy rice plants indicate that the field was infested by heterogeneous rice plants. It was due to crossing between weedy rice and cultivated rice in the direct seeded rice field (Table 2).

Status of Infestation

Weedy rice was first detected in the southern part of the Muda area in the off-season 1990 with 2 hectares of rice field being infested. After the incident, a field survey was conducted by extension staff in the off-season 1993. The result showed that serious infestation occured where 168 hectares of infested areas were identified (Table 3).

Follow-up survey was done in District IV, MADA in the off-season 1993 and it revealed that out of the 127 hectares of survey areas, 65 hectares or 51% was infested by weedy rice (Table 4). Results of the survey also indicated that, the occurance of weedy rice has close relationship with volunteer seedlings practices. Losses in yield is also reported in the range of 30 - 90%.

Continuous field survey was conducted in 1994 after the proper control programme and the result showed the incident was reduced drastically to only 81 hectares (Table 3).

Control Programme

A proper control programme was launched in the off-season 1993 to reduce the occurance of weedy rice in the Muda area. An integrated approach was suggested. Those included proper land preparation, killing of volunteer seedling, use of certified seeds, rogueing, demonstration control plots and extension campaign.

Training programme, posters and pamphlets distribution were carried out to create farmers' awareness and ability in weedy rice control. Studies on the control measures of weedy rice were conducted in the off-season 1993. Five crop establishment methods were tested, namely wet seeding, dry seeding, mechanical row seeding, mechanical transplanting and manual transplanting. Results from this study showed that manual transplanting with

100mm of standing water can reduce the infestation of weedy rice up to 100%. Under dry seeded conditions, proper land preparation coupled with stale seedbed technique using paraquat and glufosinate ammonium for preplant treatment can reduce the density of weedy rice in the infested field.

The degree of control under various crop establishment methods are in the following order: manual transplanting > mechanical transplanting and mechanical row seeding > wet seeding > dry seeding (Table 5).

Conclusions

Weedy rice appear to be a new challenge in the rice industry, especially under direct seeded rice in the Muda area and other rice granary areas in Malaysia. For control measures, an integrated approach is an important tool to combating weedy rice occurence. It is important to increase the planting of certified seeds as one of the most effective control measures. Production of good seeds among farmers in the Muda area should be increased in order to overcome the shortage of certified seeds.

Recent control measures revealed that a few chemicals are effective for controlling weedy rice. Studies on control measures should be continued by researchers in the provision of adequate technology and guidelines to overcome the weedy rice problem.

Acknowledgement

The authors wish to express their gratitude to Dato' Syed Azizan Al-Idrus - General Manager of MADA and Dato' Haji Hassan bin Lebai Mat - Head of Agricultural Division MADA for their encouragement and permission to present this paper at the 15th APWSS Conference in Tsukuba Japan.

References

Abdullah, M.Z., Azmi, M., Watanabe, H., 1994. Hypothesis Regarding The Origin of Padi Angin, A Weedy Rice Form of Rice in Malaysia.

Azmi, M., Watanabe, H., Abdullah, M.Z., 1994. Morphological Characteristics of Padi Angin. Paper presented at the 1994 Workshop on Padi Angin, Pulau Pinang, Malaysia.

Md. Zuki, I., Kamarudin, D., 1994. Status and Control of Padi Angin in the Muda Area, Malaysia (in Malay). Paper presented at the 1994 Workshop on Padi Angin, Pulau Pinang, Malaysia.

Watanabe, H., Azmi, M., Md. Zuki, I., 1994. Morphological and Ecological Variation of Weedy Rice in the Muda Area, Malaysia. Paper presented at the 1st. Quarterly Meeting under MADA/MARDI/JIRCAS Joint Research Programme, July 1994.

Table 1 : Morphological Characteristics of Weedy Rice in the Muda area

Morphological Characteristic	Plant Height (cm)	Panicle Structure	Spikelet Structure	
			Awn	Pigment
1	160 - 180	Compact	+	-
2	160 - 180	Compact	+	Rusty
3	160 - 180	Compact	+	Purple
4	160 - 180	Open	+	-
5	160 - 180	Open	-	-
6	160 - 180	Open	-	Rusty
7	100 - 120	Compact	-	-
8	100 - 120	Compact	-	Rusty

\+ present
\- absent

Table 2 : Morphological variation of weedy rice in the Muda area

Characteristics		sampled from different fields*			sampled from the monitoring field**					
		18 weedy rice variants			100 weedy rice plants			20 cultivated rice plants		
		Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum
Culm length	cm	51	120	188	82	135	164	85	92	106
Panicle length	cm	20	26	36	17	23	30	19	23	28
Number of spikelets		110	216	406	(16	111	306)***	(85	106	135)***
Number of elongated internodes		—	—	—	4	6	11	4	4	5
Flag leaf length	cm	—	—	—	15	29	57	20	29	39
Flag leaf width	mm	—	—	—	7	11	17	11	13	16
Grain length	mm	7.4	8.8	10.3	6.8	8.6	9.9	8.7	9.4	9.7
Grain width	mm	2.1	2.4	2.9	2.0	2.6	3.1	2.5	2.6	2.7
100 Grain weight	g	1.31	1.82	2.32	(0.96	1.67	2.23)***	(2.12	2.16	2.21)***
Awn length	mm	—	—	—	0	18	71	0	0	0

Note : * 18 variants' data was determined in growed plants in plant house, MARDI Bertam.

** Data was determined in growing plants in the monitoring field in I/1993.

*** ( )data was determined in growed plants in plant house, MARDI Bertam.

Watanabe, H. (1994)

Table 3 : Weedy Rice Infestation in the Muda Area Malaysia

Location (District)	Season I/93 (Hectares)	Season II/93 (Hectares)	Season I/94 (Hectares)	Season II/94 (Hectares)
I	0	2	4	4
II	15	34	66	48
III	15	4	43	17
IV	138	217	212	12
Total	168	257	325	81

Table 4 : Survey of Weedy Rice in District IV MADA (off-season 1993)

Locality	Survey Area (hectares)	Affected Area (Hectares)	% Affected
A-IV	19	10	53%
B-IV	9	6	67%
C-IV	16	2	13%
D-IV	32	0	0%
E-IV	13	9	69%
F-IV	20	20	100%
G-IV	18	18	100%
Total	127	65	51%

Table 5 : The Degree of Weedy Rice Infestation Under Various Crop Establishment (Off-season 1993)

Planting Method	The Degree od weedy Rice Infestation (%)	
	Before Control Programme	After Control Programme
Manual Transplanting	80%	0%
Mechanical Transplanting	80%	30%
Mechanical Row Seeding	80%	30%
Wet Seeding	80%	45%
Dry Seeding	80%	55%

Isolation and Characteristics of Soil Bacteria Degrading Herbicide Napropamide

S. S. Han
Dept. of Agricultural Chemistry, Wonkwang University, Iri 570-749, Korea

Abstract. This study was carried out to isolate the soil bacteria degrading herbicide napropamide [N,N-diethyl-2-(1-naphthoxy)-propionamide] from the clayey loam soil tested and to clarify the characteristics of the napropamide-degrading bacteria. Twenty strains of the gram-positive and the gram-negative bacteria were isolated and identified from the clayey loam soil tested. Most of them vigorously proliferated at 100ppm of napropamide, and two strains of *Staphylococcus* spp., *Corynebacterium* spp. II and another spp. II were very tolerant to napropamide, even at the concentration of 1500ppm. *Staphylococcus* spp. II and *Actinobacillus* spp. II of the isolated bacteria degraded more than 20% of the treated napropamide. These two strains could not utilize napropamide as a sole nitrogen source, but could use this compound as a sole carbon source. Napropamide was rapidly decomposed by *Staphylococcus* spp. II when applied once or three times, but was not when applied twice.
Key words: soil bacteria, napropamide-degrading bacteria, isolated bacteria, strains, clayey loam soil.

Introduction

Napropamide is a pre-emergence soil-treatment herbicide which is excellent for the control of annual grasses and broad leaf weeds in upland field. Nowadays in Korea, this herbicide is widely used for controlling annual weeds in arable land of various crops such as red-pepper, tomato, potato, Chinese cabagge, sesame, tobacco, tangerine and Zoysia grass. By the way, napropamide brought about crop-over injury for Italian rye grass, direct-seeded rice, and barley as succeeding crops cultivated after this chemicals was applied at both summer and winter crops(Ryang and Moon, 1991).

Our previous paper reported that napropamide degradation was remarkebly retarded in the strile soil more than in the nonsterile soil, which suggests the degradation is microbial(Han et al., 1994) and the microbial decomposition is most important in many cases(Hill and Wright, 1981).

Because the utilization of pesticide-degrading microbes is desirable to diminish negative values occurred form pesticide residue, author was to elucidate an aspect of microflora which may contribute to napropamide degradation. The present study was initiated to isolated and classified bacteria from soil tested. And the following study investigated vitality and degradation ability of isolated bacteria against napropmide, utilization of this chemicals as nutrition source for growth, and effect of repeated application on its degradation by the isolates to clarify characteristics of napropamide-degrading bacteria.

Materials and Methods

Chemicals and soil. Napropamide(99.5% purity) was obtained from Agrochemicals Research Institute, Office of Rural Development in Korea, while Devrinol WP 6% a.i. was used for degradation test. Soil texture used for bacteria isolation was a clay loam soil in upland field. Its organic matter content was 2.8% and soil pH, 5.9.

Isolation and identification of bacteria. Soil bacteria were isolated by the conventional method(Itoh, 1991) and the isolated strains were classified by taxonomy after observed 24 kinds of physiological and biochemical tests(Krieg and Holt, 1984).

Vitality of isolated bacteria against napropamide. One loopful of bacteria was innoculated at nutrient borth(NB) agar medium including napropamide ranged from 100 ppm to 3,000 ppm and then incubated at 30 °C for five days. Vitality was evaluated by four levels(vigorous, good, weak, and not grown) according to the degree of colonies' formation.

Degradation ability of naropamide by isolated bacteria. Two loops of bacteria were innoculated into NB medium in test tube and incubated at 30 °C for 48 hrs with shaking. After incubation they were individually diluted with sterilized distilled water to prepare a constant optical value at 660nm. One ml of the dilutions was transferred to 19 ml of NB medium including 20 ppm of napropamide and incubated at 30 °C for three days with shaking. Bacteria multiplication and napropamide residue were seperately measured.

Utilization of napropamide as nutrient sources by the degrading bacteria. Two strains of bacteria having over 20% of degradation were innoculated at inorganic salt media(Itoh, 1991). After five days of incubation at 30 °C, NO_3-N by Brucine method(Norman and Stucki, 1981) and glucose by dinitrosalicylic acid method(Jeong and Jang, 1992) were determined to evaluate utilization of napropamide as a carbon or a nitrogen source by *Staphylococcus* II and *Actinobacillus* I.

Effect of repeated application of napropamide on degradation and growth. A fixed quantity of preincubated *Staphylococcus* II was innoculated at NB media containing 10 ppm of napropamide when applied once. When the chemicals was applied twice and three times, the same concentration was seperately added to the same media with lapse period of seven days. At seven days after final treatment, rate of degradation and degree of propagation were investigated.

Analysis of napropamide. Residual napropamide in the culture was determined with GLC-FTD(Shimazu GC-14A) as reported previously(Han et al., 1994).

Results and Discussion

Classification of bacteria. Twenty strains of bacteria were isolated from clay loam soil in upland field which napropamide had never been applied. They were classified by morphological difference of coloniess and twenty four kinds of taxonomic characteristics. 10 strains of bacteria were classified by *Corynebacterium*, *Listeria*, *Staphylococcus* and *Streptococcus* as gram-positive bacteria, while 10 strains of

bacteria belonged to *Actinobacillus*, *Alcaligens*, *Enterobacterium* and another as gram-negative ones, as shown in Table 1.

Table 1. Clssification of bacteria isolated from clay loam soil.

Gram-positive bacteria		Gram-negative bacteria	
Corynebacterium spp.	I	*Actinobacillus* spp.	I
	II		II
	III	*Acaligens* spp.	I
Listeria spp.	I	*Enterobacterium* spp.	I
	II		II
	III		III
	IV		IV
Staphylococcus spp.	I	Another	I
	II		II
Streptococcus spp.	I		III

Table 2. Vitality of isolated bacteria against napropamide herbicide.

Strains		Concentration of napropamide (ppm)									
		100	200	300	400	500	1000	1500	2000	2500	3000
Corynebacterium spp.	I	◎	◎	◎	◎	○	△	△	×	×	×
	II	◎	△	△	×	×	×	×	×	×	×
	III	◎	◎	◎	◎	◎	◎	◎	×	×	×
Listeria spp.	I	◎	△	×	×	×	×	×	×	×	×
	II	◎	△	×	×	×	×	×	×	×	×
	III	◎	△	×	×	×	×	×	×	×	×
	IV	◎	△	△	△	△	△	△	△	×	×
Staphylococcus spp.	I	◎	◎	◎	◎	◎	◎	◎	×	×	×
	II	◎	◎	◎	◎	◎	◎	◎	△	×	×
Streptococcus spp.	I	◎	△	△	×	×	×	×	×	×	×
Actinobacillus spp.	I	◎	○	×	×	×	×	×	×	×	×
	II	◎	○	△	△	△	△	△	×	×	×
Alcaligens spp.	I	◎	×	×	×	×	×	×	×	×	×
Enterobacterium spp.	I	◎	○	○	×	×	×	×	×	×	×
	II	◎	△	△	×	×	×	×	×	×	×
	III	◎	○	○	×	×	×	×	×	×	×
	IV	◎	△	△	△	△	△	△	×	×	×
Another	I	◎	×	×	×	×	×	×	×	×	×
	II	◎	◎	◎	◎	◎	◎	◎	×	×	×
	III	◎	×	×	×	×	×	×	×	×	×

Dgree of vitality : ×; not grown, △; weak, ○; good, ◎; vigorous.

Vitality of the isolated bacteria against napropamide. The pure cultured colonies were innoculated at nutrient broth agar media including napropamide ranged from 100 to 3,000 ppm. Taking a look at Table 2, all tested strains vigourously grew at 100ppm of the chemicals. *Corynebacterium* II, *Staphylococcus* I, *Staphylococcus* II and another II were very tolerant against napropamide even at the high concentration of 1500ppm. *Listeria* IV and *Staphylococcus* II could weakly proliferated even when applied with 2,000 ppm. In these results we can see the fact that some of bacteria could survived at high concentration of napropamide.

Degradation of napropamide by the isolates. This experiment was to find degrading bacteria which have a higher activity for degradation of napropamide among bacteria from soil tested. Napropamide was more or less degraded by all tested bactera except for *Listeria* spp. III and another II. Especially *Staphylococcus* II and *Actinobacillus* I degraded 20 % of this chemicals during 72 hours' incubation(Table 3). When compared the degree of vitality of the isolates(Table 2) to the degradation ability of napropamide by them(Table 3), the tolerant and well-proliferated bacteria were not always a good napropamide-degrader. These were different from the results that fungicide chlorothalonil degraded well by bacteria tolerant against the high concentration of the fungicide (Katayama et al., 1991).

Table 3. Degradation ability of napropamide herbicide of bacteria isolated from soil.

Gram-positive bacteria			Gram-negative bacteria		
Srains		Percentage of degadation(%)	Strains		Percentage of degadation(%)
Corynebacterium spp.	I	5	*Actinobacillus* spp.	I	22
	II	8		II	10
	III	18	*Alcaligens* spp.	I	10
Listeria spp.	I	10	*Eterobacterium* spp.	I	18
	II	8		II	16
	III	0		III	16
	IV	18		IV	10
Staphylococcus spp.	I	5	Another	I	19
	II	21		II	0
Streptococcus spp.	I	6		III	8

Utilization of napropamide as carbon or nitrogen source by bacteria. The gram-positive bacterium *Staphylococcus* II and the gram-nerative one *Actinobacillus* I, which had a higher degradation ability in the proceeding experiment, were used to elucidate the utilization of naprpmide as nutrient sources. All tested two strains utilized NO_3-N by similar level when compared the inorganic salt medium without napropamide including $NaNO_3$ to the inorganic salt medium with both napropamide and $NaNO_3$. While, these strains degraded still more napropamide in the inorganic salt medium with both napropamide and $NaNO_3$ than that in the medium adding napropamide instead of $NaNO_3$(Table 4).

Table 4. Utilization of herbicide napropamide as a nitrogen source by bacteria.

Composition of culture media	*Actinobacillus* spp. I		*Staphylococcus* spp. II	
	Utilization of NO_3-N (%)	Percentage of degradation (%)	Utilization of NO_3-N (%)	Percentage of degradation (%)
Inorganic salt medium with $NaNO_3$ and without napropamide(control)	62	—	60	—
Inorganic salt medium with napropamide and without $NaNO_3$	—	4	—	7
Inorganic salt medium with both napropamide and $NaNO_3$	61	15	62	17

Table 5. Utilization of herbicide napropamide as a carbon source by bacteria.

Composition of culture media	*Actinobacillus* spp. I		*Staphylococcus* spp. II	
	Utilization of glucose (%)	Percentage of degradation (%)	Utilization of glucose (%)	Percentage of degradation (%)
Inorganic salt medium with glucose and without napropamide(control)	33	—	35	—
Inorganic salt medium with napropamide and without glucose	—	14	—	32
Inorganic salt medium with both napropamide and glucose	36	23	38	37

As shown in Table 5, utilization of glucose by two tested bacteria was not much different between the inorganic salt medium without napropamide(control) and the inorganic salt medium including both glucose and napropamide. While, percentage of naprpamide degradation by *Actinobacillus* I and *Staphylococcus* II was 14 % and 32 % in the inorganic salt media with napropamide instead of glucose and that by them was 23 % and 37 % in the medium including napropamide plus glucouse, respectively. They degraded more naprpamide in the latter medium than that in the former medium. Especially *Staphylococcus* II utilized napropamide as carbon source more than *Actinobacillus* I. These results indicate that two strains of bacteria tested were not able to use napropamide as a sole nitrogen source but able to utilize the chemicals as a sole carbon source and energy, which is catabolism-type degradation(Matsumura, 1982).

Effect of repeated application on degradation. *Staphylococcus* II degraded napropamide by 66% when applied once, 14% when did twice, and 52% when did three times, individually. While, this microbe grew gradually with lapse of time, but the growth rate was very fast when obserbed at seven days after innoculation into NB media including napropamide.

Table 6. Effect of repeated application on degradation of napropamide by *Stphylococcus* spp. II and its growth.

Percentage of degradation(%)			Degree of growth (O.D.value)[a]			
Number of times of application			Days after innoculation			
Once	Twice	Three times	0	7	14	21
66	14	52	0.112	1.343	1.543	2.000

a) Optical density was measured at 660 nm.

From the results, the tested bacterium seems to have a lag-period in degradation when napropamide was applied repeatedly and the extent of degradation seems to be considerabley associated with the degree of proliferation.

Acknowledgement: Author is grateful for financial support to Wonkwang Uuniversity in Korea.

References

Han, S. S., J. H. Jeong and C. K. Choi: 1994, Residue of Herbicide Napropamide and Change of Microflora in Upland Soil under Different Environmental Conditions, Korea Journal of Weed Science, 14(4), in press.

Hill, I. R. and S. J. L. Wright: 1978, Pesticide Microbiology, Academic press London, 79-136.

Itoh, K.: 1991, Characteristics of Microflora Degrading Insecticide Salithion in Soil. Journal of Pesticide Science, 16(1), 77-83.

Jeong, D. H. and H. K. Jang: 1992, Food Analysis, Jinro Research Printing Co., 179.

Katayama, A., H. Isemura and S. Kuwatsuka: 1991, Population Change and Characteristics of Chlorothalonil-Degrading Bacteria in Soil. Journal of Pesticide Science, 16(2), 239-245.

Krieg, N. R. and J. G. Holt: 1984, Bergey's Mannual of Systematic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore, 964.

Matsumura, F.: 1982, Biodegradation of Pesticide, Plenum Press, New York, 67-82.

Norman, R. J. and J. W. Stucki: 1981, The Determination of Nitrate and Nitrite in Soil Extractants by Untraviolet Spectrophotometry, Soil Sci. Soc. Am. J., 45, 347-353.

Ryang, H. S. and Y. H. Moon: 1991, Residual Activity and Effect of Soil-Applied Herbicides on Succeeding Crops in Vegetable Field. Korea Science & Engineering Foundation, Final Research Report, KOSEF 891-1502-037-2, 57-65.

A NEW LOW-RATE PRE-EMERGENCE HERBICIDE KPP-314 FOR RICE

K. Hirai*, T. Yano, S. Ugai#, O. Yamada#, T. Yoshimura#, M. Hori#
Sagami Chemical Research Center, Kanagawa 229, Japan
#Kaken Pharmaceutical Co., Ltd, Tokyo 103, Japan

Abstract. A series of 3-(substituted phenyl)-5-isopropylidene-1,3-oxazolidine-2,4-dione derivatives was synthesized and their herbicidal activities against various weeds were examined. The activity was mainly influenced by the substituents on the phenyl ring and the compounds with fluorine and chlorine atoms at the 2- and 4-positions exhibited the highest activity. The selectivity was found to be affected by the substituent at the 5-position and introduction of a cyclopentyloxy group increased the selectivity for rice. Among the compounds of the series, 3-[4-chloro-5-(cyclopentyloxy)-2-fluorophenyl]-5-isopropylidene-1,3-oxazolidine-2,4-dione (KPP-314) was selected as a potent herbicide in paddy field. In outdoor pot tests, KPP-314 exhibited excellent activity against annual lowland weeds by pre- and post-emergence treatment at 1.5 to 6.0ga.i./are with good selectivity between rice plant and *Echinochloa oryzicola*. In addition, the higher safety of KPP-314 to rice was observed in further evaluation trials under several conditions.

Key words. KPP-314, oxazolidinedione, herbicide, pre-emergence, post-emergence, rice, annual broadleaf weeds, *Echinochloa oryzicola*, selectivity, residual activity, safety.

INTRODUCTION

KPP-314, discovered by Sagami Chemical Research Center and Kaken Pharmaceutical Co. Ltd., is a new herbicide under development for *Echinochloa oryzicola* and broadleaf weeds control in paddy field. KPP-314 exhibits excellent herbicidal activity in both pre- and post-emergence applications at low dosage with good safety for rice. In this paper, the synthesis of KPP-314, and its physical and chemical properties, and the results of several evaluation trials will be summarized. Additionally, its mode of action will be also introduced briefly together with the structural similarity of KPP-314 comparing with other cyclicimide-type herbicides.

MATERIALS AND METHODS

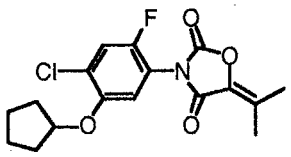
1. Synthesis of Compounds

KPP-314, 3-[4-chloro-5-(cyclopentyloxy)-2-fluorophenyl]-5-isopropylidene-1,3-oxazolidine-2,4-dione, was synthesized by reacting of 4-chloro-5-(cyclopentyloxy)-2-fluorophenylisocyanate prepared by phosgenation of the corresponding aniline with 2-hydroxy-3-methyl-3-butenoate in the presence of base.[1)] Spectral data of KPP-314 are as follows; ^{1}H-NMR (400MHz, ppm) δ: 1.64(2H, m), 1.85(2H, m), 1.90 (4H, m), 2.06 (3H,

s), 2.30(3H, s), 4.74(1H, m), 6.84(1H, d, J_{HF}=6.4Hz), 7.30(1H, d, J_{HF}=9.1Hz). IR (KBr disk, cm^{-1}): 2970, 1815, 1740, 1500, 1200. UV (CH_3CN): λ_{max}=247.5nm (ε_{max} =21850), λ_{max}=289.6nm (ε_{max}=6790). Physical and chemical properties of KPP-314 purified are summarized in Fig-1. In a similar manner, other kinds of 3-(substituted phenyl)-5-isopropylidene-1,3-oxazolidine-2,4-dione derivatives were prepared via the corresponding substituted phenylisocyanates, and their structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS spectroscopy and elemental analysis.

Fig-1. Physical and Chemical Properties of KPP-314

Chemical Structure :



Molecular Formula : $C_{17}H_{17}ClFNO_4$

Molecular Weight : 353.777

Melting Point : 104.5~105°C (corrected)

IUPAC Name : 3-[4-chloro-5-(cyclopentyloxy)-2-fluorophenyl]-5-isopropylidene-oxazolidine-2,4-dione

CAS Number : 110956-75-7

Appearance : White micro crystalline solid

Solubility (g/L) :

Water	: 0.216ppm	Methanol	: 24.8
Xylene	: 430	Isopropyl alcohol	: 12.9
Hexane	: 5.10	Petroleum ether	: 5.30
Acetone	: 740	Acetonitrile	: 330
Ethyl acetate	: 500	Diethyl ether	: 26.4

2. Herbicidal Activity

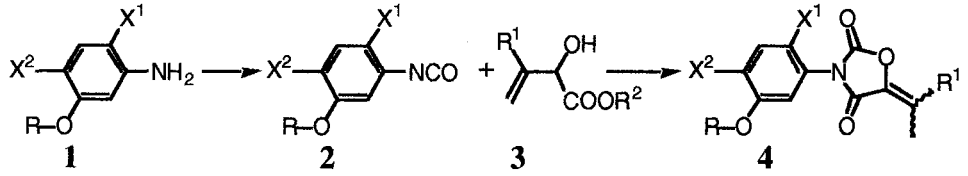
Oxazolidinedione derivatives such as KPP-314 were formulated into 10% emulsifiable concentrate and applied in the predetermined amounts per Are onto the pots which were filled with paddy field soil, and seeded with the seeds of weeds, such as *Echinochloa oryzicola*, *Cyperus difformis*, Broadleaf weeds, *Monochoria vaginalis*, *Eleocharis acicularis*, *Scirpus juncoides*, and transplanted with a rice plant. Pre-emergence treatments were conducted one day after seeding and early post-emergence treatments were conducted 4~7 days after seeding. Weed control and crop tolerance were assessed by visual rating scale of zero (no effect) to 10 (complete death) on the 7th or 14th day after treatment.

RESULTS AND DISCUSSION

1. Synthesis

Although 3-(substituted phenyl)-1,3-oxazolidine-2,4-dione without an alkylidene moiety at the 5-position of the oxazolidine ring was readily prepared by the reaction of substituted

phenylisocyanate with 2-hydroxyalkanoate under basic condition, it was difficult to be modified by the alkylidene moiety at the 5-position.[2] In the course of the studies on the synthesis of new oxazolidine heterocycles, we found that the alkylidene moiety could be easily introduced at the 5-position by addition reaction of 2-hydroxy-3-alkenoate to substituted phenylisocyanate followed by intramolecular cyclization and olefin isomerization as outlined in Fig-2. In this reaction, when R^1 was not a methyl group, the product was obtained as a mixture of E and Z forms.



1 2 3 4

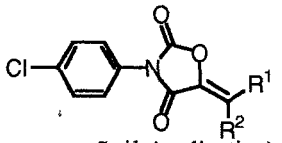
Fig-2. Synthetic Route for 3-(Substituted phenyl)-5-alkylidene-1,3-oxazolidine-2,4-dione

2. *Herbicidal Activity and Selectivity*

2.1 *Effect of 5-Alkylidene Moiety and Phenyl Ring Substituents*

Table-1 summarized the results of preliminary evaluation test using 3-(4-chlorophenyl)-5-alkylidene-1,3-oxazolidine-2,4-diones in pre-emergence soil application. Among these derivatives (**1**~**6**), isopropylidene (**1**) and 2-butylidene derivatives (**2**) showed the highest activities. 2-Hexylidene (**3**), 3-pentylidene (**5**), and cyclopentylidene (**6**) showed moderate to weak activities, while 2-octylidene (**4**) was inactive. Therefore, the lengthening of carbon chain of R^1 or R^2 showed the tendency to reduce the herbicidal activity remarkably.

Table-1. Herbicidal Activity of 3-(4-Chlorophenyl)-5-alkylidene-1,3-oxazolidine-2,4-diones

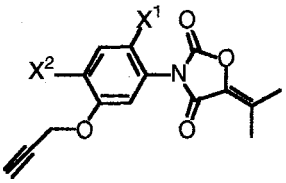


(Pre-Emergence Soil Application)

No	R^1	R^2	ga.i./are	Herbicidal Activity Ec	Pl	Ca
1	Me	Me	100	10	10	10
2	Me	Et	100	10	10	-
3	Me	Bu	100	4	6	4
4	Me	Hex	100	0	0	0
5	Et	Et	100	2	2	0
6	$-(CH_2)_5-$		100	2	4	2

Ec:*Echinochloa crus-galli*
Pl:*Polygonum lapathifolium*
Ca:*Chenopodium album*

Table-2. Herbicidal Activity of 3-(2,4-Dihalo-5-propargyloxyphenyl)-5-isopropylidene-1,3-oxazolidine-2,4-diones



(Early Post-Emergence Paddy Field Application)

No	X^1	X^2	ga.i./are	Herbicidal Activity Ec	Mv	Am
7	Cl	Cl	0.5	9	10	9
8	F	Cl	0.5	10	10	10
9	F	Br	0.5	8	10	10
F	F		0.5	9	10	10

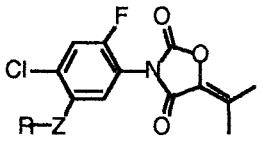
Eo:*Echinochloa crus-galli* (1L)
Mv:*Monochoria vaginalis*
Am:*Ammannia multiflora*

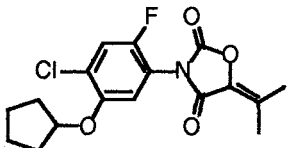
As the herbicidal activity was observed in a series of 3-(4-chlorophenyl)-5-alkylidene-1,3-oxazolidine-2,4-diones, we then proceeded to the modification of the phenyl ring system with other substituents by fixing the alkylidene moiety as an isopropylidene group and also fixing the substituent at the 5-position of phenyl ring as a propargyloxy group, because the propargyloxy group was known to be the most effective in enhancing herbicidal activity in cyclicimide-type herbicide. Table-2 shows the herbicidal activity of 3-(2,4-dihalogeno-5-propargyloxyphenyl)-5-isopropylidene-1,3-oxazolidine-2,4-dione (**7~10**) by early post-emergence treatment at 0.5ga.i./are under paddy field conditions. Among these compounds, 2-fluoro-4-chloro derivative (**8**) exhibited more potent activity against *Echinochloa crus-galli* than that of other halogen pairs of derivatives (**7**, **9**, **10**). This structure-activity relationship was similar to that of other cyclicimide-type herbicides such as 3,4,5,6-tetrahydrophthalimide and 3,4-tetramethyleneisourazole derivatives.

