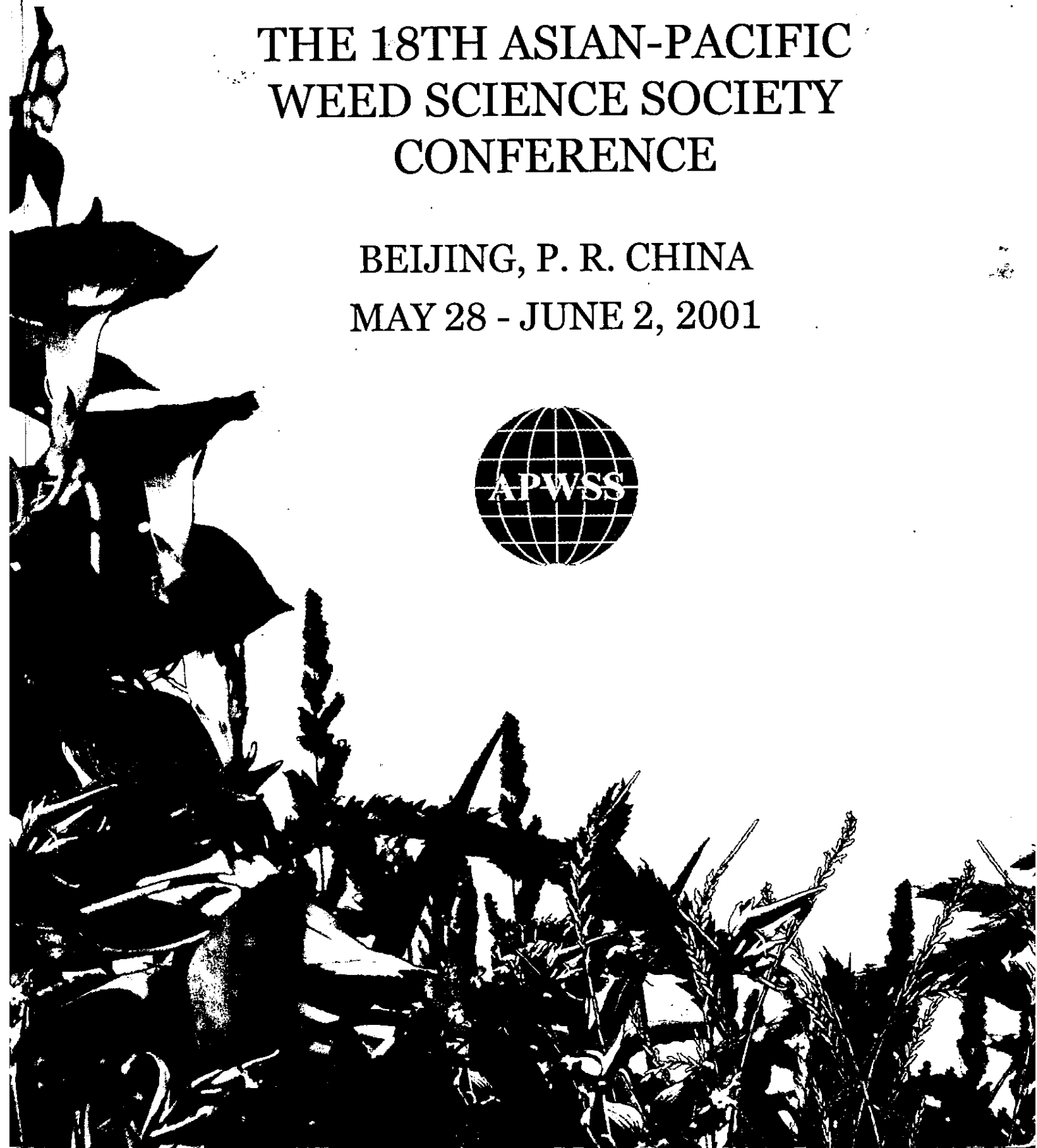


# PROCEEDINGS I

## THE 18TH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE

BEIJING, P. R. CHINA  
MAY 28 - JUNE 2, 2001



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# **THE 18TH ASIAN-PACIFIC WEED SCIENCE SOCIETY CONFERENCE**

**Beijing, P. R. China  
May 28 - June 2, 2001**



**Standards Press of China**

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

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## PRESIDENTIAL ADDRESS

Distinguished guests, ladies and gentlemen, weed science colleagues:

It is a great honor for me to give an address at the 18th Asian-Pacific Weed Science Society Conference held in Beijing, the capital of People's Republic of China. (1)

On behalf of the APWSS and the Organizing Committee of the 18th APWSS Conference, I would like to sincerely appreciate your enthusiasm and efforts to attend this conference. In particular, we have the greatest pleasure that more than 200 abstracts and 150 manuscripts covering various aspects of weed science and technology had been submitted and consequently the majority of the writings were collected to publish the proceedings before the conference opening. Furthermore, approximately 400 persons from more than 20 countries come to the conference. Especially being honored many guests from International Weed Science Society, the Weed Science Societies of America, Australia, India, Indonesia, Japan, Republic of Korea, Malaysia, New Zealand, Philippines and other countries kindly attend this conference as the distinguished scholars to address at this conference. We would warmly appreciate their presentations and supports for the development of the Asian-Pacific Weed Science Society.

Along with the chemical weed control area being steadily enlarged in global weed management in recent decades, the environmental problems, weed resistance to herbicides and other side effects in crop production induced by the intensive herbicide application are becoming severity day by day. For changing this situation, many agricultural scientists and technologists have recognized that the technical skills in weed management should be raised to continuously strengthen the approach in weed science research and technology extension, including grappling with new problems in world weed control by means of new chemicals or technology. For this reason, The 18th APWSS Conference will cover most important aspects of the weed science, focusing on the role of ecologically based weed management for sustainable agriculture in the 21st century. (2)

Many developing countries located in Asian-Pacific region, including China, need to do more works to develop the food production and protect the environment. Strengthening the research in the area of integrated weed management is becoming more and more important in promoting the regional development.

This is an exciting opportunity to gather a large number of global participants to discuss the latest development and challenges in weed science and technology. I believe all the weed science researchers and experts from various regions or countries will take this opportunity to exchange their views and learn most achievements in

weed science research and technology from each other at the forum. I sincerely expect Asian-Pacific Weed Science Society having a glorious duty to undertake more hard works for the future development in weed science and technology.

I think many of you are new comers to Beijing, the capital of People's Republic of China. And I believe you will have the opportunity to observe Beijing, a big city covering more than 16000 square kilometers, having a population of 13.8 millions and possessing historical monuments and tourist spots. I wish that all of you have pleasant days staying in Beijing and taking post conference tours in other famous places in China if possible.

I would like to sincerely thank the public-minded organizations, institutions, universities and agrochemical companies or incorporations inside and outside China for supporting this conference.

Finally, I am looking forward to the consummation of the conference.

Thank you for your kind participation and warm support!

Ze Pu Zhang,

Analytical and Testing Center,  
Chinese Academy of Agricultural Sciences,  
Beijing 100081, China

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**APPENDIX II.** List of officers at Weed Science Society of China, CSPP 2001

**AUTHOR INDEX**



## FAO TRAINING ON WEED MANAGEMENT

R. Labrada

Weed Officer Plant Protection Service FAO, Rome. ricardo.labrada@FAO.org

### SUMMARY

FAO training activities on weed management over the past four years and regions where they have been implemented are described in this paper.

At present the main areas of training are Weed Eco-biology and Competition, Herbicide Resistance and Waterweed Control. Such courses are usually organized at regional level and given by leading weed scientists actively working on relative topics of interest. To date three regional courses on weed ecology and competition have been carried out, as well as a further three on herbicide resistance. Waterweed control training is usually conducted within the framework of ongoing FAO projects on aquatic weed control.

FAO foresees the continuation of the courses on waterweed control in countries where training is still required. New courses on improved weed management in crop areas are due to start, where training should cover suitable methods for the control of existing weed complexes in the field. This will be carried out in two ways, through national training of researchers and extensionists as well as brief fellowships overseas for young weed scientists. The other area of interest in the future is training technicians from developing countries on risk/benefit assessment of Herbicide Resistant Crops (HRCs).

### INTRODUCTION - THE STATUS OF WEED MANAGEMENT

Despite the progress made in weed science over the last 40 years, weeds remain one of the major constraints to agricultural production in developed and developing countries. Losses caused by weeds in the developing world are still at least 15% annually and in certain regions, such as the arid Sahel in Africa, losses can be up to 50-60% in cereal-growing areas heavily infested with *Striga* parasitic weeds (Labrada, 1992).

In the developing world, progress on weed management is also being achieved and new weed control technologies have been introduced and adapted in several countries of Asia and Latin America. These control methods have to some extent alleviated the traditional drudgery of farmers. Herbicides are increasingly used in crops such as rice, wheat, sugarcane, cotton, soybean, sunflower and others. Already in 1991 Latin American countries were consuming 8% of the total herbicide market while Asia consumed nearly 15% (Terry, 1996). Cover crops are widely used in fallow areas and in the crop inter-rows of orchards; minimum and no tillage systems are expanding rapidly in various regions.

Despite the real progress made in weed management, several weed species continue to interfere seriously with crops, reducing their yields and quality, and farmers are still not aware of the basic elements of rational weed management.

New weed control methods introduced in the developing world have been implemented in many cases without taking account of prevailing local weed flora, its

composition and characteristics. In several places, three to four years after these introductions new troublesome species still thrive, causing subsequent crop losses in affected areas.

Examples of such phenomena are to be found in direct-seeded rice, where the use of herbicides has been unable to reduce the growth and reproduction of weedy *Oryza sativa* (red or weedy rice) and/or the incidence of the ever-present *Echinochloa*-spp.

Cases of herbicide resistance also occur increasingly in several developing countries, particularly in rice and wheat.

All these problems are the consequence of the rapid process of introduction of weed control measures. In many cases, the technology is derived from positive results achieved with a particular weed control method or chemical without taking into consideration other aspects of the prevailing weed complex, its characteristics and possibilities of new weed species becoming predominant.

It is no secret that in many developing countries there is a lack of understanding of the importance of studies on weed eco-biology, the process of weed interference and other aspects of weed behaviour.

Several weed problems together cannot be solved by using a single control method. The control of parasitic and perennial weeds, for example, requires the implementation of more than one control measure to effectively reduce existing infestation.

Nevertheless, it is considered that in order to promote improved weed management practices effectively, it is essential to train technicians and scientists in developing countries on the basic aspects of weed management, including some key control methods.

## THE CONTENT OF TRAINING ON WEED MANAGEMENT

Training on weed management in the 60-70s was based mainly on promoting safe handling and use of herbicides with scarce weed eco-biology elements.

A good basis for the development of an integrated approach to weed management could be the use of data related to weed growth cycles, rate and mode of plant reproduction, weed seed bank in soil and weed interference with crops.

Weed research programmes in many developing countries have been drastically reduced. Such reduction has also reached some International Centres of the CGIAR system. Weed research has suffered in much the same way over the past few years. In many cases it was believed that to control weeds it is enough to be familiar with the use of chemical herbicides, and many weed activities were devoted solely to herbicide testing. In the 70s, the only activity of many agricultural centres in developing countries was testing of all new herbicides in different crops. Once this herbicide "boom" was over the institutions started to abolish weed research posts. The technical justification for studies of weed population dynamics and/or seed bank in soil was not given or planned, so it was thought there was no scope for weed research.

While developed industrialized countries, which depend less economically speaking on their agricultural production, retain the work of their weed science societies and strengthen their research/extension programmes, the developing world still underestimates the importance of improved weed management.

Greater awareness of the importance of weed control is still needed, and it should be emphasized that weed management is responsible for 80% of the success in cropping.

Land preparation (no matter whether conventional, minimum or zero tillage systems are used), cultivation, use of cover crops, mulching, inter or multi-cropping plus weeding (either mechanically or manually) all have more to do with weed control than with any other aspect of cropping.

### WEED ECO-BIOLOGY AS A BASIS FOR SOUND WEED MANAGEMENT

The spread of weeds is largely determined by abiotic factors, such as climate and soil, as well as by competition and current cropping practices.

The basis of sound weed science is a knowledge of weed ecology and biology (Table 1).

Table 1. Eco-biological attributes of weed behaviour

Characteristics	Remarks
a) Diaspora Germination	Seasonality and factors intervening in their Germination and emergence
b) Plant Growth Rate	Plant phenophases up to seed setting
c) Reproduction of Diaspores	Capacity and outputs of diaspores
d) Dispersal	Biotic and abiotic factors intervening in the spread of the weed
e) Diaspora longevity	Understanding weed seed dormancy and behaviour of underground vegetative propagules

Another practical aspect for farmers to understand is when to weed, something that will surely save them and their families time. Knowledge of methods for determining weed-free periods and critical periods of weed competition will help farmers to understand the importance of early removal of weeds to minimise crop losses caused by weed competition.

In no less than 80% of developing countries there is a lack of data on weed ecology (behavioural ecology of weeds) and biology, and in half of them no studies of critical periods of weed competition have ever been carried out. (6)

The development of applied ecology has been of enormous value for better management of natural resources and improvement of agricultural production, including weed management.

In addition, the weed management component should be ecologically based if it is to be included in IPM programmes. Therefore, assistance to countries by giving training to still existing weed scientists or agronomists on methods to study weeds, their life cycle, reproduction, weed seed bank and weed competition, is essential for the sound development of weed management. (7)

The first phase of such development should avoid the inclusion of mathematical modelling in the training syllabus. It is essential first to study the weeds and to gather as many data as possible. Once this is done and the study process is established, then modelling may take place.

### ASPECTS RELATED TO THE USE OF HERBICIDES

The introduction of herbicides in nearly all countries has been carried out through

internal pesticide registration procedures. This process normally includes some field tests in order to study the effectiveness of the product in weed control and its possible phytotoxicity to crops. Only in a few developing countries have such tests included other studies of herbicide carry-over and any other indirect effect from the use of these agro-chemicals.

The problem of weed resistance is not new. In the past, Gressel (1978) presented an interesting review of this problem and presented his ideas of what might occur. Nowadays weed resistance is a problem in nearly all developed countries and is also becoming an important issue in several developing countries, particularly in Latin America and Asia where herbicides have been used for 20 or more years in various crops. In addition, the use of new molecules of imidazolines, sulfonylureas and aryloxyphenoxyalkanoic acid esters (post-emergence selective graminicides) has produced problems of weed resistance in a relatively short period of time.

## WATER WEED CONTROL

Waterweeds are a major problem in nearly all tropical and sub-tropical countries. These weeds cause problems to fishing, irrigation networks and hydroelectric power supplies and may serve as habitats for the development of vectors of human diseases, such as malaria and bilharziasis.

The introduction and spread of several plants, such as water hyacinth (*Eichhornia crassipes*) and water fern (*Salvinia molesta*), in tropical countries has been a disaster to the water bodies of these nations. These plants, particularly water hyacinth, were introduced as potential ornamentals and for taxonomic studies without the presence of their natural enemies. The latter fact has been the main cause of their rapid dissemination in the countries. Sometimes this phenomenon even touches countries with nearly temperate climate, as is the case of water hyacinth establishment in Portugal and in Tarija province in Bolivia.

Another waterweed problem in polluted water bodies of several countries is water lettuce (*Pistia stratiotes*), whose presence has increased enormously in recent years.

Affected countries urgently need training and advice on how to control these floating weeds and prevent their further dissemination. It is well known that floating aquatic weeds are good targets for biological control, but to implement such control methods training is required on safe introduction, rearing and release of effective biological control agents.

## FAO TRAINING PROGRAMME ON WEEDS

Based on the problems described above, FAO has implemented training on various aspects of weed management as an initial step towards increasing technical capabilities in the countries. Training is conducted on the three major aspects mentioned above, i.e. weed eco-biology and competition, herbicide resistance and aquatic weed control.

Since each training programme has different objectives, the duration also varies. Relevant weed research personnel conduct the training courses, which include lectures, seminars and field demonstrations and activities. The main aim is to provide an understanding of the fundamental aspects of each method of evaluation and/or control.

### Training on weed eco-biology and competition

Courses on these topics have already been conducted in three region, Latin America (Lima, 1999), Asia (Bangkok, 2000) and French-speaking Africa (Cotonou, 2000). To date, weed scientists from 24 developing countries have been trained in weed eco-biology and competition. Two additional courses are foreseen for the period 2001-02, one in Ethiopia for English-speaking African countries and the one for countries of the Middle East. The content of these courses is shown in table 2.

The content of the course is based mainly on the recommendations made by an international panel of experts in a technical consultation on weed ecology and management organized by FAO (1997a).

The advantage of the courses is that the participants discuss weed problems in their own countries and usually receive advice from the other participants. In some cases, workshops are organized on particular topics, such as the one conducted in Cotonou on *Imperata cylindrica* control or one held in Asia where participants formulated a regional project profile on weed management.

Once the cycle of courses is finished, a second phase of field research involving farmers is foreseen in pilot countries. The idea is to use data from these studies in ongoing IPM programmes.

Table 2. Content of Courses on Weed Eco-biology.

Aspect imparted	Time (hrs)	Remarks
a) Ecology & Biology damage	5.5	Weed Concept Weed characteristics & Weed Classification Weed Origin Life Cycle (Demography, seed bank, seedling recruitment and survival)
Methods for studies on weed ecology	3	Determining phenophases, plant productivity, diaspora bank
b) Weed Interference	7	Competition, Allelopathy and Parasitism Methods to study competition Methods to estimate weed crop losses in the field
c) Seminars and practice	15	
d) Field visit	7	

### Training on herbicide resistance

This type of training has only been carried out in regions where herbicides are known to be used in one major or several crops. Thus the first regional training as such was organized in Jaboticabal, Brazil (FAO, 1997b), with the participation of eight Latin American countries, while a second was carried out in Taegu, Korea (FAO, 1998), with the participation of seven Asian countries. The third course was a modest sub-regional workshop for Baltic countries (Estonia, Latvia and Lithuania) (FAO, 1999).

The content of the course is shown in table 3. In addition to lectures on herbicide resistance and demonstration of methods for detection of resistance, seminars are also included in order to discuss the status of herbicide use in each country and related

problems.

Increased use of herbicides in other developing regions will indicate whether there is a need to organize courses on weed resistance in other developing regions.

Table 3 Content of Courses on Herbicide Resistance

Aspect imparted	Time (hrs)	Remarks
Country reports	8	Data on Herbicide Use and Resistance
History & evolution of the problem	1.5	Cases of Weed Resistance
Mechanisms of evolution & population dynamics of weeds	1.5	Information on weed ecology
Weed Biology & interference	1.00	Behaviour and competition
Herbicide action and steps associated with herbicide resistance	3	An overview 1. Herbicides interfering photosynthesis. 2. Inhibitors of aminoacid synthesis 3. Inhibitors of ACCase and cell division
Mechanisms of resistance to major herbicides & approaches used to combat reduced activity:		
Methods for detection Of herbicide resistance.	3	Simple methods for detection of resistance
Herbicide behaviour in soil	1.5	Activity in soil of main herbicides
Integrated Crop Management	2	Other weed control methods
Seminars and field visit	14	

### Training on integrated water weed control

Due to severe problems caused by water weeds, several affected countries have requested technical assistance from FAO to implement control programmes, particularly of water hyacinth.

Since 1991 FAO has implemented 11 projects: two in Latin America and the rest in Africa, four of which were regional. One of the main components of these projects is strengthening national capabilities in biological control of aquatic floating weeds. In these activities national personnel receive training on methods for surveying aquatic weeds, rearing and release of relevant biological control enemies and on survey of their establishment in treated areas.

The existence of various sound rearing units of biocontrol agents, as well as personnel already trained and experienced in these activities, permit collaboration among African countries in issues related to water weed control. The content of the training programme on waterweed control is shown in table 4.

Training on waterweed control refers mainly to floating waterweeds, i.e. *E. crassipes*, *S. molesta* and *P. stratiotes*.

In this training, experience gained by other countries in waterweed control in the

region is discussed.

FAO has also promoted waterweed control in Latin America, and a regional seminar on this topic was organized in Cuernavaca, Mexico, with the participation of five countries.

Table 4 Content of Training on Water Weed Control in Africa

Aspect imparted	Time (hrs.)	Remarks
Overview of the Aquatic Weed Problem	1	Major Emphasis on biocontrol, chemical control & manual removal
Biocontrol Agents	2	Characteristics and identification
Biology of water weeds	4	Characteristics and behaviour of major floating weeds
Weed surveillance	4	Methods to monitor water weed stand
Rearing, maintenance and quality control of bioagents	12	Well known methods for reproduction and release of bioagents.
Harvesting, Storage/Transportation of biocontrol agents	10	
Monitoring and Evaluation	6	Methods to monitor establishment of bioagents.
Community Participation	8	Methods for involving riparian communities in the release of bioagents and manual removal
Video show	2	
Field Practices (rearing, release, monitoring)	20	
Seminars	12	

### **Future activities and complement**

For the immediate future, three types of training are planned:

**Improved weed management** There is a need to implement improved weed management in many developing countries, where the fundamental aspects of appropriate weed control are still not used. Instead of implementing projects on a single harmful weed species, the development of projects comprising rational control of weed complexes in economically important crops of the countries is foreseen.

Training on integrated weed management will be included in the work plan of these projects. This should be done in two ways: a national training course for various participants (researchers, extensionists, others) and brief overseas training for one or two weed scientists.

**Water weed control** Training on this aspect should continue in those countries still requiring the implementation of integrated waterweed control. The training should be included in the work plan of projects specific to this topic.

**Herbicide Resistant Crops** The issue of Herbicide Resistant or Tolerant Crops (HRCs) is gaining in importance. Developing countries should decide individually whether or not to use these transgenic crops, for which they would need tools to evaluate possible risks and/or benefits of their use.

FAO is at present finalizing guidelines for the evaluation of HRCs, which may be used by the countries as the step prior to the possible introduction of these crops. Certainly, guidelines alone will not be enough. Therefore training is essential for correct implementation of this type of evaluation.

2 Within the limited funds available, FAO will continue to assist the countries in matters related to integrated weed management. It is also important to point out that this assistance has also been made possible through the active participation of several outstanding weed scientists, who kindly agreed to share their knowledge and experience with technicians from the developing world.

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# **Weed Biology and Ecology**

## DIAGNOSTIC DEVELOPMENT AND GENETIC ANALYSIS OF WEEDY *SPOROBOLUS* SPECIES

S. W. Adkins<sup>1</sup>, G. C. Graham<sup>2</sup>, S. Shrestha<sup>1</sup> and D. S. Loch<sup>3</sup>

<sup>1</sup>School of Land and Food Sciences, University of Queensland, St. Lucia, 4072  
s.adkins@mailbox.uq.edu.au

<sup>2</sup>Centre for Identification and Diagnostics, University of Queensland, St. Lucia, 4072

<sup>3</sup>Queensland Department of Primary Industries, Redlands Research Station, Cleveland,  
4163

**Abstract:** Five introduced weedy *Sporobolus* species have become major weeds in Australia: viz., *S. pyramidalis*, *S. natalensis*, *S. fertilis*, *S. africanus*, and *S. jacquemontii*. These weeds are of most concern in dairy and beef production. An important method of spread is as contaminants of pasture seed lots where it is impossible to identify them from desirable native species. Thus, a method for species identification that can be applied to seed is required. Initially, Random Amplified Polymorphic DNA (RAPD) analysis was used to develop species-specific markers. However, because the RAPD technique was highly sensitive to a number of reaction conditions, an alternative method was sought for the development of a commercial diagnostic protocol. The nuclear ribosomal DNA Internal Transcribed Sequences (ITS) of 14 *Sporobolus* species were sequenced and restriction site maps developed. Five restriction enzymes were found that could differentiate the five Australian weedy species from the remaining native species. In the development of the diagnostic protocol an opportunity arose to study the phylogenetic relationship of these species. From the strict consensus tree, the species fell into two major groups. One, previously named the *Sporobolus indicus* complex, contained all of the weedy species. The other group was made up of the Australian natives. The species in the first group were further subdivided into five major clades, indicating that there may only be five true species in this complex. In such an analysis, *S. pyramidalis* and *S. natalensis* could be considered to be very closely related.

**Keywords:** beef production, dairy production, diagnostics, seeds, weedy grasses.

## INTRODUCTION

Five introduced species of the genus *Sporobolus* are considered to be serious weeds in the northern beef and dairy production regions of Australia. These include two giant rattail grasses (*Sporobolus pyramidalis*, *S. natalensis*), giant Parramatta grass (*S. fertilis*), Parramatta grass (*S. africanus*) and American rattail grass (*S. jacquemontii*). The undesirable characters of these plants include a) their unpalatable leaves and stems, b) their ability, as perennial tussock grasses, to crowd out desirable pasture plants, and c) their ability to increase the fire hazard. Consequently, even minor infestations can lead to significant reductions in beef and dairy production. These weedy grasses currently infest approximately 350 000 hectares with the potential to infest up to 30% of mainland Australia. Typical production losses range from 10-80% depending on the density of infestations. It is estimated that annual losses to beef production in northern Australia will be in the vicinity of \$60 million/year, if these weeds are allowed to spread to their adaptational limit. Key environmental areas are also at risk of degradation from these weeds.

Because of the genetically close relationships among these weedy species, they are very difficult to tell apart, and often difficult to recognise from desirable endemic species. This identification problem is especially true when only seed samples are available for analysis. Since one of the major modes of spread for these weeds is as contaminants of pasture seed lots used for pasture establishment, it is important that a new identification tool be developed and a protocol be established for preventing contaminated seed lots being marketed.

The use of molecular techniques to identify closely related plant species is now well documented. Several techniques exist that would potentially allow for the rapid identification of a species from a single seed. At the University of Queensland, we have been working for the past two years on genetic relationships within the genus to develop a diagnostics protocol for *Sporobolus* species. Field collections of seed from all weedy and many native *Sporobolus* species have already been made and a seed-specific DNA extraction protocol developed.

Thus, we report on: 1) The development of a random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) technique and progress towards the development of a sequence characterised amplified region (SCAR) species-specific diagnostic. 2) The sequencing of the internal transcribed sequence (ITS) region of the nuclear ribosomal DNA of a number of *Sporobolus* species and the development of a second species-specific diagnostic based on restriction site variation. 3) A study of the phylogenetic relationship of the various *Sporobolus* species within Australia with a view to using this to help direct future weed management options for these closely related native and weed species.

## MATERIALS AND METHODS

### Plant materials

Fifty-six seed samples coming from 13 *Sporobolus* species were used (Table 1). The samples were collected from a number of different locations (Shrestha, 2001) from around Australia and from Mexico (*S. indicus*), South Africa (*S. natalensis*), Indonesia and Papua New Guinea (*S. sessilis*), and Indonesia and Malaysia (*S. diandrus*). Representative plants were grown in a glasshouse at the University of Queensland (UQ) for traditional taxonomic identification.

### DNA extraction and quantification

Genomic DNA was extracted from the seed tissue of each sample using the protocol of Edwards et al. (1991). This was analysed using a Gene Quant II Pharmacia Biotech Spectrophotometer and RAPD cycling conditions modified from Yu and Pauls (1994). Amplification products were analysed on agarose gel in TBE buffer, and stained with ethidium bromide and visualised under transilluminator and photographed.

### RAPD-PCR and gel electrophoresis

RAPD amplification reactions were performed in 25 µL reaction volume containing 2.5 mM MgCl<sub>2</sub>, 2.5 µL of 10x reaction buffer (670 mM Tris-HCl, pH 8.8 at 25 °C, 166 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 4.5 % Triton X-100 and 2 mg/mL gelatin), 0.2 mM dNTPs, 0.4 µM concentration of each decanucleotide primer (OPA primers from Operon Technologies Inc., Alameda, CA. USA, and UBC primers from University of British Columbia, Oligonucleotide Synthesis Laboratory, Vancouver, British Columbia), 1.1 unit of *Taq*

polymerase (Biotech International Ltd.) and 25 ng of genomic DNA. Amplification reaction was performed in Hybaid Omne-E thermal cycler. The cycling condition used consisted of one cycle of one minute at 94 °C, 35 cycles of 10 seconds at 94 °C, 30 seconds at 38 °C and 60 seconds at 72 °C followed by 5 minutes final extension at 72 °C. The cycling condition was modified from Yu and Paulis (1994). The amplification products were analysed on a 1.5 % agarose gel in 1x TBE buffer at 12 V/cm for 2 hours. The gels were stained with ethidium bromide and visualised under a transilluminator and photographed using Polaroid film.

Table 1. *Sporobolus* species used in PCR-RAPD and ITS studies, their weedy status within Australia and their common names

<i>Sporobolus</i> species	Common name	Australian status	No. populations used	
			PCR-RAPD	ITS
<i>Pyramidalis</i>	Giant Rats Tail grass	Introduced/ Weed	5	5
<i>Jacquemontii</i>	American Rats Tail grass	Introduced/ Weed	4	5
<i>Fertilis</i>	Giant Parramatta grass	Introduced/ Weed	7	5
<i>Africanus</i>	Parramatta grass	Introduced/ Weed	5	4
<i>Natalensis</i>	Giant Rats Tail grass	Introduced/ Weed	7	7
<i>Sessilis</i>	Wire grass	Native	4	4
<i>Diandrus</i>			3	3
<i>Elongatus</i>	Slender Rats Tail grass	Native	3	5
<i>Creber</i>	Bunched Rats Tail grass	Introduced	3	3
<i>Laxus</i>	Lax Rats Tail grass	Native	2	2
<i>Carolii</i>	Fairy grass?	Native	4	4
<i>Australasicus</i>		Native	7	7
<i>Indicus</i>			2	2
<i>Pennisetum alopecuroides</i>	Swamp Foxtail	Out-species	0	1
<i>Heteropogon contortus</i>	Black Spear grass	Out-species	0	1

## PCR-ITS

PCR amplification of the ITS 1 and ITS 2 region was performed with primer pairs from Pacific Oligos (Southern Cross University, NSW, Australia). Template DNA, Taq polymerase and substrates were amplified and the products visualised following agarose gel electrophoresis and staining with ethidium bromide. ITS sequences were retrieved in a computer and edited using 'Edit View' and 'Sequence Editor' programs to produce consensus sequences of all the samples. These sequences were then transformed into a Nexus format for master alignment and phylogenetic analysis. Sequences of *P. alopecuroides* and *H. contortus* were designated as out groups for the phylogenetic analysis. The entire ITS data set of all the samples from the 13 species of *Sporobolus* and two out groups were analysed using phylogenetic analysis using parsimony.

## RESULTS AND DISCUSSION

### RAPD

From an initial screening of 120 different decanucleotide primers using *S. pyramidalis*, the best 46 amplifying primers were selected and used in a RAPD profiling experiment involving 12 species of *Sporobolus* (viz., 5 introduced weedy species and 7 native species). A marker confirmation experiment was then carried out on the 10 most promising primers using all 52 seed samples of the 12 species (i.e. 2 to 5 samples from each of species depending on availability). Six of these primers generated appropriate markers (Table 2). The most important diagnostic markers were those of UBC51<sub>490</sub> for *S. pyramidalis* and *S. natalensis*, UBC43<sub>470</sub> for *S. jacquemontii* and UBC43<sub>310</sub>, UBC43<sub>2100</sub> and UBC43<sub>2000</sub> for *S. fertilis* and *S. africanus*, and OPA20<sub>850</sub> for *S. jacquemontii*.

Table 2. Presence (+) or absence (-) of various markers in five different weedy species. +/- sign indicates markers that were not always present in all samples.

Primer	Fragment Size (bp)	<i>Sporobolus</i> Species				
		<i>pyramidalis</i>	<i>jacquemontii</i>	<i>natalensis</i>	<i>fertilis</i>	<i>africanus</i>
UBC13	2000	+	-	+	+/-	+/-
UBC43	650	+	-	+	+/-	+/-
*UBC43	470	-	+	-	-	-
*UBC43	310	-	-	-	+	+
*UBC43	2100	-	-	-	+	+
*UBC43	2000	-	-	-	+	+
UBC92	1000	+	-	+/-	+/-	-
UBC92	930	-	-	+/-	+	+/-
*UBC51	490	+	-	+	-	-
UBC75	950	+	+	+	+/-	-
OPA20	910	+	+	+/-	+	-
*OPA20	850	-	+	-	-	-

\* Useful identification markers

Reaction conditions must be strictly maintained if the same banding patterns are to be produced reliably each time. For this reason an, as yet unsuccessful, attempt was made to convert the RAPD-derived markers into a more robust SCAR assay (data not shown). Once this has been achieved, then this diagnostic protocol will be of great value in two respects. Firstly, it will permit the accurate and rapid identification of weedy *Sporobolus* species, enabling the more rational management of these species in Australian pastures. Secondly, pasture seed lots can be screened effectively to determine the presence or absence of weedy *Sporobolus* species.

## ITS

Sequencing of the nuclear ribosomal DNA Internal Transcribed Sequences (ITS) was carried out on 42 seed lots from 14 *Sporobolus* species and two out-group species. The entire ITS region (including ITS 1 and ITS 2) was 604 base pairs (bp) long except in *S. diandrus* which was 603 bp. These species-specific ITS sequences were then utilized to develop a diagnostic protocol for the five Australian weedy species. The consensus sequences were introduced into a webcutter 2URL (<http://www.firstmarket.com/cutter/cut2.html>) program and restriction site maps developed. Selection of useful restriction enzymes was then made. Out of ca. 140 enzymes

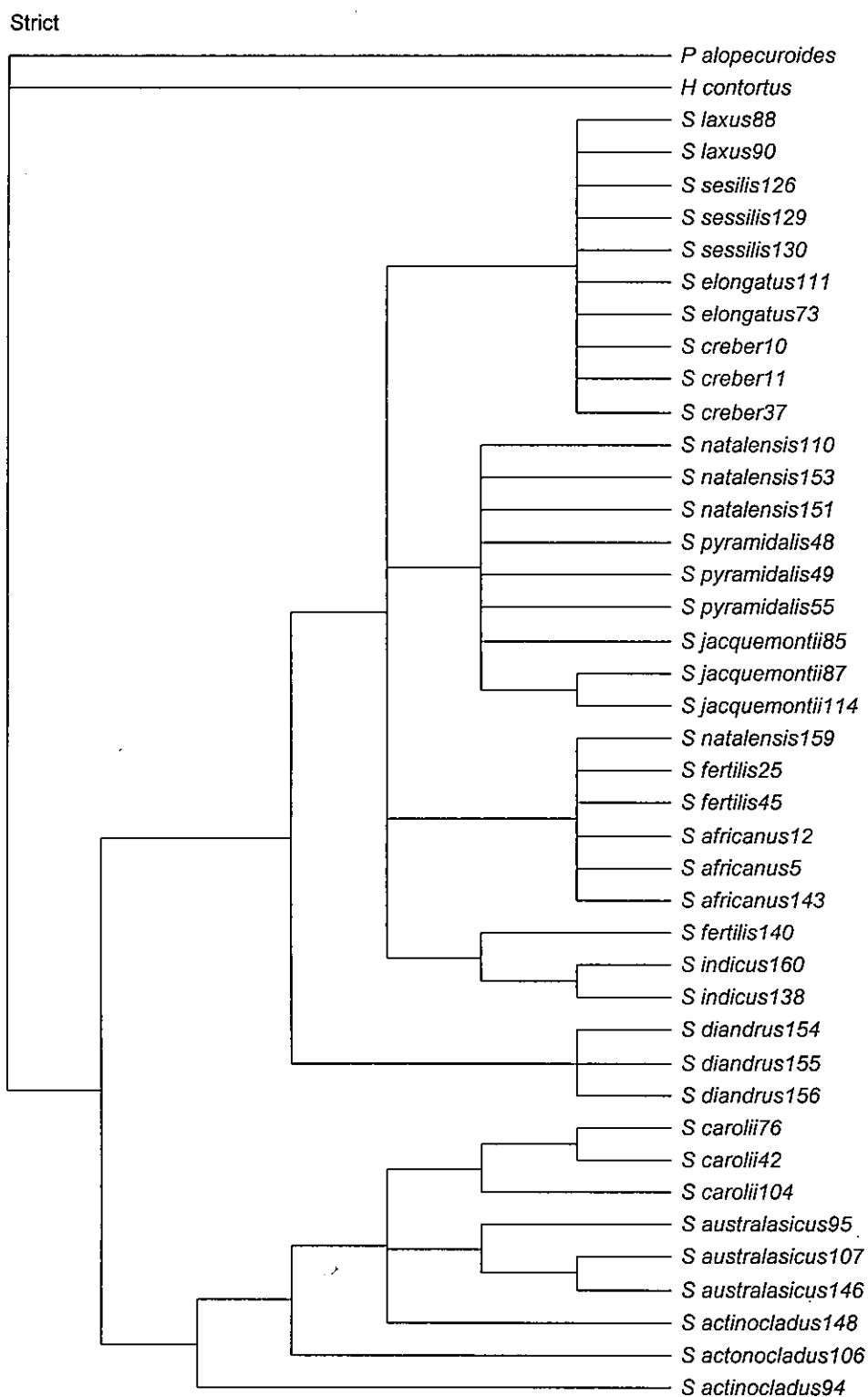


Figure 1. Strict consensus tree of 64 maximally parsimonious trees generated from entire ITS data set. For each MPT, consistency index, CI = 0.94; retention index, RI = 0.98; homoplasy index, HI = 0.05 and tree length = 488. Note that all the species of *S. indicus* complex are grouped together and all the natives form a separate group.

processed through the web cutter program, 104 enzymes were scored for having one or two restriction sites in the entire ITS sequence. Five enzymes were then selected which could differentiate eight weedy species (including the present five within Australia) from the remaining species (data not shown). This ability to select weedy species from non-weedy species will be exploited further to develop a commercially viable diagnostic procedure.

### Phylogenetics

In the development of the diagnostic an opportunity arose to study the phylogenetic relationship, at a number of different taxonomic levels, of the various species under study. Proportionate differences in the ITS sequences of the five weedy species was 0 to 2% whereas it was 21% between weedy and the native species. Heuristic analysis of potentially informative sites in the entire ITS region of the 14 species generated 64 maximally parsimonious trees, each having a tree length of 488 with a consistency index of 0.94, a retention value of 0.98 and a homoplasy index of 0.05. In the strict consensus tree for these 64 trees, the species formed two major groups. One of these groups had been previously identified as being made up of species that were morphologically very similar and therefore had been given the name of the *Sporobolus indicus* complex. The other group was made up of the Australian natives (Figure 1). The species in the first group were further subdivided into five major clades. The first consisted of four species (*S. laxus*, *S. sessilis*, *S. elongatus* and *S. creber*). A second was made of the three most serious weed species (*S. pyramidalis*, *S. natalensis* and *S. jacquemontii*). In the third clade *S. fertilis* and *S. africanus* were placed while *S. indicus* formed the fourth clade.

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## SPATIO-TEMPORAL PATTERNS OF WEED SEEDS AND SEEDLING EMERGENCE IN ARABLE PEAT AS INFLUENCED BY TILLAGE REGIMES IN KELANG, PENINSULAR MALAYSIA

B. B. Baki and R. S. Jeremiah, Jr.

Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

baki@biology.um.edu.my

**Abstract :** Tillage regimes and tillage implements are of fundamental importance in the integrated weed management strategies. Tillage, however, causes detrimental impact on soil and water conservation. Current economic and management concerns have inculcated growers interest in reducing the frequency of tillage. Field research was conducted at MARDI Research Station, Kelang, Peninsular Malaysia in 1998 –1999 to evaluate the effect of tillage regimes on weed seed distribution, seedling emergence pattern and seedling population fluxes in arable peat soil. Four tillage regimes consisting of 1 round (1RR), two rounds (2RR), 3 rounds (3RR) and 4 rounds (4RR) of rotovation with the Kubota rotary tillers. Samplings with soil cores, each measuring 15cm depth and 2 cm diameter, in the experimental plots showed that 63% of weed seeds were found in the top 5 cm soil profile while the respective 24 and 13% were located in the next 5-10 and 10-15 cm soil profiles. Irrespective of the tillage regimes, weeds seeds were evenly distributed in the first 15 cm of the soil profile. Weed seed bank density at the onset of tillage was *ca.* 140,694 seeds m<sup>-2</sup>. A 66% decline in weed seed bank population was recorded 8 weeks after tillage. Seed bank depletion due to seedling emergence was only *ca.* 0.59%. Weed seedling emergence over time registered a curvilinear response to all tillage regimes with the highest number recorded in the first 4 weeks after tillage. Thereafter, seedling emergence declined rapidly. Over a 10-week period after tillage, no less than 83% of weed seeds emerged, Plots with 4RR recorded the highest weed seedling emergence (1357 seedlings m<sup>-2</sup>). Plots with 3RR, 2RR and 1RR tillage regimes registered the respective populations of 1203, 496 and 356 seedlings m<sup>-2</sup>. We believed that the large depletion of seed bank might be attributed to inherent factors such as seed predation, decay and probably seeds being displaced elsewhere or deeper into the soil profile during tillage.

**Keywords:** Weed seed bank, tillage regimes, peat soils

### INTRODUCTION

Tillage embraces all operations of seedbed preparation that optimize soil and environmental conditions for seed germination, seedling establishment and crop growth (Lal, 1995). The primary objectives of soil tillage, according to Plaster (1992) are to reduce weed populations, improve soil conditions and the management of crop residues. Unger (1984) listed mechanical soil disturbance leading to land degradation and reduced crop production potential, *inter-alia*, as the primary disadvantages of tillage operations. Egley (1986) believes that tillage often promotes the emergence of weed seedlings in the field. Besides removing vegetation cover and residues, tillage exposes soil to rainfall, wind and increased surface run-off.

Ideally, to the agronomists and farm operators alike, tillage should provide a weed-free seedbed for planting, burying weed seedlings and suppresses the growth of weeds.



Tillage profoundly affects the distribution of weed seeds in the soil profile (Edley & Williams, 1990; Buhler & Mester, 1991, Cardina *et al.* 1991; Yenish *et al.* 1996). There are evidences that tillage stimulates germination by distributing weed seeds to positions in the profile that are favourable for germination and may at the same time induce seed scarification (Edley, 1992, Yenish *et al.* 1992). Tillage exposes seeds to light, improves soil aeration, and increases the loss of volatile inhibitors from the soil (Edley 1986).

Arable peat accounted for *ca.* 2.6 millions hectares of the total land area in Malaysia of which a sizeable area are exploited for agricultural pursuits. This paper summarizes the data generated from a five-year study on seed bank dynamics and the spatio-temporal patterns of seedling emergence of weeds on arable peat as affected by tillage regimes in Kelang, Malaysia.

## MATERIALS AND METHODS

Field experiments were conducted from July to October annually for 5 years beginning in 1995 until 1999 at the MARDI Research Station, Kelang, Malaysia. The study area was located at National Grid of 2° 59' N and 101° 31' E and at an altitude of 3.1m above sea level. The site was a well-drained arable oligotrophic peat cultivated with vegetables and fruit orchards through the years but had been undisturbed for 4 years prior to the study. At a depth down to 25 cm, the soil consisted of dark amorphous aggregates of semi-decomposed woody plant materials. At greater depths, larger woody materials were present.

Five different main plots, adjacent to each other, each measuring 60 m long and 15 m wide were chosen for the study. Each main plot was allocated for each year of the study beginning in 1995. A preliminary account of the weed flora and general coverage was taken at the main plots and surrounding area before the study was conducted. The main plot was mowed of weed cover and 4 sub-plots each measuring 7.5 m wide and 30 m long were established. Each sub-plot was subjected to different tillage regimes consisting 1, 2, 3 or 4 rounds of rotovation (hereafter referred as 1RR, 2RR, 3RR and 4RR, respectively) using rotary tillers at an operating depth of 30 cm. The rotary tiller used in this study consisted of a power shaft, transverse to the direction of travel, equipped with curve knives that slice through the soil, chop surface residue, and mix all materials in the disturbed layer. Surface residue left was minimal.