2.2 Selectivity for Rice

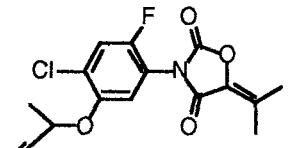
KPP-314 exhibits good safety for rice plant at the standard rate showing excellent activity against annual lowland weeds with pre- and post-emergence application. Table-3 summarizes the herbicidal activity against *Echinochloa crus-galli,* rice injury and the selectivity of KPP-314 applied by early post-emergence together with that of other 1,3-oxazolidine-2,4-diones modified by several kinds of substituents on the oxygen atom at the 5-position of phenyl ring.

Table-3. Herbicidal Activity and Selectivity of 3-(substituted phenyl)-5-isopropylidene-1,3-oxazolidine-2,4-diones





KPP-314



KPP-300

(Early Post-Emergence Paddy Field Application)

No	R	Z	Ec ED_{90}(ga.i./ha)	Rice ED_{10}(ga.i./ha)	Selectivity ED_{10}/ED_{90}	
11	Me	O	250	8	0.032	
12	Et	O	200	100	0.5	
13	Hex	O	40	4	0.1	
14	i-Pr	O	10	2.5	0.25	
15	i-Pr	S	80	40	0.5	
16	Sec-Bu	O	100	40	0.4	
17	3-Pent	O	40	30	0.75	
18	cyclo-Pent	O	20	40	2.0	KPP-314
19	cyclo-Pent	S	100	30	0.3	
20	cyclo-Hex	O	200	50	0.25	
8	Propargyl	O	25	2.5	0.1	
21	1-Butyn-3-yl	O	20	10	0.5	KPP-300

Eo:*Echinochloa crus-galli* (0.5L), Rice:Var.(Nipponbare, 1L)

ED_{90} means the value of the effective dose to give 90% weed control in the rating scale and ED_{10} means the same to give 10% rice injury, therefore, the ratio of ED_{10}/ED_{90} represents the selectivity precisely.

Although isopropyl derivative (**14**) showed the highest activities among the alkyl group substituted derivatives (**11**~**17**), severe rice injury was observed. Highly active compounds (**8**, **21**) introduced a propargyl group on the oxygen atom at the 5-positions showed poor selectivities owing to low dosages to give rice injury. On the other hand, cyclopentyl derivative (**18**), namely KPP-314 exhibited excellent efficacy (ED_{90}=20 ga.i./ha) and the highest selectivity (ED_{10}/ED_{90}=2.0) because of a moderate dosage showing rice injury. While the herbicidal activity of cyclohexyl derivative (**20**) was poor, and sulfur modified derivatives (**15**, **19**) at the 5-position of phenyl ring showed lower activities and selectivities than that of their parent compounds.

Fig-3 and Fig-4 show the herbicidal activity against important weeds in paddy field and rice injury of KPP-314 by pre- and post-emergence treatment at 5 to 20ga.i./ha and 28 to 113ga.i./ha, respectively. With pre-emergence application at the middle rate of 10ga.i./ha, the herbicidal activities against *Echinochloa oryzicola*, *Scirpus juncoides*, and annual broadleaf weeds were a little bit decreased, however, at the rate of 20g a.i./ha, all weeds subjected were enough controlled by KPP-314 with good selectivity for rice.

On the other hand, KPP-314 applied post-emergence at 28 to 113ga.i./ha was effective against *Echinochloa oryzicola*, *Cyperus difformis*, and annual broadleaf weeds, while *Scirpus juncoides* were not controlled at any dosages. Safety of KPP-314 for rice was excellent at these rates with post-emergence applications.

Fig-3. Herbicidal Activity and Rice Injury of KPP-314 by Pre-Emergence (+1)

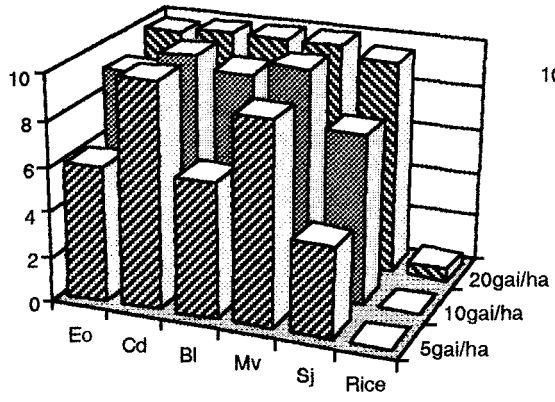
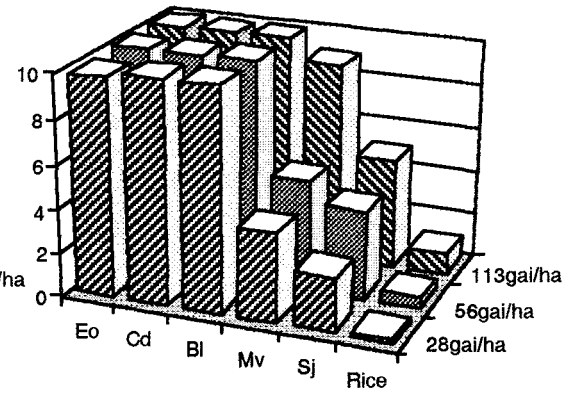


Fig-4. Herbicidal Activity and Rice Injury of KPP-314 by Post-Emergence (+7)



0~10 rating scale, Plot size:1/10000are, 14 days after treatment, Rice:transplanting rice at 2L (Koshihikari), Eo:*Echinochloa oryzicola*, Cd:*Cyperus difformis*, Mv:*Monochoria vaginalis*, Bl:Annual broadleaf weeds (Lindernia, Rotala, Elatine spp), Sj:*Scirpus juncoides*.

2.3 Residual Activity of KPP-314

Another most useful characteristic of KPP-314 is that the herbicidal activity keeps for a

long time. Fig-5 depicted the residual activity of KPP-314, chlomethoxynil (X-52), and pretilachlor (CG-113) against *Echinochloa crus-galli* by interval sowing method at the standard rate of each compound. As shown in Fig-5, KPP-314 maintained its herbicidal activity for more than 45 days after application, while the activities of X-52 and CG-113 were hastily reduced after 20 and 30 days, respectively.

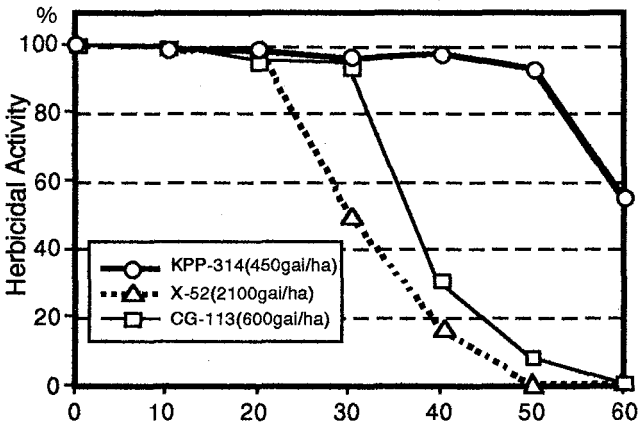


Fig-5. Residual Activity of KPP-314, X-52, and CG-113

3. Mode of Action

Potent new oxazolidinedione herbicides such as KPP-314 classified to cyclicimide-type bleaching herbicide were found to require light for expression of its herbicidal activity and also give remarkable effects on chlorophyl biosynthesis like tetrahydrophthalimide and diphenylether herbicides. In fact, KPP-314 severely inhibited growth of *Senedesmus* cells at 10^{-5}M concentration, degraded chlorophyll of the cells, and exhibited a high ethane formation.[3] Obviously, KPP-314 is a protox inhibitor showing peroxidizing herbicidal activity. Furthermore, based on crystallographic analysis, the structural similarities of KPP-314 and other protox inhibitors are also discussed comparing with that of Protogen as a substrate of Protoporphyrinogen oxidase, Protox.

REFERENCES

1) K. Hirai, T. Yano, E. Ejiri, K. Aizawa, M. Yamashita, K. Shikakura, S. Nagato, S. Ugai, T. Taguchi, H. Hirose, A. Fujita and H. Sato, Abstract of 17th Annual Meeting of Pesticide Science Society of Japan, p.46 (1992)

2) e.g. G. Morel et al., US Patent, 3201410 (1965), K. Sato et al., US Patent, 3703526 (1972), D. Mangold et al, US Patent, 3995049 (1976)

3) H. Kohno, K. Hirai, M. Hori, Y. Sato, P. Böger, and K. Wakabayashi, Z. Naturforsh., **48c**, 334 (1993)

Conversion and Secretion of Phenolic Compounds by Wheat Plant and their Antimicrobial Activities

Akio KOBAYASHI[1)], Myong Jo KIM*, Kazuyoshi KAWAZU
1)Dept. of Biotech., Osaka Univ., Suita 565, JAPAN
Dept. of Agric. Sci., Okayama Univ., Okayama 700

Abstract

The root-soil interface can be conveniently regarded as the rhizoplane, and the rhizosphere is influenced by the root. However, the detailed mechanism of the rhizosphere formation of many economically important plants is not thoroughly understood. Plants release a variety of organic compounds into the environment by way of leaf leachates, root exudates, and through decomposition of litter. The growth of microorganisms in the rhizosphere can be profoundly controlled by these compounds. A certain class of these compounds, predominantly phenolic acids, has been shown to be allelopathic to seed germination, seedling growth, and soil bacteria. However, little is known about the other biologically active compounds concerning the relationship between plants and microorganisms. On the other hand, microbial metabolites may affect plant growth conversely. In this study we were specifically interested in the compounds selectively taken up by plants and moreover in those secreted from the roots. We chose wheat plant as a model plant. Our preliminary study indicated that the wheat root took up simple phenolics selectively, so we focused on phenolic acids, i.e., *p*-coumaric acid and ferulic acid which are specially taken into the plant. Wheat plants were grown on gelrite medium at 20 °C for 8 days and the roots were then soaked in 500 μg/ml of phenolic acid solution. After incubation (20 °C for 8 h), the plants were removed from the test solution, which was found to have a pronounced antibacterial activity. Chromatographic separation enabled to isolate antimicrobial components, which were identified as 4-hydroxystyrene and 3-methoxy-4-acetoxystyrene by spectroscopic analysis. These compounds markedly inhibited the growth of some bacteria isolated from the rhizosphere. These findings suggest that plants may take up the external compounds from the roots and use them positively as allelochemicals.

Key Words : Decarboxylation *p*-Coumaric acid Ferulic acid Uptake Exudate

Introduction

Many studies have been accumulated in the plant nutrition, which deals mainly uptake of inorganic elements into plants, and rather a small number of papers have been published on uptake of organic compounds. The most papers have dealt with plant primary metabolites, plant hormones and herbicides were dealt. However, there are only a few papers on organic compounds taken into plants. For example, an antibiotic, cycloheximide produced by streptomyces is known to bring about increment of sodium ion and α-ketoglutaric acid flux in plant tissues. Our preliminary study of composit components indicated the occurrence of a variety of simple phenolics, and some components were found to be specifically taken up by plant roots. In this course of studies, we were interested in the uptake- selectivity toward simple metabolites of soil-microorganisms (phenolic acids), and their physiological effects on the plant. Recently, we established a model assay system to seek the compounds which selectively taken into wheat plant and exert physiological significance to pathogenic invasion into the plant. We chose wheat plant as a model plant. Our preliminary study indicated that the wheat root took up simple phenolics selectively, so we focused on phenolic acids, i.e., *p*-coumaric acid and ferulic acid which are specially taken into the plant. Chromatographic separation enabled to isolate antimicrobial components, which were identified as 4-hydroxystyrene, 3-methoxy-4-hydroxystyrene and 3-methoxy-4-acetoxystyrene by spectroscopic analysis. These compounds were shown to be derived from *p*-coumaric acid / ferulic acid by the feeding experiment of the ^{13}C-labeled compounds. Antimicrobial activities of the styrenes were examined (Table).

Materials and Methods

Identity of the compounds isolated was confirmed by ^{1}H-NMR and MS spectral analyses.

Plant materials (Conditions for Plant growth).

Wheat (*Triticum vulgaris*) seeds were used in the uptake experiment. The seeds were peeled and sterlized by immersing in 70% EtOH for 60sec, followed by 5 % H_2O_2 for 20 min. The seeds thus treated were rinsed three times with sterilized water. Five surface-sterilized seeds were transferred onto a medium contaning 0.1% (w/v) $MgCl_2$ and 0.2% (w/v) gelite in test tubes and incubated in the light at 20 °C for 8 days. The roots of 8-day-old plants were soaked into each test solution containing 500 μg/ml of *p*-coumaric acid, ferulic acid, and ^{13}C labeled *p*-coumaric acid, respectively at 20 °C for 8 h. The plants were removed from the test solutions, and an aliquot of the solutions was then subjected to an antimicrobial test.

Extraction and isolation of chemical compounds.
The test solution (2 l) was extracted twice with EtOAc. The organic phases were combined and concentrated *in vacuo* to afford brownish oil (175 mg), which was chromatographed on a silica gel column (Wakogel C-100) eluted in stepwise mode with 65 ml each of 20, 40, 70, 100 % EtOAc/*n*-hexane. The active fraction (40 % EtOAc elute) was further purified by a silica gel column to give active compounds, **1** (30 mg), **2** (5 mg), and **3** (2 mg).

Properties of 1
EI-MS *m/z* : 120(M^+), UVλmax (MeOH) nm (log ε) : 225(3.56), 286sh(3.37), 299sh(3.41), 313(3.45) ; ^{1}H NMR δ (500MHz, d_6-Acetone) :5.02 (1H,dd,*j*=1.01,10.8Hz), 5.58(1H,dd,*j*=1.01,17.6Hz), 6.63 (1H,dd, *j*=10.8,17.6Hz), 6.79(2H,d,*j*=8.7Hz), 7.27(2H,d,*j*=8.7Hz), 8.46(1H,s).

Properties of 2
EI-MS *m/z* : 192(M^+), UVλmax (MeOH) nm (log ε) :207(4.15), 250(3.56), 287(3.46), 315sh(3.34) ; ^{1}H NMR δ (500MHz, $CDCl_3$) : 2.31(3H), 3.85(3H), 5.23(1H,d,*j*=10.7Hz), 5.68(1H,d,*j*=17.4Hz), 6.67 (1H, d,*j*=10.7,17.4Hz), 6.98(3H).

Properties of 3
EI-MS *m/z* : 150(M^+), UVλmax (MeOH) nm (log ε) : 211(4.25), 287(4.01), 300sh(3.56), 312(3.95) ; ^{1}H NMR δ (500MHz, $CDCl_3$) : 3.86(3H), 5.03(1H,d,*j*=10.7Hz), 5.60(1H,d,*j*=17.7Hz), 6.64(1H,d,*j*=10.7, 17.7Hz), 6.77(1H), 6.89(1H), 7.09(1H).

Anti-microbial assay.
The MIC values against bacteria and fungi were determined by the serial 2-fold dilution method. Bacteria were pre-cultured in 10 ml of a nutrient-broth medium for 12 h at 27 °C on a shaker, and then diluted 100-fold with the same medium. Fungi were inoculated into 10 ml of a potato-malt extract-sucrose agar medium, and incubated at 27 °C for 7 days to from a well-expanded fungal mat with spores. The spores were suspended in 50 ml of a medium containing 0.2 % glucose, 0.1 % yeast extract, 0.1 % citric acid, and 0.37 % Na_2HPO_4. Liquid cultures or spore-suspension cultures containing the test comopund were placed in the wells of a 96-well microplate and then incubated at 27 °C for 24 h. Growth of the test bacteria was evaluated by the degree of turbidity of the culture in macrography, and the spore germination was examined under a microscope.

Synthesis of ^{13}C rabeled p-coumaric acid

In a round-bottomed flask, fitted with a reflux condenser and a thermometer, was placed 109 mg (1.05 mmol) malonic-2-^{13}C acid, 87 mg (0.71 mmol) of *p*-hydroxybenzaldehyde, and 1.5 ml of pyridine. The malonic-2-^{13}C acid is warming on a oil bath and dissolved by stirring. Piperidine (20 μl) is then added, and the mixed solution was heated to 80 °C. An internal temperature of 80-85 °C was maintained for 1 h, and finally heated under reflux (109-115 °) for additional 3 h. After being cooled the reaction mixture was poured into a beaker containing 5 ml of cold water, and then acidfied by slow addition of 2N hydrochloric acid. The resulting solution was partitioned with EtOAc, and then dried over anhydrous Na_2SO_4. After a silica gel column the labeled *p*-coumaric acid was obtained in crystalline form. The same method was applied to ^{13}C labeled feruric acid synthsis.

Results and Discussion

The EtOAc extract obtained from the feeding experiments showed pronounced antibacterial and antifungal activities. Several UV-absorbing spots were detected by silica gel TLC. Chromatographic separation enabled to isolate antimicrobial components (**1**: 4-hydroxystyrene, **2**: 3-methoxy-4-hydroxystyrene, **3**: 3-methoxy-4-acetoxystyrene), which had a variety of activities as shown in Table. Compound **1** (4-hydroxystyrene) and **3** (3-methoxy-4-acetoxystyrene) inhibited the growth of *Cladosporium herbarum* and *Aspergillus candidus* at the minimum inhibitory concentration (MIC) of 32 μg/ml. The decarboxylation of phenolic acids to corresponding styrenes is known in the studies on fungal and bacterial metabolisms (Finkle et al., 1962; Indahl and Scheline, 1968). However, there is no direct evidence that ferulic acid/*p*-coumaric acid is concerned into the corresponding styrene by the microoganisms. So, we conducted a feeding experiment with ^{13}C labeled *p*-coumaric acid under conditions with wheat culture, and found a major antimicrobial component to be ^{13}C labeled 4-hydroxystyrene/3-methoxy-4-acetoxystyrene, indicating that plants possess a unique enzyme which catalyzes decarboxylation of C_6-C_3 unit consisting of benzene and ethylene chromophores into the corresponding styrenes. Another type of decarboxylation occurs during the biosynthesis of the vinyl side chains of the I and II rings of protoporpyrin. A propionic acid side chain is decarboxylated to a vinyl group; but, unlike the mechanism formation of vinyl group described here, the porphyrin decarboxylation is reported to proceed via an oxidation reaction without occurrence of acrylic acid type of intermediate (Granick,s., and Sano,s.,). When a bacterium *K.oxytoca* was inoculated into PD-medium containing a substrate at a concentration of 500 μg/ml (750 ml, 375 mg of *p*-coumaric acid) and then incubated for 3 days at 28 °C in the dark, 223mg of *p*-hydroxystyrene was obtained from the medium by TLC (Y.Hashidoko, M.Urashima, T.Yoshida, and J.Mizutani; 1993). Hydroxystyrenes

obtained from decarboxylation of hydroxycinnamates by microoganisms have been assumed to be detoxification products for the microbe (A. R. Goodey and R. S. Tubb,). Of the many types of phytotoxic compounds released from decaying plant materials by microbial activity or leaching, the phenolic acids are probably the most common (Rice, 1974; Whittaker, 1970). Ferulic acid, as well as other phenolic acids, is produced from intermediates of respiratory metabolism via the shikimic acid pathway. Ferulic acid has been found in a variety of crop residues by a number of researchers (Guenzi and McCalla, 1966a; Lodhi, 1979; Wang et al., 1967). Borner (1960) found that the growth of aerial parts of wheat as well as the root growth of rye was suppressed at a low concentration of ferulic acid (10ppm). Detoxification of the acid into the corresponding styrene may have some biological significance, and the antimicrobial function of the styrene may be favorable to the control of the microbial flora in rhizosphere. Further studies on the decarboxylation mechanism as well as physiological significance of the styrenes are now in progress.

Acknowledgments
We are grateful to the SC-NMR Laboratory of Okayama University for 500 MHz experiments. This work was supported by a Grant-in Aid A.K for Scientific Research (No.06660136) from the ministry of Education, Science and Culture of Japan.

Refercnces

1) A. Kobayashi, Y. Koguchi, H. Kanzaki, S.Kajiyama, and K. Kawazu,
Biosci.Biotech. Biochem., **58** (1), 133-134 (1993).
2) A. Kobayashi, A. Igawa, M.J. Kim S.Kajiyama, H. Kanzaki, and K. Kawazu, The annual meeting of the Agricultural Chemical Society of Japan (Tokyo), Abstracts, p192 (1994).

3) A. R. Goodey and R. S. Tubb, *J. Gen. Microbiol.*, **128**, 2615-2620 (1982).
4) B. J. Finkle, J. C. Lewis, J. W. Corse, and R. E. Lundin, *J. Biol. Chem.*, **237**, 2926-2931 (1962).
5) Borner. H, *Bot. Rev.* **26**, 393-424 (1960).
6) Guenzi, W.D., McCalla, T.M., and Norstadt, F.A, *Agron. J*, **59**, 163- 163 (1967).

7) Rex A. Liebl and A. Douglas Worsham, *J. Chemical Ecology*, **9** (8), (1983).
8) Rice, E.L., Alleropathy. Academic Press. New York (1974).
9) Whittker, R.H., The biochemical ecology of higher plants,pp. 43-70, in E. Sondheimer and J. B. Simonc(ed). Chemical ecology. Academic Press, New York (1970).

Antioxidants in Tolerant and Susceptible Plants to Oxyfluorfen

Y. I. Kuk, J. O. Guh, E. K. Lee, and R. D. Park
Chonnam National University, Kwangju, 500-757, Korea

Abstract: Ten-day-old seedlings of 15 plant species (8 tolerants and 7 susceptibles) selected from 428 species were soaked in oxyfluorfen at 10^{-6}M for 2 hrs, kept for 24 hrs in the dark, and exposed to light for 0, 2, 4, or 6 hrs to investigate changes in the contents of antioxidants possibly protecting plants from membrane peroxidation. Tolerant rice cultivars contained more vitamin C than did susceptibles. There were no consistent trends in the contents of vitamine E, carotenoids and glutathione between tolerant and susceptible species. Contents of vitamin C and carotenoids in both tolerant and susceptible seedlings treated with oxyfluorfen generally decreased upon light exposure, while interestingly those of vitamin E and glutathione increased.
* Key words : Oxyfluorfen, Antioxidants, Tolerance

Introduction

The selectivity of herbicides to plants is determined by the morphological characters such as position of growing point, condition of root distribution and structural features of leaf itself, and by the selective absorption, translocation and metabolism of the herbicides in plants.

In a series of processes of the inhibition of protoporphyrinogen oxidase (protox), accumulation of protoporphyrinogen IX (PPIX), generation of active oxygens, peroxidation of membrane, and dying of the plants, one of action mechanisms of diphenylether herbicides suggested recently, various factors including the inhibition of protox (14), degree of PPIX accumulation (9), ability of the antioxdative enzymes (13) and antioxidants (3,5,10) to detoxificate active oxygens are expected to play an important role. Among these factors the antioxidation ability to detoxificate active oxygens resulted from oxyfluorfen treatment determines to cause the selectivity of plants to the herbicide. Plants have a lot of antioxidants which directly respond to and remove active oxygens. The most representative ones are vitamin C (3), vitamin E (5), glutathione [GSH, GSSG] (13), and carotenoids (6).

Several researchers have proposed that the water-soluble vitamin C is an antioxidant-synergist with the lipid-soluble vitamin E, and that both vitamins can act together as a powerful antioxidative system in cells (10,13). The antioxidative role can be explained by the interaction of both vitamins. Vitamin E acts as a primary antioxidant, while vitamin C reductively regenerates oxidized vitamin E (5,10,13). Finckh and Kunert (3) found that peroxdation by oxyfluorfen decreased when the ratio of vitamin C to vitamin E was appropriate (10~15:1). Orr and Hess(10) found that the antioxidant vitamin E inhibited membrane leakage in diphenyl ether treated cucumber cotyledons.

In soybean, both enhanced glutathione reductase activity and higher production of antioxidants, like glutathione and vitamin C, seem to play an important role to limit peroxidation (13). Damage of DPE herbicides oxyfluorfen and bifenox was decreased by pre-treatment of α-tocopherol, mannitol, and hydroquione(8). Ascorbate, GSH and α-tocopherol can inactivate a variety of toxic oxygen radicals and are considered the first line of defense against oxidative stress in plants (7). It is reported that glutathione content is increased by stress and acifluorfen treatment (11). Besides the role of antioxidant, glutathione in plants is conjugated with chloracetanilide, diphenyl ether and thiocarbamate by glutathione-S transferase (GST) and serves for detoxificating the herbicides (2).

This study was done to determine the change of antioxidant contents in the tolerant and susceptible plants to oxyfluorfen selected in a laboratory.

Materials and Methods

Plant materials

Eight tolerant plants [rice (*Oryza sativa* L.) cultivars (Baru, Hunan 31 and Hawon), ryegrass (*Lolium multiflorum* Lam.), tallfescue (*Festuca arundinacea* Schreb), weepinglovegrass (*Zoysia japonica* STEUD.) chinese cabbage (*Brassica pekinensis* Pupr), and mustard (*Sinapis alba*)], and seven susceptible plants [rice culivars (HP857, HP907, HP1033 and Weldpally), barnyardgrass (*Echinochlora crus-gall* Beauv), large crabgrass (*Digitaria sangunalis* (L.) Scop.) and indian zointvetch (*Aeschynomene indica* L.)] were grown in plastic pots containing vermiculite for 10 days in a growth chamber, regulated to a 12 hrs photoperiod (7,000Lux) with day and night temperatures of 28℃ and 20℃, respectively.

Treatments

The seedlings were soaked in oxyfluorfen at 10^{-6}M for 2 hrs in the dark, and then grown for 24 hrs in the dark. Finally, the plants were exposed to light for 0, 2, 4 or 6 hrs.

Analysis

Vitamin C contents were measured by dye titration according to the method of Law et al. (7). Vitamin E contents were determined by fluorospectrophotometer (Kontron SFM 25) at an excitation wavelength of 290nm and emission wavelength of 325nm (3). Carotenoid contents were measured according to the method of Ridley et al. (12). GSH and GSSG were measured according to the method of Hissin et al. (4).

Results and Discussion

1. Selection of tolerant and susceptible plants to oxyfluorfen

Total 428 plant species including 400 rice cultivars, 4 forage crops (rye, tallfescue, ryegrass and redfescue), 2 species of lawn (loard lawn and weepinglovegrass), 9 species of weed (rice flatsedge, japanese clover, spanish needles, jimsonweed, panicum, curled dock, barnyardgrass, large crabgrass, and indian jointvetch), and 15 species of vegetable crop (soybean, cucumber, garland chrysanthemum, spinach, leaf mustard, rape, radish, carrot, tomato, chinese cabbage, corn, barley, kidney bean) were tested. They were treated with oxyfluorfen (Goal EC 23.5% a.i.) at the concentrations of 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M at different leaf stages (germination stage, 1 and 3 leaf stages), seedling conditions (light, dark), methods of treatment (foliar spray, soaking). Three days after treatment, germination rate, plant height and shoot fresh weight were measured. We finally selected 8 tolerant and 7 susceptible species.

2. Content of antioxidants

The content of vitamin C in the tolerant rice cultivars have higher than in the susceptible rice cultivars, as shown in Table 1 and that in the tolerant vegetable crops chinese cabbage and mustard have higher than susceptible plants. The rest of the tolerant and susceptible plants have no quantitative difference in vitamin C contents. In the contents of vitamin E and carotenoid, there is no difference between tolerant and susceptible species.

It is reported that broad leaf plants have more content of vitamin C and E than gramineae plants but the former shows higher degree of phytotoxicity than the the

latter. They concluded that the contents of two vitamins and selectivity of plants to herbicides are little related (3,5).

Table 1. Antioxidant contents(μg/g fresh weight) in various plant species.

Plant species	Vitamin		Carotenoid	Glutathione		
	C	E		GSH	GSSG	Total
	---------- Tolerant species ----------					
Rice						
Hawon	118.7±13.2	10.9±0.3	318±20.5	210±7	400±29	610
Hunan 31	137.9±15.8	6.7±0.2	-	220±10	425±30	645
Baru	115.3± 2.5	8.4±0.6	310±25.3	236±37	467±45	703
Forage crops						
Ryegrass	68.6± 5.2	6.6±0.2	527±31.2	133±24	255±37	388
Tallfescue	98.6±18.0	10.5±0.1	-	136±15	255±22	391
Lawn						
Weepinglovegrass	91.3±12.9	6.9±0.3	-	150±18	232±36	382
Vegetable crops						
Chinese cabbage	148.3± 7.2	12.4±0.2	-	106±28	199±63	305
Mustard	296.0±14.4	25.8±0.3	337±29.4	126±8	371±30	497
	---------- Susceptible species ----------					
Rice						
HP857	97.4± 5.6	8.9±0.3	357±20.5	215±17	473±57	663
HP907	88.0± 3.2	10.3±0.4	-	190±15	415±37	605
HP1033	94.3± 7.7	8.2±0.5	-	223±20	471±50	694
Weldpally	88.8± 4.2	9.4±0.2	294±15.9	237±18	415±72	652
Barnyardgrass	103.0± 5.5	8.6±0.1	447±32.7	59±4	94±12	153
Large crabgrass	118.0± 6.4	6.6±0.1	479±30.2	109±4	203±4	312
Indian zointvetch	97.6± 7.9	7.4±0.6	-	-	-	-

In the content of glutathione (GSH and GSSG), there is no difference between tolerant and susceptible plants. It is noteworthy that the susceptile barnyardgrass contains much less glutathione compared with other plants. Breaux et al. (1) reported that more glutathione was found in plants tolerant to chloracetanilide than in susceptibles.

3. Changes in antioxidant contents upon oxyfluorfen treatment

As shown in Figure 1, at the moment of light exposure vitamin C contents in tolerant species kept in the dark for 24 hrs after oxyfluorfen treatemnt are increased in comparison with those not treated but those in the susceptible ones decreased. After light exposure, the contents of vitamin C generally decreased in most oxyfluorfen-treated species, except for some susceptible rice cultivars. Upon light exposure, vitamin C content was decreased in the susceptible rice cultivar weldpally but was increased in the tolerant talfescue.

The effects of oxyfluorfen on vitamin E content are shown in Figure 2. At the moment of light exposure vitamin E contents in the tolerant and susceptible species are not changed, except for those in the tolerant rice cultivars in which vitamin E contents decreased in comparison with the untreated. After light exposure, the contents of vitamin E generally increased in most oxyfluorfen-treated species, except for the susceptible rice cultivars which showed some decrease in the contents. It is noteworthy that after 4 hrs of light exposure vitamin E contents in barnyardgrass, large crabgrass and indian zointvetch rapidly decreased.

The content of carotenoid components (phytoene, phytofluene, and β-carotene) in the seedlings are slightly decreased, except for those of rice Baru and branyardgrass (Table 2). Thus, no difference between the tolerant and susceptible species was found in content of carotenoid components when the plants treated with oxyfluorfen were exposed to light.

Table 2. Change of carotenoid contents (% of control) at 6 hrs after treatment of oxyfluorfen in various plant species.

Plant species	Phytoene	Phytofluene	β-Carotene	Total
	---------- Tolerant species ----------			
Rice				
Hawon	88	101	95	95
Baru	124	138	105	119
Ryegrass	80	79	80	86
Mustard	97	97	95	96
	---------- Susceptible species ----------			
Rice				
HP857	78	73	53	87
Weldpally	75	65	71	81
Barnyardgrass	98	116	107	128
Large crabgrass	76	80	77	78

The change of GSH contents is shown in Figure 3. At the moment of light exposure the reduced glutathione contents in the tolerant rice cultivars are increased in comparison with the controls. After light exposure, the contents of GSH generally increased in all of oxyfluorfen-treated species. After 4 hrs of light exposure GSH contents in the susceptible rice cultivars rapidly decreased. It is noteworthy that the GSH contents in the tolerant species ryegrass and mustard dramatically increased upon light exposure. This is similar to the previous reports that glutathione content increased due to stress and oxyfluorfen treatment(11). Our results are on the same line with them.

The changes of oxidized glutathione (GSSG) content after oxyfluorfen treatment between the tolerant and susceptible plants are almost same as those of reduced glutathione (Figure 4). The contents of GSSG increased upon light exposure. No difference between the tolerant and susceptible species was found in response to oxyfluorfen treatment in the light.

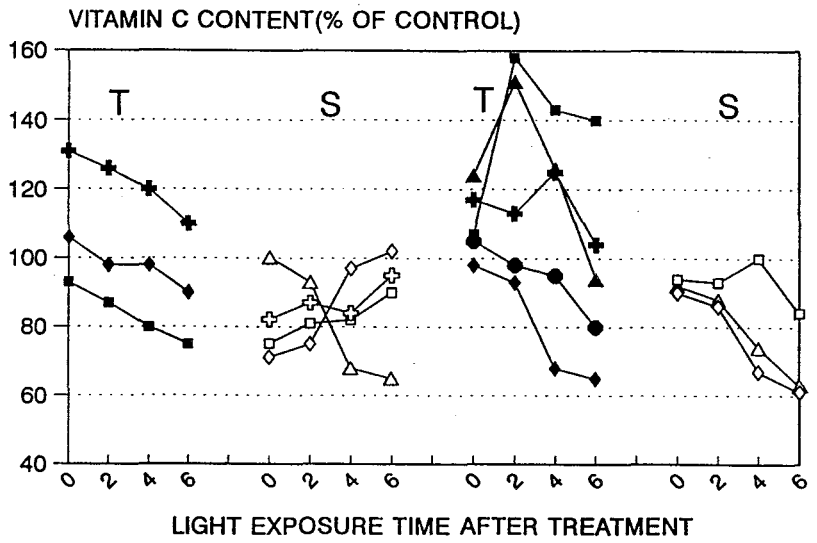
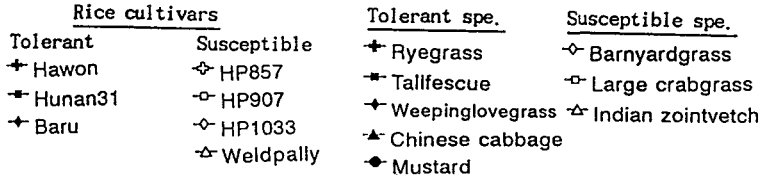


Fig. 1. Change of vitamin C contents in various plant species after treatment of oxyfluorfen.



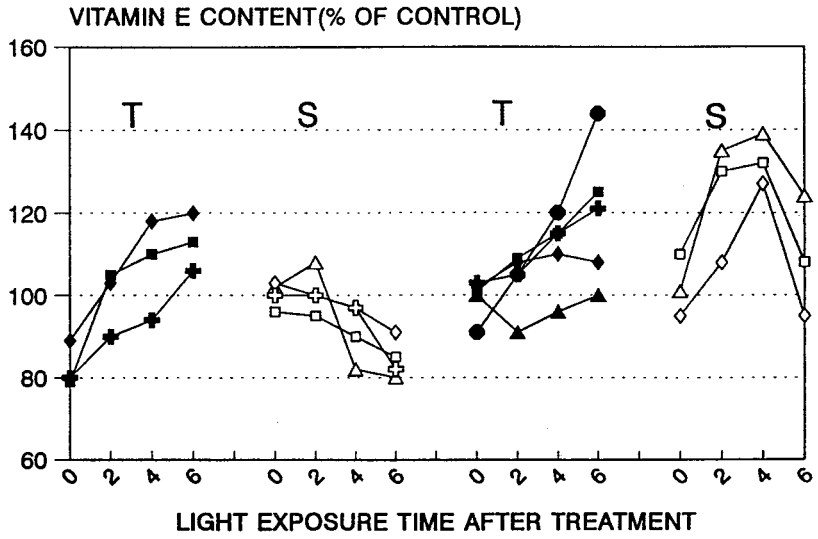


Fig. 2. Change of vitamin E contents in various plant species after treatment of oxyfluorfen.

Rice cultivars	
Tolerant	Susceptible
Hawon	HP857
Hunan31	HP907
Baru	HP1033
	Weldpally

Tolerant spe.	Susceptible spe.
Ryegrass	Barnyardgrass
Tallfescue	Large crabgrass
Weepinglovegrass	Indian zointvetch
Chinese cabbage	
Mustard	

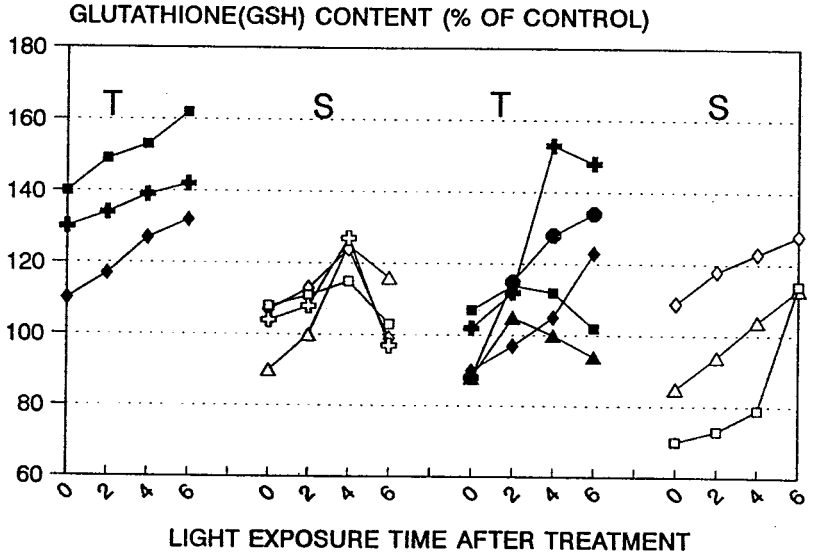


Fig. 3. Change of glutathione(GSH) contents in various plant species after treatment of oxyfluorfen.

Rice cultivars	
Tolerant	Susceptible
Hawon	HP857
Hunan31	HP907
Baru	HP1033
	Weldpally

Tolerant spe.	Susceptible spe.
Ryegrass	Barnyardgrass
Tallfescue	Large crabgrass
Weepinglovegrass	Indian zointvetch
Chinese cabbage	
Mustard	

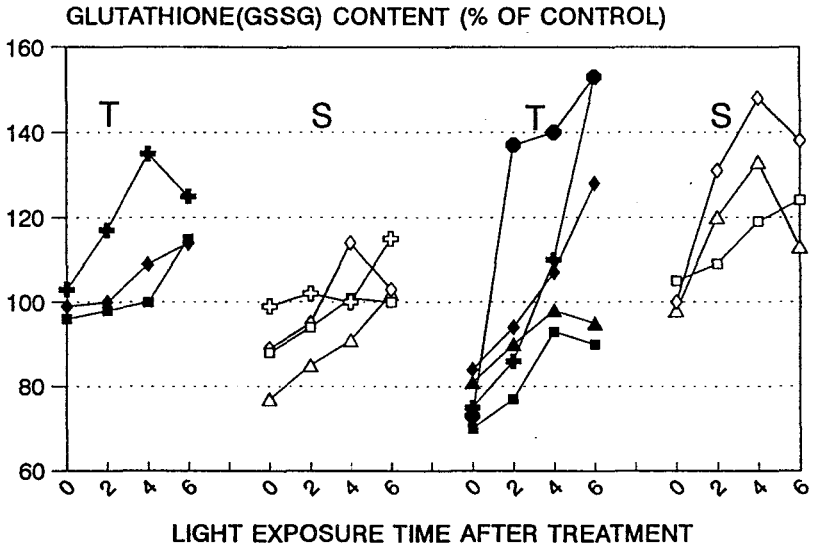


Fig. 4. Change of glutathione(GSSG) contents in various plant species after treatment of oxyfluorfen.

Rice cultivars		Tolerant spe.	Susceptible spe.
Tolerant	Susceptible	Ryegrass	Barnyardgrass
Hawon	HP857	Tallfescue	Large crabgrass
Hunan31	HP907	Weepinglovegrass	Indian zointvetch
Baru	HP1033	Chinese cabbage	
	Weldpally	Mustard	

References

1. Breaux, E. J., J. E. Patanella and E. F. Sanders. 1987. Chloracetanilide herbicide selectivity ; Analysis of glutathione and homoglutathione in tolerant, susceptible, and safened seedling. J. Agric. Food chem. 55: 474-478
2. Edwards, R. and Owen, W. J. 1986. Comparison of glutathione S-transferase of *Zea may* responsible for herbicide detoxification in plants and suspension cultured cells. Planta 169: 208-215.
3. Finckh, B. F and K. J. Kunert. 1985. Vitamin C and E : An antioxidative system against herbicide-induced lipid peroxidation in higher plants. J. Agric. Food chem. 33: 574-577.
4. Hissin, P. J. and R. Hilf. 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74:214-226.
5. Kunert, K. J. and P. Boger. 1984. The diphenyl ether herbicide oxyfluorfen : Action of antioxidants. J. Agric. Food chem. 32: 725-728.
6. Lambert, R. and P. Boger. 1984. Peroxidative activity of oxyfluorfen with regard to carotenoides in Scenedesumus. J. Agric. Food chem. 32:523-526.
7. Law, M. Y., S. A. Charles and B. Halliwell. 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. Biochem. J. 210 : 899-903.
8. Matsumoto, H., S. Kojima and K. Ishizuka. 1990. Characteristics of herbicidal injury by diphenyl-ether herbicides oxyfluorfen and bifenox in *Lamela pausicostata* Hegeim. Weed research, Japan 35(1): 36-43.
9. Matsumoto, H. and S. O. Duke. 1990. Acifluorfen - methyl effects on porphyrin synthesis in *Lemna pausicostata* Hegelm. 6746. J, Agric. Food chem. 38(11): 2066-2071.
10. Orr, G. L. and F. D. Hess. 1981. Characterization of herbicidal injury by acifluorfen-methyl in excised cucumber (*Cucumis sativus* L.) cotyledons. Pestic. Biochem. Physiol. 16:171-178.
11. Rennenberg, H. 1982. Glutathione metabolism and possible biological roles in higher plants. Phytochemistry 21 : 2771-2781.
12. Ridley, S. M. and J. Ridlet. 1979. Interaction of chloroplasts with inhibitors-location of carotenoid synthesis and inhibition during chloroplast development. Plant physiol. 63:392-398.
13. Schmidt, A. and K. J. Kunert. 1986. Lipid peroxidation in higher plants. The role of glutathion reductase. Plant physiol. 82: 700-702.
14. Witkowski D. A. and B. P. Halling. 1989. Inhibition of plant protoporphyrinogen oxidase by the herbicide acifluorfen - methyl. Plant physiol. 90:1239-1242.

PERSISTENCE AND ITS SIMULATION OF PENDIMETHALIN IN SOIL

Young-Hee Moon, Yong-Hwi Kim, Young-Sok Kim and Yoon-Tae Kim[1]

Department of Agricultural Chemistry, Chonbuk National University
Chonju 560-756, Korea

Abstract. The present experiments were made to gain further information on the persistence of the herbicide pendimethalin in soil under Korea conditions. In the laboratory experiments, degradation of pendimethalin in soil followed first-order reaction kinetics. The rate of loss was influenced by soil temperature and soil moisture content. The half-life was 148.7, 48.5 and 26.1 days at 10, 18 and 25°C and 109.5, 70.3 and 50.3 days at 20, 40 and 60 % of field water capacity, respectively. The adsorption distribution coefficient (Kd) was 19.72. In a field study prepared in autumn with undisturbed soil columns (30 X 10 cm d.i.), pendimethalin residues were largely confined to the top 2 cm of soil over a 2 month period. Half-life of pendimethalin was about 35 and 70 days in spring and autumn, respectively. Predicted persistence value obtained by a computer model of herbicide behaviour coincided with the observed persistence value.
Key words: Pendimethalin, Soil, Mobility, Persistence, Simulation.

Introduction

In general, persistence and mobility of herbicides in soil are greatly influenced by environmental conditions such as temperature, rainfall, soil moisture, soil characteristics, etc. Therefore, computer models that take account of variations in environmental data, soil characteristics, and degradation and adsorption properties of the chemical, such as those decribed Walker and Barnes[6], Nicholls *et al.*[3] and Walker[5], can be very useful in understanding the variability of the pesticides behaviour in soils under practical field conditions.

Pendimethalin [N-(1-ethylpropyl)-2,6-ditro-3,4-xylidine] is used for selective control of annual weeds in crops such as corn, soybeans, peas and several vegetable crops. Persistence of pendimethalin is influenced by soil temperature and moisture conditions and soil type[10]. However, there were few reports on relationship between the degradation of pendimethalin in soil and environmental conditions in Korea.

In the present experiments for further information of the degradation and mobility of pendimethalin in soil, the detailed studies were performed on the effects of temperature and soil moisture on rates of loss in laboratory incubation. Persistence and movement of residues in soil were also measured under natural conditions in the field. The data were evaluated using a computer model of herbicide persistence.

Materials and Methods

Soil and Herbicide

The soil used was sampled from Agricultural Experiment Farm at Chonbuk national University, Chonju, Chonbuk in Korea. It was collected from the 0-20 cm horizon. The main properties of the soil were as follows: kaoline clay mineral, clay loam (34.3 % clay content), pH(H_2O) 7.29, 1.36 % organic mater, 1.4 g/cc bulk density, 37.3 % field moisture capacity. The herbicide used was a commercial emulsifiable concentrate formulation of pendimethalin (31.7% a.i.) and analytical grade pendimethalin.

Laboratory study

A freshy soil sample was passed through a 2-mm mesh sieve and duplicate amounts (10g) were dried for 5 hrs at 110°C to determine soil moisture content. A suspension of

[1]Present Address: Pesticide Residue Lab., Hankook Samkong Co., Ltd., Pyungchang, Chongro, Seoul 110-102, Korea

the commercial formulation of pendimethalin in water was added to separate 1 kg samples of soil to give an initial concentration of 8.0 mg a.i./kg-dry soil. For the effect of temperature on the degradation, the three samples with soil moisture content of 60 % of field capacity were incubated at 10, 18, and 25 °C. For the effect of soil moisture content on the degradation, the other 3 samples with moisture contents of 20, 40 and 60 % of field capacity were incubated at 18°C. At designated intervals during the incubation period, duplicate about 40 g of subsamples were stored under frozen condition until they were analyzed.

Herbicide adsorption

Amounts of air-dry soil (5g) were weighed into 100ml conical flasks and mixed with 50 ml water solutions of emulsifiable concentrate formulation of pendimethalin. There were duplicate samples with initial concentrations of 1, 5, 10, 50 and 100 ug/ml. The samples were shaken on a vertical shaker for 8 h. The samples were centrifuged at 10,000 rpm. The supernatant was extracted with n-hexane. The hexane extract was used for determination of pendimethalin.

Field experiment

For the mobility experiment, the outdoor study was made using a mini-lysimeter system. The lysimeter system comprised PVC tubes (10 cm i.d., 30 cm long) containing undisturbed soil from the field site. The columns of soil were collected in early September, 1992 by driving the tubes into the soil until the soil surface was approximately 1 cm below the top of the tube. The columns were vertically buried into the ground. The soil surface inside the columns was level with that ouside. Pendimethalin was applied to the surface of 3 columns on October 5, 1992. A suspension of the commercial formulation in water (2 ml) was pipetted dropwise to the surface of each column to give rate of 2.38 kg a.i./Ha. One whole column of soil was removed immediately after application of the herbicide and single columns were removed at 20 and 69 days. The columns of soil were divided into successive 2-cm segments from the surface downwards using a sectioning apparatus. Each soil samples was mixed thoroughly and then kept frozen until they were analyzed.

For persistence of pendimethalin in field soil, uncropped plots (5 X 1.3 m) in the Farm were prepared and pendimethalin was sprayed to the soil surface of duplicate plots at 2.38 kg a.i./Ha. on March 11, and October 5, 1992. Immediately after application, 10 cores (3cm i.d. to a depth of 10 cm) were taken from each plot at random positions. The cores from each plot were bulked together, thoroughly mixed by several times through a 2-mm sieve and the total weight of sieved soil recorded. The samples were frozen until they were analyzed. Further soil sample were taken at the designated intervals.

Herbicide analysis

Duplicate 40 g amounts of the sampled soils were shaken for 1 h on a vertical shaker and then filtered. The concentration of pendimethalin in the extracts was determined directly by a Shimadzu GC-14A model equipped with FTD gas chromatograph. A glass column (1.2m x 3 mm i.d.) packed with 3 % SE-30 was used and the operating temperatures of injection port, column and detector were 250, 245 and 270 °C, respectively.

Results and Discussion

The straight line relationships obtained from the effect of soil temperature and soil moisture content on the degradation of pendimethalin showed close correspondence to first-order degradation kinetics ($r>0.96$) as shown in Fig. 1. With the effect of temperature half-lives were 148.7, 48.5 and 26.1 days at 10, 18 and 25 , respectively. There was a remarkable effect of temperature on degradation rate of pendimethalin.

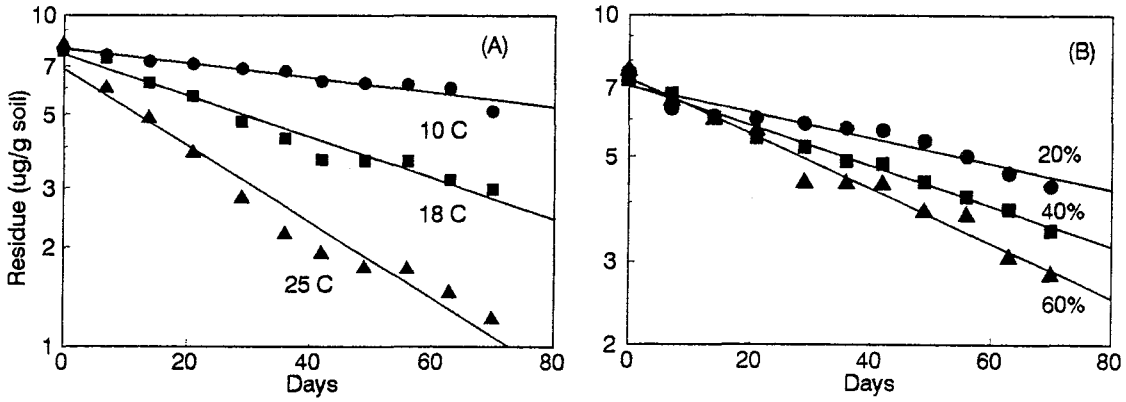


Fig. 1. Effect of soil temperature(A) and soil moisture(B) on the degradation of pendimethalin.

In some studies[8,9], the effect of soil temperature on herbicide degradation were often characterized using the Arrhenius equation. Appropriate data in the results of the degradation were fitted to the Arrhenius equation by linear regression analyses of the logarithm of the half-lives against the reciprocal of the absolute temperature as shown in Fig. 2-A. The Arrhenius activation energy derived from the slope of the lines was 81.7 KJ/mol. The value of activation energy was 27-69 KJ/mol and 68-78 KJ/mol in the effect of soil temperature on the degradation of simazine[9] and alachlor[1,8], respectively.

In the effect of soil moisture content, half-life of pendimethalin at 20, 40 and 60 % of field water capacity were 109.5, 70.3 and 50.3 days, respectively. In some experiments[1,8,9], the effect of soil moisture on herbicide degradation rates was characterized using an empirical equation: $H=AM^{-B}$ (H : half-life at moisture content M, A and B : constants). The value of the constants A and B derived from the present data were 382.6 and 0.66, respectively (Fig.2-B). The slope of the line (B) gives a measure of the moisture depencence of degradation. The B value in the effect of soil moisture was 1.33-2.07 for alachlor in 5 soils and 0.03-1.28 for simazine in 16 soils[8,9].

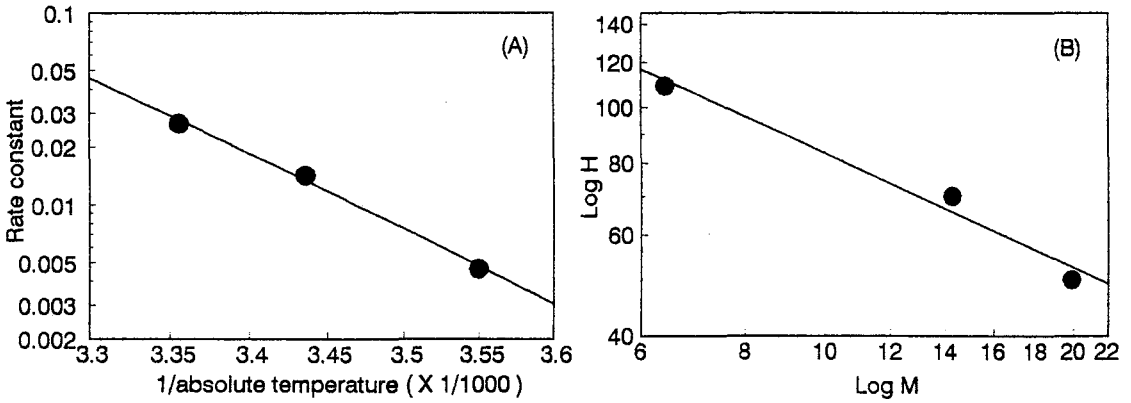


Fig. 2. Arrhenius plots(A) and Empirical plots(B) for the degradation of pendimethalin in the soil.
H:Half-life, M:soil moisture.

The adsorption isotherm for pendimethalin in the soil was defined by linear regression analysis of the amount adsorbed (ug/g) against the equilibrium concentration (ug/ml) as shown in Fig. 3. The adsorption distribution coefficient (Kd) was unexpectedly high value of 19.72. The Kd value of ethoprophos in the same soil was 0.27 in our previous study[2]. In herbicide adsorption experiments[4,7,8], the Kd value of alachlor, metolachlor and napropamide was 0.52-13.47, 0.48-10.94, and 2.8-15.9, respectively. It seems that the adsorption rate of pendimethalin is very high, while herbicide adsorption was positively correlated with content of soil organic matter and clay.

The result of pendimethalin mobility in field soil was summarized in Fig. 4. The pendimethalin was distributed mostly in 0-2 cm soil depth, while little found in 2-4 cm soil depth. The narrow mobility of pendimethalin may be due to the high adsorption rate.

In the period of field experiment, the rages of highest and lowest temperatures were 10-20°C and 0-10°C in spring, and 0-20 °C and -10-10°C in autumn, respectively. In general, the temperature gradually increased in spring and decreased in autumn.

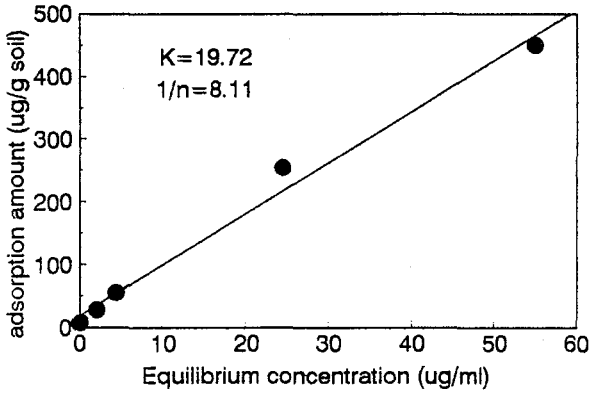


Fig. 3. Adsorption isotherm of pendimethalin by soil.

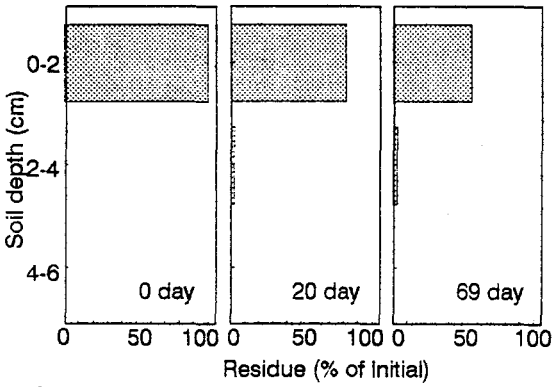


Fig. 4. Vertical distribution of pendimethalin in semi-lysimeter soil.

Results from the persistence experiment are shown in Fig. 5. The amount of residues declined progressively throughout the experiment. The rates of loss reflected differences in weather conditions, particularly temperature. The degradation was much faster in the application in March than in the application in October. The half-life was about 35 and 70 days in spring and autumn, respectively.

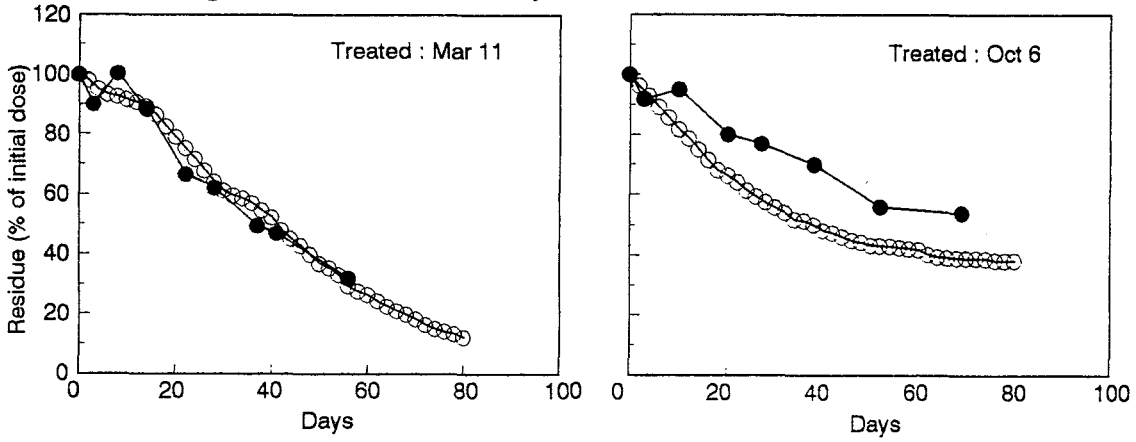


Fig. 5. Persistence of pendimethalin in the field soil. Observed data (●), Predicted data (○).

The constants derived from the laboratory experiments, the value of activation energy and the of A and B in empirical equation, were used in conjugation with the appropriate weather data and soil properties in the models of pesticide persistence described by Walker and Barnes[6]. The results from the persistence model are shown in Fig. 5. As previous reports on persistence prediction for some herbicides [1,3,8,9], the observed residues of pendimethalin at different times were predicted with reasonable accuracy by a computer model.

References

1. Moon, Y. H. & A. Walker. 1991. The degradation and mobility of alachlor in a sandy loam soil. Brighton Crop Protection Conference -Weeds. P:499-506.
2. Moon, Y. H., Y. T. Kim, Y. S. Kim & S. K. Han. 1993. Simulation and measurement of degradation and movement of insecticide ethoprophos in soil. Korean J. Environ Agric. **12(3)**:209-218.
3. Nicholls, P. H., A. Walker & R. J. Baker. 1982. Measurement and simulation of the movement and degradation of atrazine and metribuzin in a fallow soil. Pestic. Sci. **13**:484-494.

4. Peter, C. J. & J. B. Weber. 1985. Adsorption, mobility and efficacy of alachlor and metolachlor as influenced by soil properties. Weed Sci. **33:**874-881.
5. Walker, A. 1987. Evaluation of a simulation model of herbicide model of herbicide movement and persistence in soil. Weed Res. **27:**143-152.
6. Walker, A. & A. Barnes. 1981. Simulation of herbicide persistence in soil, a revised computer model. Pestic. Sci. **12:**123-132.
7. Walker, A., P. A. Brown & P. R. Mathews. 1985. Persistence and phytotoxicity of napropamide residues in soil. Ann. appl. Biol. **106:**323-333
8. Walker, ., Y. H. Moon & S. J. Welch. 1992. Influence of temperature, soil moisture and soil characteristics on the persistence of alachlor. Pestic. Sci. **35:**109-116.
9. Walker, A. *et al.* 1983. EWRS Herbicide-Soil Working Group : Collaborative experiment on simazine persistence in soil. Weed Res. **23**: 373-383.
10. Zimdahl, R. L., P. Catizone & A. C. Butcher. 1984. Degradation of pendimethalin in soil. Weed Sci. **32:**408-412.

RELATIONSHIP BETWEEN CONCENTRATION OF THENYLCHLOR IN SOIL WATER AND ITS HERBICIDAL ACTIVITY

M.Onoe[1], K.Kobayashi[2], D.J.Lee[2], Y.Yogo[3] and H.Sugiyama[3]
[1]Hokko Chemical Industry Co., Ltd., Atsugi, Kanagawa 243, Japan
[2]Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305, Japan
[3]National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan

Abstract. The herbicidal activity of soil-applied thenylchlor[2-chloro-*N*-(3-methoxy-2-thienyl methyl)2',6'-dimethylacetanilide] on *Echinochloa crus-galli* var. *formosensis* was more remarkable in sandy loam than in light clay. These soils were separated into soil water and centrifuged-soil by centrifugation with a stainless double tube, and the amount of thenylchlor in these fractions were determined. Growth inhibition was well correlated with the concentration in soil water, but not with the amount in centrifuged-soil. The adsorption coefficient was greater in the light clay. The downward mobility of thenylchlor was greater in the sandy loam, and the herbicidal activity in the layers of each soil also closely corresponded to the concentration in each soil water. It was suggested that the herbicidal activity of thenylchlor was dependent upon the concentration in soil water, and that the concentration of thenylchlor in soil water was dominated by adsorption in the soil.
Key words : thenylchlor, concentration in soil water, adsorption by soil, downward mobility in soil, herbicidal activity.