To assess the rate and magnitude of seedling emergence in the field, three permanent quadrats, each measuring 0.5 m x 0.5 m were established within each sub-plot. Each quadrat was divided into 2 sub-quadrats measuring 0.25 m x 0.5 m. Weed seedling populations were censused periodically at 2, 4, 6, 8 and 10 weeks after tillage. In one sub-quadrat (=disturbed plots), weed seedlings were identified by species, counted and removed at each census using the methods of Cardina & Sparrow (1996) and Cardina *et al.* (1996). Care was taken to prevent secondary soil disturbance when seedlings were removed. In the other sub-plots (undisturbed plots), weed seedlings were identified by species, counted and marked at each interval. New emergence and mortality were noted. Seedling mortality occurring between counting dates were not determined.

As for seed bank characterization and assessment, three soil samples were taken randomly from each sub-plot prior to tillage. Soil samples were obtained with a soil sample probe using aluminium sleeves measuring 5.0 cm in diameter and 15 cm deep. Subsequent soil samples were taken at 2, 4, 6, 8 and 10-week intervals after tillage

corresponding with seedling emergence census. At each sampling interval one soil sample was taken within 5 cm from the edge of each quadrat within each sub-plot. The soil column was divided into 3 layers: 0-5, 5-10, and 10-15 cm according to the depth. Sub-samples were stored in plastic bags at ambient temperature of 27° C prior to analysis for weed seeds. Each of these sub-samples was mixed with water to facilitate separation of soil aggregates in the sample (Dessaint *et al.*, 1997). Seeds were extracted by washing through Endecotts laboratory test sieves with 250µm, 500µm and 1 mm mesh. Seeds were identified and counted by species using dissecting microscope or table mounted magnifying lenses. Seeds resisting gentle pressure by fine-tipped forceps were considered viable (Mulugeta & Stoltenberg, 1997).

The data of seedling and seed populations from the 5 main plots of the 5-year study was collated to generate diversity indices, describing species richness in the seed bank and emerged seedling phase based on Shannon-Weiner Function. Comparisons of species diversity among different phases of the weed populations were made using the Sorenson's Index of Similarity. Weed seed bank density and seedling emergence data were subjected to analyses of variance with treatment means separated using the Least Significant Differences (LSD) tests at  $P=0.05$ . Cumulative emergence data for weed seedling emergence were described using regression equations. Adequacy of fitted curves was determined by the coefficient regression. Analyses of the data were done using Statgraphics Version 5.0 (1991).

## RESULTS AND DISCUSSION

### Weed flora spectrum

Thirty-two weed species belonging to 13 families were observed in the study plots and surrounding area prior to tillage (Table 1). Of these species, 17 were broadleaves, 8 were grasses and 7 were sedges. *Panicum repens* L. occurred predominantly in all plots except plot 4 throughout the 5-year study. In the seed phase, only 13 weed species from 8 families were recorded from soil samples with notable absence of 8 species of broadleaves, 3 species of grasses and 1 sedge species, previously present in the mature-plant phase (Table 1). We believed that the absence of certain species of weed seeds could be attributed to differences in seed fecundity, seed viability and life span and perhaps seed predation among species. Baki *et al.* (1997) forwarded similar lines of argument explaining the spatio-temporal heterogeneities in occurrence of weed seeds in arable peat in Malaysia.

Sedges dominated the seed bank with seed density estimated to be in excess of 43,400 seedsm<sup>-2</sup> or 49.5% of total seed counts. The broadleaves contributed ca. 33,780 seedsm<sup>-2</sup> (37.8%) and grasses with 12,240 seedsm<sup>-2</sup> or 17.7%, respectively. Only 10 weed species contributed substantially to the soil seed bank based on accumulated abundance of >90% of the total counts. Of these, 5 species were heavily represented in the counts, viz. *Cyperus compressus* L. (24.7%), *Cleome rutidosperma* DC. (15.6%), *C. distans* L.f. (11.2%), *Ageratum conyzoides* L. (10.2%) and *C. polystachyos* (10.2%). Other weed species registered abundance values of <3.7% or <3,300 seedsm<sup>-2</sup>.

The mean density of weed seeds within the first 15 cm soil depth was ca. 89,455 seedsm<sup>-2</sup> of which 22,086 seedsm<sup>-2</sup> were those of *C. compressus*, 13,937 seedsm<sup>-2</sup> were *C. rutidosperma*, 11,697 seedsm<sup>-2</sup> were *C. distans*, 9,999 seedsm<sup>-2</sup> were *A. conyzoides* and 9,125 seedsm<sup>-2</sup> were *C. polystachyos*. Invariably, there were insignificant ( $P<0.05$ )

time- and space-mediated differences in population counts of weed seeds before and after tillage operations during the 5-year study.

In total, across all sub-plots and sampling dates, 19 weed species from 11 families were recorded as weed seedlings with clear dominance of broadleaves, registering 13 species (Table 1). The dominant presence of broadleaves in terms of absolute abundance with seedling density of *ca.* 790 seedlingsm<sup>-2</sup> with *Ludwigia hyssopifolia*, *C. rutidosperma*, *A. conyzoides* and *Amaranthus gracilis* contributing >90% of accumulated abundance of emerged seedlings (Table 2). Time- and space-mediated insignificant ( $P < 0.05$ ) differences in seedling counts or emergence prevailed after tillage operations during the tenure of the study (Table 2).

Seed banks are not influenced by rates of input and losses, but are also historically dependent on weed flora seed input and dynamics in recent years. Dessaint *et al.* (1997) reported that communities that were dominated by annuals and/or those experiencing frequent disturbance often displayed high similarity between the populations of soil seed bank with vegetation. Frequent disturbances allow only certain species to mature and replenish the seed bank. In the current study area, cultivation had ceased for 7 years prior to this experimentation and a shift in dominance among weed species may have occurred as a result, but temporally the seed bank would require greater time to exhibit this change. However, soil seed bank diversity was relatively high, registering the Shannon-Weiner function re values of 2.318. In the same vein, emerged seedling displayed lower diversity spectrum, registering the parallel value of 1.849 (Table 3). The relationship between the mature above-ground flora, soil seed bank, and emerged seedling did not show high similarity based on Sorenson's Index values although all phases were spatially similar (Table 3). The seed bank's species affinity reflected only 60.5% of the mature weed population (Table 3). The parallel value between the seed bank and seedling populations was 66.0%. Species affinity between mature weed populations with their seedling counterparts was greater with Sorenson's Index of Similarity value of 0.76 (Table 3). Invariably, seedlings emergence are determined by several environmental factors (Prostko *et al.* (1997) and their eventual establishment are subjected to an environmental sieve (*sensu* Harper, 1977). As such, at times, time- and space-discrimination by certain species against others do prevailed.

### **Tillage, weed seed bank population and distribution patterns**

Tillage is the primary cause of vertical seed movement in agricultural soils (Roberts, 1963; Cousens & Moss, 1990). The extent and depth of weed seed distribution is directly related to the amount of soil disturbance (Dryer, 1995). Weed seed bank density prior to tillage was estimated at 140,694 – 140,702 seedsm<sup>-2</sup> but decreased logarithmically with depth. Invariably, depth-mediated differences in seed bank populations were observed in different tillage regimes (Fig 1). Irrespective of tillage regimes, albeit inconsistencies in the rates of population decay, seeds density decreased significantly with time after tillage (Table 2). Depending on tillage regimes and 8 weeks later, these decays ranged from 69% to 78% of the initial populations prior to tillage. Seeds prevailing in the top 5 cm were lost through germination or dessication (Clements *et al.*, 1996) or consumed by predators or decayed more easily (Yenish *et al.*, 1992).

Seeds were most abundant in the first 5-cm of soil profile, registering a density of 88,576 seedsm<sup>-2</sup> or 63% of the total seed counts within the first 15cm depth (Fig.2a). The parallel figures for the next 5-10 cm and 10-15 cm soil profiles were 33,786 and

18,377 seeds/m<sup>2</sup>, representing some 24 and 13% of weed seeds within the top 15 cm of soil, respectively. Four species dominated the seed bank, viz: *Cyperus compressus*, *C. ruidosperma*, *C. distans*, *A. conyzoides*, and *C. polystachyos*.

After tillage, seeds were distributed rather evenly within the soil profile (Fig 2b.). Similar observations were made by Clements *et al.* (1996) and Vanasse & Leroux (2000) in their study to characterize floristic diversity, size and vertical distribution of weed seeds under different tillage systems. Tillage, arguably, caused the vertical mixture of soil layers within the soil profile and may have buried a substantial amount of seeds deeper within the profile as shown by this study.

### **Tillage, seedling emergence pattern and magnitude**

A curvilinear response was observed for weed seedling emergence from 2 to 4 weeks after tillage followed by a decline thereafter in all tillage regimes (Fig 3). Both time- and species-mediated variations were registered in seedling emergence time, arguably due to different degree of dormancy, or other events of disturbance at a smaller scale or the effect of heterogeneous environment prevailing at the time of experimentation. Plots subjected to 4 rounds of tillage (4RR) registered the highest number of emerged seedlings vis-a-vis other tillage regimes 10 weeks after tillage. Seedling emergence 4 weeks after tillage operations for tillage regimes of 2RR and 4RR had reached 83% of the total seedling emergence recorded 10 weeks after tillage.

We believed that the spatial heterogeneity of both seed bank population counts and seedling emergence pattern and magnitude in this study was probably caused by the patchy but extensive presence of *P. repens*. *Panicum repens* with its extensive and robust creeping rhizomes (Holm *et al.*, 1977) impeded thorough tillage measures limiting soil inversion to some extent. The presence of heaps of rotting rhizomes of *P. repens*, apparently prevented seedling emergence leading us to believe that some kind of allelopathic mechanism was operating in the system. Overall, a very patchy or mosaic patterns of seedling emergence prevailed.

Weed seedling emergence was generally higher in disturbed plots than in undisturbed plots up to 6 - 10 weeks after tillage operations (Table 4). Increased emergence in disturbed plots can be attributed to secondary soil disturbance following sequential seedlings removal from the soil. This action may have brought soil to the surface and thus exposing seeds to light and better soil aeration. Seedling removal may have lead to reduced competition pressure among emerged seedlings. Although the argument based on time-mediated increase in canopy cover (Fenner, 1995) might induce dormancy in some seeds on the soil surface, thereby reducing germination and seedling emergence in undisturbed plots, this may have not been a prevalent factor as competition pressure would not have been very intense at this early stage following tillage. In fact, during the first 10 weeks after tillage, seedling mortality was generally low at ca. 8.25% (Fig. 4). No concrete reasons can be forwarded to explain the variations in seedling mortality in plots subjected to different tillage regimes. However, it is fashionable to argue that differences in fitness prevailed among seedlings emerging at different point in time and space. This argument, perhaps explain the spatio-temporal dynamics of emerged seedlings and established plant populations observed in this study.

The number of seeds that give rise to seedlings and those becoming non-viable influence the magnitude of seed depletion (Forcella, 1992). Prior to tillage, average seed bank density was ca. 140, 694 seeds/m<sup>2</sup>. The mean seedling emergence in all tillage regimes was ca. 843 seeds/m<sup>2</sup>. This translated into seed bank depletion through

emergence at only 0.59%, representing a relatively marginal change in seed bank density. On species *per se*, *Fimbristylis acuminata* registered the highest depletion value of 23.5% attributed to seedling emergence, followed by *Phyllanthus debilis* and *Euphorbia hirta* at the respective values of 1.43 and 1.28%. Values estimated for other species were generally low (Table 5). Table 6 show the relationship between seed bank depletion and seedling emergence as influenced by tillage regimes.

Tillage operations may have dual effect on seed bank reserves, namely either burying seeds deeper into the soil profile or bringing them near or to the soil surface. While certain species experienced serious depletion of seed bank reserves following tillage, others displayed monumental increase. *Panicum repens*, for example, registered a 97.3% decrease in seed bank population after tillage operations. The respective parallel figures for *F. dichotoma*, *A. gracilis* and *Asystasia intrusa* were 89.4, 84.0 and 70.0%. *Euphorbia hirta*, *F. acuminata*, *P. debilis* and *C. distans* are three species registering a respective increase of 323.3, 228.6, 116.5 and 92.6% in seed bank reserves after tillage. Seed immigration from neighbouring fields is one possible reason for this increase.

The results of this study indicate that tillage treatments have a marked influence on weed seed bank. Fields subjected to 3 or 4 rounds of tillage registered more seedling emergence than their counterparts receiving 1 or 2 rounds of tillage operations thereby leading to parallel decrease in seed bank populations. We believe that these results could be exploited in weed management operations by spraying a more judicious rate of non-selective herbicide with better adaptation to actual weed density to remove any emerged young weed seedlings after 3 or 4 rounds of tillage. A notable missing link in this study is the failure to predict seedling emergence based on vertical distribution of weed seeds. We suggest that spatial relationships between years should be investigated in fields with a more diverse management inputs, especially soil tillage, pre- and post-emergence and pre- and within crops herbicides treatment regimes. This is very relevant to the patchy nature of seedling emergence pattern observed in this study. A further avenue of research activity would be to model patch dynamics of weed seed bank and seedlings emergence pattern.

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## ECOPHYSIOLOGICAL TRAITS OF RED RICE (*ORYZA SATIVA* L.) AND POSSIBLE EFFECTS OF INTROGRESSION ON WEEDINESS

N. R. Burgos<sup>1</sup>, L. Estorninos<sup>1</sup>, E. N. Cable<sup>1</sup>, D. R. Gealy<sup>2</sup>, and P. A. Counce<sup>3</sup>

<sup>1</sup>Department of Crop, Soil, and Environmental Sciences, University of Arkansas,  
Fayetteville, AR, USA 72704. nburgos@uark.edu

<sup>2</sup>Dale Bumpers National Rice Research Institute, USDA-ARS, Stuttgart, Arkansas, USA

<sup>3</sup>Rice Research and Extension Center, Stuttgart, Arkansas, USA

**Abstract:** Red rice (*Oryza sativa* L.) is a major weed in rice in many countries worldwide. Understanding the physiology of red rice and the impact of introgression on the genetic diversity and competitiveness of this weed is crucial for devising weed control strategies. Experiments were conducted to understand some physiological traits that contribute to competitiveness of red rice and to detect genetic convergence of red rice with rice. At panicle initiation (PI), red rice and rice produced equivalent amounts of shoot biomass, which contained similar amounts of N regardless of soil fertility levels. At two weeks after heading, red rice removed more N from soil and produced more biomass than cultivated rice at N rates from 0 to 200 kg ha<sup>-1</sup>. Under full sunlight, Katy red rice photosynthesized at similar rate as 'Wells' or 'Bengal' rice. Under 50% shade, photosynthesis rate of Katy red rice declined 18% whereas those of 'Bengal' and 'Wells' declined 34% and 46%, respectively. Stuttgart strawhull produced the most tillers, panicles, and shoot biomass. In terms of these parameters Katy red rice, which was a suspected hybrid of cultivated rice and red rice, showed intermediate characteristics between the Stuttgart strawhull and cultivated rice. Genetic analysis of 23 red rice accessions exhibited substantial genetic diversity, resulting in four genotypic clusters. There was strong indication that Katy red rice was a product of natural hybridization between red rice and rice but not of the cultivars tested. The effect of introgression on genetic diversity and weediness of red rice still needs to be studied for better weed management.

**Key words:** Biomass, genetic diversity, nitrogen uptake, outcrossing, photosynthesis, shade adaptation, tillering

## INTRODUCTION

Wild rice (*Oryza* spp.) is a serious weed in many countries including Argentina, Brazil, Colombia, Costa Rica, Gambia, Guyana, Indonesia, Korea, Mexico, Peru, and the United States (Holm et al. 1997). In Arkansas, the major rice-producing state in the USA, red rice (*Oryza sativa* L.) is the most troublesome weed in rice (Dowler, 1997). Molinate is the only herbicide that has some activity on red rice, and its use is limited to preplant incorporated application for dry-seeded rice. In general, red rice cannot be selectively controlled by rice herbicides. Red rice has the competitive edge over cultivated rice because red rice grows faster, is generally taller, and produces more tillers and biomass than domesticated rice (Diarra et al. 1985). One red rice plant can reduce the yield of rice up to 272 kg ha<sup>-1</sup>, depending on the cultivar (Kwon et al. 1991). Economic loss from red rice is exacerbated by the reduction in quality of harvested rice due to red rice seed contamination. Red rice breaks easily during milling and it requires

extra milling procedures to remove the red pericarp. Contaminated produce commands a lower price than pure rice grain.

Although rice and red rice are predominantly self-pollinated, natural hybridization between the two have been documented (Beachell et al. 1938; Langevin et al. 1990; Oka and Chang 1959; Oka and Morishima 1971). In agricultural ecology, indications are that gene transfer occurs more strongly from cultivated rice to the wild types as plant traits converge more toward domesticated traits such as reduced dormancy and shattering in the wild types (Oka and Chang 1959). In the southern United States a red rice accession, which looked more like domesticated rice, was found in a field planted to 'Katy' rice. This supposedly red rice hybrid has less shattering and is shorter than the predominant strawhull red rice biotypes in the region. The overall effect of introgression on the weediness of red rice is not yet fully understood. This issue becomes more critical with the release of rice cultivars resistant to glyphosate, glufosinate, or imazethapyr. This new technology is so far the best tool for red rice management. The lifespan of this technology, however, depends greatly on what introgression does to red rice populations.

We conducted experiments in an attempt to understand some physiological traits that contribute to the competitiveness of red rice and to determine if we can detect genetic convergence of red rice populations with rice.

## **MATERIALS AND METHODS**

### **Comparative N uptake**

Experiments were conducted in the summer of 1999 and 2000 at the Rice Research and Extension Center (RREC), Stuttgart, Arkansas, USA to compare nitrogen uptake between red rice and cultivated rice. 'Drew' rice and Stuttgart strawhull red rice were seeded at 90 kg ha<sup>-1</sup> in alternate rows, and seedlings were thinned to a ratio of 3:1 rice:red rice. 'Drew' is a long grain cultivar developed in Arkansas and released in the US in 1998. Stuttgart strawhull is the predominant red rice type in Arkansas. Each plot had eight rows, 19 cm apart, 2.44 m long. Two metal squares 76.2 by 76.2 cm, 30.5 cm deep, were installed per plot, each enclosing two rows of rice and two of red rice. Pelletized <sup>15</sup>N urea was applied in each quadrat at 0, 50, 100, 150, and 200 kg ha<sup>-1</sup> before permanent flood. Treatments were replicated four times and arranged in a randomized complete block design. Above-ground biomass was harvested at panicle initiation (PI) from one quadrat and at two weeks after heading from the second quadrat. Plant parts were separated into top and bottom leaf, culm, and panicle. Nitrogen content was determined.

### **Response of red rice to shade**

A field study was conducted in the summer of 2000 at the Stuttgart location to compare photosynthetic rates and other agronomic parameters of rice and red rice in response to shade and nitrogen application. We utilized a split-split plot design with the main plots being rice cultivar or red rice biotype, shade as subplots, and nitrogen level as sub-subplots. Two rice cultivars ('Wells' and 'Bengal') and two red rice types (Stuttgart strawhull and Katy red rice) were transplanted into the field in a noncompetitive setup. Each microplot had two rice or red rice plants, spaced 50 cm apart. Nitrogen was applied pre-flood at 0, 100, and 200 kg ha<sup>-1</sup>. 'Wells' is a long grain cultivar developed in Arkansas and released in the US in 1999. 'Bengal' is a medium



grain developed in Louisiana, USA and released in 1993. Katy red rice is a suspected red rice-rice hybrid, collected from a field planted to 'Katy' rice. At ten days after anthesis, shade treatments were implemented for one week and photosynthesis readings were taken two days after shade application. The crop was damaged by early frost, which prevented the development of grains to full maturity and made the kernels brittle thereby causing threshing problems. Only the number of tillers and panicles and biomass were obtained at harvest. The study will be repeated in the summer of 2001.

### **Genetic diversity of red rice**

We employed the randomized amplified polymorphic DNA (RAPD) fingerprinting technique to analyze the genetic relationship between some red rice accessions and rice cultivars. DNA was extracted from 'Bengal', 'Katy', and 'Kaybonnet' rice as well as from 23 red rice accessions. 'Katy' and 'Kaybonnet' are long grain cultivars also developed in Arkansas; the former was released in 1989 and the latter was released in 1995. Fifteen random 10-base primers were used to amplify various regions of the genome by polymerase chain reaction (PCR). Mackill's (1995) procedure was followed with some modifications. PCR products were separated by electrophoresis in a 1% agarose gel. PCR was repeated three times to ensure reproducibility of results. Only DNA fragments that appeared in three runs were scored for presence (1) or absence (0) in a particular accession. The genetic distance between any pair of genotypes was calculated as  $GD = 1 - (A/N)$ , where A is the total number of bands shared by two genotypes and N is the total number of bands scored between the two genotypes. To determine natural grouping among genotypes, the matrix of GD values was subjected to hierarchical cluster analysis using the average linking method. Clustering and multidimensional scaling (MDS) analysis was performed using SAS software.

## **RESULTS AND DISCUSSION**

### **Comparative N uptake**

At panicle initiation, red rice and rice produced equivalent amounts of shoot biomass regardless of N rate, and biomass increased with N level (Fig. ). At two weeks after heading, red rice produced more shoot biomass than rice at all N levels. Without added N, red rice produced almost twice as much shoot biomass as rice (830 vs. 470 g m<sup>-2</sup>) two weeks after heading. This indicated that even with limited nutrient supply, red rice was still able to produce more shoot dry matter than rice. Panicle biomass for red rice was less than that of rice at 0 kg ha<sup>-1</sup> N, surpassed the panicle weight of rice at 50 kg ha<sup>-1</sup> N, and became 2 to 3.5 times more than rice at 100 to 200 kg ha<sup>-1</sup> N (Fig. 2). For red rice to attain faster growth and higher dry matter accumulation, it has to obtain and utilize resources more efficiently than cultivated rice. Nitrogen uptake by shoot tissue was similar between rice and red rice at PI (Fig.3). This coincided with the trend in shoot biomass production at PI. At two weeks after heading, red rice shoots contained more N than did rice at all N levels. Further, the magnitude of difference in N uptake between red rice and rice was lesser than the difference in shoot biomass production. This indicated that not only did red rice remove more N from soil but it also utilized N more efficiently for biomass production than rice. Effective N utilization may be related to nutrient mobilization in the plant. Two weeks after heading, red rice had a higher percentage of total N in the flag leaf than rice whereas rice still had almost 40% of N in the bottom leaves (Fig. 4).