Introduction

Thenylchlor[2-chloro-*N*-(3-methoxy-2-thienyl methyl)-2',6'-dimethyl acetanilide] is a newly developed α-chloroacetamide herbicide for annual and perennial paddy weed control in transplanted rice paddy fields[3-6]. The adsorption of thenylchlor in soil was affected by the soil characteristics, especially the organic matter and clay contents, in a manner similar to other α-chloroacetamide herbicide, *e.g.* butachlor[*N*-(butoxymethyl)-2-chloro-2', 6'-diethylacetanilide] and pretilachlor[2-chloro-2',6'-diethyl *N*-(2-propoxyethyl) acetanilide][5]. The downward mobility of the herbicide in soil was relatively slight and was affected by the soil characteristics[5]. We revealed that the herbicidal activity of butachlor, pretilachlor, mefenacet[(2-benzothiazol-2-yloxy)-*N*-methylacet anilide] and metolachlor[2-chloro-2'-ethyl-*N*-(2-methoxy-1-methylethyl)-6'-methylacetanilide] was well correlated with the concentration of water-soluble ingredients in soil[8-11], and suggested that herbicidal activity of soil applied herbicide was dependent upon the concentration in soil water. It is well-known that the adsorption in soil and activity of soil applied herbicide is influenced by soil characteristics, but it has been little information on relationship between herbicidal activity and the performance in soil. The objective of the present study was to elucidate the relationship between performance of thenylchlor in soil and its herbicidal activity.

Materials and Methods

Soils and bioassay. Light clay and sandy loam were used in the experiments. The characteristics of these were shown in Table 1.

Table 1. Characteristics of the soils used in the experiments.

Soil texture	Organic matter (%)	CEC (me/100 g)	pH H_2O	pH KCl	Sand (%)	Silt (%)	Clay (%)	Maximum water capacity (% of dried soil)
Light clay	2.41	20.5	5.51	4.61	44.3	29.7	26.0	63.8
Sandy loam	0.43	10.8	6.13	4.91	67.0	22.7	10.3	49.5

Effect of thenylchlor on the growth of *E. crus-galli* was examined in soil applied with thenylchlor, in soil water separated from the soil, as described later and in aqueous solution of thenylchlor. The plants were placed in an incubator (25 ℃, 14 hr-illumination at 4 Klux) for 3 days, and the shoot length was measured.

Extraction and determination of thenylchlor in soil. The soil previously applied with thenylchlor solution was put in a inner tube and centrifuged with double tubes(Fig. 1) at 13,000 x g for 40 min to separate it into the solution collected in the bottom of the outer tube and the soil remaining in the inner tube by the modified method of Seto[7], : the soil water was generally considered as plant available soil water(hereinafter the former and the latter was abbreviated as available soil water and centrifuged-soil, respectively).

The amount of thenylchlor in each fractions were determined by gas chromatographic (GC) analysis. The detectable limit of thenylchlor was 0.008 ppm, and the recoveries from the centrifuged-soil and the available soil water were over 90 %, respectively.

Downward mobility of thenylchlor in soil The downward mobility of thenylchlor in soil was determined according to the method of Hikawa and Lee[1] using soil column with a minor modification. The soils were adjusted to 7 cm in height and water was added to 3 cm from the soil surface. Thenylchlor dissolved in acetone was applied at a dosage of 540 g/ha to the paddy water(final acetone concentration was less than 0.1 %). After 24 hours, the paddy water flowed from the bottom of the column at 3 cm/day for 10 days, and the upper 5 cm soils in the column was separated into every 1 cm section. The herbicidal activity of each soil layer was assayed with *E. crus-galli*, and the amount of thenylchlor in the available soil water and in the centrifuged-soil in each section were determined.

Adsorption and desorption of thenylchlor in and from soil. Adsorption of thenylchlor in soils were determined according to the method of Kuwatsuka[3].

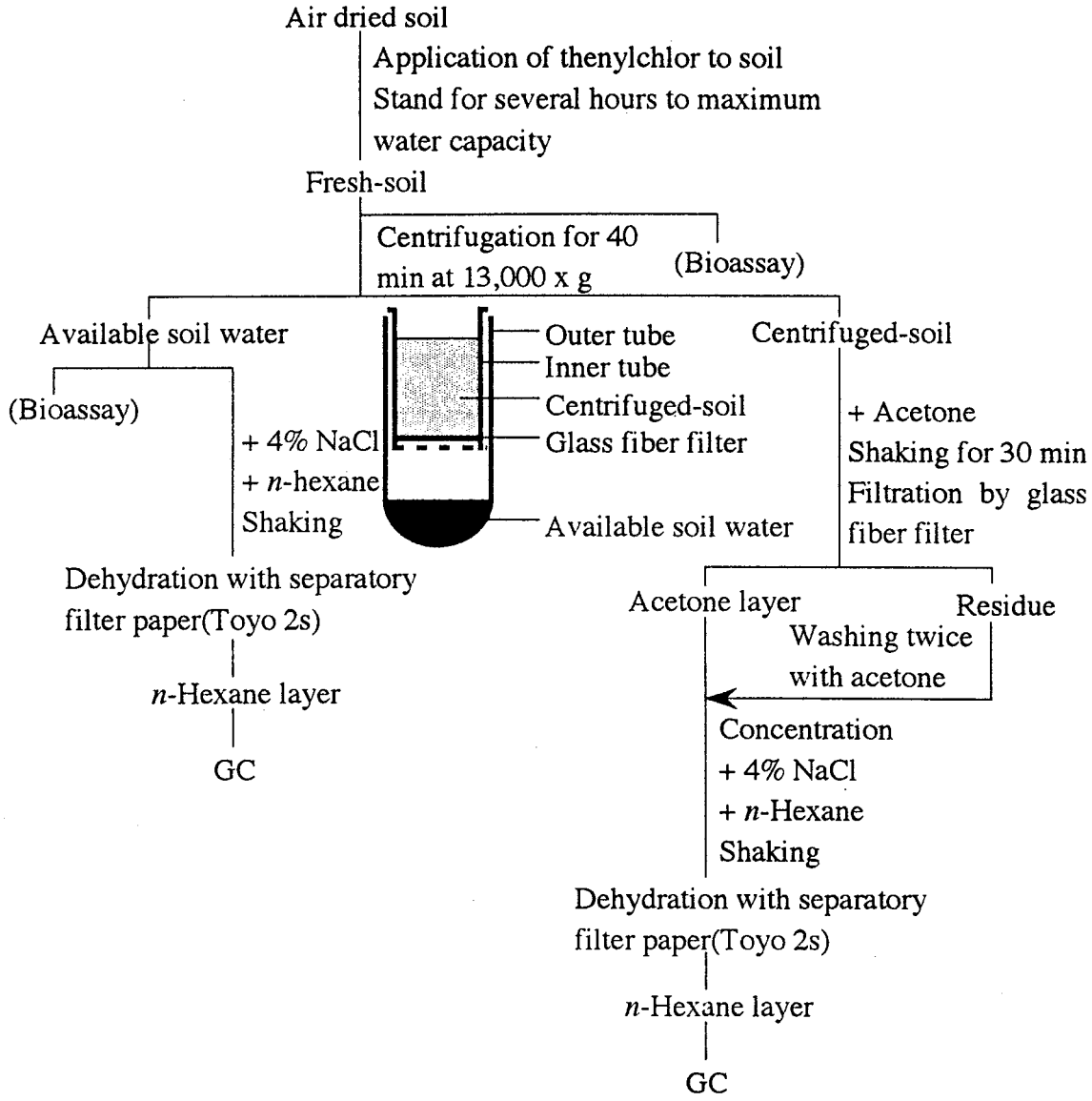


Fig. 1. Scheme for determination of thenylchlor content in centrifuged-soil and available soil water.

Each mixture of thenylchlor solution at the various designated concentrations and soil was shaken for 2 hours at 25°C in the dark. The adsorption of thenylchlor was calculated from the decrease in the concentration in the liquid phase.

Desorption of thenylchlor in the soil was determined as follows. The soil applied with thenylchlor solution was centrifuged to separate into the centrifuged-soil and the available soil water. Just after the separation, a same volume of available soil water, which had previously collected by the centrifugation from the non-treated soil was added to the centrifuged-soil and the available soil water was collected again. The procedures were repeated further two more times, and the amount of thenylchlor in the centrifuged-soil and the available soil water were determined.

All treatments were conducted with three replicates.

Results and discussion

Herbicidal activity of thenylchlor on *E. crus-galli* was more remarkable in the sandy loam than in the light clay(Table 2). The metabolites of thenylchlor in soil has no herbicidal activity. The amount of thenylchlor in the available soil water were greater in the sandy loam at each concentration applied, whereas in the fresh- and the centrifuged-soil it was greater in the light clay. The growth of *E. crus-galli* in the aqueous solution of thenylchlor was inhibited to be a similar extent to those in

Table 2. Growth of *E. crus-galli* and amount of thenylchlor in the light clay and the sandy loam applied with various concentrations of solution.

Concentration applied (μM)	Amount of thenylchlor						Plant growth	
	Fresh-soil (nmol/ml)		Centrifuged-soil (nmol/ml)		available soil water (nmol/ml)		Shoot length (% of control)	
	LiC*	SL*	LiC*	SL*	LiC*	SL*	LiC*	SL*
0.1	0.399	0.197	0.645	0.331	ND**	ND**	101.5	99.8
0.3	0.538	0.307	0.847	0.509	0.018	0.041	85.3	56.8
1.0	1.481	1.192	2.296	1.986	0.043	0.119	66.4	35.9
3.0	7.414	4.973	11.368	8.277	0.237	0.570	25.4	25.6

* LiC : light clay, SL : sandy loam, ** ND : not detected

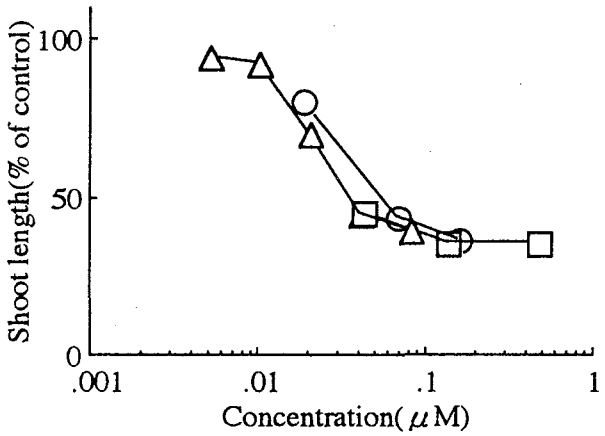


Fig. 2. Growth of *E. crus-galli* in available soil water and in aqueous thenylchlor solution.
—○— available soil water from light clay
—□— available soil water from sandy loam
—△— aqueous thenylchlor solution

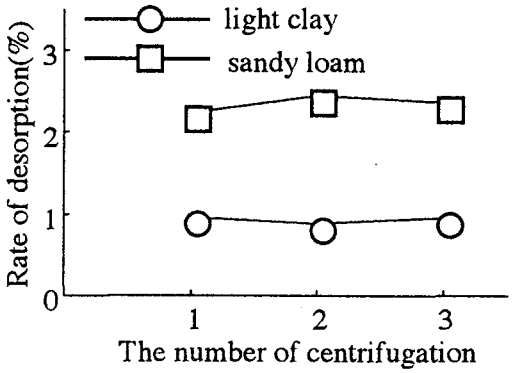


Fig. 3. Rate of desorption of thenylchlor from the soils previously applied with the herbicide.

$$\text{Desorption} = \frac{\text{Total amount in available soil water}}{\text{Total amount in fresh-soil}} \times 100$$

the available soil water separated from both soils previously applied with thenylchlor (Fig. 2), supporting that the herbicidal activity of thenylchlor applied to soil was induced by the concentration in available soil water but not by the amount in fresh-soil. The rate of desorption of thenylchlor in soil was greater in the sandy loam, suggesting that the leaching of thenylchlor was higher in sandy loam. Actually, the downward mobility of the herbicide in soil is greater in the sandy loam(Table 3), and these results supported that the herbicidal activity of thenylchlor was dominated by its concentration in plant available soil water.

Table 3. Downward mobility of thenylchlor in soil column.

Depth (cm)	Amount of thenylchlor: Fresh-soil (nmol/ml) LiC*	SL*	Centrifuged-soil (nmol/ml) LiC*	SL*	Available soil water (nmol/ml) LiC*	SL*	Plant growth: Shoot length (% of control) LiC*	SL*
0-1	8.01	3.73	12.30	6.00	0.28	0.38	22.1	26.4
1-2	3.06	4.46	4.61	7.06	0.12	0.42	29.8	28.1
2-3	0.78	3.26	1.17	5.17	0.28	0.29	100.0	28.1
3-4	0.52	1.83	0.76	2.82	0.02	0.27	98.2	29.8
4-5	0.29	0.52	0.44	0.83	0.01	0.05	95.0	72.4

* LiC : light clay, SL : sandy loam

The soil sorption coefficient(Kd) values for thenylchlor was higher in light clay than in sandy loam(Fig. 4). Comparing the soil sorption constant(Koc) values between two soils, the values was slightly greater in the light clay(Fig. 4). It thus was suggested that the concentration of thenylchlor in the available soil water is primarily dominated by the organic matter content in soil. Consequently it might be possible to reach tentative conclusions that herbicidal activity of soil-applied herbicide such as thenylchlor is induced by the concentration in the available soil water, and that the concentration in soil water is dominated by the balance of the adsorption in and the desorption from soil.

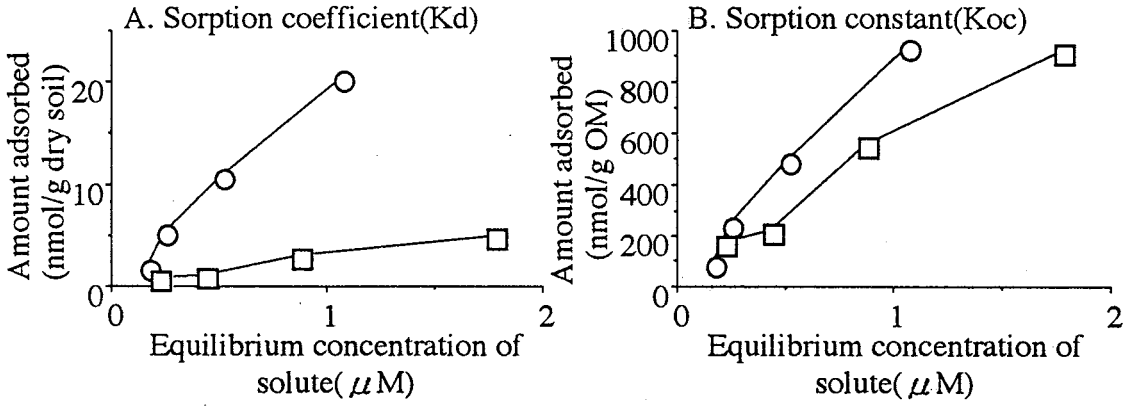


Fig. 4. The adsorption isotherm of thenylchlor in light clay(○) and sandy loam(□).

Acknowledgement : We are grateful to Tokuyama Co., Ltd. for the gift of thenylchlor.

References

1)Hikawa, M. and I. Lee 1981. Assay of herbicidal efficacy. In "Methods in pesticide science, Ⅲ. Herbicide(in Japanese)" ed. by J. Fukami, Y. Uesugi, K. Ishizuka and C. Tomizawa, Soft Sci. Inc., Tokyo., pp 43-63.

2)Kuwatsuka S. 1981. Herbicide in soils. In "Methods in pesticide science, IV. Environmental chemistry and analysis(in Japanese)" ed. by J. Fukami, Y. Uesugi, K. Ishizuka and C. Tomizawa, Soft Sci. Inc., Tokyo., pp 166-185.

3)Ogasawara, M., M. Konnai, T. Takematsu, S. Kato and M. Ishizaki 1989. Herbicidal activity of new α-chloroacetamide derivatives(in Japanese with English summary). Weed Res. Japan, **34**, 131-137.

4)Ogasawara, M., M. Konnai, T. Takematsu, S. Kato and M. Ishizaki ,1989. Properties of new herbicide, NSK-850(in Japanese with English summary). Weed Res. Japan, **34**, 138-145.

5)Ogasawara, M., K. Ishikawa and M. Konnai 1989. Effect of depth of flooding water on rice injury cased by α-chloroacetamide herbicides(in Japanese with English summary). Weed Res. Japan, **35**, 102-108.

6)Ogasawara, M. 1992. Herbicidal property of new potent α-chloroacetamide, NSK-850(in Japanese). Chemical Regulation of Plants, **27**, 212-221.

7)Seto, M. 1980. Rate of CO_2 evolution from soil in relation to content of soil water and amount of dissolved organic carbon in soil solution(in Japanese with English summary). Jap. J. Ecol., **30**, 385-391.

8)Sugiyama, H., K. Komamiya and K. Kobayashi 1990. Butachlor and pretilachlor concentration in paddy soils in relation to phytotoxicity to rice plant(in Japanese with English summary). Weed Res. Japan, **35**, 116-121.

9)Sugiyama, H., K. Komamiya and K. Kobayashi 1990. Changes in the concentration of residues of pendimathalin applied to soil and effect on plant growth(in Japanese with English Summary). Weed Res. Japan **35**, 122-128.

10)Sugiyama, H., K. Komamiya and K. Kobayashi 1990. Mefenacet concentration in the paddy soil and its phytotoxicity to rice plant(in Japanese). Weed Res. Japan, **35**, 180-182.

11)Sugiyama, H. and K. Kobayashi 1993. Herbicidal activity and the residual state of metolachlor in upland soil. Weed Res. Japan, **38**, 300-306.

ACTION MECHANISM OF A HERBICIDE, FLUTHIACET-METHYL

T. Shimizu[1], N. Hashimoto[1], T. Nakao[1], H. Mizutani[1], T. Unai[1], M. Yamaguchi[2], H. Abe[1], I. Nakayama[1]

[1]Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., Kikugawa-cho, Ogasa-gun, Shizuoka, 439 Japan
[2]K-I Chemical Research Institute Co., Ltd., Fukude-cho, Iwata-gun, Shizuoka, 437-12 Japan

Abstract. A novel isourazole herbicide, fluthiacet-methyl promoted the leakage of electrolytes from cotyledons of velvetleaf and cotton plants that are sensitive to this compound. It induced the accumulation of protoporphyrin IX (Proto IX) in cotyledons of cotton and inhibited chlorophyll (Chl) biosynthesis in cotyledons of velvetleaf and cotton at low concentrations (I_{50} values, 10-12 nM). Fluthiacet-methyl was converted to its urazole by glutathione S-transferase that had been partially purified from velvetleaf. The urazole inhibited protoporphyrinogen oxidase (Protox, E.C. 1.3.3.4) from some plants, including velvetleaf, at low concentrations (I_{50} values, 5.1-11 nM), whereas fluthiacet-methyl was not as potent. The effects in vivo (electrolyte leakage and inhibition of Chl biosynthesis) of fluthiacet-methyl were correlated with the inhibition of Protox activity by the urazole and not with the action of fluthiacet-methyl itself. From these results, it is concluded that fluthiacet-methyl inhibits Protox activity after conversion to the corresponding urazole by glutathione S-transferase. It is in this way that fluthiacet-methyl exerts its effect as a light-dependent peroxidizing herbicide.

Key words. fluthiacet-methyl, KIH-9201, light-dependent peroxidizing herbicide, protoporphyrinogen oxidase (E.C. 1.3.3.4), chlrophyll biosynthesis

Introduction

We have developed a novel isourazole-type herbicide, namely, fluthiacet-methyl (chemical name, methyl [[2-chloro-4-fluoro-5-[(5,6,7,8-tetrahydro-3-oxo-1H,3H-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]acetate; experimental code name, KIH-9201; Fig. 1) for control of weeds in fields of soybean and corn (Yamaguchi et al. 1988). This herbicide kills weeds that are sensitive to it at an extremely low application rate (5-10 g/ha) and it is especially effective against velvetleaf (Miyazawa et al. 1993, Kobayashi et al. 1994). The herbicide possesses an isourazole chemical structure similar to those of experimental isoimide herbicides; which have been shown to have the action mechanism of same as the N-phenylimide light-dependent peroxidizing herbicides (Hoshi et al. 1993). In the present report, we describe the mechanism of action of fluthiacet-methyl as a light-dependent peroxidizing herbicide in higher plants, as elucidated by an examination of four physiological parameters: electrolyte leakage; inhibition of Chl biosynthesis; accumulation of porphyrins; and inhibition of Protox activity. We also show the relationship between the mechanism of action of fluthiacet-methyl and the newly confirmed enzymatic isomerization of this compound (Mizutani et al. 1994b), which is catalyzed by glutathione S-transferase (GST).

Materials and Methods

Chemicals

Fluthiacet-methyl and the derivatives of this herbicide tested in this study (Fig. 1) were synthesized by the previous reported methods (Yamaguchi et al. 1988). Other compounds were purchased from commercial sources.

Measurement of leakage of electrolytes

Cotton (Gossypium hirsutum L. var. Cocher) and velvetleaf (Abtilon theophtasti Medic) were used in the experiment. Etiolated cotyledons (0.5-1 g fr wt) of these plants were incubated in 25 ml of distilled water that contained a compound to be tested at 27 °C for appropriate periods of time under illumination from fluorescent lamps (15,000-18,200 lux). The conductivity of the solution was then measured with a conductivity meter.

Measurement of Chl biosynthetic activity

The biosynthesis of Chl was monitored in terms of the greening of etiolated cotyledons of cotton and velvetleaf. Chl was extracted with 80% acetone from cotyledons that have been incubated as described above for the measurement of electrolyte leakage. Total Chl contents were determined by measuring the absorbance at 665 and 649 nm with a spectrophotometer.

Extraction and analysis of porphyrins

Porphyrins were extracted with basic acetone from etiolated cotton cotyledons, that had been treated with fluthiacet-methyl, and analyzed by HPLC. Porphyrins were detected by fluorescence above 510 nm (with excitation at 410-430 nm) and they were identified by co-chromatography with the authentic standards (Shimizu et al. 1995).

Preparations of Protoporphyrinogen IX (Protogen IX) and of etioplasts, and the assay of Protox activity

Protogen IX was prepared by reducing Proto IX with sodium amalgam. Two kinds of reducing agent, namely, dithiothreitol (DTT) and sodium isoascorbate were used in order to avoid auto-oxidation of Protogen IX (Shimizu et al. 1995). Etioplasts were prepared from the etiolated leaves of corn (Zea mays L. var. Pioneer 3352), cotton, soybean [Glycine max (L.) Merr. var. Deltapine 506], velvetleaf and cocklebur (Xanthium strumarium L.), which turned slightly green upon standing in room light. The etioplasts were sonicated with Tween 20 [final concentration, 0.5 % (v/v)], just before assays of enzymatic activity. The assay of Protox activity was carried out basically as described by Jacobs and Jacobs (1982), but, again, two reducing agents were employed (Shimizu et al. 1995).

Reactions of fluthiacet-methyl with SH-compounds and identification of reaction products

Fluthiacet-methyl at 10 mM was incubated at room temperature (25 °C) with an SH-compound, namely, DTT, glutathione (GSH) or cysteine (1 mM), in 20 mM Tris-HCl (pH 8.0) for appropriate periods of time. The solution was then analyzed directly by HPLC using ODS column. The reaction product was identified by liquid chromatography-positive-ion frit FAB ionization mass spectrometry with LC/MS.

Purification of glutathion S-transferase (GST) and reaction of fluthiacet-methyl with GST/GSH

GST from shoots of green velvetleaf was partially purified by ammonium sulfate precipitation, with subsequent anion-exchange column chromatography on DEAE-Toyopearl 650 M, gel-filtration column chromatography on Cellulofine GC-700 m, anion-exchange column chromatography on a Mono Q column, gel-filtration column chromatography on Superose 12. 1-Chloro-2,4-dinitrobenzene was employed as the substrate for assays. The enzymatic reaction of fluthiacet-methyl with GST/GSH was examined at 30 °C in a reaction mixture (2 ml) of the following composition: 100 mM potassium phosphate buffer (pH 6.5), 1 mM GSH, 10 μM fluthiacet-methyl and purified GST. The reaction product was identified by HPLC, after pre-treatment of the mixture with a SEP-PAK C_{18} cartridge.

Quantitation of protein

Proteins were quantitated by the method of Bradford (1976) using bovine serum albumin as the standard.

Results and Discussion

The leakage of electrolyte from both etiolated and green cotyledons of cotton that had been treated with fluthiacet-methyl at 27 °C and 15,000 lux occurred after a delay of approximately 12 hr. The leakage from etiolated velvetleaf cotyledons treated with fluthiacet-methyl or its urazole were increased with increasing concentrations of the compounds (Fig. 2). The Chl contents of etiolated cotyledons of cotton and velvetleaf were significantly reduced by treatment with fluthiacet-methyl or the urazole (Fig. 3). Fluthiacet-methyl induced the accumulation of porphyrins (Fig. 4A), which were deduced to be Proto IX and mesoporphyrin IX from the results of co-chromatography with authentic standards, and the accumulation was dose-dependent (Fig. 4B). These results suggested that both fluthiacet-methyl and the urazole are light-dependent peroxidizing herbicides.

The Protox activity of corn was inhibited by fluthiacet-methyl and its derivatives. The strength of the inhibition by fluthiacet-methyl was similar to that by oxadiazon and rather greater than that by nitrofen when DTT was used as the reducing agent (Table 1). However, inhibition by fluthiacet-methyl was weaker than that by nitrofen when DTT was replaced by sodium isoascorbate (Table 1). The urazole was the most potent inhibitor among the tested compounds. The free acid of the urazole was as potent as oxadiazon, and the free acid of fluthiacet-methyl was almost as potent as fluthiacet-methyl when sodium isoascorbate was used as the reducing agent. The inhibitory potencies of the urazole and the free acid of the urazole were unchanged when we tested enzymes from different sources, indicating that there was no difference in the sensitivity of the Protox from different plants to these two inhibitors (Table 2). Fluthiacet-methyl was decomposed to an unknown compound, which had the same retention time as the urazole on HPLC, upon reaction with an excess amounts of an SH compound at pH 8.0. However, sodium isoascorbate did not decompose fluthiacet-methyl. The unknown compound gave molecular ion peaks at m/z 404 with fragment peaks at m/z value of 372, 344, 300, 216, 172, and 127 on LC/MS. The m/z peaks at 372 and m/z 344 corresponded to the characteristic ion peaks that are considered to be those of the des-sulfur derivative and des-carboxymethyl derivative of the authentic urazole, respectively. Other fragment peaks (m/z 300, 216, 172 and 127) were also found in the analysis of the authentic urazole, revealing that the unknown compound was the urazole. However, chemical isomerization hardly occurred at pH 6.5, enzymatic isomerization by GST was observed at pH 6.5 with GST that had been partially purified (507-fold; specific activity, 5.32 μmol min^{-1} mg $protein^{-1}$) from green shoots of velvetleaf (Table 3). These results strongly suggested that fluthiacet-methyl inhibited Protox activity after conversion to its urazole by GST. The finding by Mizutani et al. (1994a), namely, that the urazole is the main metabolite in fluthiacet-methyl-treated plants, supports this suggestion.

Correlations among the concentration required for 50 % of the maximum leakage (L_{50}), the concentration required for 50% inhibition (I_{50}) of Chl biosynthesis and of Protox activity were examined using velvetleaf (Table 4). Fluthiacet-methyl and the urazole induced electrolyte leakage to almost the same extent (L_{50}: 17 and 20 nM, respectively from Fig. 2). The potency for inhibition of Chl biosynthesis of fluthiacet-methyl and that of the urazole were nearly identical (I_{50}: 10 and 12 nM, respectively from Fig. 3). However the abilities of these compounds to inhibit Protox activity were very different. The I_{50} value of fluthiacet-methyl was 830 nM, whereas that of the urazole was 5.4 nM, which was close to the values of L_{50} and I_{50} for the inhibition of Chl biosynthesis. By contrast, the L_{50} value of leakage and the I_{50} values of the inhibition of Chl biosynthesis and of the inhibition of Protox activity by the free acid of the urazole were 310 nM, 220 nM and 100 nM, respectively. These results indicated that the effects in vivo (electrolyte leakage and the inhibition of Chl biosynthesis) of fluthiacet-methyl were correlated with the effects in vitro (the inhibitions of Protox activity) of neither fluthiacet-methyl itself nor the free acid of the urazole, but they were correlated with the effects in vitro of the urazole. Therefore, the urazole, which is derived from fluthiacet-methyl, is considered to exert its inhibitory effect to Protox activity before it is hydrolyzed to its free acid.

In conclusion, it appears that fluthiacet-methyl is a Protox inhibitor, and the major active form of this compound for the inhibition in vivo of Protox activity is its urazole. Moreover, it is confirmed that glutathione S-transferase plays an important role in the conversion of fluthiacet-methyl to the urazole. Since there were no differences in the sensitivity of Protox to the urazole among plant species, the difference in sensitivity to fluthiacet-methyl between tolerant crops (corn and soybean) and sensitive

weeds is assumed to depend on other factors, such as uptake, translocation and degradative metabolism of the compound. In addition, differences in substrate specificities of glutathione S-transferase among plant species might also be another factor in the determination of sensitivity.

Acknowledgement: We acknowledge Miss Hitomi Sasano for her skilled technical assistance.

References

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. **72**: 248-254.

Hoshi, T., K. Koizumi, Y. Sato and K. Wakabayashi. 1993. Hydrolysis and phytotoxic activity of N-aryl-3,4,5,6-tetrahydroisophthalimides. Biosci. Biotech. Biochem. **57**: 1913-1915.

Jacobs, N. J. and J. M. Jacobs. 1982. Assay for enzymatic protoporphyrinogen oxidation, a late step in heme synthesis. Enzyme **28**: 206-219.

Kobayashi, K., T. Miyazawa, M. Yamaguchi and S. Shigematsu. 1994. Characterization of a new compound, KIH-9201, as a new post-emergence herbicide, and its application in the U.S.A. Weed Res. Japan **39** (Suppl.) : 14-15 (in Japanese).

Miyazawa, T., K. Kawano, S. Shigematsu, M. Yamaguchi and K. Matsunari. 1993. KIH-9201, a new low-rate post-emergence herbicide for maize (Zea mays) and soybean (Glycine max). in Proceedings of Brighton Crop Protection Conference - Weeds. Edited by The British Crop Protection Council. pp. 23-28. Major Print Ltd, Nottingham.

Mizutani, H., Y. Nakahira, T. Unai, K. Ishikawa, Y. Yusa and M. Yamaguchi. 1994a. Abstract from the 19th Meeting the Pesticide Science Society of Japan (in Japanese). p. 129.

Mizutani, H., T. Unai, T. Shimizu, K. Ishikawa, Y Yusa and M. Yamaguchi. 1994b. Abstract from the 19th Meeting the Pesticide Science Society of Japan (in Japanese). p. 128.

Shimizu, T., N. Hashimoto, I. Nakayama, T. Nakao, H. Mizutani, T. Unai, M. Yamaguchi and H. Abe. 1995. A novel isourazole herbicide, fluthiacet-methyl is a potent inhibitor of protoporphyrinogen oxidase after isomerization by glutathion S-transferase. Plant Cell Physiol. **36** (4), in press.

Yamaguchi, M., Y. Seto, T. Yoshimoto, T. Miyazawa, T. Kanbe and S. Kato. 1988. Thiadiazacyclononanes and their use for herbicides. Jpn. Kokai Tokkyo Koho JP 63-264489 (in Japanese).