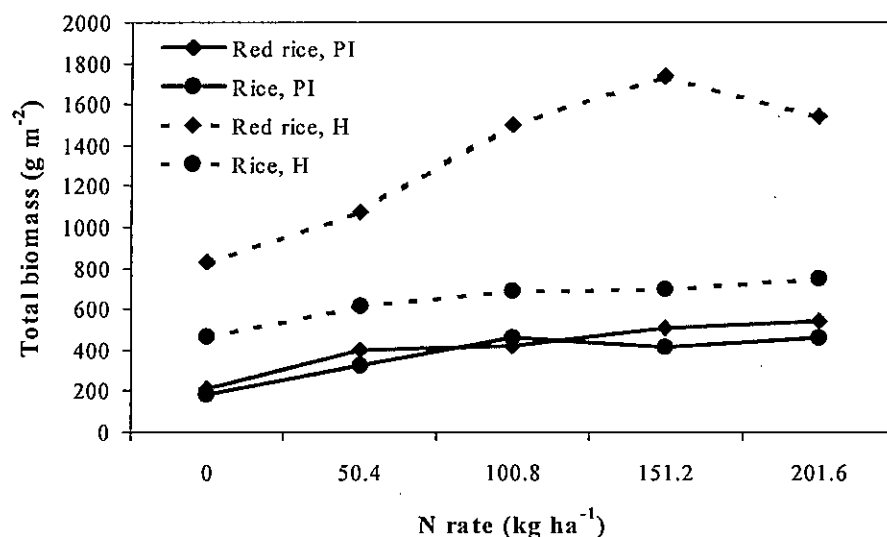


Figure 1. Total shoot biomass of 'Drew' rice and Stuttgart strawhull red rice in response to N levels at Stuttgart, Arkansas, USA. Regression equations are as follows. Red rice, PI:  $Y=199 + 61.32 \ln(X)$ ; Rice, PI:  $Y=178 + 50.1 \ln(X)$ ; Red rice, H:  $Y=772 + 10.24X - 0.03X^2$ ; Rice, H:  $Y=461 + 49.32 \ln(X)$ . PI = panicle initiation, H = two weeks after heading.

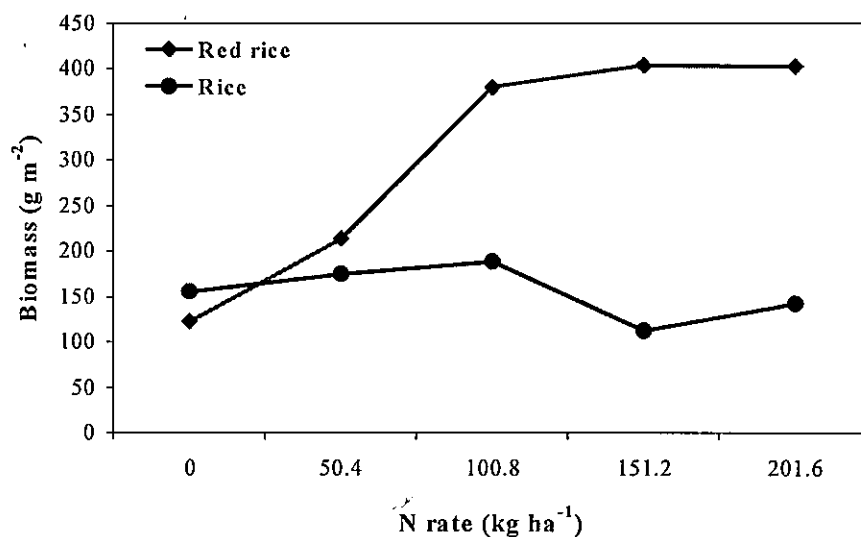


Figure 2. Panicle biomass of 'Drew' rice and Stuttgart strawhull red rice harvested two weeks after heading, as affected by N levels, Stuttgart, Arkansas, USA. Regression equation for red rice:  $Y=103 + 53 \ln(X)$ . N rate did not affect panicle biomass of rice.

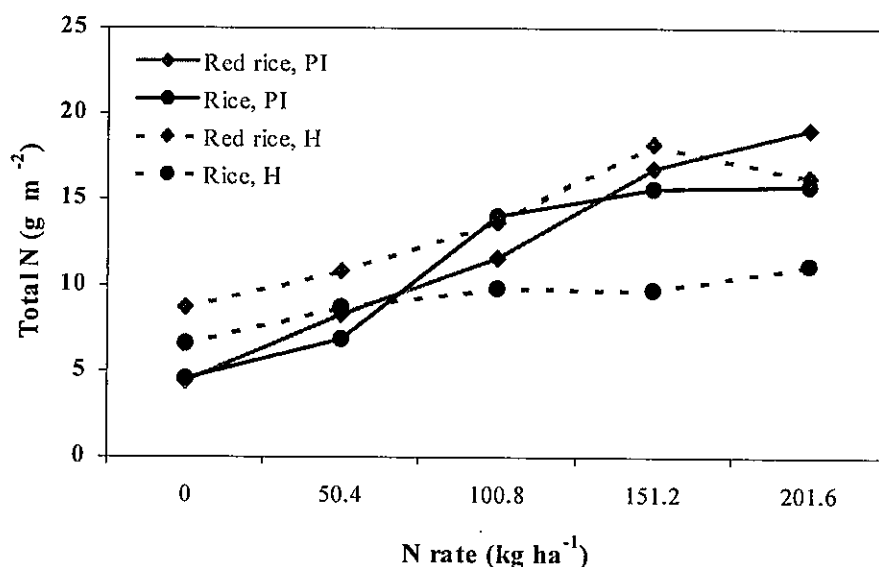


Figure 3. Total N in shoot tissue of 'Drew' rice and Stuttgart strawhull red rice, expressed in terms of N removed m<sup>-2</sup> of ground area at Stuttgart, Arkansas, USA. Regression equations are as follows. Red rice, PI:  $Y=4.6 + 0.08X$ ; Rice, PI:  $Y=3.5 + 2.1 \ln(X)$ ; Red rice, H:  $Y=8.3 + 0.07X - 0.0002X^2$ ; Rice, H:  $Y=6.5 + 0.68 \ln(X)$ . PI = panicle initiation, H = two weeks after heading.

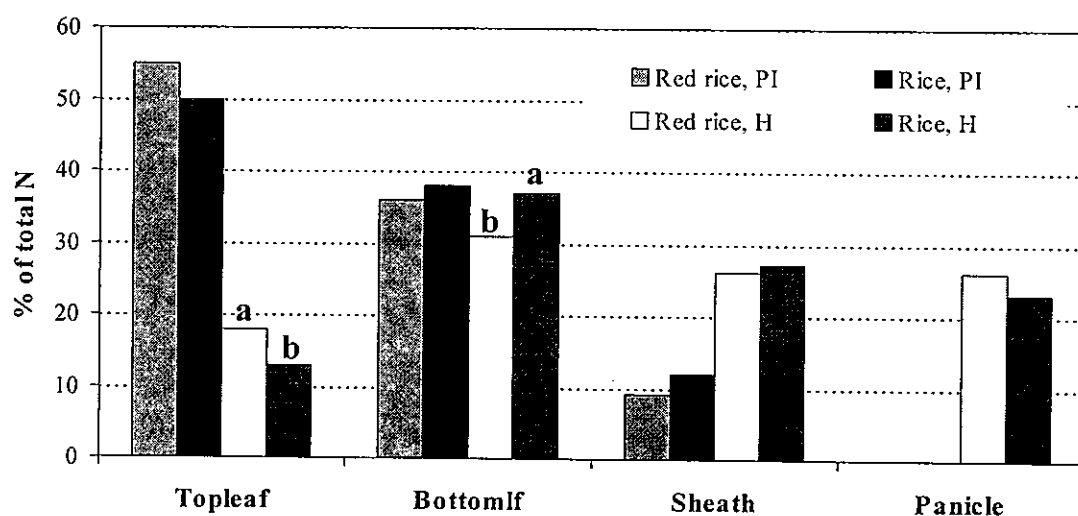


Figure 4. N distribution in shoot tissues of 'Drew' rice and Stuttgart strawhull red rice harvested at panicle initiation and two weeks after heading at Stuttgart, Arkansas, USA. PI = panicle initiation, H = two weeks after heading.

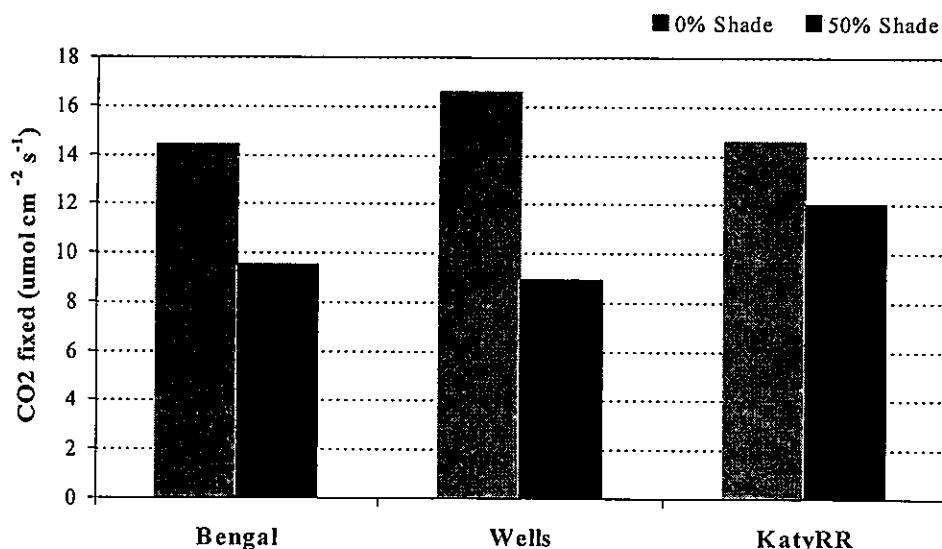


Figure 5. Photosynthetic response of rice cultivars ('Bengal' and 'Wells') and red rice (Katy RR) to shade, averaged over N levels. Shade treatment was implemented at 10 days after anthesis, and data were taken two days after shading. To compare shade treatments under the same cultivar, LSD = 2.2; to compare cultivars within the same shade treatment, LSD = 2.1.

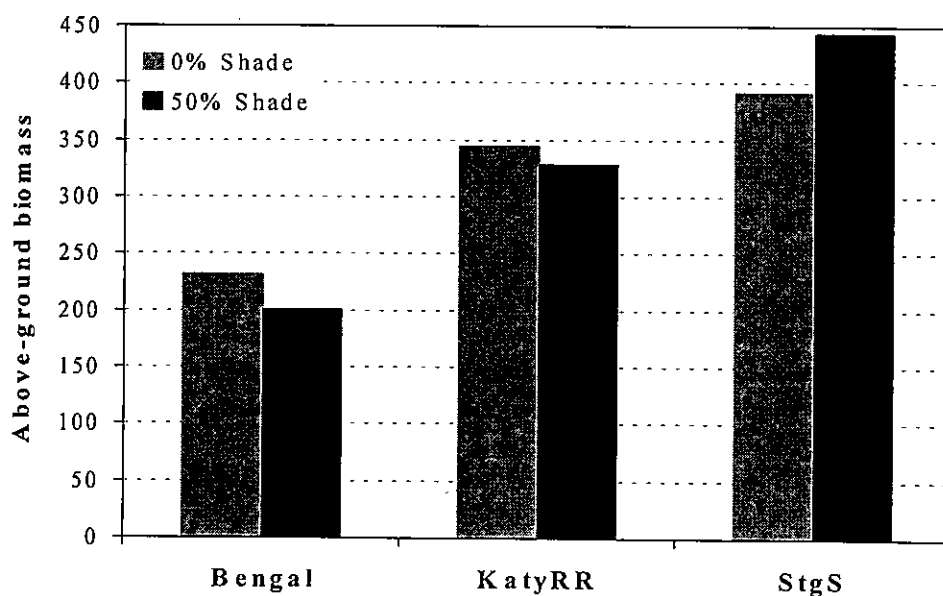


Figure 6. Biomass of 'Bengal' rice and red rice (KatyRR and Stuttgart strawhull) at harvest as affected by shade, averaged over N rates, Stuttgart, Arkansas, USA. To compare any pair of means, LSD = 59.

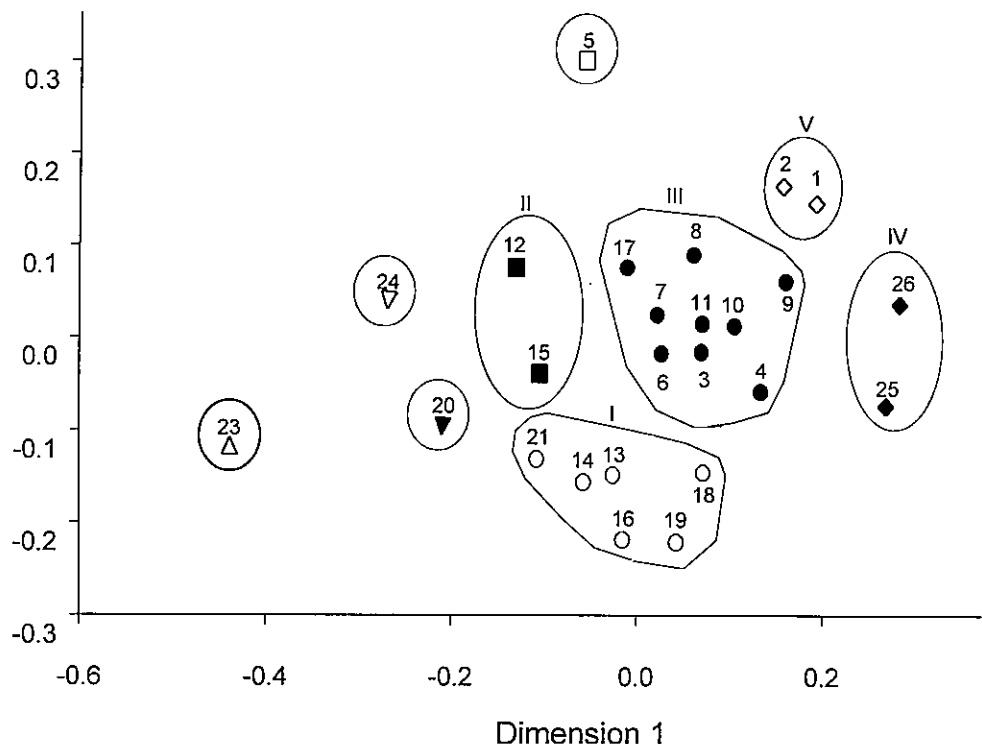


Figure 7. Distribution of 23 red rice accessions and three rice cultivars based on cluster analysis and Multidimensional Scaling (MDS) generated from 15 random primers. Group I (awnless strawhull red rice) = AR1994-7 (21), MS1996-5 (19), AR1995-7 (18), ARSgt-S (16), AR1994-15A (14), and LA1995-12 (13); Group II (awned blackhull red rice) = AR1994-17C (12) and AR1995-1 (15); Group III (awned blackhull or strawhull red rice) = AR1994-11D (3), LA-3 (4), AR1994-5A (6), MS-4 (7), AR1994-19A (8), LA1995-13 (9), AR1995-4 (10), LA1995-14 (11), and AR1994-10A (17); Group IV (long small grain rice cultivars) = Kaybonnet rice (25) and Katy rice (26); and Group V (awned blackhull red rice) = TX-4 (1) and ARSgt-B (2). Outliers were Bengal (23) - medium grain rice, AR1994-8 (22) - awned blackhull red rice, ARKaty-RR(24) - awnless strawhull red rice, AR1996-1 (5) - awnless strawhull red rice, and MS1996-7 (20) - awned blackhull red rice. Numbers in parentheses correspond to the 26 populations labeled on the graph.

### Differential response to shade

Stuttgart strawhull flowered earlier, and therefore was shaded earlier than the rice cultivars and Katy red rice. Under full sunlight, 'Wells' showed marginal advantage over 'Bengal' in photosynthesis rate, which could be a consequence of varietal improvement (Fig. 5). There was no difference, however, in photosynthesis rate between Katy red rice and 'Wells' or between Katy red rice and 'Bengal'. Photosynthesis data for Stuttgart strawhull was lost due to unexpected circumstances. When placed under 50% shade for two days, photosynthesis rates of rice and red rice declined but the magnitude of reduction was higher in 'Bengal' (34%) and 'Wells' (46%) than in Katy red rice (18%). Nitrogen level did not affect photosynthesis. Averaged over N rates and shade, 'Bengal' and Katy red rice produced 47 to 48 panicles

per plant (Table 1). Stuttgart strawhull red rice produced more panicles than 'Bengal' or Katy red rice. The same trend was observed with tiller number (data not shown). Consequently, Stuttgart strawhull produced the highest above-ground biomass, 'Bengal' the least, and Katy red rice was intermediate (Fig. 6). This experiment showed that some characteristics of Katy red rice had deviated from that of true red rice and became closer to cultivated rice. The intermediacy of Katy red rice supports the theory of natural hybridization and introgression of rice genes into red rice (Oka and Chang 1959). From this standpoint, it appeared that Katy red rice would be less competitive than Stuttgart strawhull. It does not mean, however, that the hybrid weedy rice would be easier to control than true red rice. This needs further investigation.

Table 1. Tiller production of rice and red rice averaged over shade treatment and nitrogen levels, Rice Research and Extension Center, Stuttgart, Arkansas, USA.

Cultivar	Panicle number
Bengal	47
Katy red rice	48
Stuttgart strawhull	76
LSD <sub>0.05</sub>	10

### Genetic diversity of red rice

The dendrogram resulting from the cluster analysis and MDS showed that the accessions were distributed into five clusters with five outliers, which did not belong to any group (Fig.7). The resulting clusters corresponded to some degree to morphological similarities among populations. Group I contained six strawhull, awnless type red rice accessions. Group II contained the awned blackhull red rice types, AR1994-17C and AR1995-1. Group III were awned types comprised of four strawhull MS-4, LA-3, AR1994-11D, and LA1995-14 and five blackhull AR1995-4, AR1994-10A, AR1994-19A, AR1994-5A, and LA1995-13. Group IV consisted of the long grain cultivars 'Katy' and 'Kaybonnet'. This is not surprising since 'Katy' is one of the parents of 'Kaybonnet'. Group V contained the awned blackhull red rice TX-4 and ARSgt.-B. The suspected hybrid Katy red rice (ARKaty-RR) did not cluster with any of the red rice accessions, or the rice cultivars. Our data did not show that any of the rice cultivars included in this analysis was a parent of Katy red rice. However, analysis of these same entries plus some offsprings of intentional hybridization between rice and red rice showed that Katy red rice clustered with the intentional hybrids (Gealy et al. 2000). Their genetic data had indirectly demonstrated that Katy red rice was a product of introgression although data did not show that Katy is one of the parents of Katy red rice. Discrimination of red rice genotypes was most strongly affected by the presence or absence of awns. With the use of RAPD primers, hull color was not a consistent discriminator between genotypes as there were clusters containing both strawhull and blackhull red rice types. Further, our data showed that genotypes were not delimited by location, as illustrated by the awned blackhull red rice from Texas (TX-4) and Arkansas (ARSgt-B), which appeared to be genetically similar. Some accessions from Louisiana (LA-3, LA1995-13, LA1995-14) and Mississippi (MS-4) were genetically similar to

several accessions from Arkansas. This is indicative of occasional seed movement across states, perhaps due to contamination of rice seed.

## Conclusion

Among the ways by which red rice defeats rice are higher N uptake and better shade adaptation. Reduction in plant size, tillers, and panicle numbers in red rice as a result of introgression may be beneficial for cultivated rice with respect to reduced competitiveness. However, the red rice hybrid would still have the red pericarp and other undesirable traits associated with weediness, such that weed control would remain critical. Roguing vegetative red rice that looks more like rice may become extremely difficult.

## ACKNOWLEDGMENTS

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## LONGEVITY OF BURIED *CIRSIIUM ARVENSE* SEED IN FOUR NEW ZEALAND SOILS

T. K. James and A. Rahman

AgResearch, Ruakura Research Centre, Hamilton, New Zealand

trevor.james@agresearch.co.nz

**Abstract:** *Cirsium arvense* (L.) Scop. is a perennial thistle which dies back completely each winter in New Zealand and regrows from its lateral roots each spring. Originally from Europe it is now abundant throughout New Zealand and many other countries world wide. It is a problem weed in both crops and pastures and can produce dense infestations that exclude most other flora. The viability of *Cirsium arvense* seed buried for several years at 0 – 2, 4 – 6 and 19 – 21 cm depths was evaluated in four different soil types. Seed samples buried in nylon mesh bags were removed after 1, 2, 3, 5, 11 and 16 years burial and their viability determined by germination in the glasshouse. After 16 years only small numbers of viable seed were found in the surface (0 – 2 cm) layer in the different soils. In this layer it took from 8 to 16 years for the viable seed numbers to fall to 1% of the original amount, depending on soil type. At the 4 – 6 and 19 – 21 cm depths the amount of viable seed recovered after 16 years burial was considerably greater. The seed was least viable after being buried in a clay soil prone to wetting and drying. From the data collected over the 16 year period it is predicted that the seed buried at the 4 - 6 cm depth in this soil would take up to 17 years to decline to less than 1% of the original amount while that buried at 19 – 21 cm may take up to 26 years. However, in a sandy soil, the buried seed was found to maintain a higher degree of viability. The other two soils, a peat soil and a silt loam gave intermediate results.