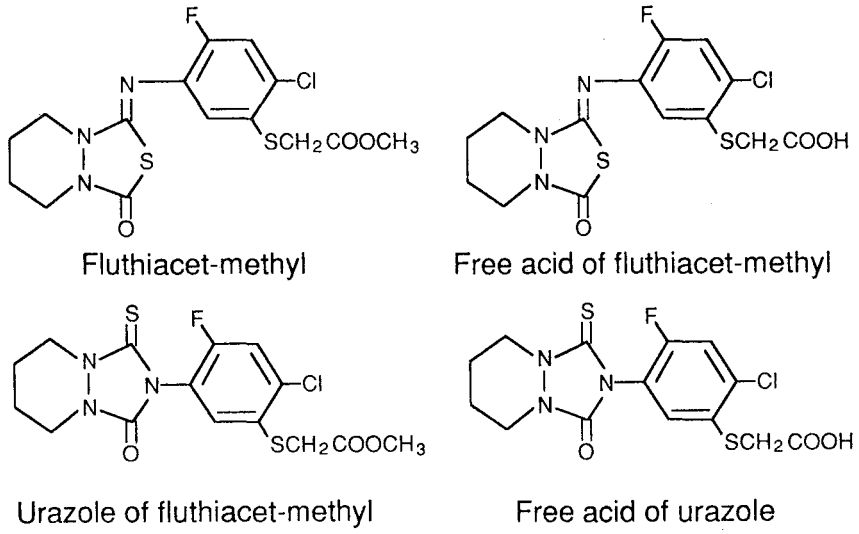


Fig. 1. Fluthiacet-methyl and the derivatives tested in this study.

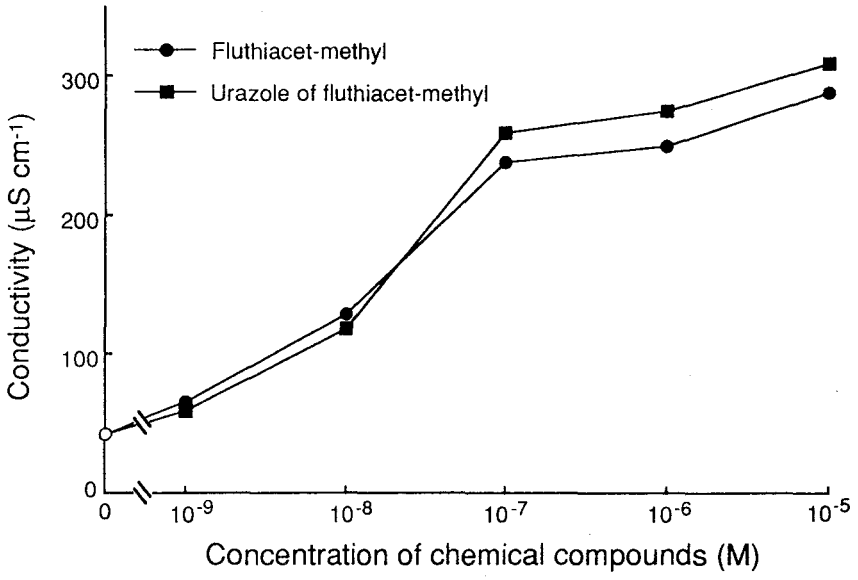


Fig. 2. Leakages of electrolytes from etiolated velvetleaf cotyledons treated with fluthiacet-methyl and its urazole (18,200 lux, 27 °C, 18-hr incubation)

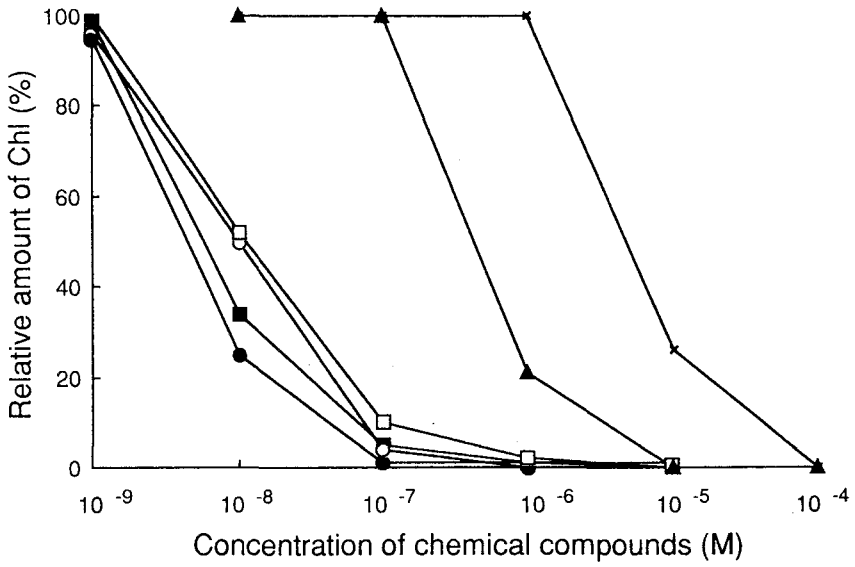


Fig. 3. Chlorophyll contents of cotyledons treated with fluthiacet-methyl and its urazole. Chl was extracted after a 24-hr incubation at 27 °C and 18,200 lux. Total Chl contents of cotyledons of the control samples of cotton and velvetleaf were 380 and 330 μg per g tissue, respectively.

●, Cotton treated with fluthiacet-methyl; ■, cotton treated with urazole; ▲, cotton treated with oxadiazon; ×, cotton treated with nitrofen; ○, velvetleaf treated with fluthiacet-methyl; □, velvetleaf treated with urazole.

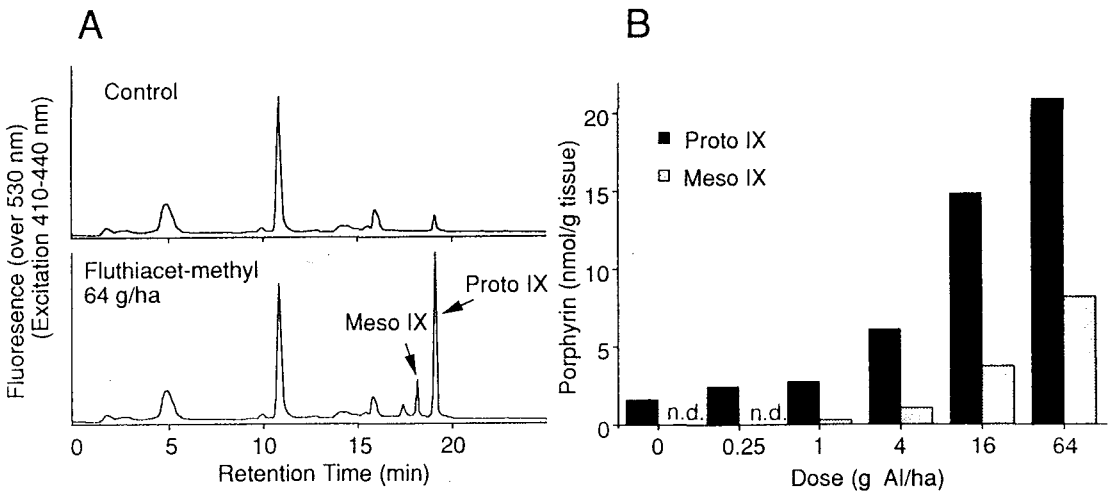


Fig. 4. Accumulation of porphyrins in etiolated cotton cotyledons treated with fluthiacet-methyl. Etiolated cotton seedlings were sprayed with fluthiacet-methyl, at the doses described in the Fig. B, and allowed to stand for 48 hr in darkness. Then porphyrins were extracted from cotyledons and analyzed by HPLC (Fig. A).

Proto IX, protoporphyrin IX; Meso IX, mesoporphyrin IX; n.d., not detected.

Table 1. Inhibition of Protox activity of corn seedlings by fluthiacet-methyl (FLU), free acid of fluthiacet-methyl (FAFLU), urazole of fluthiacet-methyl (URA), free acid of urazole of fluthiacet-methyl (FAURA), oxadiazon (OXA) and nitrofen (NIP) with dithiothreitol or sodium-isoascorbate as the reducing agent. Protox activities of control samples ranged from 0.3 to 0.49 nmol min^{-1} mg $protein^{-1}$.

Reducing agent	I_{50} (nM)					
	FLU	FAFLU	URA	FAURA	OXA	NIP
Dithiothreitol	110	350	12	110	130	1000
Sodium isoascorbate	1500	2000	10	120	120	840

Table 2. Inhibition of Protox activity from various plants by urazole of fluthiacet-methyl and its free acid. Dithiothreitol was used as the reducing agent.

Enzyme source	I_{50} (nM)	
	Urazole	Free acid
Cotton	11	66
Velvetleaf	5.4	100
Cocklebur	5.1	100
Corn	9.1	110
Soybean	8.1	66

Table 3. Requirements for enzymatic isomerization of fluthiacet-methyl.

Condition	Isomerization [a]
Complete	162
- Glutathion	n.d. [b]
- Glutathion S-transferase (GST)	n.d.
Boiled GST	n.d.

a) nmol of urazole produced per 200 min per mg protein.
b) n.d., not detected.

Table 4. I_{50} values of fluthiacet-methyl and its derivatives for the inhibition of Protox activity and of chlorophyll biosynthesis, and L_{50} values for electrolyte leakage.

Compound	I_{50} (nM)		L_{50} (nM)
	Protox	Chlorophyll	
Fluthiacet-methyl	830	10	17
Urazole	5.4	12	20
Free acid of urazole	100	220	310

Effect of Glyphosate on Growth of Indica Rice Callus and Regenerated Plants

S. Surawattananon*[1], C. Wongwattana[2], K. Namwongprom[1], K. Sangnil[3], M. Na Nakorn[1] and S. Cha-um[1]

[1] Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand.
[2] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.
[3] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Abstract Seeds of indica rice (*Oryza sativa* L.) cv. NMS 4 were sterilized and cultured on Murashige and Skoog (MS) agar medium supplemetned with 2 mg/L of 2,4-D to induce callus. The suitable medium to proliferate was MS containing with 2 mg/L of 2,4-D. Induced callus of NMS 4 were then treated with glyphosate at the concentration ranging from 10^{-7} to 10^{-3} M. The results showed that the concentration of glyphosate required to inhibit growth of callus by 50% (ID_{50}) was 10^{-4} M. However, regenerated plants at all concentrations could not grow further. By contrast to the regenerated plants *in vivo* culture when treated with glyphosate at 0, 50, 100, 500 and 1000 mg/L, it was found that plantlets at 500 mg/L produced a high number of tillers but could not survive at 1000 mg/L. It could be concluded that the effect of glyphosate is likely to be *in vitro* than *in vivo* culture.

Key words: glyphosate, N-phosphonomethylglysine, indica rice callus, *Oryza sativa* L.,

Introduction

Glyphosate (N-phosphonomethylglysine) is a non-selective herbicide that leaves no active soil residue and has low toxicity in mammals, fish and aquatic invertibrates (Folmar et al.,1979). It is a potent inhibitor of the shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) [EC 2.5.1.19], for aromatic amino acids biosynthesis (Steinrücken and Amrhein, 1980). Glyphosate in plant can blocks this synthesis and leads growth inhibition (Haderlie et al., 1977). It also reduces ion uptake (Brecke and Duke, 1980), inhibit chlorophyll formation (Kitchen et al., 1981), accelerates chlorophyll degradation (Lee, 1981), modifies chloroplast ultrastructure (Campbell et al., 1976), alters the metabolism of phenolic compounds (Duck and Hoagland, 1978), induces chromosomal aberrations (Boyle and Evans, 1974), promotes metabolism of indole-3-acetic acid (Lee, 1982) and affects respiration and inhibits photosynthesis (Sprankle et al. 1975). These additive effects may be responsible for the ultimate death of plants.

There are several papers reported about the effect of glyphosate on growth of plant and plant cell culture, such as, carrot and soybean suspension culture (Gresshoff, 1979) and tobacco callus (Lee et al., 1983). The objective of this study, is to demonstrate the effect of glyphosate on growth of indica rice callus and regenerated plants both *in vitro* and *in vivo* culture.

Materials and Methods

Chemical

Analytical grade glyphosate (47.7% ae) was in isopropylamine salt form and sterilized by membrane filter to prepare for the required stock solution.

Plant material and explant preparation

Mature seeds of indica rice (*Oryza sativa* L.) cv. NMS 4 were dehusked and surface sterilized with 70% (v/v) ethanol for 2-3 min, then with 15% clorox for 30 min, and rinsed 3 times in sterile distilled water.

Callus induction and proliferation

Sterilized seed were placed on Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962) supplemented with 2 mg/L 2,4-D. The cultures were incubated at 25±2 °C in dark and light (38 μ mol m^{-2} s^{-1}, 16 h).

Induced callus were cultured on MS medium containing 2 mg/L 2,4-D for callus proliferation .

Plant regeneration

Proliferated callus were cultured on MS medium with 1 mg/L kinetin at 25±2 °C in the same condition as callus induction.

Effect of glyphosate on growth of callus

Callus were treated with glyphosate at 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M. Effect of glyphosate on growth was determined in terms of callus fresh weight and inhibition percentage.

Effect of glyphosate on growth of regenerated plant *in vitro*

Regenerated plants were cultured on MS medium with 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M of glyphosate. Effect of glyphosate on growth of regenerated plant was determined as the percentage of inhibition, tiller height, number of tillers, root length and number of roots.

Effect of glyphosate on growth of regenerated plant *in vivo*

Regenerated plants were transferred to pots and treated with 0, 50, 100, 500 and 1000 mg/L of glyphosate at 4 weeks after transplanting. Effect of glyphosate on growth of regenerated plant *in vivo* was determined as plant height, number of tillers and toxicity level.

Results

Effect of glyphosate on growth of callus

Growth inhibition by glyphosate on callus culture was shown in Table 1. There was no effect at 10^{-7}, 10^{-6} and 10^{-5} M. At 10^{-4} M, the inhibition was 20% and the concentration required to inhibit growth by 50% (ID_{50}) was 10^{-3} M.

Effect of glyphosate on growth of regenerated plant *in vitro*

The inhibition of growth by glyphosate in *in vitro* regenerated plant showed that at 10^{-4} and 10^{-3} M, the inhibition were 100%. Glyphosate had some effects on tillering, tiller height, number of root and root length. At 10^{-5} M, number of tillers was significantly higher than other concentrations but tiller height was less than that of the others. The results also showed that this concentration induced length of root but number of root was limited (Table 2).

Effect of glyphosate on growth of regenerated plant *in vivo*

The lower concentrations of glyphosate (50 and 100 mg/L), the less toxic to plants. At the highest concentration (1000 mg/L), the toxicity appeared in one week as leaves burned first and then died. At 500 mg/L of glyphosate, the toxicity was less than that of 1000 mg/L as leaves chlorosis and the severe toxicity showed at 4 weeks. Besides that plant produced a high number of tillers (Table 3). This table also showed that untreated plant was higher than glyphosate treated plants

Discussion

Glyphosate inhibits growth of plants by inhibiting EPSP synthase. Suwanwong (1990) reported that growth of carrot cell suspension in 10^{-4} M glyphosate was inhibited at early period after treatment. In this experiment, the concentration of 10^{-4} M glyphosate inhibited 50% of growth of rice callus, but inhibited 100% of growth of *in vitro* regenerated plants. It seemed to be that plantlets had vascular system for translocating glyphosate to another part of plants. In case of *in vivo* treated to regenerated plants, the concentration that killed all of plantlets was 1000 mg/L ($6x10^{-2}$ M). Comparision between *in vitro* and *in vivo* treated by glyphosate, the effect on growth of *in vitro* treated plantlets was largely than *in vivo* treated. The medium for *in vitro* treated plantlets was supplemented with glyphosate, thus plantlets could absorb continuously. In *in vivo* treated, glyphosate was sprayed once to plantlets, so only glyphosate that spreaded on leaves could be absorbed into plants. The amount of absorbed glyphosate in *in vitro* treated might be greater than in *in vivo* treted and caused more severe than *in vivo* treated.

References

Boyle, W.S. and J.O. Evans. 1974. Effect of glyphosate and ethephon on meiotic chromosomes. J. Hered. 65:250.

Brecke, B.J. and W.D. Duke. 1980. Effect of glyphosate on intact bean plants (*Phaseolus vulgaris* L.) and isolated cells. Plant Physiol. 66:656-659.

Campbell, W.F., J.O. Evans and S.C. Reed. 1976. Effect of glyphosate on chloroplast ultrastructure of quackgrass mesophyll cells. Weed Sci. 24:22-25.

Duke S.O. and R.E. Hoagland. 1978. Effect of glyphosate on metabolism of phenolic compounds. I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots. Plant Sci. Lett. 11:185-190.

Folmar, L.C., H.O. Sanders, A.M. Julin. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertibrates. Arch. Environ. Contam. Toxicol. 8: 269-278.

Gresshoff, P.M. 1979. Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine. Aust. J. Plant Physiol. 6:177-185.

Harderlie, L.D., J.M. Widholm and F.W. Slife. 1977. Effect of glyphosate on carrot and tobacco cells. Plant physiol. 60:40-43.

Kitchen, L.M., W.W. Witt and C.E. Rieck. 1981. Inhibition of chlorophyll accumulation by glyphosate. Weed Sci. 29:513-516.

Lee, T.T. 1981. Effect of glyphosate on synthesis and degradation of chlorophyll in soybean and tobacco cells. Weed Res. 21:161-164.

Lee, T.T. 1982. Mode of action of glyphosate in relation to metabolism of indole-3-acetic acid. Physiol. Plant. 54:289-294.

Lee, T.T., T. Dumas and J.J. Jevnikar. 1983. Comparison of the effects of glyphosate and related compounds on indole-3-acetic acid metabolism and ethylene production in tobacco callus. Pesticide Biochem. Physiol. 20:354-359

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Sprankle, P., W.F. Meggit and D. Penner. 1975. Absorption, action and translocation of glyphosate. Weed Sci. 23:235-240.

Steinrücken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimate -3-phosphate synthase. Biochem. Biophys. Res. Comm. 94:1207-1212.

Suwanwong, S. 1990. Studies on the selection of carrot cells tolerant to several herbicides and the mechanism of their tolerance. Doctor thesis. University of Tsukuba.

Table 1 Effect of glyphosate on growth of NMS4 callus

Conc. of glyphosate (M)	Fresh weight of callus (mg)	Inhibition %
0 M	126.28	0
10^{-7} M	199.10	0
10^{-6} M	150.37	0
10^{-5} M	152.64	0
10^{-4} M	112.82	20
10^{-3} M	60.26	50

Table 2 Effect of glyphosate on growth of *in vitro* treated plantlets

Conc. of glyphosate (M)	Inhibition %	Tiller height (cm)	No. of tillers	Root length (cm)	No. of roots
0 M	0	23.75 a[1]	2.95 b	18.11 ab	11.25 b
10^{-7} M	0	24.47 a	2.68 b	12.21 b	15.45 a
10^{-6} M	0	23.33 a	2.15 b	15.55 b	13.33 ab
10^{-5} M	10	13.88 b	6.80 a	21.78 a	3.41 c
10^{-4} M	100	0 c	0 c	0 c	0 d
10^{-3} M	100	0 c	0 c	0 c	0 d

[1] Values followed by same letters are not significantly different at $P < 0.01$ (DMRT 's test)

Table 3 Effect of glyphosate on growth of regenerated plant *in vivo* treated plantlets

Conc. of glyphosate	0 week			1 week			2 weeks			3 weeks			4 weeks		
	height	tillers	toxic.[2]	height	tillers[NS]	toxic.	height	tillers[NS]	toxic.	height	tillers[NS]	toxic.	height	tillers	toxic.
(mg / L)	(cm)			(cm)			(cm)			(cm)			(cm)		
0	45.51	1.2	1	53.74 ab[1]	1.3	1.0a	56.75 b	1.3	1.0a	56.34 b	1.3	1.0a	63.38 bc	1.3 a	1.0a
50	45.73	1.3	1	52.59 a	1.2	1.0a	54.02 b	1.3	1.0a	54.64 b	1.3	1.0a	59.26 b	1.3 a	1.0a
100	41.78	1.2	1	46.52 a	1.2	1.0a	47.19 a	1.3	1.0a	47.41 a	1.4	1.0a	53.54 b	1.4 a	1.0a
500	42.23	1.2	1	43.34 a	1.2	1.6 b	43.45 a	1.2	2.5 b	43.49 a	1.7	2.7 b	43.52 a	2.6 b	3.3 b
1000	43.02	1.2	1	42.87 a	1.2	3.1 c	41.85 a	1.3	3.9 c	41.69 a	1.2	4.2 c	41.15 a	1.2 a	4.6 c

[NS] = Nonsignificant

[1] Values followed by same letters are not significantly different at $P < 0.01$ (LSD's test)

[2] Toxic. = toxicity levels

1 = no toxicity

2 = partial leaf burn (1/3 of total)

3 = partial leaf burn (2/3 of total)

4 = total leaf burn

5 = plant die

Effect of Glufosinate on Growth and Ammonia Accumulation in Indica Rice Callus

S. Surawattananon*[1], C. Wongwattana[2], K. Namwongprom[1], K. Sangnil[3], M. Na Nakorn[1] and K. Siripun[1]

[1] Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand.
[2] Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.
[3] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Abstract Seeds of indica rice (*Oryza sativa* L.) cv. LPT 123 were sterilized and cultured on Murashige and Skoog (MS) agar medium supplemetned with 2 mg/L of 2,4-D to induce callus. The induced callus were treated with glufosinate at the concentration ranging from 10^{-8} to 10^{-4} M. The results showed that the inhibition of callus growth was observed at 10^{-5} M and was 50% at $5x10^{-5}$ M. It was also found that callus when treated with 10^{-5} and 10^{-4} M of glufosinate had high ammonia content. These would indicating the effect of glufosinate on ammonia accumulation in this callus.

Key words: glufosinate, DL-homoalanin-4-yl(methyl)phosphinate, *Oryza sativa* L., indica rice callus

Introduction

Glufosinate (DL-homoalanin-4-yl(methyl)phosphinate) is a non-selective herbicide with a rapid contact action and minor portion as systemic effect. Glufosinate has no activity via soil (Smith, 1988). It is characterized as a strong inhibitor of glutamine synthetase (GS) [EC 6.3.1.2], the enzyme for ammonia assimilation in higher plants (Manderscheid and Wild, 1986) and would also block the assimilation of ammonia produced in plant metabolism (Leason et al., 1982). In consequence of this enzyme inhibition, there is a rapid accumulation of ammonia in leaves (Wild and Manderscheid, 1984) which is not momentary but maintain until the death of plants. The toxicity of ammonia accumulation is assumed to be the primary factor of the herbicidal activity (Tachibana et al., 1986). Other effects reported are inhibition of photosynthesis (Wild and Manderscheid, 1984) and increased potassium ion permeability of leaf tissue (Köcher and Lötzsch, 1985).

There are several papers reported on the effect of glufosinate on growth of carrot cells (Suwanwong et al., 1989) and on ammonia accumulation (Suwanwong et al., 1990). The objective of this study is to determined the effect of glufosinate on growth and ammonia accumulation in indica rice callus

Materials and Methods

Chemical

Analytical grade glufosinate (94.4% purity) was in ammonium salt form and sterilized by membrane filter to make a solution.

Explant preparation and callus induction

Hulled seeds of indica rice (*Oryza sativa* L.) cv. LPT 123 were disinfected first by immersion in 70% (v/v) ethanol for 1 min, then in 15% clorox for 30 min, followed by rinsing with sterile distilled water for 4-5 times.

The sterilized seed were then cultured on Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962) supplemented with 2 mg/L of 2,4-D. The cultures were placed in dark at 25 ± 2 °C for 10-12 days.

Effect of glufosinate on growth of callus

Callus were cultured on the MS medium with 2 mg/L of 2,4-D supplemented with glufosinate at 0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M in the same condition as callus induction. Fresh weight of callus was determined as the effect of growth.

Effect of glufosinate on ammonia accumulation in callus

Callus were treated with glufosinate to give a final concentration of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. Callus were collected and ground with liquid nitrogen, then extracted with 10 mM HCl (Desmaison et al., 1984). The crude extracts were deproteinized with 3% sulfosalicylic acid, then placed at room temperature for 15 min following with centrifuged at 3,000 x g for 15 min. The supernatants were used

for ammonia analysis by the method of Weatherburn (1967). Ammonia accumulation was defined in term of μg NH_3/g fresh weight.

Results and Discussion

The result showed that callus growth of indica rice cv. LPT 123 was inhibited depening upon the glufosinate concentration (Fig. 1). The dramatic decrease in fresh weight markedly shown when treated at higher concentration than 10^{-7} M. The concentration that inhibit 50% of growth was about $5x10^{-5}$ M (Fig. 2). In all case, it was found that survival callus was later turned brown and black. The similar results was reported by Suwanwong (1990) that glufosinate also inhibited on the growth of carrot cell suspension but at the ID_{50} of $2x10^{-6}$ M.

The increasing of ammonia content in callus cell was significantly observed when treated with glufosinate at the concentration higher than 10^{-7} M. (Fig. 3). At 10^{-6} 10^{-5} and10^{-4} M to the 10^{-7} M, the ammonia content was about 2, 4 and 6 μg NH_3/g fresh weight at 20 days. Similar to the report of Suwanwong et al. (1990), ammonia accumulated gradually increased with increasing concentration of glufosinate.

Glufosinate is a strong inhibitor of GS, the first enzyme in the assimilation of ammonia and causing the accumulated ammonia (Wild and Manderscheid, 1984 and Tachibana et al., 1986). In this study when callus were treated with 10^{-6}, 10^{-4} and 10^{-5} M of glufosinate, the ammonia content in treated callus was about 5, 10 and 15 times higher than untreated callus, respectively. The remarkable decrease of growth also showed at these concentrations. These finding showed that glufosinate involved the accumulation of ammonia and the toxicity of these ammonia which in turned inhibition of growth of rice callus.

References

Desmaison, A.M., M.H. Marcher and M. Tixier. 1984. Changes in the free and total amino acid composition of ripening chesnut seeds. Phytochemistry. 23(11):2453-2456.

Köcher, H. and K. Lötzsch. 1985. Uptake, translocation and mode of action of the herbicide glufosinate-ammonium in warm climate weed species. Proc. 10th Asian Pacific Weed Science Soc. Conf. pp.193-198.

Leason, M., D. Cunliffe, D. Parkin, P.J. Lea and B.J. Mifin. 1982. Inhibition of pea leaf glutamine synthetase by methionine sulphoximine, phosphinothricin and other glutamate analogues. Phytochemistry. 21(4):855-857.

Manderscheid, R. and A. Wild. 1986. Studies on the mechanism of inhibition of phosphinothricin of glutamine synthetase isolated from *Triticum aestivum* L. J. Plant physiol. 123:135-142.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Smith, A.E. 1988. Persistence and transformation of the herbicide [14C] glufosinate-ammonium in prairie soils under laboratory conditions. J. Agric. Food Chem. 36:393-397.

Suwanwong, S. 1990. Studies on the selection of carrot cells tolerant to several herbicides and the mechanism of their tolerance. Doctor thesis. University of Tsukuba.

Suwanwong, S., K. Usui and K. Ishizuka. 1989. Selection of tolerant cells from carrot suspension culture to bensulfuron methyl, glyphosate and glufosinate. Weed Res. Japan. 34(4):315-321.

Suwanwong, S., K. Usui and K. Ishizuka. 1990. Glufosinate tolerance in carrot cell suspension culture. Weed Res. Japan. 35(1):53-60.

Tachibana, K., T. Watanabe, Y. Sekizawa and T. Takematsu. 1986. Accumulation of ammonia in plants treated with bialaphos. J. Pesticide Sci. 11:33-37.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971-974.

Wild, A.and R. Manderscheid. 1984. The effect of phosphinotricin on the assimilation of ammonia in plants. Z. Naturforsch. 39c:500-504.

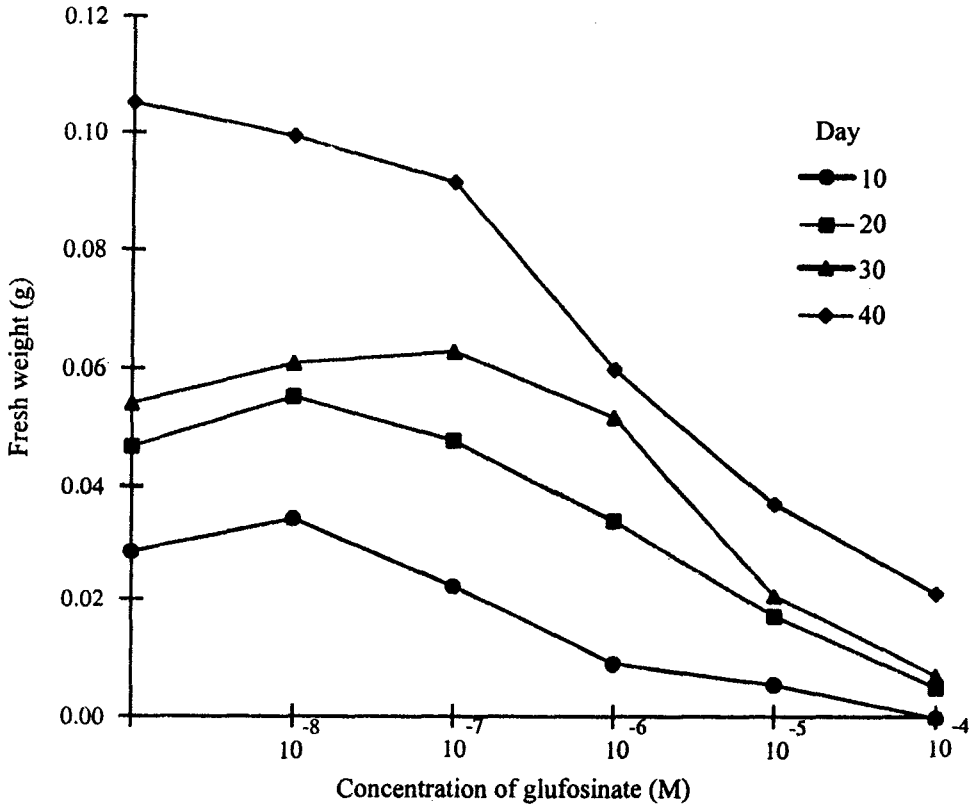


Fig.1 Effect of glufosinate on growth of LPT123 callus

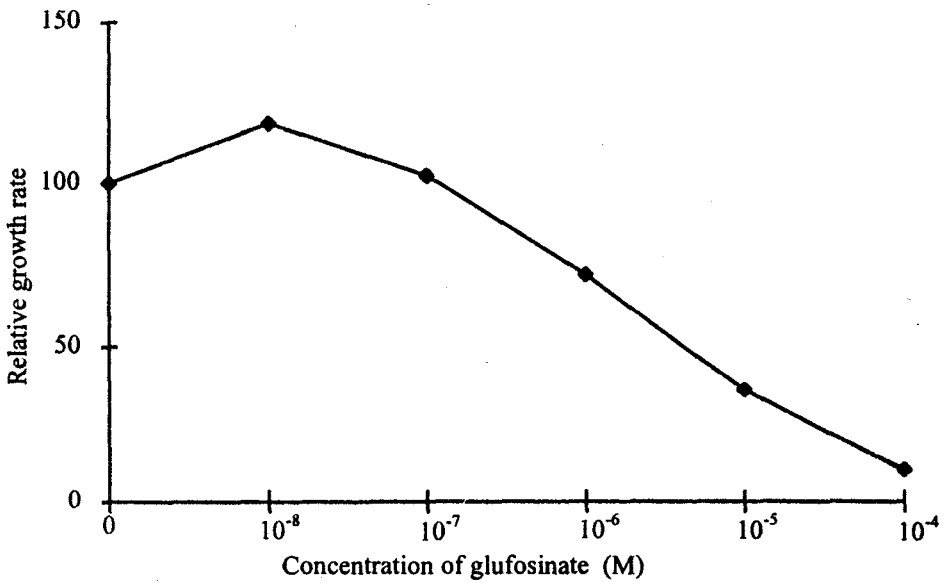


Fig.2 Effect of glufosinate on growth of LPT123 callus.

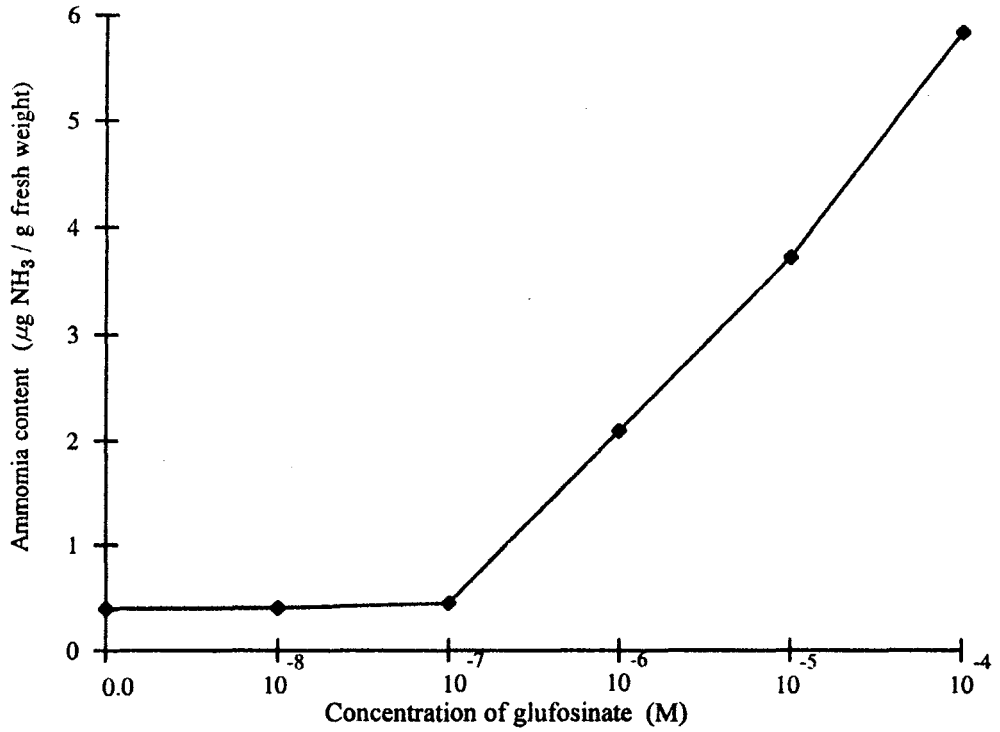


Fig. 3 Effect of GLU on ammonia accumulation in LPT123 callus

POSTEMERGENCE APPLICATION OF ACIFLUORFEN FORMESAFEN AND LACTOFEN FOR BROADLEAF WEED CONTROL IN SOYBEAN

R. Suwanketnikom and E. Panyakit

Department of Agronomy, Kasetsart University, Bangkok 10900, Thailand

Abstract. Experiments were conducted to determine the effect of herbicide rates and stages of plant growth on the efficacy of acifluorfen, fomesafen and lactofen for broadleaf weed control and their phytotoxicity to vegetable soybean cultivars. Herbicides provided good broadleaf weed control when applied at two weeks after soybean germination. The efficacy of herbicides was in the order lactofen > acifluorfen = fomesafen. The susceptibility of weeds to herbicides was in the order *Trianthema portulacastrum* > *Amaranthus hybridus* > *Euphorbia heterophylla*. Phytotoxicity of herbicides to soybean was in the order lactofen > acifluorfen > fomesafen and at high rate herbicides were more phytotoxic to soybean than at low rate. The tolerance of soybean to herbicides was in the order S.J.5 > white Lion > KPS 205 > KPS 305

Key words: lactofen, acifluorfen, fomesafen, broaleaf weeds, vegetable soybean (*Glycine max* (L.) Merr.)

INTRODUCTION

Acifluorfen, fomesafen, and lactofen are diphenyl ethers available for broadleaf weed control in soybean. Acifluorfen is sodium salt with very high water solubility. Fomesafen is methyl sulfonyl salt can soluble in water at 50 mg/l. Lactofen is ester of ethoxy methyl oxoethyl with very low water solubility, 0.1 ppmw (Weed Science Society of America, 1989). Herbicidal acitivity of these herbicides might be difference.

Amaranthus hybridus L., *Trianthema portulacastrum* L., and *Euphorbia heterophylla* L. are three main broadleaf weeds in soybean grown in Central Thailand. They are very strong competitors for vegetable soybean. The tolerance of these weeds to diphenyl ethers might be difference. Furthermore, at different stage of growth these weeds might show different response to diphenyl ethers. White Lion, KPS 205, and KPS 305 are vegetable soybean grown in Thailand. They are less competitive to weeds because the plants are too short and too small canopy. Farthermore, they might susceptible to diphenyl ethers.

The objectives of these experiments were to determine the effect of herbicide rates and stages of plant growth on the efficacy of acifluorfen, fomesafen, and lactofen for broadleaf weed control and their phytotoxicity to vegetable soybean cultivars.

MATERIALS AND METHODS

A field soybean, S.J.5 and three vegetable soybean, White Lion, KPS 205, and KPS 305 were planted in Tropical Vegetable Research Center Field, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, 85 km Northwest of Bangkok. The soil is clay (Typic Hyphestalfs) with 2.7 % organic matter and pH 7.1. Experiments were conducted during November 1990 until February 1991. The soybean seed were sown with 50 x 12.5 cm spacing in a 3 x 4 m plot and three to four seed per hill. At five days after germination plants were thinned to two plants per hill. Acifluorfen (Blazer 2EC), fomesafen (Flex 2EC), and lactofen (Corra 2EC) with the rates listed in Table 1 were applied with a spray volume of 500 l/ha at one, two, and three weeks after soybean germination. During herbicide application the soil was wet and the temperature was 30 ± 5 C.

At ten days after soybean germination, haloxyfopmethyl (Gallant 2EC) at the rate of 200 g/ha was applied for grasses control. Fertilizer 15-15-15 at 187.5 kg/ha was applied before planting and at 30 days after soybean germination. Irrigation system was furrow. The recommended insecticides were applied when necessary. Hand weeding of control plot was done at two and four weeks after soybean germination.

Experiments were designed in Randomized Complete Block with four replications.

RESULTS AND DISCUSSION

At one week after soybean germination *A. hybridus* did not germinate. Medium and high rate of acifluorfen and fomesafen gave good and fair control of *T. portulacastrum* and *E. heterophylla* respectively. While all rates of lactofen provided excellent control of *T. portulacastrum* and good control of *E. heterophylla*.

At two weeks after soybean germination medium and high rate of all herbicides gave good control of all broadleaf weeds at one week after application (Table 1). However, the control efficiency of herbicides was reduced at two and four weeks after application

(Table 1). When herbicides were applied on three weeks old soybean, the control efficiency of herbicides was reduced less than application on two weeks old soybean.

It was observed that *E. heterophylla* was more tolerance to herbicides than other two weeds (Table 1). This might be due to more wax covered on leaf surface of *E. heterophylla* than on *A. hybridus* and *T. portulacastrum* leaf surface. Furthermore, the stem of *E. heterophylla* and *A. hybridus* are erected while *T. portulacastrum* is prostrate. Therefore less spray droplet could be retained on *E.heterophylla* leaves than the leaves of other two weeds. Since diphenyl ethers are contact herbicides (Weed Science Society of America, 1989). They need more spray droplet to cover all over the whole plants.

Lactofen provided higher efficacy for broadleaf weed control than fomesafen and acifluorfen (Table 1) which was similar to the other reporsts (Bates et al, 1986; Haris et al, 1986). This might be due to the difference in physical and chemical properties of herbicides. Lactofen is ester with very low water solubility while fomesafen and acifluorfen are salts with low and high water solubility respectively (Weed Science Society of America, 1989). Therefore lactofen might be absorbed into leaves of weeds and crop more than fomesafen and acifluorfen respectively.

When herbicides were applied to one week old soybean the vegetable soybean, White Lion, KPS 205, and KPS 305 were more susceptible to herbicides than S.J.5, the field soybean. Two and three weeks old soybean were more tolerant to herbicides than one week old soybean. The phytotoxicity of herbicides to two weeks old soybean was in the order S.J.5 < White Lion < KPS 205 < KPS 305 (Table 2). The young plants were more susceptible to diphenyl ethers than the old plants which was similar to the reports of Lee and Oliver (1982), Retizinger and Roger (1986), and Trammell et al (1986). Furthermore, high rate of herbicides were more phytotoxic to the plants than low rate (Table 2).

Yield of S.J.5 and White Lion soybean did not affect by diphenyl ethers, while yields of KPS 205 and KPS 305 were reduced by certain herbicides when herbicides were applied to two weeks old soybean (Table 3).

Acknowledgement : The authors express their sincere appreciation to Tropical Vegetable Research Center, Kasetsart University for supporting this study.

REFERENCES

Bates, M.R., H.B. Hagwood, R.L. Nicholes, C.A. Trammell, and E.J. Spyhaski. 1986. Comparative efficacy of lactofen, acifluorfen, bentazon and acifluorfen-bentazon tank mixed for weed control in soybean. Proc. South, Weed Sci. Soc. 39 : 96-99.

Haris, J.R., B.J. Gossett, and T.R. Murphy. 1986. Response of broadleaf weed and soybean to diphenylether herbicides. Proc. South. Weed Sci. Soc. 39 : 474-477.

Lee D.S. and L.R. Oliver. 1982. Efficacy of aciflurofen on broadleaf weed, times and method of application. Weed Sci. 30 : 520-526.

Retzinger, E.J. and R.L. Rogers. 1986. Efficacy of fomesafen alone and in tank mixture. Proc. South Weed Sci. Soc. 39 : 89-92.

Trammell, C.A., M.A. Bates, R.L. Nichols, H.E. Shepherd, and F.R. Taylor. 1986. Lactofen efficacy related to weed size at time of application. Proc. South. Weed Sci. 39 : 97-101.

Weed Science Society of America. 1989. Herbicide Handbook. Champaign, Illinois. 301 p.

Table 1 The control of *A. hybridus*, *T. portulacastrum*, and *E. heterophylla* at one, two and four weeks after application of diphenyl ethers on two weeks old soybean.

Herbicide	Rate g.ai /ha	% Weed control[1/]								
		AMAHY[2/]			TRTPO			EUPHE		
		week								
		1	2	4	1	2	4	1	2	4
Acifluorfen	50	36 f[3/]	11 f	0 c	41 g	14 f	0 e	30 f	10 f	0 c
	100	61 bc	40 b	10 b	52 f	16 f	0 e	39 de	15 e	0 c
	200	63 b	41 b	16 b	53 ef	24 d	0 e	41 d	16 e	0 c
Fomesafen	50	35 f	12 f	0 c	55 e	21 e	0 e	36 e	15 e	0 c
	100	56 d	20 e	0 c	60 d	41 c	5 c	50 c	20 d	0 c
	200	58 cd	30 c	0 c	65 c	42 c	5 c	61 b	22 cd	0 c
Lactofen	12.5	52 e	21 de	0 c	56 e	27 d	4 d	48 c	20 d	0 c
	25	56 d	23 d	0 c	61 d	42 c	5 c	58 b	23 c	0 c
	50	62 bc	40 b	9 b	80 b	53 b	16 b	60 b	39 b	5 b
Weeded		95 a	85 a	85 a	95 a	85 a	85 a	95 a	85 a	85 a
Non-weeded		0 g	0 g	0 c	0 h	0 g	0 e	0 g	0 g	0 c

1/ % Weed control, 0 = no control, 100 = complete control.

2/ AMAHY = *Amaranthus hybridus* was 1 to 2 cm height with 2 to 3 leaves.

TRTPO = *Trianthema portulacastrum* was 3 to 5 cm height with 4 to 6 leaves.

EUPHE = *Euphorbia heterophylla* was 4 to 8 cm height with 4 to 5 leaves.

3/ Means in the same column followed by the same letter are not significantly different at 5 % level by DMRT.

Table 2 Phytotoxicity of diphenyl ethers on S.J.5, White Lion, KPS 205 and KPS 305 soybean at one, two and four weeks after application on two weeks old soybean.

Herbicide	Rate	% Phytotoxicity[1/]											
		S.J.5[2/]			White Lion			KPS 205			KPS 305		
	g.ai/ha	Week after application											
		1	2	4	1	2	4	1	2	4	1	2	4
Acifluorfen	50	8 cd[3/]	4 c	0 b	10 e	5 c	0 b	12 b	5 b	0 c	11 e	5 d	0 b
	100	10 b	4 c	0 b	14 d	10 ab	0 b	20 a	10 b	4 b	15 d	10 c	0 b
	200	14 a	9 b	0 b	15 cd	10 ab	0 b	22 a	11 a	5 a	20 c	14 b	5 a
Fomesafen	50	4 d	0 d	0 b	5 f	0 d	0 b	5 c	0 c	0 c	5 f	0 e	0 b
	100	5 de	0 d	0 b	7 f	0 d	0 b	6 c	0 c	0 c	6 f	0 e	0 b
	200	6 be	0 d	0 b	18 bc	5 c	0 b	10 b	5 b	0 c	10 e	5 d	0 b
Lactofen	12.5	13 b	9 b	0 b	20 b	9 b	0 b	19 a	9 b	0 c	21 c	10 c	4 a
	25	16 b	11 b	0 b	20 b	10 b	0 b	20 a	10 a	4 ab	25 b	15 b	5 a
	50	23 a	15 a	5 a	25 a	15 a	6 a	21 a	13 a	5 a	31 a	20 a	6 a
Weeded		0 f	0 d	0 b	0 g	0 d	0 b	0 d	0 d	0 c	0 g	0 e	0 b
Non- weeded		0 f	0 d	0 b	0 g	0 d	0 b	0 d	0 d	0 c	0 g	0 e	0 b

1/ 0 = no phytotoxicity, 100 = complete kill.

2/ At 2 weeks each cultivar has two trifloriate leaves with the height of 15 to 18 cm.

3/ Means in the same column followed by the same letter are not significantly different at 5 % level by DMRT.

Table 3 Yield of S.J.5, White Lion, KPS 205, and KPS 305 soybean when diphenyl ethers were applied at two weeks old.

Herbicide	Rate g. ai/ha	S.J.5	White Lion	KPS 205	KPS 305
		(kg/ha)			
Acifluorfen	50	2084 ab[1/]	1580 cd	2205 bc	2231 c
	100	2080 ab	1461 cd	2180 c	2487 bc
	200	2205 ab	2350 a	1543 d	2725 ab
Fomesafen	50	1935 ab	1655 bcd	2582 ab	2258 c
	100	2053 ab	1273 de	2250 abc	2491 bc
	200	2559 a	1677 bcd	2476 abc	2319 bc
Lactofen	12.5	2270 ab	2137 ab	2342 abc	2233 c
	25	2242 ab	1944 abc	2447 abc	2387 bc
	50	2116 ab	1891 abc	2193 bc	2459 bc
Weeded	-	2523 a	1773 bcd	2600 a	2977 a
Non-weeded	-	1536 b	963 e	1505 d	1349 d
C.V. (%)		11.5	21.6	13.0	11.9

[1/] Means in the same column followed by the same letter are not significantly different at 5 % level by DMRT.

ACCUMULATION OF ANTIFUNGAL 5-(8Z-HEPTADECENYL)RESORCINOL AND ITS HOMOLOGS, SPECIFIC TO ETIOLATED RICE SEEDLINGS

Yoshikatsu SUZUKI*, Hiroshi HYAKUTAKE, Yoshiki KONO, and Akira SAKURAI

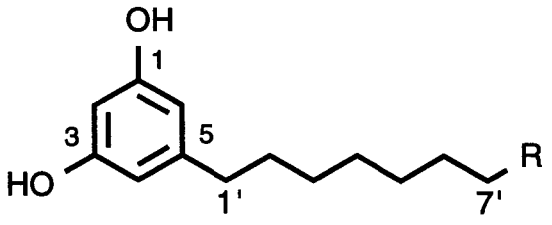
The Institute of Physical and Chemical Research (RIKEN)
Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan

ABSTRACT: 5-Alkylresorcinol homologs (ARs) composed of $C_{13:0}$, $C_{15:1}$, $C_{15:0}$, $C_{17:1}$, and $C_{17:0}$ carbon chains were obtained from etiolated rice (*Oryza sativa*, CV. RD-25) seedling extracts and exhibited high antifungal activity against the rice blast fungus, *Pyricularia oryzae*. Changes of ARs accumulation under several seedling conditions suggested that these substances may play a role in defending against fungal microbes during the period that the germs are under the ground. That is, ARs : 1) are newly synthesized after germination, 2) reach an effective concentration at an early seedling stage, 3) accumulate specifically to the etiolation plants, 4) accumulate much more in the seed portion, and 5) decrease rapidly when the etiolated seedlings are exposed to light. Interestingly, Indica- and Africa-type cultivars (*O. sativa* and *O. glaberrima*) and weedy-type wild rices (*O. rufipogon* and *O. sativa*) tested accumulated ARs, while the Japonica-type cultivars tested did not or trace amounts.

KEY WORDS : resorcinol, *Oryza sativa*, antifungal substance, *Pyricularia oryzae*, *Oryza* spp

INTRODUCTION

Disease defending substances specific to a seedling stage and different from the subsequent stages are assumed to be present in most crops. Because a seedling stage that mechanically weak germs generally situate in high soil-microbe density is one of the most infectious one among plant life cycle. Cyclic hydroxamates (1) which are widely distributed as sugar conjugates to a seedling stage of *Gramineae* plants have been presumed to play a role in defending against insects and microbes, especially in high contents of corn (2), wheat, and rye. Also, these metabolites reach to a maximum level within a week after germination, followed by decrease with age in corn and wheat. We attempted to identify disease-defending substances displaceable by cyclic hydroxamates from seedling plants of *Gramineae* cereals which contain little or no detectable amounts : *i.e.*, rice and sorghum (3). We report here on identification of 5-(8Z-heptadecenyl)resorcinol ($AR_{17:1}$) and its homologs as antifungal substances from etiolated rice seedlings and finding that these accumulations are specific to etiolated seedlings.



($AR_{13:0}$) R = $-(CH_2)_5CH_3$
($AR_{15:1}$) R = $-CH=CH(CH_2)_5CH_3$
($AR_{15:0}$) R = $-(CH_2)_7CH_3$
($AR_{17:1}$) R = $-CH=CH(CH_2)_7CH_3$
($AR_{17:0}$) R = $-(CH_2)_9CH_3$

Fig. 1 Structures of resorcinol homologs (ARs) in the etiolated rice seedlings

MATERIALS AND METHODS

Preparation of rice seedlings Rice seeds (*Oryza sativa* L cv. RD-25 : Indica-type) were imbibed at 30°C for 2 days in the dark . The imbibed seeds were transferred on vermiculite and incubated at 20 to 27°C in the dark or light conditions. The other cultivars and the *Oryza* spp. were also grown similarly.

Extraction and solvent partitioning of antifungal substances. The shoot tissues of 7-day-old etiolated rice seedlings (20-25°C) were homogenized with a) 80% MeOH or with b) water for one min. MeOH was added to the latter homogenate after one hr. After one day each filtrate was concentrated and partitioned, giving ether soluble neutral fraction (N- or TN-fraction), ethyl acetate soluble acidic fraction (A- or TA-fraction), and n-butanol soluble fractions (B- or TB-fraction), respectively.
Isolation for antifungal substances (ARs). The TN fraction was purified on Sephadex LH-20 column (ϕ 3x50 cm) with MeOH and then silica plates (Merck, 0.5 mm thickness of silica gel F_{254}) developed with 7% MeOH-$CHCl_3$.

Separation between saturated and unsaturated ARs. A mixture of ARs was charged on Silica gel plates (0.25 mm thickness, 20 cm X 20 cm) impregnated with $AgNO_3$ (15%)) and the plates were developed with benzene-ethyl acetate (85/15) at 10 cm height followed by 15 cm height. NMR spectra were recorded with tetramethylsilane as an internal standard, using a JEOL TNM-EX 270 instrument. IR spectra were measured as films with a Shimadzu IR-430 spectrometer. FAB and remote-charge fragmentation mass data were measured with a JEOL JMS-HX/HX110A Tandem Mass Spectrometer .

Quantitative analysis of ARs by GC-MS . Seedling samples (*ca*, 1 g fw.) were homogenized with MeOH (50 ml) and the filtrate was concentrated. The residue was placed on ODS cartridge column (Absorbex RP-18 : 400 mg, Merck) using water (4 ml). The column was successively eluted with water (4 ml), 50% (4 ml), 75% (4 ml), and 100% MeOH (3 ml). MSTFA (50 µl) was added to a concentrated MeOH eluate or purified ARs and the mixture stood for an hour at room temperature. One µl of the sample was injected into GC equipped with MS spectrometer (Hitachi M-80 spectrometer at 20 eV, using DB-1 column (ϕ 0.53 mm X 15 m, 1.5 µm film thickness, column oven temperature : 230°C) with 10 ml/min. of He gas flow rate. Total amounts of ARs were calculated from the mass chromatograms of common base peak at m/z 268, showing a linear relationship between 40 and 200 ng.

Spore germination assay *Pyricularia oryzae* P_2 was grown on oatmeal-agar medium at 25°C in the dark for 7 to 10 days. Spores were collected by agitating with distilled water from further 2-day-old cultures under fluorescent light after removal of the spores from the culture plate. The spore suspension was filtered through a tissue paper and adjusted to a density of about 10^5 spores/ml by addition of distilled water. Thirty µl of the spore suspension was added to a 30 µl of test sample (15% EtOH solution) and it was mixed well with toothpick and incubated at 25°C under the dark for 5 hr. The percentages of germinating and non-germinating spores were determined by a microscopy.

RESULTS AND DISCUSSION

Antifungal activity of etiolated rice seedling extracts. All except for n-butanol fractions (B and TB) possessed the high activities which are sufficient to inhibit completely the spore germination in the fresh weight concentration. The most of activities for each fraction seemed to be due to constitutive antifungal substances because an increase of the activity by the injury treatment was small. Only ethyl ether soluble acidic fraction (TA) may contain induced antifungal substances together with constitutive ones (Table 1).

Isolation and structure determination of active substances (ARs). Fractionation of the TN fraction by column chromatography on Sephadex LH-20, followed by preparative TLC on silica gel gave a 64 mg of syrupy , active substance (ARs) from the shoot tissues (400 g fw). ^1H- and ^{13}C-NMR and GC-MS data (4,5,6,7) showed that

active substance is a mixture of five 5-alkylresorcinols (ARs) with saturated carbon chains of C_{13} ,C_{15}, and C_{17} and with mono-(Z)-olefinic chains of C_{15} and C_{17}, respectively (4). In addition, it was also shown that $AR_{17:1}$ comprised about 50% of the five homologs by GC-MS analysis. Positions of the olefinic group for $AR_{15:1}$ and $AR_{17:1}$ were determined as a mixed state by remote-charge fragmentation FAB data (data not shown) to be the 8-position in both case (Fig. 1).

Table. Antifungal activity of each fraction obtained with/without injury treatment on spore germination of Pyricularia oryzae

	Inhibition %		
Conc.*1	N (TN)*2	A (TA)*2	B (TB)*2
1.0	89.7 (96.1)	98.3 (100)	0 (7.5)
0.5	49.2 (68.4)	74.4 (100)	/
0.25	57.8 (32.0)	41.6 (100)	/
0.125	41.0 (4.5)	0 (48.7)	/

Rice (var. RD-25) shoots of 8-day-old plant grown at 20-25oC in the dark were used for extraction.
Values represent the mean of four replicates.
*[1]Conc.1.0 represents a fresh weight concentration
*[2]Parenthes represent samples with injury treatment

Antifugal activity of ARs. Separation of ARs by TLC impregnated with $AgNO_3$ gave $AR_{17:1}$ (containing $AR_{15:1}$, 2%) and a mixture of $AR_{13:0}$, $AR_{15:0}$, and $AR_{17:0}$ in total 45.6% yield. The unsaturated one ($AR_{17:1}$) exhibited slightly higher antifungal activity than the saturated ARs. The $[ED]_{50}$ was 40 μg/ml for $AR_{17:1}$, 50 μg/ml for saturated ARs, and 45 μg/ml for ARs (data not shown), respectively.

ARs accumulation under several seedling conditions. To explore a role of ARs in defense of rice plants against microbes, a time course on ARs accumulation in etiolated seedlings and changes of ARs accumulation contents under several seedling conditions were investigated.
A time course experiment (Fig. 3) using a whole of etiolated seedlings (25°C) showed that ARs were absent for a period up to 3 days, first detected at 4 day, increased linearly with the periods, and lead to a concentration of 126 μg/g fw at 10 day. This result showed that ARs were newly produced after germination, but not present in the seeds and that ARs contents lead to a concentration of 50 μg /g fw comparable to $[ED]_{50}$ at 6 day.

ARs accumulation contents varied by rice seedling conditions and rice cultivars. Figs 4 and 5 showed that ARs accumulation was specific to etiolated seedlings. Content of ARs accumulation in green seedlings was *ca.* one twentieth of that in the etiolated ones (Fig.4). Similar specificity was observed between the seed portions, between shoot portions, and between seed and shoot portion in green seedlings (Fig. 5). The content for seed portion in the green seedlings , 34 μg/g wt, was near to $[ED]_{50}$. Such an unexpected, fairly high accumulation is probably due to a dark circumstance of the seed portion. In addition to this, high accumulation at an early stage of etiolated seedlings suggested that ARs may play a role in defending rice plants against microbes during a period that the germs are under the ground. Accumulated ARs, however, rapidly decreased about a half at one day and one third at 2 day when the etiolated seedlings were exposed to light (Fig. 6). This result clearly showed that light condition, at least, enhanced ARs metabolisms, though it is unclear whether biosynthetic potential of ARs was suppressed.

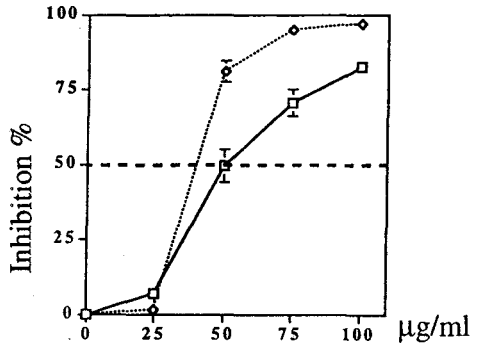


Fig.2. Inhibitory Activity of ARs on Spore Germination of *Pyricularia oryzae* P_2

Date represent an average of two samples
(o) : C17:1), (□) : sat. ARs

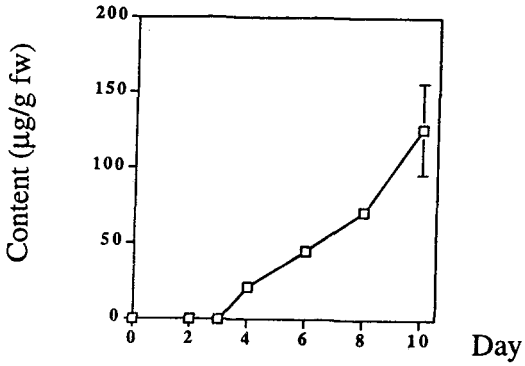


Fig. 3 Time Course of ARs Accumulation in Etiolated Rice Seedlings

Data represent an average of three samples
Etiolated whole plant (cv. RD-25) at 25°C

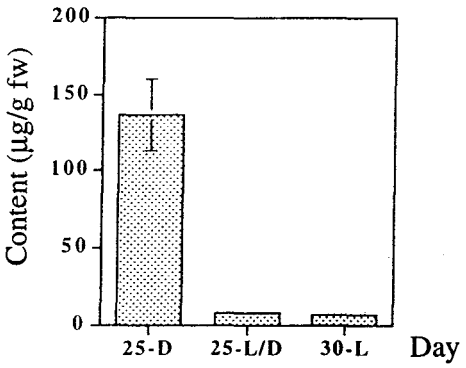


Fig. 4 Effect of Light on ARs Accumulation in Rice Seedlings

25-D: continuous dark at 25°C
25-L/D: light/dark(16/8) at 25°C
30-L: continuous light at 30°C

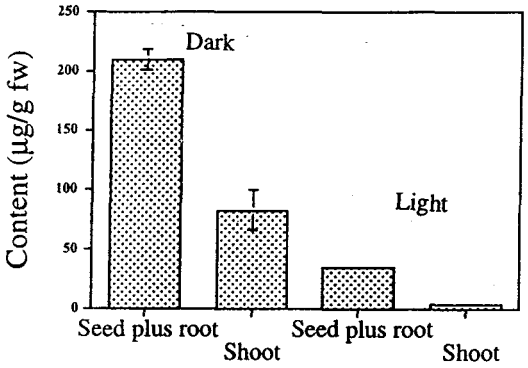


Fig. 5 ARs Content in Each Portion of Rice Seedlings under Dark or Light Conditions

cv. RD-25 at 30°C for 12 days
Data represent an average of two samples

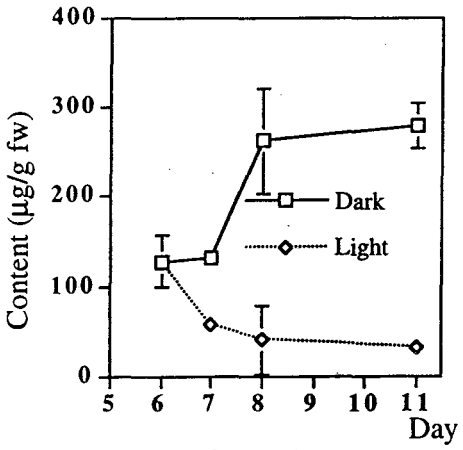


Fig. 6 Change of ARs Content in Etiolated Rice Seedlings by Exposure to Light

Whole plants (cv. RD-25) at 27°C
Data represent an average of three samples

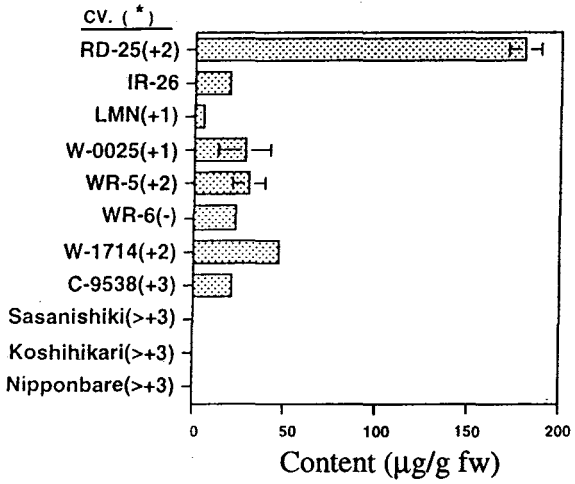


Fig. 7 Comparisons of ARs Content in Etiolated Whole Plants of Caltivated and Weedy-type Wild Rices

Etiolated seedlings at 30°C for 12 days
*Susceptibility against *Pyricularia oryzae* P_2

As described above, we studied on ARs accumulation for an Indica-type cultivar, RD-25, which was resistant against *Pyricularia oryzae* P_2. In Fig. 7, ARs accumulation contents in etiolated seedlings of the other cultivars and weedy-type wild rices (*Oryza* spp.) which have varying susceptibility were summarized. A positive tendency that low susceptible cultivars and weedy-type wild rices have ARs accumulation potential was observed except for C-9538. These contents were 5 to 50 μg/gwt for Indica-and Africa-type cultivars and weedy-type wild rices except for CV. RD-25, whereas these were trace for the Japonica-type cultivars which were highly susceptible.