**Keywords:** *Cirsium arvense*, buried seed, seed longevity, weed seed, seed viability.

### INTRODUCTION

*Cirsium arvense* (L.) Scop. (Canada thistle, creeping thistle, Californian thistle, perennial thistle) is a perennial herbaceous plant which dies back completely each winter in New Zealand and regrows from its lateral roots each spring. It is indigenous to Europe, but now widespread throughout New Zealand and many other countries world wide. Its presence here was first recorded near Nelson in 1895 (Allan 1940) and *Cirsium arvense* has been reported as a problem weed since the early 1900's. It is a perennial weed with an extensive root system that sends up many aerial shoots. These shoots usually form dense patches which suppress other plants and are difficult to control. It is a weed of significant economic importance in both cropping and pastoral situations.

*Cirsium arvense* reproduces both vegetatively and sexually. Within a confined area it is commonly accepted that most of its reproduction is vegetative, forming large, single sex colonies that can grow at a rate of 12.2 m/year (Chancellor 1970). The production of seed is complicated by its dioecious nature. Seed production is quite variable and is usually constrained by the availability of pollen (Lalonde and Roitberg 1994). However, up to 27 seeds/capitulum, 600 seeds/stem or 30,000 seeds/m<sup>2</sup> have been reported (Chancellor 1970; Bostock and Benton 1979). In their native habitat *Cirsium arvense* seed are often heavily predated by insect larvae but in New Zealand



this weed has few natural biological control agents. Furthermore, the seed is only loosely attached to the pappus and most viable seed falls within meters of the parent plant. Distances of 10 m and 61 m have been reported from Holland and Canada respectively (Chancellor 1970).

As these seeds are not required to sustain the presence of the local infestation, their main role is in colonising new areas or in recolonisation after eradication. In the latter case persistence of seed in the soil seedbank is very important. This persistence is influenced by a number of factors, two of the most important being seed burial depth and soil type (Forcella 1992; Roberts and Feast 1972). Other factors include differences in soil moisture, temperature, light and aeration within the soil profile, all of which influence seed survival by processes such as ageing, fungal parasitism and faunal predation (Buhler et al. 1998). Therefore, knowledge of the persistence of *Cirsium arvense* seed in a variety of different soils is important in determining the likelihood of reinfestation of this weed after successful eradication.

This paper reports the results of an experiment, aimed at evaluating the effect of burial depth, burial time and soil type on the viability of *Cirsium arvense* seed. The experiment was replicated at four field sites with similar climate but widely varying soil characteristics.

## MATERIALS AND METHODS

Four soil types commonly found in the Waikato region of New Zealand were selected for this study (Table 1). The Horotiu and Dunmore soils are both well drained, the Hamilton clay loam is moderately well drained and the Rukuhia peat is very poorly drained. In June 1981 for each site, 90 sets of 250 (0.26 g) freshly gathered *Cirsium arvense* seed were each mixed with heat sterilised soil (60 g) collected from the site and 60 sets were placed in fine nylon mesh bags (0.25 mm mesh). Thirty tubes for each site (25 cm length of perforated, 6 cm diameter plastic drainpipe) were filled with non-sterilised soil from the site. During this process, a seed/soil bag was placed in each tube at 4 - 6 cm and another at 19 - 21 cm from the top end of the pipe. The top 2 cm of the pipe was left free of soil and an unbagged seed/soil mixture was placed there, separated from the soil below by a layer of fine nylon mesh but uncovered at the top. The pipe sections were then buried vertically in the appropriate soil type with their tops flush with the soil surface. The tubes were arranged in a regular 10 x 3 matrix at 20cm centres. The burial sites were in permanent pasture that were regularly grazed or mown.

Table 1. Descriptions and some characteristics of the soils used for this study

Soil	% sand	% clay	% OC**	pH	CEC* meq/100g	Field capacity (% v/v)
Horotiu sandy loam	61	15	8.7	5.4	37.4	44.8
Dunmore silt loam	54	17	9.5	5.5	51.7	66.7
Rukuhia peat	24	16	49.1	4.6	103.6	60.7
Hamilton clay loam	29	31	4.6	5.6	28.2	36.8

\*OC = Organic carbon; \*\*CEC = Cation exchange capacity.

At 2 - 4 monthly intervals for the first 3 years after burial, emerged seedlings were counted and removed from the tops of the pipe sections. After 1, 2, 3, 5, 11 and 16 years, 3 randomly selected pipes were retrieved from each site in June or July and the viable

seed from each depth assessed. Seed viability was determined by germination in an unheated glasshouse. The contents of each nylon bag and the 0 - 2 cm layer were spread thinly (2-4 mm) on paper towels laid over damp vermiculite in a tray. At approximately monthly intervals, emerged *Cirsium arvense* seedlings were counted and removed. The soil was then thoroughly mixed and the procedure repeated until no further seedlings emerged (4 - 6 months).

## RESULTS AND DISCUSSION

The viability of the original seed (collected in autumn 1981), as determined by the Official Seed Testing Station, Palmerston North, was 70% and within the range found by Amor and Harris (1974) when they tested fresh seed from several locations in Victoria, Australia. This value was used to calculate the initial number of viable seeds (175) buried at each depth.

Results from the four sites show that seed viability was affected primarily by burial depth. The *Cirsium arvense* seed mixed in the top 2 cm of the soil initially disappeared at a much faster rate than that at the 4 - 6 and 19 - 21 cm depths (Figure 1). Some of the surface seed was lost through germination in the first two years of the experiment (Figure 2). In the Hamilton clay loam and the Dunmore silt loam soils, germination accounted for about 14% of the viable seed while in the other two soils it accounted for less than 5% of the seed. A few seed germinated in the field in the Hamilton clay loam soil during the third year but no seed emerged at the other three sites. By the end of the experimental period only 1% or less of the original viable seed remained in the surface burial layer.

The disappearance of the seed placed at the 4 - 6 cm depth was much slower than that from the surface layer, with 1% to 21% of the original viable seed remaining in the soil after 16 years. At the 19 - 21 cm depth between 7% and 57% remained viable after 16 years. At both depths seed disappeared fastest from the Hamilton clay loam soil and persisted longest in the Horotiu sandy loam soil. The Rukuhia peat and the Dunmore silt loam soils were intermediate in this respect (Figure 1).

The weed seed bank normally declines exponentially and this is readily explained in terms of biological processes (Lewis 1973; Thompson and Makepeace 1983; Rahman et al. 1998). Therefore, an exponential model was used to examine these results and regression lines were fitted to logarithmic transformed data. From the regression equations the times for the amount of viable seed to be reduced to 1% of the original viable number were calculated and are presented in Table 2, together with the  $R^2$  of the fit. These data along with plotted results (Figure 1) show that the decline of the *Cirsium arvense* seed at all depths is consistent with exponential decay.

Table 2. Predicted time (years) for viable *Cirsium arvense* seed numbers to be reduced to 1% of original viable seeds in the four soil types at three depths.

Soil Depth (cm)	Soil type							
	Horotiu		Dunmore		Rukuhia		Hamilton	
	Years	$R^2$	Years	$R^2$	Years	$R^2$	Years	$R^2$
0 - 2	14	0.9619	16	0.6361	16	0.8995	8	0.9231
4 - 6	>50	0.8949	29	0.9811	29	0.8663	17	0.9915
19 - 21	>50	0.8423	45	0.7252	40	0.8777	26	0.9283

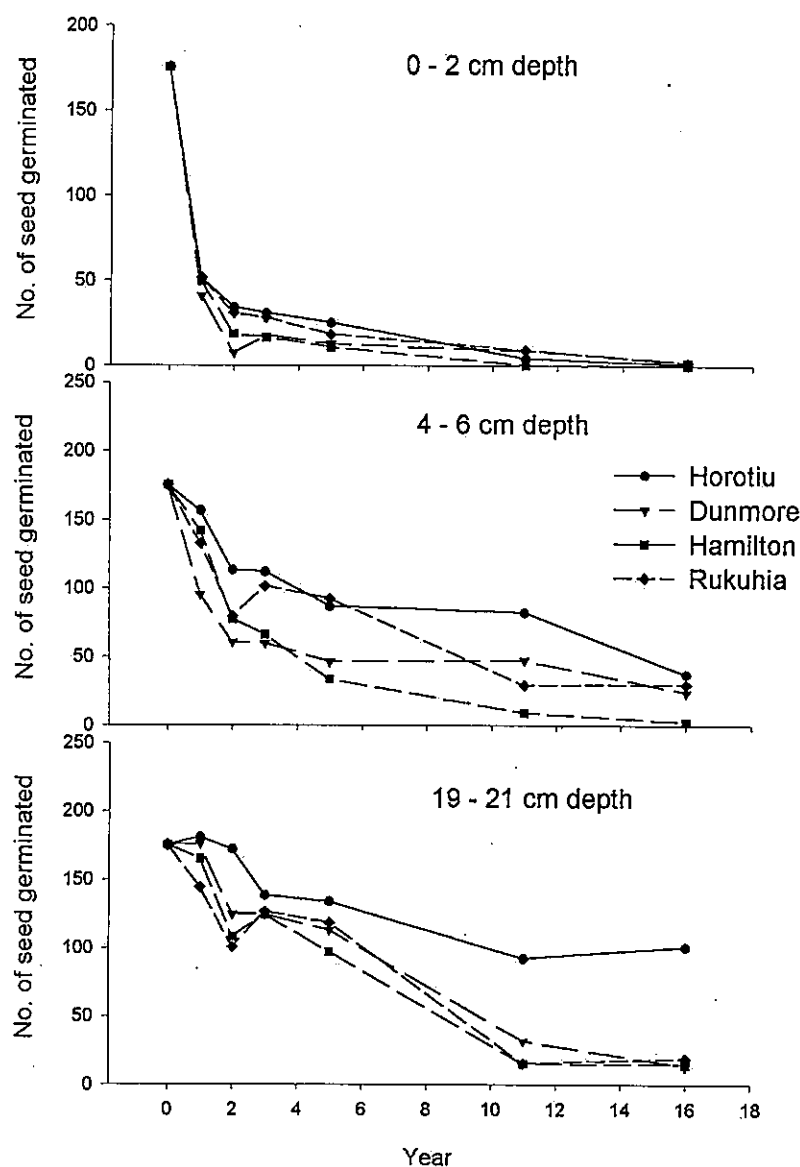


Figure 1. Percentage of the originally viable *Cirsium arvense* seed which germinated after recovery from being buried at 0 - 2 cm, 4 - 6 cm and 19 - 21 cm depths in four different soils (average of three replicates).

The seed of *Cirsium arvense* is clearly quite stable in a range of soil environments and remains viable for extended periods of time. This seed was most persistent in the Horotiu sandy loam where, over the 16 year period of this study, it underwent little decay at the 4 - 6 cm and the 19 - 21 cm depths. This indicates that it would take some considerable time for the amount of viable seed to fall below 1% of the original amount in this soil. However, to extrapolate past the 50 year mark from these limited data is untenable, as the potential error becomes very large. Although this soil has the highest sand content, none of the measured soil characteristics truly set it apart from the others and thus it is difficult to understand why the deeply buried seed at this site remained viable in such large quantities. The site with the shortest persistence of *Cirsium arvense* seed was on the Hamilton clay loam soil which had the lowest field capacity and was prone to severe fluctuations of wetting and drying. This could be the most probable

reason for the short persistence of the seed at this site. Seeds of other weed species have also been found to have shorter persistence in this soil (James and Rahman 1999; 2000). Of the other two soils included in this study, the Dunmore silt loam soil is a hill country soil derived from volcanic ash but in many respects is similar to the Horotiu sandy loam soil. The Rukuhia peat is a recently developed peat soil with a very high organic carbon content and is frequently waterlogged. Of the four soils the Rukuhia peat is the most different and it would have been easy to explain differences in the persistence of seed in this soil. The absence of any differences indicates that *Cirsium arvense* seed is able to resist the normal dynamics involved in the ageing and persistence of seed.

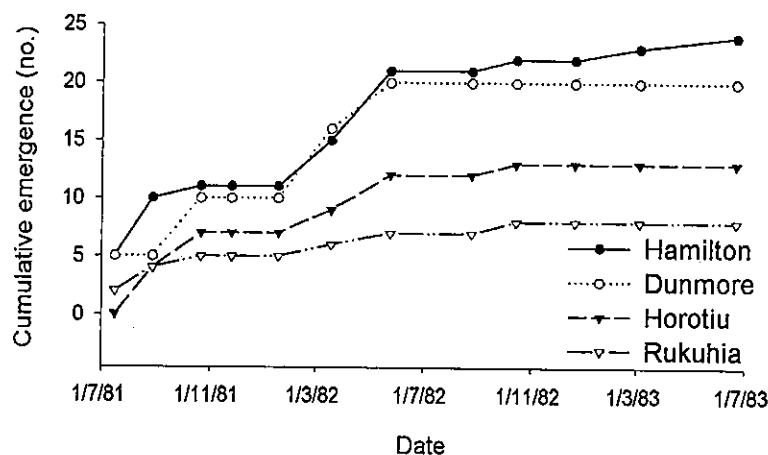


Figure 2. Cumulative emergence of *Cirsium arvense* in the field, from the 0 – 2 cm depth, during the first 2 years, in four different soils (average of three replicates).

Our results from three of the four soils in this experiment show viable seed persisting for 26 to 45 years. These results are similar to those of Toole (1946) who found that *Cirsium arvense* seed remained viable for 21 years when buried 20 cm deep in soil under natural conditions. It also explains the appearance of new infestations of this weed. These could occur from previous occurrences of the weed and do not have to be the result of fresh, wind borne seed. Therefore, after successful eradication of this weed, ongoing vigilance and control measures will be required for several years, particularly if the soil is disturbed.

### ACKNOWLEDGEMENTS

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## WEED DIVERSITY AS AFFECTED BY RICE CULTIVATION METHOD

Y. C. Ku

National Crop Experiment Station, RDA, Suwon, 441-100, Korea. [kuyc@rda.go.kr](mailto:kuyc@rda.go.kr)

**Abstract:** Weed species diversity is very important factor on the ecologically based weed management for sustainable agriculture in the 21st century. Weed species and diversity is the strongly affected by cultivation method and control method. Weed diversity index of the paddy rice field showed 0.79-0.92 in the non-fertilizer with non-herbicide application and 0.14-0.62 in the fertilizer with herbicide application. Dominant weed species in non fertilizer with non-herbicide application were *Scirpus juncooides*, *Eleocharis congesta*, *Echinochloa crus-galli*, *Potamogeton distinctus* and *Monochoria vaginalis*. On the other hand, in herbicide application, *Monochoria vaginalis* and *Sagittaria trifolia* showed 44%, 44% on importance value respectively. Rice productivity without using the fertilizer and herbicide was reduced to 22-33% by comparison with conventional method at first year but at the second year reduced by 51-90% of rice productivity

**Key words:** Weed, species diversity, cultivation method, dominant, rice

### INTRODUCTION

Weed species diversity of rice field has not only disadvantage but also favorable factors on the basis of rice production. It is difficult to clear-cut differentiation between gain and loss. Weed diversity has both faces to agricultural ecosystem. Weed species diversity should be evaluated by separation from the rice productivity.

For thousands of years, the weeds in rice field regarded as target of control. But the weed population of the field was changed continuously by the farmer endeavor. Agricultural ecosystem is quite different from the natural environment ecosystem.

Recently, abundant agrochemical application, development of agricultural machine, change of cropping system and cultivation methods caused disappear or extinction of the specific weeds, uniformity and simplicity of weed species. Although weed species diversity decreased, the weed population of noxious weeds was increased. Weed diversity can affect sustainable rice production.

This experiment conducted to know the conditions of weed diversity and to retain weed diversity on several rice cultivation methods.

### MATERIALS AND METHODS

This study was conducted to evaluate of weed diversity in rice field at National Crop Experiment Station, Suweon, Korea from 1999 to 2000. Rice cultivation methods was none fertilizer with non herbicide application, organic and none herbicide and conventional method.

In none fertilizer with none herbicide, fertilizer and herbicide was not used at all. Organic and none herbicide treatment applied organic fertilizer 10 ton/ha before transplanting, conventional method using the fertilizer and herbicide was applied nitrogen, phosphate and potassium 110,55 and 47 kg/ha respectively. Herbicide used 1521g ai/ha at the 10 days after transplanting by pyrazosulfuron+molinatate. Infant seedling which grown 10 days was planted by transplanting machine at 30×14cm

distance at May 20. Weed sampling was done 2 times with 50×50 quadrat (0.25m<sup>2</sup>) at the 60 days after transplanting and weed diversity was calculated by the Simpson's index.

## RESULTS AND DISCUSSION

### Yearly changes in dominant weed species and importance value

Table 1 shows the yearly changes in dominant weed species and importance value. In 1999, dominant weed species and importance value were *Monochoria vaginalis* (33%), *Eleocharis congesta* (26%), *Scirpus juncoides* (15%), *Cyperus difformis* (15%) and *Echinochloa crus-galli* (9%). Its importance value and dominance index were 98%, 0.21 respectively. While in 2000, dominant weed species and importance value were *Scirpus juncoides* (24%), *Eleocharis congesta* (21%), and *Echinochloa crus-galli* (14%) but *Scirpus juncoides* and *Echinochloa crus-galli* were increased compared to 1999 but *Monochoria vaginalis* and *Cyperus difformis* were decreased. Simpson's dominance index was 0.13-0.21.

Table 1. Yearly Changes in dominant weed species and importance value as affected by successive non-fertilizer with non-herbicide

Weed Species	1999	2000
<i>Monochoria vaginalis</i>	33	5
<i>Eleocharis congesta</i>	26	21
<i>Scirpus juncoides</i>	15	24
<i>Cyperus difformis</i>	15	4
<i>Echinochloa crus-galli</i>	9	14
<i>Ludwigia prostrata</i>	1	4
<i>Sagittaria trifolia</i>	1	3
<i>Rotala indica</i>	0.6	3
<i>Potamogeton distinctus</i>	0.6	11
<i>Aeschynomene indica</i>	0.6	3
Simpson dominance index*	0.21	0.13

\*Simpson's dominance index =  $\sum (n/N)^2$ ; n: importance value of individuals in species; N: total individual importance value

In organic fertilizer and non-herbicide treatment, dominant weed species and importance value were *Monochoria vaginalis* (26%), *Eleocharis congesta* (24%), *Scirpus juncoides* (18%) and *Echinochloa crus-galli* (16%). But second year *Scirpus juncoides* and *Echinochloa crus-galli* were increased 2-5% compared to 1999. Simpson's dominance index was 0.08.

In pyrazosulfuron+molinate herbicide application, dominant weed species were *Echinochloa crus-galli* (90%), *Lindernia procumbens* (4%), *Monochoria vaginalis* (3%) and *Sagittaria trifolia* (2%) in 1999. *Monochoria vaginalis* and *Sagittaria trifolia* were also dominant weed species but *Echinochloa crus-galli* did not occur in 2000.

On the other hand, Simpson's dominance index was greatly lowered to 0.38 due to decrease of *Echinochloa crus-galli* (Table 3).

### Changes of weed flora as affected by cultivation method

Table 4 shows change of weed flora as affected by cultivation method. Based on morphological characteristics of weeds distribution rate of grasses, broadleaves and

sedges were 14:34:52 in 2000 and 15:47:38 in case of organic and none herbicide. While fertilizer and herbicide application covered 93% grasses in 1999 but changed broadleaves due to control of *Echinochloa crus-galli* by herbicide application (pyrazosulfuron + molinate) in 2000.

Table 2. Yearly changes in dominant weed species and importance value as affected by successive organic fertilizer with non-herbicide

Weed Species	1999	2000
<i>Monochoria vaginalis</i>	26	16
<i>Eleocharis congesta</i>	24	9
<i>Scirpus juncoides</i>	18	20
<i>Echinochloa crusgalli</i>	16	21
<i>Cyperus difformis</i>	7	9
<i>Lindernia procumbens</i>	4	6
<i>Sagittaria trifolia</i>	1	7
<i>Fimbristylis niliacea</i>	0.1	-
<i>Potamogeton distinctus</i>	0.1	8
Simpson dominance index	0.19	0.08

Table 3. Yearly changes in dominant weed species and importance value as affected by successive herbicide treatment.

weed species	1999	2000
<i>Echinochloa crus-galli</i>	90	-
<i>Lindernia procumbens</i>	4	10
<i>Monochoria vaginalis</i>	3	44
<i>Sagittaria trifolia</i>	2	43
<i>Rotala indica</i>	-	-
Simpson's dominance index	0.86	0.38

Table 4. Yearly change of weed flora as affected by cultivation method.

Cultivation method	Year	Composition of the weed flora (%)		
		Grasses	Broadleaves	Sedges
No fertilizer and herbicide	1999	9	39	52
	2000	14	34	52
Organic and no herbicide	1999	16	34	50
	2000	15	47	38
Fertilizer and herbicide	1999	93	5	2
	2000	-	100	-

#### Weed species weed number and diversity as affected by cultivation

Table 5 shows yearly changes weed species and diversity as affected by cultivation method. Weed species and number was the greatest in the none fertilizer with none herbicide application with 13-16, 236-796 no./m<sup>2</sup> and the least in the fertilizer and herbicide application with 3-4, 14-22 no./m<sup>2</sup>. Weed diversity was the highest in the none fertilizer with none herbicide application with 0.79-0.87, followed by the organic and no herbicide treatment with 0.81-0.92 and the least in the fertilizer and herbicide treatment



with 0.14-0.62. This results indicate that weed diversity is strongly affected by herbicide application.

Table 5. Yearly changes weed species, weed member and diversity as affected by cultivation method

Cultivation method	Weed species		Weed number		Weed diversity	
	'99	'00	'99	'00	'99	'00
No fertilizer and herbicide	13	16	236	796	0.79	0.87
Organic and no herbicide	13	14	207	513	0.81	0.92
Fertilizer and herbicide	4	3	22	14	0.14	0.62

Diversity index = 1-dominance index

### Yearly Changes of rice yield as affected by cultivation method

Table 6 shows yearly changes of rice yield as affected by cultivation method. Yield loss as affected by weed competition was shown big difference among the cultural methods. Rice yield without using the fertilizer and herbicide was reduced by 22-33% comparison with conventional method at first year but at the second year reduced by 51-90% rice yield. This result indicate that herbicide is very important factor to increase rice yield.

Table 6. Yearly changes of rice yield as affected by cultivation method.

Cultivation method	Rice Yield (ton/ha)			
	1999	Index	2000	Index
No fertilizer and herbicide	3.95	(67)	0.45	(10)
Organic and no herbicide	4.61	(78)	2.15	(49)
Fertilizer and herbicide	5.93	(100)	4.43	(100)

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## EFFECT OF LONG TERM NO-TILLAGE APPLIED GLYPHOSATE HERBICIDE ON WEED COMMUNITY OF IRRIGATED LOWLAND RICE

Z. Lamid<sup>1</sup>, E. S. Saragih<sup>2</sup>, and A. D. Tambijo<sup>1</sup>

<sup>1</sup> SAIAT, P.O. Box 34 Padang 25001, Indonesia.

<sup>2</sup> PT. Monagro Kimia, Jl. M.H. Thamrin 57, Jakarta 10350, Indonesia

**Abstract:** This study was conducted at farmer's field Solok, West Sumatra Indonesia from wet season (WS) 1994/1995 to WS 2000 (15 consecutive cropping seasons) to determine the performance of weed community as well as rice grain yield as affected by long term no-tillage practice. No-tillage (NT) applied with glyphosate herbicide at the rate of 1,44 kg ae/ha (in formulation of Polaris 240 AS for 12 cropping seasons and Polado 240/105 AS for 3 cropping seasons) and farmer's practice (intensive tillage, IT) were field evaluated following plot demonstration with three replicates. Results showed that targeted weeds species to be controlled by herbicide application in terms of visual distribution was *Fimbristylis littoralis* Gaudich (sedge community) and then shifted to *Ischaenum timorensense* Kunt starting from third up to 15<sup>th</sup> cropping. Meanwhile, rice grain yields of NT were consistently higher up to 12<sup>th</sup> cropping than IT and then fluctuated by cropping seasons. However, average of 15-cropping seasons, NT gave 450 kg rice grain than IT. It concluded that continuous application of glyphosate herbicide on NT practice might change predominant weed community from sedge to relatively pure grass and did not affect rice grain yield or it would not yet be initiated IT up to 15<sup>th</sup> cropping of NT irrigated lowland rice.

**Key word:** No-tillage, irrigated rice, glyphosate, weed community.

### INTRODUCTION

Irrigated lowland rice cultivation is usually prepared by intensive tillage or conventional tillage prior to planting. Intensive tillage systems (plowing twice, harrowing once and leveling) that commonly practised by farmers create puddling condition that easiable transplanting rice, part of weed control and incorporation of organic matters (weeds and previous rice straw) (De Datta 1981). Pudding condition on a certain type of lowland sawah could be achieved by irrigation water without intensive tillage systems (Hakim *et al*, 1986). Therefore, no-tillage systems may be applicable in such condition.

Successful application of no-tillage systems in the field is limited by the infestation of weeds and regrowth of previous rice ratoons, and managable water irrigation as well (Bangun, 1995; Utomo *et al*. 1995). Thus, spraying systemic and post emergence herbicides those non-biological activity or non-residual effects in the soil (recently Glyphosate) are a must to control them (Lamid *et al*. 1997). Partial research result showed that application of Glyphosate herbicide at recommended rate (1,44 kg ae/ha) may speed up weeds and ratoon decay or decomposition without any residual effects on rice crop (Lamid *et al*. 1995; Ardjasa *et al*. 1994). Using the same herbicide and rate as well on long term trials resulted in unaffected soil carbon, nitrogen and phosphorus but tended to increase availability of phosphorus to be uptaken by rice crop (Lamid *et al*. 1997). On the other hand, Lamid *et al*. (1999) reported that continuous no-tillage practices applied with same herbicide and rate too, resulted in shifting targeted

weed community starting from the third cropping to succeeding cropping seasons. However, long term demonstration of the said tillage practice needed to be monitored whether there will be consistent changing of weed community or resistant development of a certain weed species and rice grain yield performance when practiced on irrigated lowland rice.

Objectives of field evaluation were to determine weed community by group and response of grain yield of irrigated lowland rice as due to continuous no-tillage applied glyphosate herbicide.

## MATERIALS AND METHODS

Field evaluations were conducted at farmer's field (FF) of Solok (West Sumatera), Indonesia from wet season (WS) 1994/95 to WS 2000 (15-succeessive cropping seasons). Study area was characterized by semi-technical irrigated systems and alluvial soil. No-tillage applied glyphosate herbicide (Polaris 240 AS for first 12 cropping seasons and Polado 240/105 AS for remaining croppings) at recommended rate (1.44 kg ae/ha) and farmer's practice (intensive tillage) were tested using plot demonstration with three replications.

No-tillage systems followed that the area was kept dry to moist condition for 20 days after harvesting of previous rice crop, and then applied with herbicide in spraying volume of 500 l water/ha using knapsack sprayer (15-liter capacity) or 10 days before rice transplanting. Soil was kept dry for another 5 days and then flooded. Meanwhile, intensive tillage was done through plowing, harrowing and leveling.

Rice seedlings (cv. Cisokan) at 21-days old were transplanted on plot demonstration (3 seedlings/hill) and spaced 25 by 25 cm between and within crop rows and hills, respectively. Crop was fertilized with Urea, TSP and KCl at the rates of 200, 150 and 100 kg/ha, respectively. One-third of Urea, and all TSP and KCl were braodcasted at palnting. A one-half of the remaining Urea was topdressed at 21 and 42 days after transplanting (DAT). Carbofuron (17 kg/ha Curater 3-G) was also broadcasted at planting and periodical spraying of Chlorfiriphos (2 cc Kiltop/l water) was done to control insects infestation. Leaf or neck blast disease was controlled by spraying Isoprotiolan (2 cc Fujiwan/l water) at maximum tillering, booting and flowering stages. Meanwhile, manual weeding was done at 21 and 42 DAT.

Data collected were weed dryweights by groups (grass, broadleaf and sedge) at 21 DAT (before first weeding), and yield of irrigated lowland rice at very cropping season.

## RESULTS AND DISCUSSION

### Weeds

Demonstration area was occupied with 12 weed species which 3, 6 and 3 species belonged to grass, broadleaf and sedge community, respectively before applying treatment. Earlier report indicated that targeted weed species to be controlled by glyphosate herbicide was *Fimbristylis littoralis* Gaudich (abundant visual distribution) and then followed by *Scirpus juncoides* Roxb, *Limnocharis flava* (L.) Buch, *Monochoria vaginalis* (Burm.f) Presl. and *Enchinocloa colona* (L.) Link (respectively frequent) (Lamid *et al*, 1999). Other weed species on the other hand was minor important or observed on rare distribution such as; *Ischaenum timorense* Kunth, *Ludwigia octovalvis* (Jacq) Raven, *L. ascendens*(L.) Hara, *Cyperus halpan* L. and *Leersea hexandra* Sw.

Observation made at after treatment or before first weeding, dry weights of weed group by continuous application of glyphosate herbicide from season to season on plots of no-tillage (NT) and Intensive tillage (IT) varied with cropping seasons (Table 1). For grass group, two species (more population of *I. timorensis* than *L. hexandra*) were observed with their inconsistent total dryweights with succeeding cropping seasons; 0.00 to 35.69 g/sqm under no-tillage and 0.00 to 29.17 g/sqm for intensive tillage. However, average of 15-cropping seasons indicated that those weed dry weights were 10.66 g/sqm, more than those from intensive tillage plot (5.33 g/sqm).

Table 1. Dry weights of weed groups on long term no-tillage (NT) applied glyphosate herbicide and intensive tillage (IT) before first weeding, FF. Solok (West Sumatera), Indonesia WS 1994/95-2000.

Planting Season	Grasses (g/sqm) <sup>1)</sup>		Broadleaf (g/sqm)		Sedges (g/sqm) <sup>2)</sup>	
	NT	IT	NT	IT	NT	IT
I. WS 94/95	13.80	0.00	5.40 <sup>3)</sup>	0.00	0.80	0.00
II. DS 1995	0.79	0.00	0.00	0.00	12.99	0.00
III. WS 1995	23.36	0.00	0.00	0.00	0.00	0.00
IV. WS 95/96	0.00	0.00	0.00	0.90	0.00	0.00
V. DS 1996	9.44	9.75	0.00	0.00	0.20	0.00
VI. WS 96/97	10.47	23.37	0.00	0.00	0.00	0.00
VII. DS 1997	18.77	29.17	0.00	0.00	0.00	0.00
VIII. WS 97/98	0.00	0.00	0.00	0.00	0.0	0.00
IX. DS 1998	3.06	0.00	0.00	0.00	0.31	0.00
X. WS 1998	5.05	4.98	0.96 <sup>4)</sup>	0.00	0.92	3.00
XI. WS 98/99	4.66	5.10	0.73	2.12	1.17	1.46
XII. DS 1999	4.21	3.91	0.59	2.33	0.93	1.42
XIII. WS 1999	35.69	0.00	1.99	0.00	0.86	0.00
XIV. WS 99/00	13.86	3.62	0.75	1.13	0.23	0.42
XV. WS 2000	16.72	0.00	1.07	0.00	0.38	0.00
Average	10.66	5.33	0.77	0.43	1.25	0.42

<sup>1)</sup> Coverage *I. timorensis* > *L. hexandra*; <sup>2)</sup> Only *F. littoralis*; <sup>3)</sup> Individual *L. octovalvis* > *L. flava* > *M. vaginalis*; <sup>4)</sup> Individual *C. baghallensis* > *L. octovalvis*

Broadleaved weed species on no-tillage systems was still observed at planting season I which more individual numbers in order of *L. octovalvis*, *L. flava* and *M. vaginalis* with their total dryweights of 5.40 g/sqm (Table 1). Weeds disappeared up to planting season IX and then appeared at succeeding cropping seasons. This was due to those weed seeds were brought through irrigation water to plot demonstration. Meanwhile, broadleaved weed species on intensive tillage plot was occasionally observed only at planting season IV at light weight (0.90 g/sqm) and then disappeared up to 10<sup>th</sup> cropping season, and redetected up to 14<sup>th</sup> cropping season. Data generally stated that continuous no-tillage systems would eliminate existing broadleaved weed population (average of total dryweight from 15-planting seasons were 0.77 g/sqm while on plot of intensive tillage were 0.43 g/sqm).

Dry weights of sedge weed group also varied with planting seasons and tillage systems which consisted only by one species, *F. littoralis* (initially was a targeted weed species to be controlled). The weed was inconsistently observed at a certain planting seasons under no-tillage systems while only at 10<sup>th</sup> planting season up to 14<sup>th</sup> of

intensive tillage systems. Based on average dryweight of 15-planting seasons indicated that no-tillage plot was 1.25 g/sqm and 0.42 g/sqm from intensive-tillage plot. They both were lower weight per unit area which assumed that unaffected to rice crop.

Performance of weed dryweight on no-tillage plot showed that weed species belonged to grass group, particularly predominated by *I. timorensis* (based on its dry weight), weightier than sedge and broadleaved weed groups. Therefore, initial predominant-weed species (*F. littoralis*) was shifted to *I. timorensis* starting from 3<sup>rd</sup> planting season onward. This was due to nodal stolon of the said weed species was capable of regrowing to produce new sprouting shoot as well as root since it was approaching soil surface. On the other hand, it was assumed that there was a specific enzyme inside the node which capable of resisting mode action of herbicide. Even though formerly expanded leaves were toxified by Glyphosate herbicide. Furthermore, detected broadleaf weed species at 10<sup>th</sup> cropping season was *Commelina beghalensis* L. which undetected before experiment going on. This species might resist up to 15<sup>th</sup> cropping season. It indicated that the same capability of said species as *I. timorensis* in responding to continuous herbicide application in NT practice.

### Grain Yield of Rice Crop

Rice grain yields varied with planting seasons and tillage systems (Table 2). No-tillage rice cultivation produced grain yields of 2133 to 5400 kg/ha depending on seasonal cropping, relatively higher than intensive tillage (1517 to 5007 kg/ha) when observed up to 10<sup>th</sup> cropping season. The succeeding cropping seasons, NT gave inconsistent lower yield than IT. Data indicated that no-tillage systems seemed to be profitable in producing obtainable grain yield rather than intensive tillage. Data also

Table 2. Grain yields of rice crop on long term no-tillage (NT) applied glyphosate herbicide and intensive tillage (IT), FF. Solok (West Sumatera), Indonesia WS 1994/95-2000.

Planting Season	Grain Yield (kg/ha) <sup>1)</sup>	
	NT	IT
I. WS 94/95	2977	2830
II. DS 1995	2133	1517
III. WS 1995	2637	2770
IV. WS 95/96	3100	2517
V. DS 1996	4380	3233
VI. WS 96/97	4473	3263
VII. DS 1997	4413	3237
VIII. WS 97/98	4877	4067
IX. DS 1998	5400	5007
X. WS 1998	4727	4397
XI. WS 98/99	3927	4033
XII. DS 1999	4310	3963
XIII. WS 1999	3930	3880
XIV. WS 99/00	4437	4223
XV. WS 2000	4243	4277
Average	3998	3548

<sup>1)</sup> 14% water content

indicated that grain yields of rice during dry season were much higher by no-tillage cultivation than intensive tillage one. This probably was due to lessened soil evaporation from undisturbed soil of no-tillage systems and increased water use efficiency by crop and nutrient uptake as well.

Average of 15-planting seasons showed that no-tillage rice cultivation produced 3998 kg grain yield/ha, 450 kg or 13% higher than under intensive tillage cultivation. When they were seasonal observation which supported by obtainable grain yield, there were not decreasing or lower than intensive tillage (common farmer's practice). Data concluded that long term no-tillage applied with glyphosate herbicide at recommended rate was still applicable and might be recommendable up to 15-cropping seasons or presumably more.

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## POPULATION DYNAMICS AND GROWTH OF WEEDS IN RAINFED RICE-ONION SYSTEMS IN RESPONSE TO CHEMICAL AND CULTURAL CONTROL METHODS

M. C. Casimero<sup>1</sup>, A. M. Baltazar<sup>2</sup>, J. S. Manuel<sup>2</sup>, S. R. Obien<sup>1</sup> And S. K. De Datta<sup>3</sup>

<sup>1</sup>Philippine Rice Research Institute, Munoz, Nueva Ecija, Philippines.

mcasimero@philrice.gov.ph

<sup>2</sup>University of the Philippines Los Banos, College, Laguna, Philippines

<sup>3</sup>Virginia Polytechnic and State University, Blacksburg, VA, USA

**Abstract:** A field study was conducted to determine the effects of tillage in combination with chemical control and handweeding on the population dynamics, growth and control of weeds in rainfed rice-onion systems. Twelve weed species were associated with onion but only *Cyperus rotundus* L., *Echinochloa colona* (L.) Link and *Trianthema portulacastrum* L. were dominant in terms of density and fresh weight. Tillage alone did not affect the growth of the three most dominant weeds. However, a shift in weed dominance was observed in response to weed control methods. Pre-plant application of glyphosate (1.1 kg ai/ha) at 14 days before transplanting (DBT) followed by one handweeding (HW) was as effective as the application of oxyfluorfen (0.06 kg ai/ha) followed by three HW (farmer's practice) in controlling weeds. In conventional tillage, comparable bulb yield was obtained in plots applied with pre-plant glyphosate at 14 DBT followed by one or two HW and the farmer's practice. In deep and shallow tillage, bulb yield in plots treated with glyphosate followed by one HW was lower than the farmer's practice but was comparable with the weedfree check and those applied with glyphosate followed by two HW. Percentage yield loss was comparable between the application of glyphosate at 14 DBT followed by one HW and the farmer's practice. As weed density in the field was high, 60 mandays were needed to handweed a hectare of onion. Farmers can save 60% in weed control costs when glyphosate was applied at 14 DBT followed by one HW compared with the farmer's practice.

**Key words:** Chemical weed control, cultural weed control, population dynamics, rice-onion systems, weed growth

## INTRODUCTION

Planting rainfed rice during the wet season followed by onion in the dry season is among the dominant cropping patterns adopted by farmers in Nueva Ecija province, Central Luzon, Philippines. This system is particularly attractive because of its profitability, high demand for onion in the local market, good export potential and its complementarity with rice. The rice by-products such as rice straw and rice hull are used during the onion season. Rice straw is used as mulch for the "Lasona" or cluster type while rice hull is burned to sterilize the soil and make the soil more friable and suitable for the Yellow Granex and Red Creole onions.

Onion is a profitable dry season crop with export potential in the Philippines. It ranks third both in area planted and volume of production, and second in monetary value. Onion is grown in 4,781 hectares, the largest area planted to non-rice crop in Nueva Ecija province during the dry season. The volume of production reached 52,086 mt, valued at US \$ 26 M in 1999, was mostly exported to other Asian markets (Alviola

and Casimero 2000). In 1980's, onion yield was about 8 t/ha but this has increased to about 10-12 t/ha in the 1990's. This yield can still be increased to 20 t/ha if pest and weed management technologies are improved to give farmers a competitive edge in both local and foreign markets (Gaskell 1994; Baltazar et al. 1999).

Onion cannot compete effectively with weeds because it slowly emerges from the soil giving time for weeds to germinate and establish first. The seedlings have a relatively low maximum growth rate ( $0.12 \text{ day}^{-1}$ ) compared with the  $0.18 \text{ day}^{-1}$  of weeds (Brewster 1994). Likewise, onion with its narrow, erect leaves and short stature cannot easily out shade weeds. Its shallow and low density roots per unit volume of soil makes it less competitive for water and soil nutrients. Depending on the duration and intensity of crop-weed competition, yield loss in onion can range from 40-80% (Brewster 1994).

Weed control in onion largely depends on herbicide use as this has been proven to be more economical than other control methods. It is however, more desirable if chemical control can be integrated with cultural methods to provide a longer term of weed control. Tests on herbicide usage combined with mechanical and physical methods showed better grass and broadleaf weed control with significant increases in bulb yield. In the Philippines, the integration of chemical with mechanical and physical control methods like rice straw mulch (5.0 cm) followed by one handweeding at 30 days after planting; handweeding 2-4 times at 10-60 days after planting and fluazifop, 0.1-0.2 kg ai/ha followed by handweeding were proven to be the best combinations to control weeds (Baltazar 1996). Tillage as a component of the integrated weed management strategy needs to be studied. Soil disturbance due to tillage stimulates germination of weed seeds. When followed by a non-selective control, it can effectively minimize weed problem in onion on a long term basis. In a fallow field, Egley and Williams (1990) induced weed emergence with frequent tillage for 4 years. Afterwards, tillage had no effect implying that the weed seeds had been depleted. Purple nutsedge (*C. rotundus*) was eradicated from a field in Alabama by disk harrowing at weekly or biweekly intervals for 5 months (Smith and Mayton 1938). Field studies done for three years in Nueva Ecija province Philippines likewise reduced the tuber and shoot density of purple nutsedge in straw mulched onions (Baltazar et al. 2000). Weed emergence stimulated by soil tillage reduced weed populations allowing mechanical control to partially replace herbicides.

This study was done to evaluate the effects of different tillage practices during the fallow period of the rice-onion cropping system combined with chemical and mechanical control methods on the population dynamics, growth and control of weeds in rice-onion cropping system and to identify the optimal combinations of mechanical and chemical weed control to reduce weed populations in a rice-onion cropping system.

## MATERIALS AND METHODS

The experiment was established in the IPM-CRSP experimental field in Bongabon, Nueva Ecija during the 1998-1999 dry seasons. The seedling establishment and other cultural management practices implemented in this study followed that of the recommended cultural management practices for onion in the Philippines except for the land preparation and weed control methods that were used as variables. The treatments involved three tillage practices (conventional, deep and shallow tillage) as main plot and six herbicide by handweeding combinations comprised the subplots namely; T1 - pre-



plant glyphosate at 1.1 kg ai/ha followed by oxyfluorfen at 0.06 kg ai/ha applied at 6 DAT; T2 - pre-plant glyphosate at 1.1 kg ai/ha + 1 handweeding at 30 DAT; T3 - pre-plant glyphosate at 1.1 kg ai/ha + 2 handweeding at 30 and 60 DAT; T4 - Farmer's practice (oxyfluorfen at 0.06 kg ai/ha applied at 6 DAT followed by handweeding at 30, 45 and 60 DAT); T5 - weedfree check and T6 - unweeded check. The main plots, measuring 30m x 6m, were composed of the three tillage practices. The conventional tillage (15 cm deep) was done using a small power tiller. Deep tillage was done using a four wheeled tractor that had about 20cm deep furrow slice. Shallow tillage was done using a carabao driven plow having a furrow slice of about 10cm deep. For each tillage practice, two passes of plowing and harrowing were done. Purple nutsedge was allowed to grow until 8-10 leaf stage before glyphosate was applied. Pre-plant glyphosate application at 1.1 kg ai/ha was done at two weeks before planting followed by another harrowing prior to land leveling.

The experiment was laid out in split plot in Randomized Complete Block design (RCBD) with three replications. The data gathered include weed count and weed fresh weight at 30, 60 and before harvest, yield, yield loss and cost of weed control. The data were analyzed using Analysis of Variance (Anova) and means were compared using the Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSIONS

### Weed population dynamics

**Weed species associated with onion** The weeds associated with onion in the tillage experiment were dominated by annual broadleaves, grasses and a perennial sedge. Twelve weed species were found but only three were observed to be more dominant in terms of density and fresh weight (data not shown). The first weeds to germinate at 2 to 3 days after transplanting were the annual broadleaves like *Trianthema portulacastrum*, *Portulaca oleracea*, *Heliotropium indicum*, *Eclipta. alba*, and *Physalis angulata*. The annual grasses such as *Echinochloa colona*, *Digitaria ciliaris*, *Eleusine indica*, *Oryza sativa* (volunteer rice), and *Dactyloctenium aegyptium*. *C. rotundus* started to germinate at about 5 days after transplanting. The weeds germinated almost at the same time in the three tillage practices.

**Effect of tillage depth on changes in weed population** No significant difference was observed in the density of *C. rotundus*, *T. portulacastrum* and *E. colona* in all tillage practices (Figure 1). The effect of tillage alone in controlling the weeds was not very apparent as it takes sometime before the reduction in weed density due to tillage can be observed. Smith and Mayton (1938) reported that the population of purple nutsedge and other weeds can be reduced to manageable levels by plowing or disking at intervals of three weeks or less for two years.