ARs have been known to be present in some cereal plants of *Gramineae* (rye, wheat, and barley) and some plant families (*Anacardiae, Ginkgoaceae,* or *Proteaceae*) as a deleterious substance in animal breeding (8,9) Among five ARs, $AR_{15:1}$ is already known as bilobol from *Gingko biloba* (10), however, $AR_{17:1}$ does not appear to have been recorded. It is the first finding that ARs were identified from the rice plants and had antifungal activity. There are a few differences for ARs between in rice and the other *Gramineae* plants. In the latter, ARs are originally present in these seeds and consist of components with relatively longer carbon chains of C_{13} to C_{29}, which accompany the corresponding mono- and di-unsaturated ARs as minor components (11), while in the rice plant as clarified in our study, ARs are newly produced with germinating and consisted of relatively shorter carbon chains of C_{13} to C_{17}, whose predominant one was the mono-unsaturated component of C_{17}.

Some antimicrobial substances have been also isolated from seedlings of *Gramineae* cereal plants, *i.e.*, hordatines from etiolated barley seedlings by Stoessel (12), coixindenes from etiolated adlay seedlings by Ishiguro *et. al.* (13), and avenacins from oat roots by Crombie *et. al.* (14) However, it remains unclear whether accumulations of these antimicrobial substances are specific to etiolated plants. As already mentioned above, in wheat and rye, ARs are originally present in these seeds, while cyclic hydroxamates appear upon germination under both the light and dark condition. ARs in some rice cultivars and weedy-type wild rices may play a role in defending against microbes as cyclic hydroxamates in wheat and rye seedlings .

REFERENCES

1) H. M. Niemeyer, Phytochemistry, (1988), **27**, 3349.
2) R. M. Couture, D. G. Routley, and G. M. Dunn, Physiol. Pl. Path., (1971), **1**, 515
3) C. Tang, S. H. Chang, D. Hoo, and K. H. Yanagihara, Phytochemistry, (1975), **14**, 2077 .
4) J. R. Cannon and B. W. Metcalf, Aust. J. Chem., (1971), **24**, 1925.
5) J. L. Occolowitz, Anal. Chem., (1964), **36**, 2177 .
6) M. V. Sargent, S. Wangchareontrakul, and A. Jefferson, J. Chem. Soc., Perkin Trans I, (1989), 431.
7) J. W. de Haan and L. M. van de Ven, Organic Magntic Resonances, (1973), **5**, 147.
8) E. Wenkert, E.M. Loeser, S.N. Mahapatra, F. Schenker, and E.M. Wilson, J. Org. Chem., (1964), **29**, 435 .
9) D. E. Briggs, Phytochemistry, (19740, **13**, 987.
10) S. Furukawa, Sci. Pap. Inst. Phys.Chem. Res., Tokyo, (1935), 26, 178.
11) A. Kozubek, J. Chromatography, (1984), **295**, 304.
12) A. Stoessel, T. Letters, (1966), 2287.
13) Y. Ishiguro, K. Okamoto, H. Sakamoto, and Y. Sonoda, Biosci. Biotech. Biochem., (1993), **57**, 866.
14) L. Crombie, W. M. L. Crombie, and D. A. Whiting, J. Chem. Soc.,Chem. Commun., (1984), 244 and 246.

Differential Action of Mefenacet to Rice Plant and Barnyardgrass

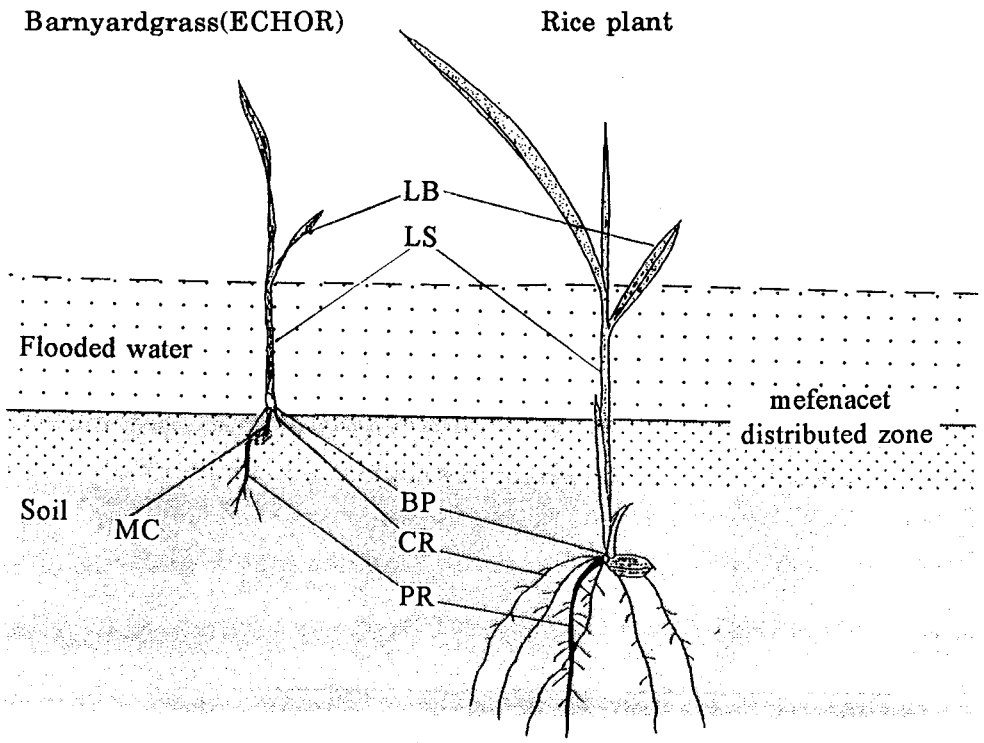
K. Ukawa*, N. Minegishi, C. Ueno, S. Ito, T. Goto and K. Yasui
Nihon Bayer Agrochem K. K. Yuki Research Center,
9511-4, Yuki, Ibaraki 307, Japan

Abstract. Mefenacet is highly effective against barnyardgrass (ECHOR) with a good selectivity to transplanted rice. This selectivity mainly depends on a so-called "placement selectivity". To clarify the differential action of mefenacet to rice plant and ECHOR, its influence when applied at different application sites of the plants was investigated. The action of mefenacet was observed as a growth suppression to rice plant and ECHOR, and mefenacet provided the highest activity when applied at the basal part of coleoptile and leaf sheath. The second highest activity was by the application at coronal and primary roots of both plants and at mesocotyl of ECHOR. Degree of the activity, however, was higher in ECHOR than in rice plant irrespective of application site. Mefenacet moderately suppressed growth of ECHOR, whereas it did only a little damage to rice plant by leaf sheath application at the upper part. The application of mefenacet at leaf blade had little influence on either plant. In paddy fields, mefenacet is distributed in a limited zone such as flooded water and surface layer of soil, and the main sites of ECHOR relating to the action of the herbicide are in the same zone. This direct contact with the sites provides a high activity to ECHOR. On the contrary, the basal part of leaf sheath of rice plant comes in little contact with mefenacet because of transplanting. Some difference in selectivity to mefenacet between rice plant and ECHOR also contributed to the action.

Key words. differential action, application sites, rice plant, barnyardgrass, mefenacet

Introduction

Mefenacet is characterized as a grass herbicide for the excellent control of barnyardgrass (*Echinochloa oryzicola* VASING : ECHOR) from preemergence up to 3 leaf stage with good selectivity to transplanted rice, and it is widely used in rice field of Japan nowadays. The high effectiveness and good selectivity of mefenacet are considered to mainly depend on a so-called "placement selectivity". Namely, by the water surface application of mefenacet, it distributes in a limited zone such as flooded water and surface layer of soil (ca. 1 cm in thickness) because of its low water solubility and high soil adsorption (1). In rice plant transplanted at 2-3 cm in depth, shoot apex in the basal part of coleoptile and leaf sheath, coronal root and primary root locate below the distributed zone of mefenacet, though 1st leaf blade and leaf sheath are in flooded water. On the other hand, mesocotyl of ECHOR elongates up to soil surface and forms shoot apex in the basal part of coleoptile and leaf sheath on soil surface. The leaf sheath, coleoptile, mesocotyl and a part of coronal and primary roots locate in the distributed zone of mefenacet (Fig. 1). To clarify the differential action of mefenacet to rice plant and ECHOR, the influence of the herbicide when applied at different sites of the plants was investigated.



LB : 1st leaf blade LS : 1st leaf sheath MC : mesocotyl CR : coronal root
PR : primary root BP : basal part of coleoptile and leaf sheath

Figure 1 Growth of rice plant and barnyardgrass and distribution of mefenacet in paddy

Materials and Methods

Activity of mefenacet at different application sites. Rice plant (*Oryza sativa* cv. Nipponbare) and ECHOR at the 2.0 and 3.0 leaf stage were used in this test. Lanolin paste containing mefenacet of 30, 3 and 0.3 μg/mg were prepared. Each 1 mg of the paste was applied to the plants in a form of ring at 1 mm wide with a syringe. Application sites were center part of the 1st leaf blade, 1st leaf sheath and imperfect leaf at 2 cm upper from the basal part, basal part of coleoptile and leaf sheath, mesocotyl (only ECHOR), coronal root at 1 cm below from the basal part, and primary root at 1 cm below from seed. After application, test plants were cultured for 2 weeks in Hoagland solution in a growth chamber controlled at 25 ± 1 °C, 80 ± 5 % relative humidity for day (12 hours) / night with light intensity of 6 klx. Activity of mefenacet was visually evaluated.

Activity of mefenacet in different transplanting depth. Rice plant and ECHOR at the 2.0 and 3.0 leaf stage were transplanted on soil surface (0 cm in depth : only roots were buried into soil) and at 2 cm in depth in plastic pots of 1/2,000 are filled with clay soil and puddled, and then carefully watered to 2.5 cm in depth. Mefenacet at the rate of 1,000 g a.i./ha was applied by water surface treatment on the same day. Activity of mefenacet to rice plant and ECHOR was visually evaluated at 5 days after application.

Activity of mefenacet by different duration of treatment. Roots and leaf sheath of rice plant and ECHOR at the 3.0 leaf stage were soaked in 1 ppm solution of mefenacet for 1, 3, 6, 12, 24 and 48 hours. After rinsing the test plants for 1 minute, they were cultured in Hoagland solution in a growth chamber controlled at 25 ± 1 °C, 80 ± 5% relative humidity for day (12 hours) / night with light intensity of 6 klx. Activity of mefenacet was visually evaluated at 1 week after soaking.

Results and discussion

Activity of mefenacet at different application sites. The action of mefenacet was observed as a growth suppression to rice plant and ECHOR, and mefenacet provided the highest activity when applied at the basal part of coleoptile and leaf sheath. The second highest activity was by the applications at coronal and primary roots of both plants and at mesocotyl of ECHOR. Degree of the activity, however, was higher in ECHOR than in rice plant irrespective of application site. Mefenacet caused severe growth suppression to ECHOR by the application at 1st leaf sheath, while it caused only a little damage to rice plant by the application at 1st leaf sheath and imperfect leaf. The application of mefenacet at leaf blade had little influence on either plant (Table 1). The test results support that the activity of mefenacet was provided by the uptake via basal part of coleoptile and leaf sheath (2) and roots (1). The little damage caused by mefenacet to rice plant suggests lower uptake via leaf sheath or faster degradation of the herbicide in the rice plant than in ECHOR. It is supposed that uptake of mefenacet via 1st leaf sheath contributed to the stable activity to ECHOR.

Activity of mefenacet in different transplanting depth. Growth of rice plant and ECHOR transplanted on soil surface was clearly influenced, when 1st leaf sheath, basal part and roots of them were in main distributed zone of mefenacet. The degree of the influence of mefenacet to ECHOR was somewhat higher than that to rice plant. In case of the transplanting of the both plants at 2 cm in depth when their 1st leaf blade and 1st leaf sheath and imperfect leaf of rice plant were in the same zone of mefenacet, a clear difference was observed on growth response between rice plant and ECHOR. Mefenacet suppressed the growth of ECHOR, whereas it did not provide a clear damage to rice plant (Table 2). Growth of ECHOR was influenced by the application of mefenacet not only at basal part and roots but also at 1st leaf sheath. The similar test results were obtained in the test with lanolin paste, too.
Accordingly, it became obvious that the difference in growth response of rice plant and ECHOR to mefenacet at leaf sheath of the plants was one of the important factors to the high effectiveness and good selectivity of mefenacet in paddy.

Activity of mefenacet by different duration of treatment. Growth of ECHOR was damaged 20% by the treatment of 1 ppm solution of mefenacet for 1 hour. The degree of the damage became higher as times passed after treatment. On the other hand, the damage of rice plant by mefenacet was observed by the treatment for 12 hours or longer, and the damage was still slight in comparison with that of ECHOR (Fig. 2).
In this test, a speed of action and level of activity provided by mefenacet was confirmed as a clear difference between rice plant and ECHOR. It suggests that this difference contributes to the high effectiveness and good selectivity of mefenacet.

Table 1 Activity of mefenacet to rice plant and ECHOR at the different application sites of plants

Application site	Dosage rate	Rice plant		ECHOR	
	μg	2LS	3LS	2LS	3LS
1st Leaf blade	30.0	30	0	20	10
	3.0	20	0	10	10
	0.3	10	0	10	0
1st Leaf sheath and	30.0	10	10	80	50
imperfect leaf* at 2 cm	3.0	10	10	80	30
upper from basal part	0.3	0	10	30	10
Basal part of	30.0	90	90	90	90
coleoptile and leaf	3.0	85	90	90	90
sheath	0.3	50	20	90	90
Mesocotyl	30.0			90	85
	3.0			90	80
	0.3			85	40
Coronal root	30.0	-	40	-	50
	3.0	-	40	-	40
	0.3	-	40	-	10
Primary root	30.0	60	70	90	80
	3.0	40	30	90	70
	0.3	30	20	70	50

Visual evaluation ; 100 (perfectly killed) - 0 (no effect)
* ; only rice plant
- ; not tested

Table 2 Activity of mefenacet to rice plant and ECHOR at the different transplanting depths in soil

Product	Dosage rate (g ai/ha)	Rice plant				ECHOR			
		2LS		3LS		2LS		3LS*	
		0 cm	2 cm	0 cm	2 cm	0 cm	2 cm	0 cm	2 cm**
Mefenacet	1000	60	0	60	0	80	50	80	50

* ; leaf stage of test plant at application time
** ; transplanting depth

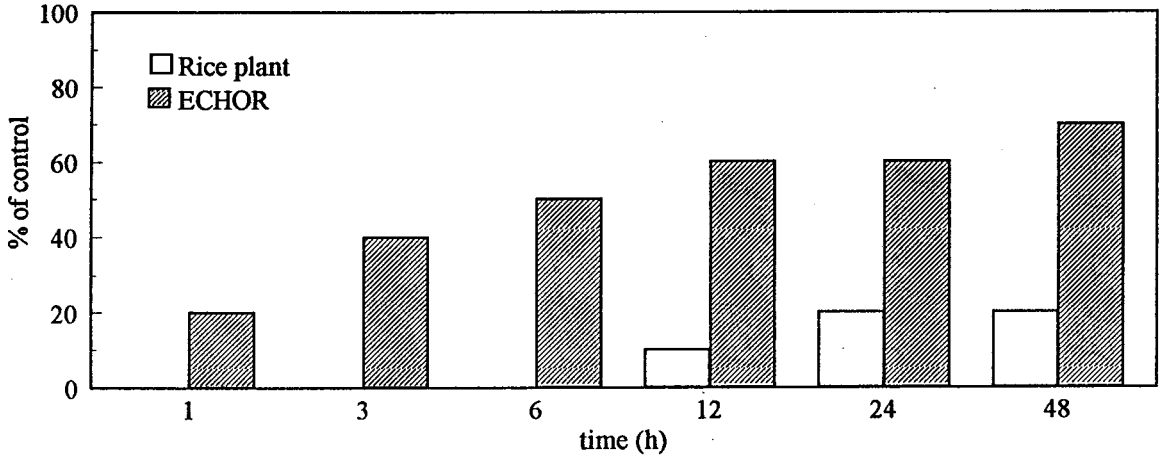


Figure 2 Growth response of rice plant and ECHOR to mefenacet by the different duration of treatment

From the above mentioned results, it can be concluded that the high effectiveness and good selectivity of mefenacet depends on not only " placement selectivity" but also the difference in sensitivity to mefenacet between rice plant and ECHOR. Especially, it is considered that the different sensitivity at leaf sheath of both plants to mefenacet participates to the efficacy and selectivity.

The difference in uptake, translocation or degradation of mefenacet at leaf sheath of rice plant and ECHOR is under investigation.

References

1. Schmidt, R. R., Eue, L., Forster, H., Mues, V. and Aya, M. 1984. Overdruk Med. Fac. Landbouww. Rijksuniv. Gent

2. Krauskopf, B., Fedtke, C. and Feucht, D. 1989. Proc. 4^{o} EWRS Mediterranean symposium

Application of Phenylcarbamates to Produce Male and Female Plug-seedlings of Asparagus

H. Watanabe*, M. Endo, T. Kodama[1], M. Yoshikawa[2] and H. Iwamura[3]
Yokohama Research Center, Mitsubishi Chem. Corp., Aoba-ku, Yokohama 227, Japan
1) Tochigi Plug Center, Dia-Topy Inc., Okubo, Tochigi-shi 328-02, Japan
2) Kyoto Prefecture Research Institute of Agriculture, Ayabe-shi 623-02, Japan
3) Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Abstract. Using a highly active phenylcarbamate to induce flowers in asparagus, we established a complete method to separate the male and female plants of asparagus plug-seedlings. The separated male and female plants of asparagus by this method showed quite characteristic growth profiles in the field cultivation depending on their sex.
Key words: asparagus, flower induction, phenylcarbamate, Ospar, plug-seedling

Introduction

Asparagus (*Asparagus officinalis* L.) is a dioecious species, and the male plants are preferred for commercial production, especially because of their higher productivity and of no useless matter consumption by fruit bearing. But it is impossible to distinguish the sex until the flowering which normally starts in the second or third year after sowing. Therefore the artificial enhancement of earlier flower induction before transplanting has been studied to enable the seedling sex distinction at earlier growth stage for obtaining only the male seedlings.

Hsung (1985) first tried to bring about early flowering of seedlings of asparagus by controlling temperature and day length. Abe *et al.* (1986, 1987) found that the herbicides of s-triazine or phenylurea type significantly initiated flower induction of asparagus seedlings, and then tested various compounds of triazine derivatives. Yanosaka *et al.* (1989) reported that some N-phenylcarbamates also had stronger activity to induce flowers of asparagus than s-triazines and others.

In this report, we first screened about 300 compounds of phenylcarbamates, s-triazines and phenylamides, then selected an n-propyl N-(3,4-dichlorophenyl) carbamate called Ospar as the highest compound of flower inducer for asparagus seedlings. Next, we established a complete method to produce the separated male and female plug-seedlings of asparagus by distinction of the sex of flowers induced by Ospar under specific conditions. Then we examined the growth profiles of the male and female plants as compared with the mixed plants controlled in the field cultivation.

Materials and Methods

1) Plant materials

The seeds of *Asparagus officinalis* L. *cv.* Mary Washington 500W, Pole Tom and Welcome were purchased from Tane-no-Sakata Company, Yokohama, and those of *cv.* Accel and Shower were from Takii Seed Company, Kyoto, and those of *cv.* Green Tower was from Kyowa Seed Company, Tokyo, Japan.

2) Measurements of Flower induction activity

The chemicals tested were dissolved in dimethyl sulfoxide, and the solution was diluted with distilled water to an appropriate concentration so that the final concentration of the organic solvent did not exceed 0.5% (v/v). A hundred seeds of asparagus were placed in a Petri dish (90 mm diameter) with two layers of filterpaper and 20 ml of the test solution. The seeds were incubated at 25 C for 8 days under the dim light.

The germination rates (%) were noted at the end of the incubation. The germinated seeds were throughly washed with tap water, planted in Vermiculite containing liquid fertilizer, Hyponex®, and grown for 2 weeks at 25 C under a 12 h period of light from fluorescent lamps (National FL 20SS, 80W/m^2). The flowering rate was expressed by the percentage of the plants with flowers in the germinated seeds.

3) Production system of plug-seedlings

We adapted the sex distinction procedure using flower inducer of asparagus to the production system of plug-seedling, Sunny Plug®, developed by Dia-Topy Inc. The seeds of asparagus were sown in #200 plastic plug-tray with peat-moss-rich medium using automatic seeding machine. The plug-tray containing seeds was placed for 8 days in germination room maintained at 25 C of temperature and above 95 % of relative humidity, then transfered to greenhouse. The germinated seedlings were grown there for more 20 days under appropriate conditions of temperature, water and liquid fertilizer controlled, and then distinguished the sex by observing the induced flowers on the apexes of the first shoots of the seedlings.

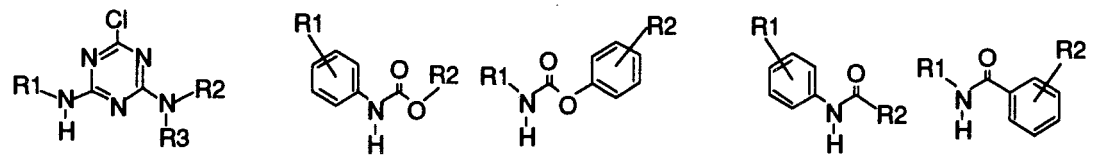
4) Growth observation in the field

The separated male and female plug-seedlings of asparagus were transplanted into the 40-liter planter (size: 50cm x 45cm x 40cm height) which placed outsides at Yokohama Research Center of Mitsubishi Chemical Corp., Yokohama-shi, and also into the test field of Chutan Laboratory of Kyoto Prefecture Research Institute of Agriculture at Ayabe-shi. Growth of the seedlings and the harvest were surveyed as compared with those of the control plug-seedlings without the chemical treatment for flower induction.

Results and Discussion

1) Screening of active compounds for flower induction of asparagus

Using *Asparagus officinalis* L. *cv.* Mary Washington 500W, we measured flower-inducing activity of various kinds of derivatives of s-triazines, phenylcarbamates and phenylamides as shown in Fig. 1. As a result of the assay of 72 compounds of s-triazines, 140 compounds of phenylcarbamates and 43 compounds of phenylamides, we got several active compounds shown in Fig. 2. In those active compounds, n-propyl N-(3,4-dichlorophenyl)carbamate, compound (3), was found to be the strongest compound as a flower inducer for asparagus, which showed more than 90% of flowering rate at the treatment concentration of 100 μM. We also examined some herbicides of phenylcarbamates, s-triazines, phenylamides, phenylureas and others as shown in Fig. 3, but did not find more powerful compound than n-propyl N-(3,4-dichlorophenyl) carbamate mentioned above. We named this compound Ospar, and tried to apply it as a candidate of flower inducer to the new production system for male and female plug-seedlings of asparagus.



s-Triazines Phenylcarbamates Phenylamides

Fig. 1 Structure of compounds tested for flower-inducing activity in asparagus

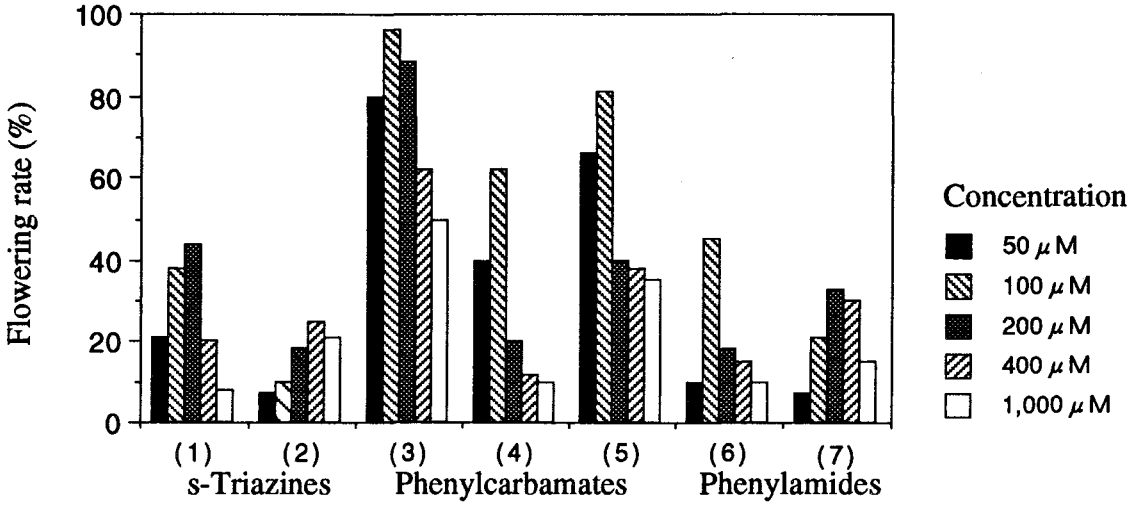


Fig. 2 Flower-inducing activity of active s-triazines, phenylcarbamates and phenylamides.

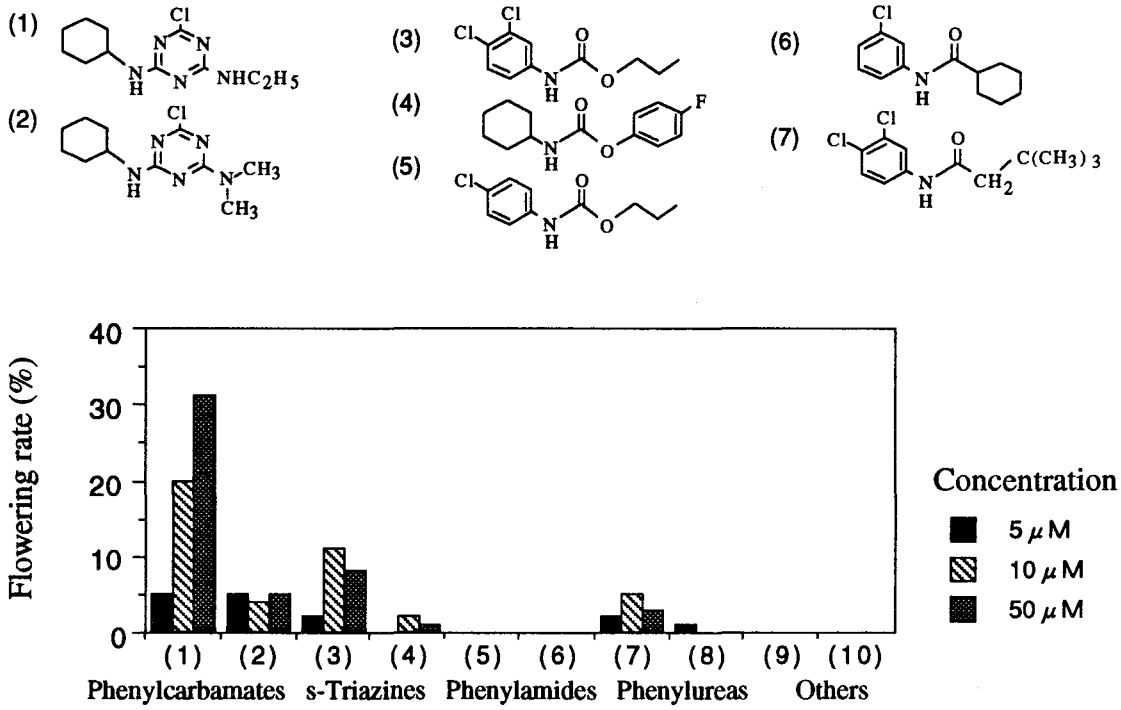
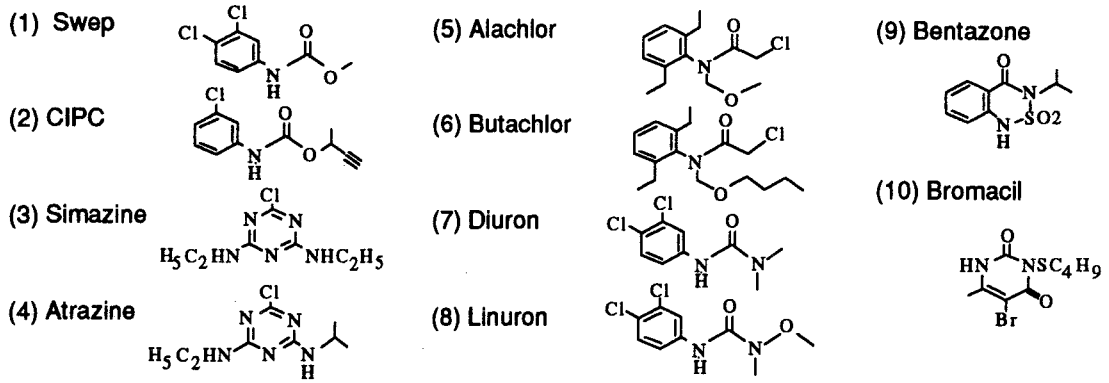


Fig. 3 Effects of herbicides on flower-inducing activity in asparagus.