**Effect of control methods on weed population change** Among the three weed groups, the broadleaves were more dominant in terms of population density than the grasses and sedges early in the season. Low population of sedges was observed as *C. rotundus* was outgrown by the faster growing broadleaf and grass weeds. The application of oxyfluorfen at 6 DAT significantly reduced the broadleaf weed population but not the sedges and grass weeds at 60 DAT. Significantly lower weed population was observed in plots applied with pre-plant glyphosate followed by oxyfluorfen and the farmer's practice. More weeds were observed in the weed control treatments where a follow up handweeding was done once or twice during the

midseason. Since weeds are dynamic, a very rapid replacement of the weeds removed by handweeding was observed. At harvest, plots applied with pre-plant glyphosate followed by oxyfluorfen had lower weed population than those with pre-plant glyphosate followed by one or two handweeding, the farmer's practice and the unweeded check.

The pre-plant application of glyphosate was not effective in controlling *C. rotundus* as indicated by the comparable weed density with the unweeded check (Figure 2A). Baltazar et al. (1999) reported similar observations in the same area in 1998. Pre-plant application of glyphosate at 14-21 days before planting either before or after land preparation did not control *C. rotundus* effectively. At 60 DAT, the density of *C. rotundus* in plots sprayed with glyphosate followed by oxyfluorfen at 6 DAT was comparable with the weedfree check and the unweeded check. The low density of *C. rotundus* in the unweeded check and in plots sprayed with glyphosate followed by oxyfluorfen was due to shading by other weeds. *C. rotundus* is a poor competitor of weeds that can grow very fast in a short time like *T. portulacastrum* and *E. colona*. In treatments followed by handweeding at 30 DAT, higher density of *C. rotundus* was obtained. The broadleaf weeds and grasses were effectively controlled in these treatments so that no competition for *C. rotundus* was present. The density of *T. portulacastrum* was significantly higher in all weed control treatments than the weedfree check at 30 DAT (Figure 2B). The pre-plant application of glyphosate and early post-emergence application of oxyfluorfen provided a good control of *T. portulacastrum* until 15 DAT after which there was a sharp decline in weed control and a consequent increase in the

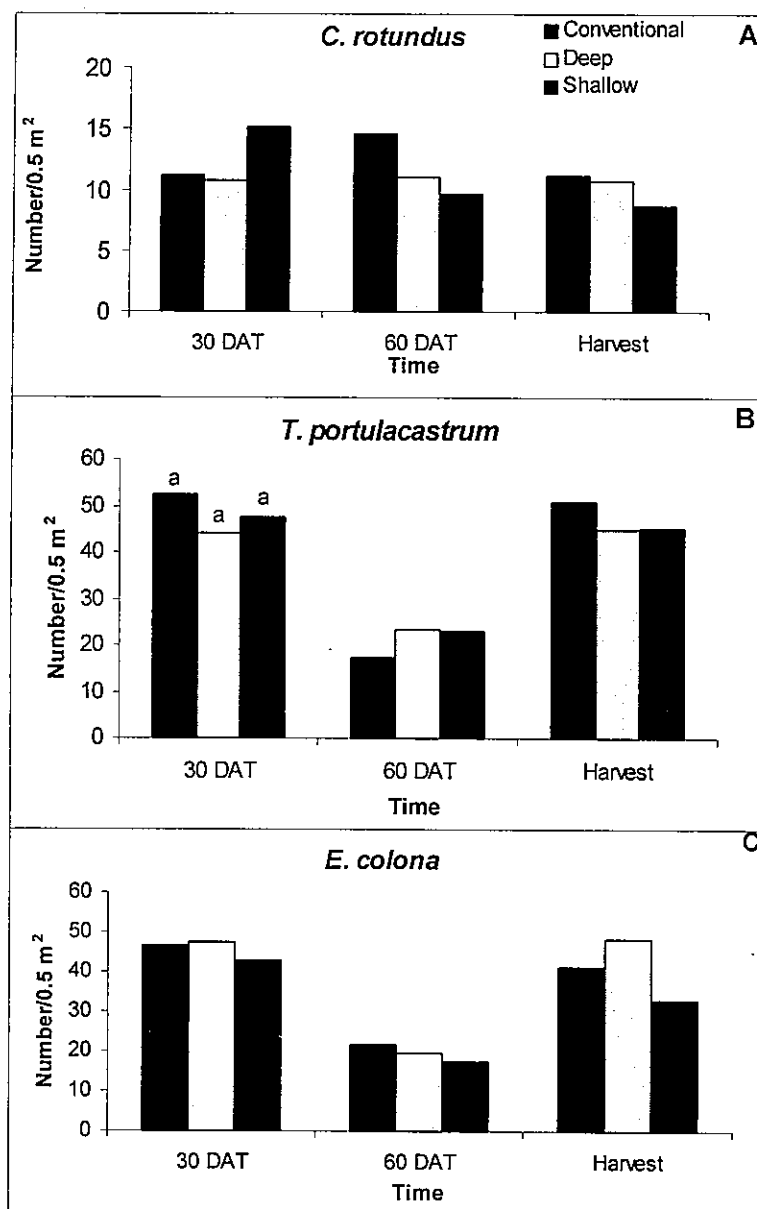


Figure 1. Density of weeds as affected by tillage at various sampling periods.

the unweeded check and in plots sprayed with glyphosate followed by oxyfluorfen was due to shading by other weeds. *C. rotundus* is a poor competitor of weeds that can grow very fast in a short time like *T. portulacastrum* and *E. colona*. In treatments followed by handweeding at 30 DAT, higher density of *C. rotundus* was obtained. The broadleaf weeds and grasses were effectively controlled in these treatments so that no competition for *C. rotundus* was present. The density of *T. portulacastrum* was significantly higher in all weed control treatments than the weedfree check at 30 DAT (Figure 2B). The pre-plant application of glyphosate and early post-emergence application of oxyfluorfen provided a good control of *T. portulacastrum* until 15 DAT after which there was a sharp decline in weed control and a consequent increase in the

density was observed because of new flushes of the weed. At 60 DAT, plots sprayed with pre-plant glyphosate followed by early post-emergence application of oxyfluorfen had lower *T. portulacastrum* density as there was no succeeding flushes of the weed. In contrast, higher *T. portulacastrum* density was noted in plots sprayed with glyphosate followed by one or two hand-weedings and the farmer's practice. At harvest more *T. portulacastrum* was observed in plots sprayed with glyphosate followed by one handweeding compared with the other treatments.

The *E. colona* density in plots sprayed with pre-plant glyphosate followed by oxyfluorfen, glyphosate followed by one or two handweedings and the farmer's practice including the unweeded check was higher the farmer's practice (Figure 2C).

Early post-application of oxyfluorfen was not effective in controlling grasses as this herbicide is more effective against broadleaf weeds. Similarly, the preplant application of glyphosate did not provide sufficient control of grasses that could last until 30 DAT. Handweeding at 30 and 45 DAT however, effectively reduced the *E. colona* population at 60 DAT. Plots handweeded once or twice and the farmer's practice had significantly lower grass weed density at 60 DAT. Similar trend with that at 60 DAT was observed in the density of *E. colona* at harvest. All weed control treatments had significantly higher *E. colona* density than the weedfree check.

### Growth of weeds

**Weed growth as affected by tillage depth** Tillage did not significantly affect the fresh weight of the three dominant weeds throughout the season (Figure 3). *T. portulacastrum* and *E. colona* were more dominant than *C. rotundus*. *C. rotundus* had higher fresh weight during the mid-season and at harvest compared with the early stages. This happens as the weeds that germinate early in the season are the broadleaves like *T.*

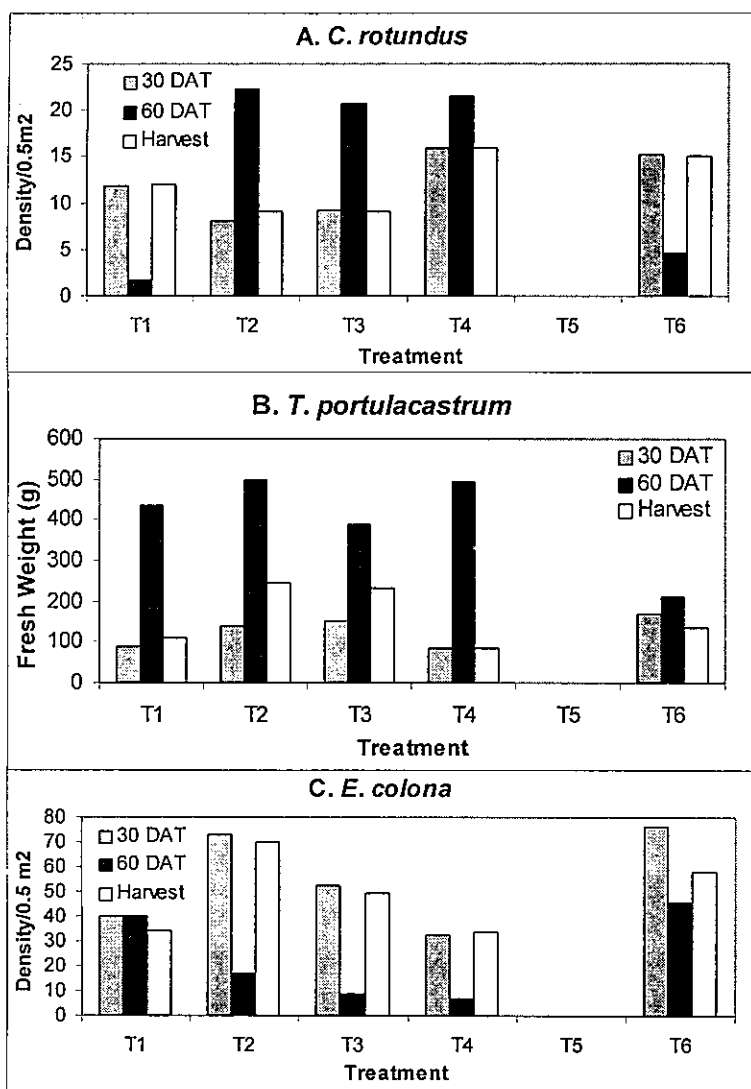


Figure 2. Density of weeds as affected by weed control methods.

*portulacastrum* followed by the grasses like *E. colona* and *C. rotundus* cannot compete effectively with these rapidly growing weeds. *C. rotundus* became more dominant during the mid-season and at the later stages of onion season as the broadleaves and the grasses had been efficiently controlled by herbicides and hand-weeding. Moreover, the herbicides that are currently being used by farmers are not effective on *C. rotundus* so that the only direct control method available to them is hand-weeding and to some extent interrow cultivation.

**Weed growth as affected by weed control methods** The follow up handweeding done at 30 and 45 DAT reduced the fresh weight of weeds during the midseason. Oxyfluorfen alone applied at 6 DAT was not effective in controlling the weeds. Apparently, the

residual activity of oxyfluorfen was not long enough to provide a season long control of weeds which implies that handweeding is a necessity at 30 DAT. Across weed classes, there was no difference in the weed weights when glyphosate or oxyfluorfen was followed by one, two, or three handweedings. The subsequent weeding operations done at 60 DAT as in the farmer's practice did not help in controlling the weeds contrary to their belief that more handweedings done provides a more effective weed control. Economically, this practice is not beneficial as the farmers are increasing the cost of weed control but control efficacy is not improved. The three dominant weeds, *T. portulacastrum*, *E. colona* and *C. rotundus*, did not differ in the fresh weights when treated with the various weed control treatments. With *C. rotundus*, all treatments having pre-plant application of glyphosate followed by oxyfluorfen or handweeding had comparable fresh weight with that of the farmer's practice (Figure 4A). None of the treatments controlled the weed as effectively as the weedfree check. Significantly higher fresh weight of *C. rotundus* were obtained at 60 DAT in plots applied with pre-plant glyphosate followed by one or two handweedings compared with plots applied with pre-plant glyphosate followed by oxyfluorfen and the unweeded check. Removal of the broad-leaf weeds and the grasses favored the establishment of *C. rotundus* which

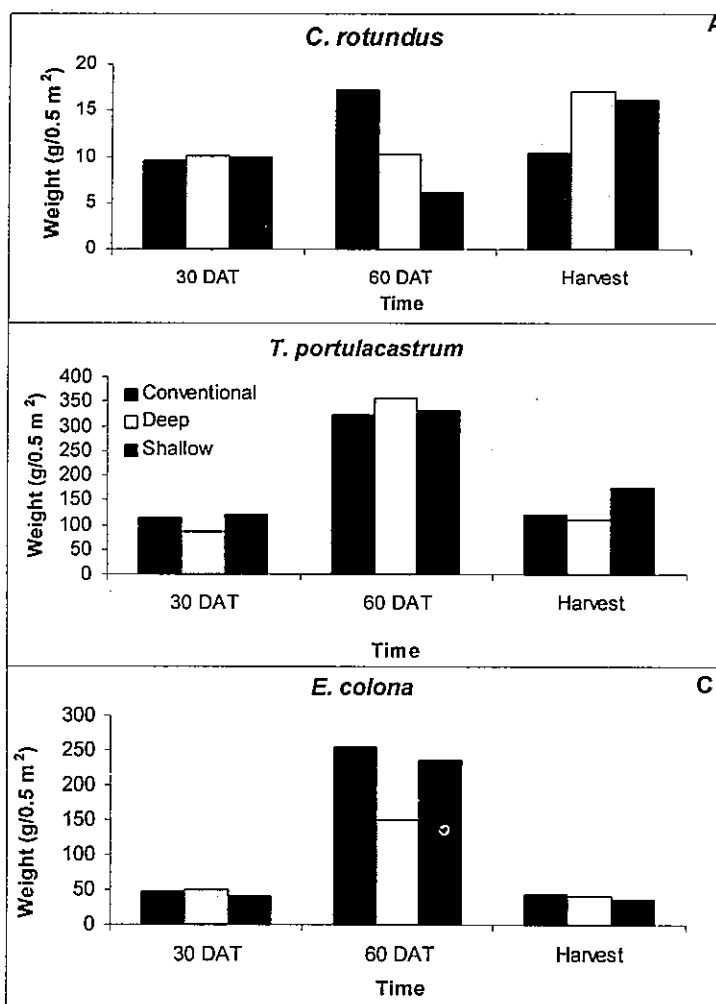


Figure 3. Fresh weight of weeds as affected by tillage at various sampling periods.

increased both in density and fresh weight during the mid-season and later stages of onion. At harvest, no significant variation was observed in plots applied with pre-plant glyphosate followed by one or two handweeding compared with the farmer's practice.

Oxyfluorfen provided a very good control of *T. portulacastrum* up to 30 DAT because of its long residual activity. The fresh weight of *T. portulacastrum* at 30 DAT was significantly lower in plots applied with pre-plant glyphosate followed by oxyfluorfen and the farmer's practice compared with the unweeded check (Figure 4B). Pre-plant application of glyphosate failed to efficiently control *T. portulacastrum*. Except for the weedfree check, all other treatments had high *T. portulacastrum* fresh weight at 60 DAT. At harvest, plots sprayed with glyphosate followed by one or two handweeding had significantly higher fresh weight compared with the farmer's practice and plots sprayed with glyphosate followed by oxyfluorfen.

At 30 DAT, plots applied with glyphosate followed by one or two handweeding had higher *E. colona* fresh weight compared with the farmer's practice (Figure 4C). The follow up handweeding at 30 DAT in these treatments effectively controlled *E. colona* while in the plots applied with pre-plant glyphosate followed by oxyfluorfen, *E. colona* fresh weight significantly increased at 60 DAT. No significant variation was observed in all treatments at harvest

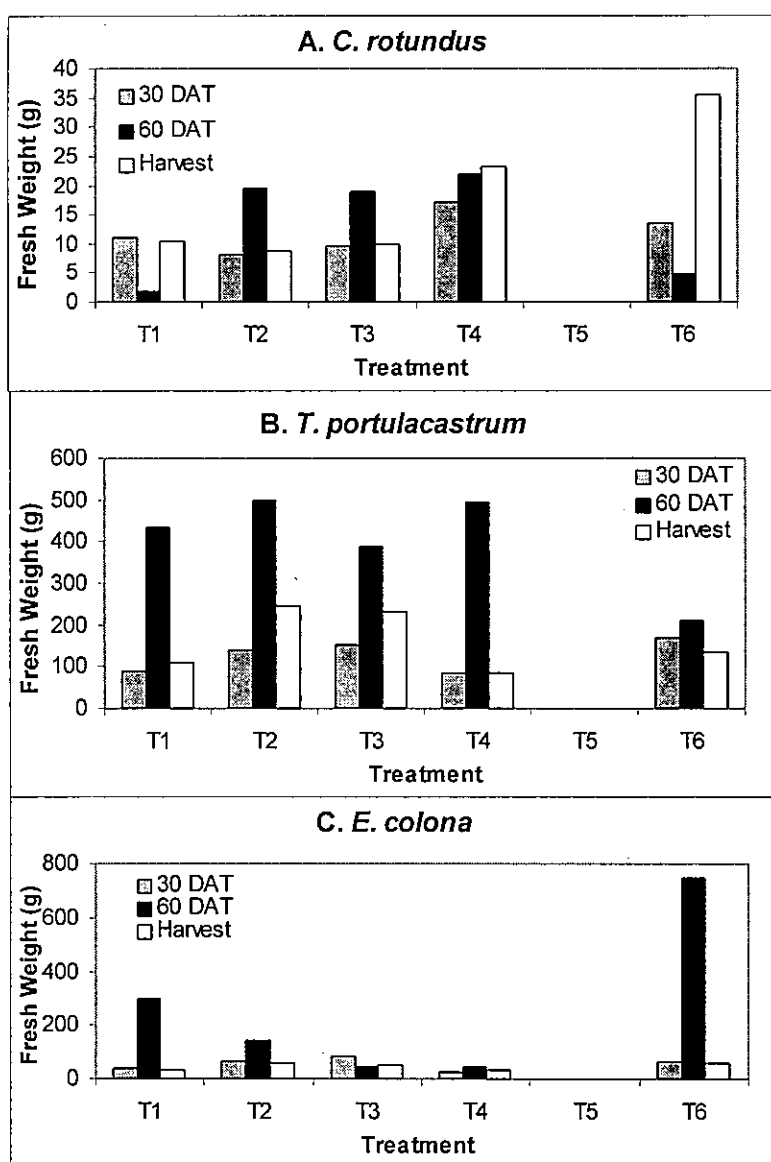


Figure 4. Fresh weight of weeds in response to various weed control methods.

**Yield** Higher yields were obtained in the conventionally tilled plots compared with deep and shallow tillage though not significantly different. Higher density of *C. rotundus* was obtained in the shallow and deep tillage during the mid-season which coincided with the bulb formation of onion which could have provided competition for resources in the forming bulbs. Hewson and Roberts (1973) reported that competition from weeds reduces the number, size, longevity and chlorophyll content of onion leaves. If bulbing has started by the time weeds are removed, bulb growth depends solely on the weed-impaired leaf stand present at the time of weeding, and yield is reduced.

The highest yield was obtained in the farmer's practice but did not significantly differ from the yield of the weedfree plot and plots sprayed with glyphosate followed by two handweedings (Table 1). The yield obtained from plots sprayed with glyphosate followed by one handweeding was comparable with the weedfree check and plots sprayed with glyphosate followed by two handweedings. Significantly lower yields were obtained in plots sprayed with glyphosate followed by oxyfluorfen and the unweeded check. This indicates that one handweeding was as effective in controlling weeds as the weedfree check and the application of glyphosate followed by two handweedings.

**Yield reduction** The highest yield reduction was observed in the unweeded check (78.9%). Application of glyphosate followed by oxyfluorfen had 71.9% which was comparable with the unweeded check. Significantly lower yield reduction was obtained in plots sprayed with glyphosate followed by one or two handweedings, farmer's practice and the weedfree check. This result reaffirms the observation that the application of glyphosate followed by one handweeding is as effective as glyphosate followed by two handweedings and the farmer's practice in controlling weeds. Keeping the field weedfree may not be necessary as there is no significant yield reduction is obtained with two handweedings only compared with the weedfree check which had four handweedings.

Table 1. Yield, yield loss, weed control costs, % of total production cost and reduction in total weed control costs of various weed control methods in the tillage experiment.

Treatment	Yield (t/ha)	%Yield Loss	Total Weed Control Cost (Php/ha) <sup>1</sup>	% of Total Cost <sup>2</sup>	% Reduced in Weed Control
Glyphosate fb Oxy	3.3 b	71.9 a	1,338.25	2.7	90.2
Glyphosate fb 1 HW	10.0 a	15.1 b	5,187.00	10.4	62.2
Glyphosate Fb 2 HW	11.8 a	6.8 bc	9,401.25	18.8	53.12
Oxyfluorfen fb 3 HW	12.5 a	6.8 bc	13,657.25	27.3	-
Weedfree	11.5 a	0.0 c	15,067.00	30.1	-
Unweeded Check	2.4 c	78.9 a	-	-	-

1. US \$ = P38.00; 2. Total production cost of farmers = P50,000

**Cost of weed control** Farmers spend about 10-20% of the total cost of production to weed control and this was verified in the field study conducted. As the density of

weeds is high in Bongabon, about 60 mandays are needed to handweed a hectare of onion which amounts to P5,000 (10%) when pre-plant glyphosate is applied followed by one handweeding. With pre-plant glyphosate followed by two handweeding, the cost is about P9,000.00 (19%). The cost of weed control in current farmer's practice is about P13,000 (27%). Farmers can save from 50-60% in the cost of weed control when one to two handweeding are done per season. Weed control is important during the first 30 to 45 days after transplanting so that the handweeding operation has to be done during this period. Weeds that grow after this period can be controlled by the subsequent interrow cultivation. Therefore, the handweeding done by farmers after this period is no longer necessary as this only increases the cost of production.

### ACKNOWLEDGMENT

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## THE TAXONOMY AND DISTRIBUTION OF *ECHINOCHLOA* SPECIES (BARNYARD GRASSES) IN THE ASIAN-PACIFIC REGION, WITH A REVIEW OF PERTINENT BIOLOGICAL STUDIES.