2) Stability of the effect of Ospar among varieties of asparagus

We examined the difference of the flower-inducing activity of Ospar among some kinds of varieties of asparagus. Ospar was applied to 6 varieties of asparagus which mainly cultivated in Japan, and the result was shown in Table 1. Ospar was found to have a quite stable activity of flower induction to all of the asparagus varieties tested.

Table 1 Effect of Ospar on 6 major varieties of asparagus

variety of asparagus	Flowering rate (%)				
Ospar conc. (μM)	50	100	200	400	1000
Asparagus officinalis L.					
cv. Mary Washington 500W	79	<u>95</u>	86	62	49
cv. Pole Tom	85	<u>92</u>	90	77	61
cv. Welcome	88	<u>97</u>	90	75	70
cv. Accel	80	<u>92</u>	88	60	42
cv. Shower	<u>90</u>	85	70	72	50
cv. Green Tower	90	<u>98</u>	89	85	65

The value underlined means the maximum flowering rate of the asparagus variety.

3) Application of Ospar to the production system of plug-seedlings, Sunny Plug®

At first Ospar was treated into the plug-medium just after sowing of the seeds, but such treating method needed comparatively large amount of compound and resulted in growth retardation of asparagus seedlings afterward in the field (data were not shown). Then appropriate amount of Ospar was, therefore, coated around the asparagus seeds. Table 2 summarized the germination rate, flowering rate and top fresh weight using the coated seeds with Ospar at various concentrations. The seeds of *Asparagus officinalis* L. *cv.* Mary Washington 500W were used in this experiment. Approximately 0.3 to 0.4 mg of Ospar coated around one seed gave quite high flowering rates over 90% with no inhibitory effect to seed germination or rare influence on top fresh weight.

Table 2 Germination rate, flowering rate and top fresh weight of asparagus plug-seedlings using Ospar-coated seeds

Conc. of coating soln.	Amount of coated Ospar	Germination rate	Flowering rate	Top fresh weight
%	mg / seed	%	%	mg / seedling
Control (not coated)		94	0	284±28
1.0	0.07	95	71	244±25
5.0	0.19	97	78	247±18
10.0	0.27	97	89	240±35
15.0	0.34	93	92	258±20
20.0	0.40	95	95	252±23
30.0	0.65	92	95	240±10

4) Growth of the separated male and female plants of plug-seedlings

Plug-seedlings which induced flowers by Ospar coated around seeds were separated according to the sex, and then transplanted in 40-liter planter. Top fresh weight and root dry weight of the male, female, and control plants which were not treated with Ospar were measured at 3-months' intervals after transplantation, and shown in Table 3. It was clarified that the separated plug-seedlings by Ospar grew with no delay or restraint of the growth, and did better than the control plants by contraries.

Table 3 Growth of the separated male and female plants of plug-seedlings

	At transplanting	3 months after	6 months after	9 months after
Top fresh weight			g / plant	
Control	0.3	88.3	243.1	240.9
male	0.3	92.0	302.9	328.0
Female	0.3	88.7	276.5	282.5
Root dry weight				
Control	0.5	112.6	355.1	337.8
Male	0.5	120.0	389.2	394.1
Female	0.5	110.8	390.4	405.5

Asparagus variety: *Asparagus officinalis* L. *cv.* Mary Washington 500W
Ospar treatment: 0.34 mg/seed

Next, the separated plug-seedlings used above were transplanted and grown for 4 years in the test field of Kyoto Prefecture Research Institute of Agriculture, and the quality and quantity of the harvest of asparagus were survayed. The total amount of harvest for the last 3 years was summarized in Fig. 4. The size classification of the harvest, which influenced quality and price of asparagus, was summarized in Fig. 5.

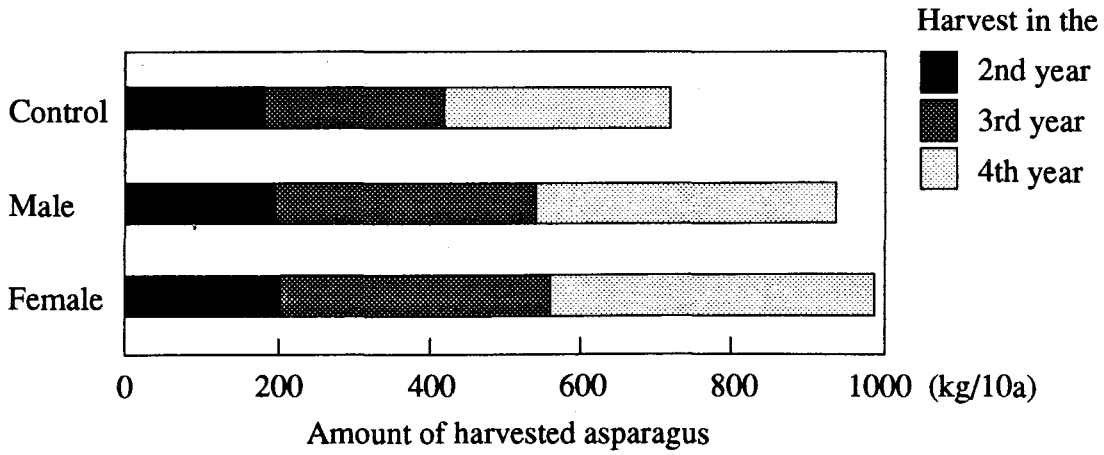


Fig. 4 Total amount of harvest of asparagus during 4 years' cultivation in the field.
Asparagus variety: *Asparagus officinalis* L. *cv.* Mary Washington 500W

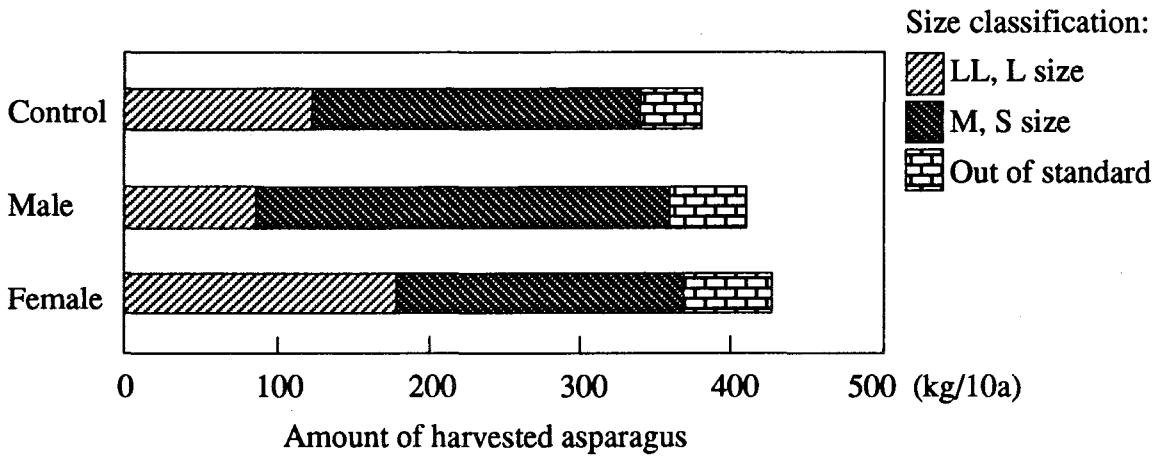


Fig. 5 Size classification of the harvest of asparagus.
Asparagus was harvested in the 4th year of cultivation.
Asparagus variety was *Asparagus officinalis* L. *cv.* Mary Washington 500W.
Size classification was accorded with the standard of Kyoto Central Market of Vegetables.

The total amount of harvested asparagus of the separated male and female plug-seedlings obviously increased as compared with that of the control plug-seedlings. Table 3 indicated that the plug-seedlings treated with Ospar grew more prosperously than the control in the early growth stage, which was considered to contribute to such remarkable augmentation of the harvest.

The size of harvest of the female plants was clearly found to be bigger than that of the male as shown in Fig. 5. It was also observed that the peak-time of harvest of male plants came at more than 2 weeks before that of female plants (data were not shown). Such characteristic differences of growth between the male and female plants must enable the asparagus growers to choose the sex of seedlings combined with their cultivation method, the climate or their purpose of marketing.

In conclusion, we selected an N-phenylcarbamate compound, Ospar, as a flower inducer of asparagus plug-seedlings. Ospar-coated asparagus seeds flowered quite stably, grew prosperously, and brought much augmentation of the harvest. The male and female plug-seedlings showed characteristic growth profiles in the field cultivation depending on their sex, which must give some valuable options in its field cropping to the growers.

References

1. Abe, T. and T. Kameya (1986) Promotion of flower formation by atrazine and diuron in seedlings of *Asparagus.*, Planta, *169*: 289;-291.
2. Abe, T., R. Shimizu, H. Iwamura and T. Kameya (1987) Flower induction by atrazine analogues in seedlings of *Asparagus officinalis.*, Physiol. Plantarum, *70,* 228-230.
3. Hsung, L. (1985) Effects of temperature, day length and cultivar on super early flowering (tip-flower) character of asparagus seedlings., Proceedings of Sixth International Asparagus Symposium, 298-306.
4. Yanosaka K., H. Iwamura and T. Fujita (1989), Flower induction in seedlings of Asparagus officinalis L. by N-phenylcarbamates., Z. Naturforsch., *44c*, 226-232.
5. Yoshikawa, M., N. Hashimoto and H. Iwamura (1995), Characteristics of carbamate-induced male and female plants in field cropping. J. Japan. Soc. Hort. Sci. *64 (Suppl. 1)*, 238-239.

Photosynthetic Inhibitory and Uncoupling Activities of Grandinol Analogs

K. Yoneyama*[1], M. Ogasawara[1], T. Saruta[1], M. Konnai[1], H. Miyoshi[2] and S. Yoshida[3]

[1]Weed Science Center, Utsunomiya University, Utsunomiya 321, Japan
[2]Faculty of Agriculture, Kyoto University, Kyoto 606, Japan
[3]The Institute of Physical and Chemical Research, Wako 351-01, Japan

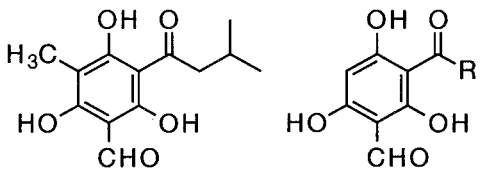
Abstract. Acylphlorophenones including grandinol, an inhibitor of seed germination and photosynthesis in *Eucalyptus* sp., were examined for their inhibition of photosynthesis and uncoupling activity in both *in vitro* and *in vivo* assays. Most of the acylphlorophenones were found to be potent inhibitors of photosynthesis and uncouplers of oxidative phosphorylation. In general, they inhibit photosynthesis at slightly lower concentrations than that needed for uncoupling.

Key Words: grandinol, photosynthesis, uncoupling of oxidative phosphorylation, *Eucalyptus* sp.

Introduction

Grandinol was originally isolated from leaf extracts of *Eucalyptus grandis* as an inhibitor of cress seed germination [3]. In addition, it also inhibited photosynthetic electron transport at reducing side of photosystem II [12]. Through extensive studies on the occurrence of grandinol within the genus, it was found that some of *Eucalyptus* trees contained grandinol [1]. Therefore, grandinol seems to play important roles in biological and/or biochemical processes in some *Eucalyptus* trees.

Structure-activity studies of grandinol-related compounds clearly demonstrated that two electron-withdrawing groups on the phloroglucinol nucleus were essential for the inhibition of both germination [2] and PS II [11], while the optimal length of the side chain or lipophilicity of the molecule were different between these two activities. For example, in the acylphlorophenone derivatives, their germination inhibitory activity dropped markedly when the side chain length exceeded C3 [2], while PS II inhibitory activity reached a maximum with a C6 side chain [11].



grandinol acylphlorophenones

Fig. 1. Structures of grandinol and acylphlorophenones.

From the chemistry of grandinol and its related compounds, they seemed to uncouple oxidative phosphorylation like other phenols. Therefore, we first examined their effects on mitochondrial respiration *in vitro*. In addition, their effects on cellular leakage from cucumber cotyledon discs were determined to evaluate their uncoupling and PS II inhibitory activities *in vivo*.

Materials and Methods

Chemicals

All of the acylphlorophenone derivatives used in this study were synthesized as reported earlier [2, 13]. Other chemicals including PCP (pentachlorophenol) and diuron were purchased from Wako Pure Chemical Industries, Ltd. or Tokyo Kasei Kogyo Co. Ltd.

Mitochondrial respiration measurements

Mitochondrial respiration measurements were conducted as reported previously [8]. Mitochondria isolated from the liver of adult Wister rats were used for the experiments. Mitochondrial respiration with 10 mM succinate as the respiration substrate was measured with a Clark-type oxygen electrode at 25°C, the final mitochondrial protein concentration in the medium being 0.7 mg/ml. The incubation medium consisted of 200 mM sucrose, 2 mM $MgCl_2$, 1 mM EDTA, 2.5 μM rotenone, and 2.5 mM potassium phosphate buffer (pH 7.4) in a total volume of 2.5 ml. The uncoupling activity of each compound was estimated from the concentration, C_{200} (M), at which the respiration rate was twice that of state 4 respiration [8]. The log of $1/C_{200}$ was used as the index of the uncoupling activity. The $\log(1/C_{200})$ was measured at least twice, and averaged. The standard deviation was within ±0.08. The maximum rate of respiration was evaluated with 0.7 mg of mitochondrial protein per milliliter in terms of nanomoles of O consumed per milligram of protein per minute. The respiration rate in each experiment was normalized to adjust possible differences among individual experimental conditions by use of the rate value with 30 nM SF6847 (2,6-di-*t*-butyl-4-(2,2-dicyanovinyl)phenol) as the standard, and this rate was expressed by the relative value, V_R; V_R = (rate induced by each compound - rate (state 4)) / (rate induced by SF6847 - rate (state 4)) [5].

Electrolytes leakage from cucumber leaf discs

Electrolytes leakage from cucumber (*Cucumis sativus* L.) leaf discs was measured according to the method of Yanase and Andoh [10] with minor modifications. Each ten cotyledon leaf discs, *id.* 10 mm, were floated on 20 ml of deionized water containing test compound, 10 μM paraquat and 100 ppm Tween 20. These were kept at 25°C in the dark for 14 hr and then transferred into continuous white light of 450±10 μEinstein (μE) m^{-2} sec^{-1} at 25°C. The conductivity of the bathing solution was measured at the beginning of the light exposure and every 2 hr afterward. The experiments were repeated three times with three replications.

Results and Discussion

Mitochondrial respiration

All of the acylphlorophenone derivatives including grandinol were found to be potent uncouplers of mitochondrial respiration as shown in Table 1. In general, uncoupling potency, $\log(1/C_{200})$, increased with an increase in the side chain length, and reach a maximum with the C7 side chain. Further lengthening of the side chain decreased the potency. These results indicated that the structural requirements were very similar in both uncoupling of oxidative phosphorylation and PS II inhibition [11], presumably due to the *in vitro* bioassay conditions where the chemicals easily approach the receptors. The maximum rate of respiration, V_R, induced by the acylphlorophenone derivatives were more than 70% of that induced by 30 nM SF6847, except for the compounds having shorter side chains (C1 and C2).

Other types of grandinol-related compounds tested, in particular, compounds having normal alkyl side chain, e.g. *N*-octyl-3-nitro-2,4,6-trihydroxybenzamide and *N*-octyl-3-nitro-2,4,6-trihydroxythiobenzamide [6, 7], also strongly uncoupled mitochondrial oxidative phosphorylation, and their potencies were parallel with their PS II inhibitory activities, i.e., more potent PS II inhibitors exhibited stronger uncoupling activities (data not shown).

Table 1. Uncoupling potency of acylphlorophenones in isolated rat-liver mitochondria.

Side chain (R)		$\log(1/C_{200})$	V_R
C1	Methyl	4.92	0.46
C2	Ethyl	5.66	0.38
C3	Propyl	5.92	0.75
C4	Butyl	6.28	0.88
C5	Pentyl	6.13	0.75
C6	Hexyl	6.80	0.75
C7	Heptyl	6.89	0.88
C8	Octyl	6.80	0.79
C9	Nonyl	6.55	0.79
C10	Decyl	6.24	0.71
grandinol		6.30	0.83

Electrolytes leakage from cucumber leaf discs

Cellular damage induced by phytotoxic compounds can be evaluated by monitoring electrolytes leakage from leaf discs [4]. This method has been utilized to determine many types of herbicidal damages; in particular, damages caused by herbicides which require light for action, e.g. bipyridiniums, protox inhibitors and PS II inhibitors, were clearly and quantitatively determined by measuring changes in conductivity of bathing solution in the light [9, 10]. When cucumber cotyledon discs were illuminated in the bathing solution containing 10 μM paraquat, the conductivity of the solution began to rise after 4 hr of illumination due to the cellular disruption caused by paraquat (Fig. 2). Such a rise in the conductivity was canceled by diuron in a dose-dependent manner, since diuron blocked photosynthetic electron transport and thus stopped the electron flow to paraquat (Fig. 2). These phenomena were observed only in the light. On the other hand, PCP, a typical uncoupler of oxidative phosphorylation, destroyed plant cells even in the dark, and the rise in the conductivity was not affected by illumination [9]. Therefore, in the light, PS II inhibitors decrease the paraquat phytotoxicity and thus the rise of conductivity of the bathing solution, while uncouplers increase it even in the dark.

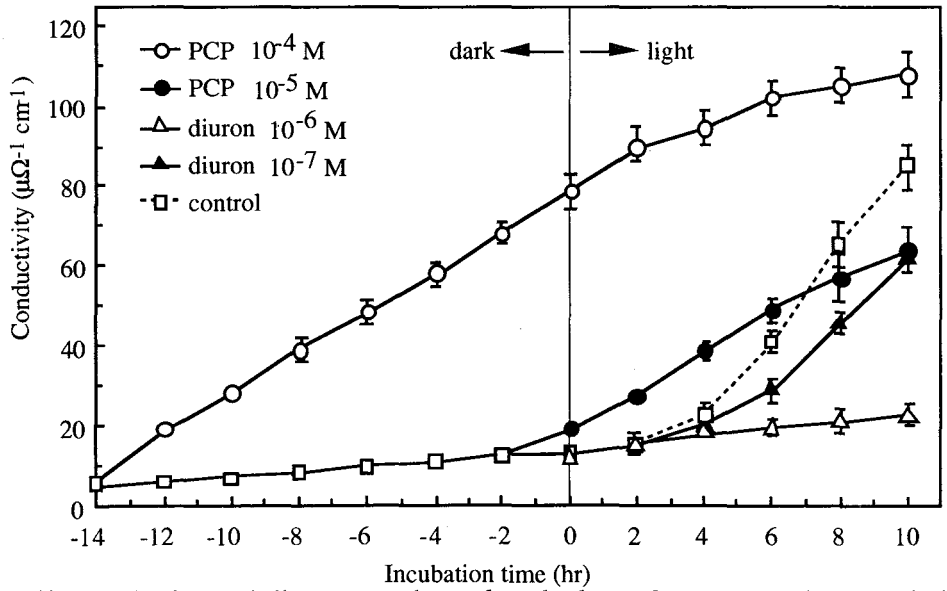


Fig. 2. Effects of PCP and diuron on electrolyte leakage from cucumber cotyledon leaf discs in the presence of 10 μM paraquat in the dark and in the light.

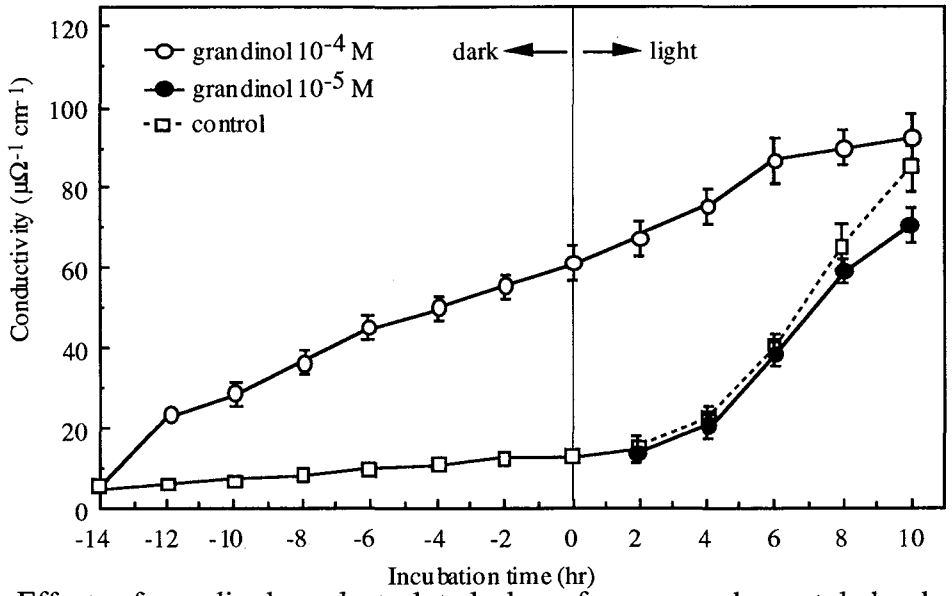


Fig. 3. Effects of grandinol on electrolyte leakage from cucumber cotyledon leaf discs in the presence of 10 μM paraquat in the dark and in the light.

Fig. 3 shows the effects of grandinol on electrolyte leakage from leaf discs. Grandinol at 10^{-4} M uncoupled oxidative phosphorylation and at 10^{-5} M inhibited PS II, since the conductivity was higher than that of control even in the dark but the rise of conductivity was suppressed in the light as compared to the control. All of the other acylphlorophenones also exhibited both activities. In general, these compounds inhibited PS II at slightly lower concentrations than that needed for uncoupling. Therefore, we could estimate uncoupling potency and PS II inhibitory activity of the compounds relative to that of PCP (10^{-4} M) and diuron (10^{-6} M), respectively.

$$\text{Relative uncoupling potency} = \frac{(\text{Cond4 of sample} - \text{Cond4 of control}) \times 100}{\text{Cond4 of } 10^{-4}\text{M PCP} - \text{Cond4 of control}}$$

(Cond4; conductivity at 4 hr after onset of illumination)

$$\text{Relative PS II inhibitory activity} = \frac{(\text{Cond10} - \text{Cond4 of control}) - (\text{Cond10} - \text{Cond4 of } 10^{-6}\text{M diuron}) \times 100}{(\text{Cond10} - \text{Cond4 of control}) - (\text{Cond10} - \text{Cond4 of sample})}$$

(Cond10, 4; conductivity at 10 and 4 hr after onset of illumination, respectively)

Relative uncoupling and PS II inhibitory activities of the acylphlorophenones were depicted in Figs. 4 and 5, respectively.

Uncoupling potency of the acylphlorophenones seemed to depend on their side chain length or lipophilicity, and reached a maximum with a C5 side chain (Fig. 4). Although the phloropropiophenone derivative had a short C2 side chain, it was as active as the C5 derivative, indicating that this compound may interact with the receptor in a manner slightly different from that of the other acylphlorophenones. In contrast, the optimal length of side chain for PS II inhibition was C3 (Fig. 5) and was different from that observed in the bioassay using isolated chloroplasts [11]. This difference in optimal length of side chain or lipophilicity for PS II inhibition between the two assay systems clearly demonstrated that relatively lower lipophilicity of chemicals is preferable for the penetration and transport in intact plants, and thus for herbicidal activity.

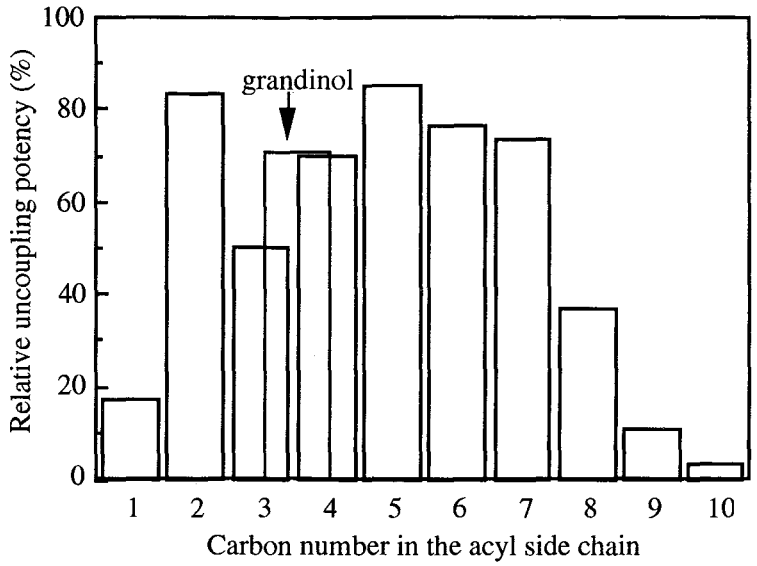


Fig. 4. Relative uncoupling potencies of acylphlorophenones at 10^{-4} M as compared to PCP (10^{-4} M).

Although the optimal side chain length or lipophilicity for uncoupling of oxidative phosphorylation was found to be different from that for PS II inhibition (cf. Figs. 4 and 5), it seemed difficult to separate these two activities through chemical modification of acylphlorophenones. Among the other grandinol-related compounds, however, we found several compounds showed quite strong PS II inhibition with very weak uncoupling potency. For example, *N*-(4'-phenoxy)-3-nitro-2,4,6-trihydroxybenzanilide at 10^{-6} M completely inhibited PS II but did not uncouple oxidative phosphorylation even at 10^{-5} M (data not shown). Therefore, we are trying to find grandinol-related compounds which specifically inhibit PS II without uncoupling activity.

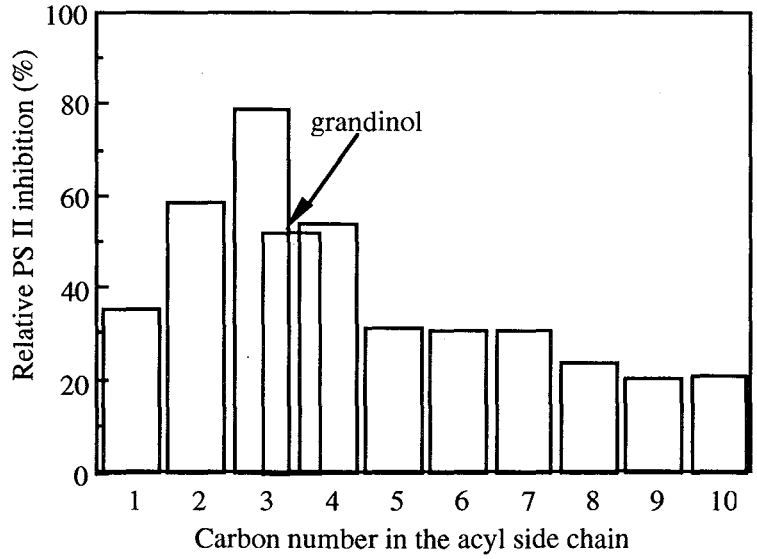


Fig. 5. Relative PS II inhibitory activities of acylphlorophenones at 10^{-4} M as compared to diuron (10^{-4} M).

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan [General (C) No. 04806018].

References

1) Bolte, M. L., J. Bowers, W. D. Crow, D. M. Paton, A. Sakurai, N. Takahashi, M. Ujiie and S. Yoshida 1984. Germination inhibitor from *Eucalyptus pulverulenta*. *Agric. Biol. Chem.*, **48**, 373-376.
2) Bolte, M. L., W. D. Crow, N. Takahashi, A. Sakurai, M. Uji-ie and S. Yoshida 1984. Structure/activity relationship of grandinol: a germination inhibitor in *Eucalyptus*. *Agric. Biol. Chem.*, **49**, 761-768.
3) Crow, W. D., T. Osawa, D. M. Paton and R. R. Willing 1977. Structure of grandinol: a novel root inhibitor from *Eucalyptus grandis*. *Tetrahedron Lett.*, 1073-1074.
4) Duke, S. O. and W. H. Kenyon 1993. Peroxidizing activity determined by cellular leakage, *In* "Target Assays for Modern Herbicides and Related Phytotoxic Compounds", ed. Böger, P. and G. Sandmann. Boca Raton, Lewis Publisher, pp. 61-66.
5) Guo, Z.-J., H. Miyoshi, T. Komyoji, T. Haga and T. Fujita 1991. Quantitative analysis with physicochemical substituent and molecular parameters of uncoupling activity of substituted diarylamines. *Biochim. Biophys. Acta*, **1059**, 91-98.
6) Honda, I., K. Yoneyama, M. Konnai, N. Takahashi and S. Yoshida 1990. Synthesis of 3-nitro-2,4,6-trihydroxybenzamide and thiobenzamide derivatives as highly potent inhibitors of photosynthetic electron transport. *Agric. Biol. Chem.*, **54**, 1071-1072.
7) Honda, I., K. Yoneyama, H. Iwamura, M. Konnai, N. Takahashi and S. Yoshida 1990. Structure-activity relationship of 3-nitro-2,4,6-trihydroxybenzamide derivatives in photosynthetic electron transport inhibition. *Agric. Biol. Chem.*, **54**, 1227-1233.
8) Miyoshi, H., H. Tsujishita, N. Tokutake and T. Fujita 1990. Quantitative analysis of uncoupling activity of substituted phenols with a physicochemical substituent and molecular parameters. *Biochim. Biophys. Acta*, **1016**, 99-106.
9) Yanase, D. and N. Andoh 1987. A new simple bioassay to evaluate photosynthetic electron-transport inhibition utilizing paraquat phytotoxicity. *Pestic. Biochem. Physiol.*, **38**, 92-98.
10) Yanase, D. and N. Andoh 1993. The paraquat bioassay to eveluate photosynthesis inhibition, *In* "Target Assays for Modern Herbicides and Related Phytotoxic Compounds", ed. Böger, P. and G. Sandmann. Boca Raton, Lewis Publishers, pp. 257-262.
11) Yoneyama, K., T. Asami, W. D. Crow, N. Takahashi and S. Yoshida 1989. Photosynthetic electron transport inhibition by phlorophenone derivatives. *Agric. Biol. Chem.*, **53**, 471-475.
12) Yoshida, S., T. Asami, T. Kawano, K. Yoneyama, W. D. Crow, D. M. Paton and N. Takahashi 1988. Photosynthetic inhibitors in *Eucalyptus grandis*. *Phytochemistry*, **27**, 1943-1946.
13) Yoshida, S., M. Ujiie, N. Takahashi, M. L. Bolte and W. D. Crow 1985. Synthesis of deuterated grandinol. *Agric. Biol. Chem.*, **49**, 211-213.

Author Index

THE ASIAN-PACIFIC WEED SCIENCE SOCIETY
THE WEED SCIENCE SOCIETY OF JAPAN
THE JAPAN ASSOCIATION FOR ADVANCEMENT OF PHYTO-REGULATORS