P. W. Michael

Department of Crop Science, Faculty of Agriculture, University of Sydney, New South  
Wales, Australia 2006. pwjemichael@hotmail.com

**Abstract:** Lists of *Echinochloa* (barnyard grass) taxa (species, subspecies, varieties, forms) are collated from the most recent taxonomic treatments of the genus for various countries (or areas) throughout the Asian-Pacific region, followed by a discussion of the taxonomic problems raised. Special attention is given to the naming of forms within the very variable *E. colona* and *E. crus-galli* and to the epithets *caudata*, *crus-pavonis*, *hispidula*, *phyllopogon*, and *zelayensis* which appear to have often been misused. A case is put for alternative (but legitimate) names to be used where it is considered appropriate, for example, *E. glabrescens* and *E. crus-galli* var. *formosensis* are true synonyms and may be used interchangeably without confusion. Comments are given on new taxa that have not yet been described. Recent biological studies of the genus pertinent to the taxa occurring in the Asian-Pacific Region are reviewed. A key to the taxa with notes on distribution within the region is presented.

**Key words:** Asian-Pacific region, barnyard grasses, distribution, *Echinochloa*, taxonomy

### INTRODUCTION

In this paper I present a much-considered taxonomic point of view of *Echinochloa* in the Asian-Pacific region in the hope that eventually some of the confusion and contradictions noted in various treatments of the genus can be overcome. One cannot repeat too often that without an informed taxonomy it is difficult to compare our work with the work of others in our own countries or in other parts of the world.

This is essentially an updated version of my papers (Michael 1983, 1991, 1994), concentrating on the Asian-Pacific region. A most useful additional bibliography may be found in Yabuno and Yamaguchi 1996. Appropriate references will be cited in the notes to the various species now listed.

### *ECHINOCHLOA* IN THE ASIAN-PACIFIC REGION LISTED BY REGION OF ORIGIN

#### World tropics

*E. colona* (L.) Link

#### Eurasia

*E. crus-galli* (L.) Beauv. ssp. *crus-galli* var. *crus-galli*

#### Asia (including South-east Asia, Indonesia, New Guinea and adjacent islands)

*E. caudata* Roshev.

*E. crus-galli* (L.) Beauv. ssp. *crus-galli* var. *praticola* Ohwi



- E. crus-galli* (L.) Beauv. ssp. *hispidula* (Retz.) Honda var. *hispidula*  
*E. crus-galli* (L.) Beauv. ssp. *hispidula* (Retz.) Honda var. *austro-japonensis* Ohwi  
*E. esculenta* (A. Br.) H. Scholz  
*E. glabrescens* Munro ex Hook. f.  
*E. oryzicola* (Vasing.) Vasing.  
*E. oryzoides* (Ard.) Fritsch  
*E. persistentia* Z. S. Diao  
*E. picta* (Koen.) P. W. Michael  
*E. praestans* P. W. Michael  
*E. stagnina* (Retz.) Beauv.

### Australia

- E. dietrichiana* P. W. Michael  
*E. elliptica* P. W. Michael et Vickery  
*E. inundata* P. W. Michael et Vickery  
*E. kimberleyensis* P. W. Michael et Vickery  
*E. lacunaria* (F. Muell.) P. W. Michael et Vickery  
*E. macrandra* P. W. Michael et Vickery  
*E. telmatophila* P. W. Michael et Vickery  
*E. turneriana* (Domin.) J. M. Black

### Africa

- E. pyramidalis* (Lam.) Hitchc. et Chase

### North America

- E. microstachya* (Wieg.) Rydb.

### South America

- E. crus-pavonis* (H. B. K.) Schult.  
*E. polystachya* (H.B. K.) Hitchc. var. *polystachya*

## KEY TO *ECHINOCHLOA* IN THE ASIAN-PACIFIC REGION

NB Spikelet length measurements do not include awns.

### A. Annuals

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|---|----------------------|
| 1. Spikelets 3 to 5mm long.   | 2.                   |
| 1. Spikelets less than 3 mm or greater than 5mm long.   | 15.                  |
| 2. Ligule a line of bristles or fine short cilia.   | 3.                   |
| 2. Ligule absent, or the ligular regions bearing a few cilia or fine pubescence.  | 4.                   |
| 3. Numerous long bristles at nodes of inflorescence. Panicle spindle-shaped, up to 15 cm long. Spikelets narrowly elliptical. Awns of lower lemma up to 30mm long, of second glume up to 10mm long. | <i>E. elliptica</i>  |
| 3. No long bristles along main axis or branches of panicle. Panicle narrow, linear. Spikelets broadly ovate or ovate-elliptical.  | <i>E. turneriana</i> |

4. Spikelets broadly ovate, crowded along the often incurved branches of the inflorescence. Fertile florets and caryopses markedly humped, so that the second glume often appears to be shorter than the spikelet. Mature fertile florets not easily deciduous. 5.
4. Fertile floret and caryopses not markedly humped. 6.
5. Spikelets brownish at maturity. Commonly awnless, sometimes awned. Caryopses brownish. *E. esculenta*
5. Spikelets pale green at maturity, awnless. Caryopses whitish. *E. frumentacea*
6. Essentially obligate weeds of rice. Close tufted erect habit. Greatly resemble rice before flowering. 7.
6. Not obligate weeds of rice, but all growing in wet places and often occurring in rice. Plants more or less spreading at base. 9.
7. Spikelets 3.5 to 5mm long. 8.
7. Spikelets 3 to 3.5mm long. Lower lemma convex, hard, and shiny. Awnless or less frequently awned. Occasionally found on banks and fallow land where it assumes a spreading habit. *E. glabrescens*
8. Spikelets broadly ovate to ovate. Inflorescence hanging almost horizontal at maturity. Spikelets nearly always awned. Awns sometimes as long as 50mm. Lower glume 0.33 to 0.5 the length of spikelet. Collar region of leaves rarely with tufts of hairs. Caryopses ovate, embryo 0.7 to 0.8 the length of the caryopsis. *E. oryzoides* and *E. persistentia*
8. Spikelets ovate-elliptical. Inflorescence more or less erect at maturity. Spikelets awned or awnless. Lower glume 0.5 to 0.66 length of spikelet. Lower lemma often convex, hard, and shiny. Collar region of leaves often with tufts of hairs. Caryopses oblong, embryo often 0.9 or more the length of the caryopsis. *E. oryzicola*
9. Lemma and palea of fertile floret acute or acuminate with stiff tip. Panicle spreading, erect. Caryopses yellowish. Spikelets 3 to 3.5mm long. *E. microstachya*
9. Lemma of fertile floret with withering tip sharply differentiated from the body of the lemma. Panicle erect or nodding. Spikelets short- or long-awned, sometimes mostly awnless, but if so, there are always a few awned spikelets at the ends of the branches. 10.
10. Inflorescence strongly drooping at maturity, sometimes bending over as much as 180°. Spikelets crowded with short, curved awns, mostly 3 to 10mm long, but can be up to 15mm long. *E. crus-pavonis*
10. Inflorescence often nodding but not strongly drooping at maturity. 11.
11. Spikelets narrowly elliptical, up to 4.2mm long, awns of lower lemma almost always up to 40mm long. Awn on the second glume up to 7mm long or longer. Bristles on spikelets not spreading. Leaf sheaths glabrous. *E. telmatophila*
11. Spikelets broadly ovate to elliptical, never narrowly elliptical.

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- |  |     |   |
|--|-----|---|
| Almost awnless, short-awned or long-awned.   | 12. |   |
| 12. Spikelets ovate or ovate-elliptical up to 5mm long. Panicle linear, anthers 1mm or more long.  | 13. |   |
| 12. Spikelets broadly ovate, ovate, or ovate-elliptical, 3 to 4mm long. Long bristles abundant along main axis and branches of panicle. Panicles various often pyramidal. Anthers generally less than 1mm long.  | 14. |   |
| 13. Spikelets ovate, uniformly 3mm with strongly spreading bristles up to 1mm long. Long bristles prominent at point of attachment of racemes and along main axis. Panicles not becoming purplish.   |     | <i>E. dietrichiana</i>                                |
| 13. Spikelets 3.5 to 5mm long, with few or no bristles on main axis and/or branches of panicle.  |     | <i>E. inundata</i>                                    |
| 14. Spikelets broadly ovate or ovate, awnless except at the ends of branches, short-awned or long-awned. Caryopses ovate. Panicles of variable length, more or less erect, often pyramidal, sometimes nodding, branches never obviously whorled. Long panicles, often with secondary branches on lower primary ones. |     | <i>E. crus-galli</i> var.<br><i>crus-galli</i>        |
| 14. Spikelets ovate-elliptical, short or long awns. Caryopses more or less oblong. Panicles rarely pyramidal, erect or nodding, branches often whorled, more or less erect except for the lowermost ones.  |     | <i>E. crus-galli</i> var.<br><i>hispidula</i>         |
| 15. Spikelets 5mm long or longer.  | 16. |   |
| 15. Spikelets 3mm long or shorter.   | 19. |   |
| 16. Spikelets with awns up to 90mm long. Anthers more than 1.5mm long. Ligule a line of bristles or cilia.   | 17. |   |
| 16. Spikelets awnless or awned. Ligule absent, rarely a line of short cilia.   | 18. |   |
| 17. Anthers 1.5 to 2mm long. Palea of lower floret about half the length of the lemma, sometimes absent. Lower floret neuter.  |     | <i>E. kimberleyensis</i>                              |
| 17. Anthers 2 to 2.8mm long. Palea of lower floret about length of lemma. Lower floret staminate.  |     | <i>E. macrandra</i>                                   |
| 18. Spikelets awnless, ovate, very finely pubescent. main axis and short branches of inflorescence without bristles.   |     | <i>E. lacunaria</i>                                   |
| 18. Spikelets awned, ovate, panicles hanging more or less horizontally at maturity. Awns up to 50mm long. Obligate weed of rice.   |     | <i>E. oryzoides</i>                                   |
| 19. Palea of lower floret absent or poorly developed. Spikelets dense, 1mm broad, with awns up to 45mm long. Panicles up to 20cm long.   |     | <i>E. caudata</i>                                     |
| 19. Palea of lower floret fully developed.   | 20. |   |
| 20. Spikelets broadly ovate to ovate, awnless with panicle not more than about 15cm long.  | 21. |   |
| 20. Spikelets ovate-elliptical to elliptical, usually with short awns. Inflorescence close, short with more or less erect branches, but rarely very long, up to 28cm with secondary branches on lower primary ones.  |     | <i>E. crus-galli</i> var.<br><i>austro-japonensis</i> |

21. Spikelets regularly arranged in rows. First glume regularly half the length of the spikelet. Caryopses whitish. Long bristles mostly absent from main axis and branches of inflorescence, occasionally a few scattered along the branches and clustered at the nodes. *E. colona*
21. Spikelets irregularly arranged. First glume about one-third length of spikelet. Caryopses brownish. Long bristles along main axis and branches of inflorescence present or absent. *E. crus-galli* var. *praticola*

## B. Perennials

All species have spikelets 3mm or more long. Ligular bristles are always present and obvious, especially in the lower leaves. The lower floret is often staminate. Plants may have long creeping rhizomes and/or stolons and spongy floating stems. Sometimes the rhizomes are much shortened and thickened.

1. Spikelets awnless or with short awns or long cusps. Spikelets crowded, very finely pubescent or for the most part glabrous, with short bristles and short awns or long cusps. Inflorescence often more than 40cm long. Secondary branches often closely appressed to primary branches of inflorescence. Plant often up to 4m tall with stout culms. *E. pyramidalis*
1. Spikelets awned, awns often long. 2.
2. Spikelets elliptical or lanceolate, up to 5mm long with bristles up to 1mm long and with long narrow lower glumes. Floating; often with long culms. *E. stagnina*
2. Spikelets obovate, broadly ovate or ovate. 3.
3. Spikelets obovate, usually more than 5mm long in South America but in plants so-called in Australia only 3.5 to 4mm. Awns 3 to 30mm long. Leaf sheaths glabrous. *E. polystachya* var. *Polystachya*
3. Spikelets broad-ovate or ovate, about 4mm long. Awns rarely frequent and never exceeding 15mm. 4.
4. Spikelets finely pubescent. Ligular bristles obvious in all leaves. Stout culms, up to 3.6m tall with broad leaves, uppermost leaf blades as much as 20mm wide. *E. praestans*
4. Spikelets with bristles up to 0.5mm long. Ligular bristles often not apparent in uppermost leaves. Culms generally less than 1m, uppermost leaf blades not exceeding 10mm wide. *E. picta*

## NOTES ON THE SPECIES LISTED IN ALPHABETICAL ORDER

*E. caudata* This riverine non-weedy species with densely packed small ovate-lanceolate dark olive-brown spikelets with long awns, first described from the Argun river in eastern Siberia, has so far only been recorded for that region and the Far East coastal region (along the Amur River) of the Russian Federation. It is perhaps likely to occur in parts of Heilongjiang and Jilin provinces of China and the far north-east part of inner Mongolia. Chromosome counts on material collected in the Khabarovsk and Primorsk regions show that it is tetraploid  $2n=36$  (Charkevich 1885; Sokolovskaya and Probatova 1977). Unfortunately this name has often been used in error at various

taxonomic levels for long-awned forms of *E. crus-galli* var. *crus-galli* and *E. crus-galli* var. *hispidula*.

***E. colona*** Widespread weed throughout the tropics and sub-tropics to warm-temperate areas. There is much variation in this species which has not yet been properly documented. Growth habit varies from low spreading forms to an erect close-tufted form which is common in wetland rice. Purplish bands occur commonly in a range of the low spreading forms. There is considerable variation in photoperiodic response among the various forms, some being insensitive. The general consensus is that *E. colona* is hexaploid ( $2n=54$ ). Other differing chromosome counts as tabulated, for example by Devesa et al. 1991 may well have been made on other taxa. It is indeed possible that there are undescribed species which have some of the features of *E. colona* and some of *E. crus-galli*. The hexaploid from Sri Lanka described by Yabuno 1985 may be one such. A rice mimic which I have collected in Karnataka, South India may be another. Both have close erect panicle-branches, characteristic of some forms of *E. colona*.

***E. crus-galli*** *E. crus-galli* var. *crus-galli* in its many forms occurs in the cool and warm-temperate parts of the Asian-Pacific region. In sub-tropical and more tropical areas it is replaced by *E. crus-galli* var. *hispidula* with its crowded, less pyramidal inflorescences. It is true that sometimes the distinction between these two varieties (or subspecies) becomes blurred especially, it seems, in Japan, but I believe that in the Asian-Pacific region there is a strong case for distinguishing between them. *E. crus-galli* var. *pratensis* is abundant in upland or drier situations in eastern Asia and occurs here and there in other parts of the region including Australia. *E. crus-galli* var. *austro-japonensis* extends from southern Japan to South-east Asia, New Guinea and the Pacific islands. I believe that the epithets *zelayensis* and *mitis* which are based on names given to North American taxa, neither of which occur in China, should be abandoned for east Asian material or indeed elsewhere in Asia. They and the var. *brevisetula* used by Chen Shouliang 1990 and based on Hitchcock 1950 are probably forms of *E. crus-galli* var. *crus-galli*.

Similarly, plants described as *E. occidentalis* (syn. *E. spiralis*), a form with very crowded greenish spikelets about 3mm long. in Charkevich 1985 should also be referred to *E. crus-galli* var. *crus-galli*. The epithet *hispidula* has unfortunately often been used for the species *E. oryzicola* and/or *E. oryzoides*. It appears that the four varieties of *E. crus-galli* are all hexaploid ( $2n=54$ ).

***E. crus-pavonis*** This South American species with softly-bristled spikelets and very strongly nodding inflorescences, occurs here and there in wet places in Australia and New Zealand but as far as I know has not yet been recorded for Asia. It occurs mainly in the south of the USA, extending into Mexico. This name has sometimes been used in China but must be abandoned because it is inappropriate. Chromosome number  $2n=36$  (Gould et al. 1972).

***E. dietrichiana*** This newly described species (Michael 1999) from coastal north-eastern and northern Australia is non-weedy. Its place in wet areas has often been taken over by exotic aggressive species like *Panicum maximum* and *Brachiaria mutica*.

***E. elliptica*** This species from northern Australia has shown some weedy potential in rice where it grows and seeds prolifically.

***E. esculenta* and *E. frumentacea*** These two hexaploid ( $2n=54$ ) species, commonly grown under cultivation, but sometimes weedy in rice have been treated in great detail by Yabuno 1962, 1987, the former mostly under the synonym *E. utilis*. Both species are

very variable (Obara 1938; De Wet et al. 1983), probably reflecting their presumed origin from the very variable *E. crus-galli* and *E. colona* respectively.

Other cultivated forms of *Echinochloa* apparently related to *E. oryzicola* have been noted from south-west China (Yamaguchi et al. 1996).

✓ *E. glabrescens* This hexaploid,  $2n=54$  (Yabuno 1966), is a common weed of rice in many countries -- the Indian sub-continent, South-east Asia, China and Japan. It is often known as *E. crus-galli* var. *formosensis*, a legitimate alternative.

*E. inundata* This Australian native grass occurs after flood rains especially in inland Queensland and New South Wales, but is only rarely weedy in rice in the Murrumbidgee Irrigation Area. Chromosome count  $2n=72$  (Knox and Michael unpub.).

*E. kimberleyensis* Non-weedy Australian native grass growing here and there in swamps in north-western Australian and in north Queensland.

*E. lacunaria* Native Australian grass occurring spontaneously after flooding of low lying areas in south-western New South Wales.

*E. macrandra* Native Australian grass, once called *E. stagnina*. Chromosome counts were made on this species (W662) by Yabuno 1970 ( $2n=108$ ).

*E. microstachya* This North American plant is occasionally weedy on the edges of rice fields in the Murrumbidgee Irrigation Area in Australia. It has been recorded in New Zealand and occasionally in Europe but not yet in Asia.  $2n=36$  (Gould et al. 1972). It is often known as *E. muricata* var. *microstachya*, a legitimate alternative.

*E. oryzicola* and *E. oryzoides* It is appropriate to consider these two rice mimics together. *E. oryzicola* has been thoroughly examined by Yabuno 1966. It is tetraploid ✓( $2n=36$ ). *E. oryzoides* ( $2n=54$ ) is considered by Yabuno (1984) to be so closely related to *E. crus-galli* that it is better to think of it as *E. crus-galli* var. *oryzoides*. *E. phyllopogon* is a synonym of *E. oryzoides* and not the valid name for *E. oryzicola* as some of us taxonomists once believed!! To make matters worse, some authors have not distinguished *E. oryzicola* from *E. oryzoides*. *E. oryzicola* is common in rice field in the Indian sub-continent, in China and Japan and occurs in Malaysia and Indonesia. It has not yet been recorded for Australia. *E. oryzoides* is a weed of rice in New South Wales, the Philippines, in South-east Asia and the Indian sub-continent, in China and I believe must also occur in Japan where it was one of the taxa of *Echinochloa* collected by Faurie (more than 100 years ago). Perhaps it has been overlooked as just another form of *E. crus-galli* which, indeed, could be argued.

*E. persistentia* This species similar to *E. oryzoides* must be examined further.

*E. picta* This species is common in India, Pakistan, Sri Lanka, Malaysia, Philippines, less so in Indonesia, and has been collected in north Queensland. Chromosome number is  $2n=126$  (Yabuno 1970). I suspect there are two forms which may each warrant specific or varietal status □ one with large spikelets, about 4mm long and the other with spikelets only 3mm long. I have seen the latter form only from Malaysia (collected by K. Itoh), Mandalay (Burma) and Cikarang (Indonesia).

*E. polystachya* var. *Polystachya* Weedy in northern Australian originating from the introduction of Aleman Grass from South America. The Australian material has smaller spikelets than typical South American material and requires further examination.

*E. praestans* First described from New Guinea but later found in Northern Australia. It perhaps also occurs in Kalimantan. A huge grass growing in deep water like the following.

*E. pyramidalis* Introduced as a potential useful perennial grass to Australia and

India many years ago. Recently found wild growing in one of the north coastal rivers of New South Wales. A very variable deep water species. Chromosome numbers various (Yabuno 1968).

*E. stagnina* This species was first described from India and I believe that it is distinctly Asian and can perhaps, through I am not yet certain, be satisfactorily distinguished from African material. It occurs in India, Indonesia and the Philippines. ✓ Chromosome counts of  $2n=36$  have been shown by Yabuno 1968 in material from West Bengal (Strain 60-1) and also from the Philippines reported in Tanesaka 1986. In contrast, examination of typical material from West Africa, known as *E. stagnina* or sometimes *E. scabra* has shown it to be hexaploid ( $2n=54$ ). Morphological features may well enable us to satisfactorily distinguish at least some of the African material from the Asian. The name *E. scabra* would be available for the West African material. The first record of *E. stagnina* for Puerto Rico in McKenzie et al. 1993 may indeed be closely related to West African material.

*E. telmatophila* Non-weedy species from the east coast of Australia, extending from around Sydney to Queensland. Found also in New Zealand.  $2n=54$  (Knox and Michael unpub.)

*E. turneriana* Non-weedy species growing in low-lying areas after floods in inland Australia.  $2n=108$  (Dundas and Britten 1978)

## BIOLOGICAL STUDIES AND TAXONOMIC DIFFICULTIES

Taxonomist rely much on the work of other biologists to help in the clarification of difficult taxa. Feng Jiuhuan and Zhang Tingbi (1993), in their study of the cytology of Chinese *Echinochloa*, give details of collection and specimen numbers so that it should be possible to check their specimens. Similarly Ju Yu-Xing et al. 1986 in their study on the leaf epidermis of Chinese *Echinochloa* have given voucher specimen numbers for the taxa used. Both of these studies use the current treatment of *Echinochloa* in Chen Shouliang 1990. On the other hand there is no record of voucher specimens being kept in the work on morphological and isozyme variation in *Echinochloa* by Ass et al. 1999. Unfortunately the study by Hilu 1994 using Random Amplified Polymorphic DNA markers is marred by confusion between *E. oryzoides* and *E. oryzicola*. It is pleasing indeed that Nakatani et al. 1998 confined their study on photoperiodic sensitivity to the one species, *E. oryzicola*, in contrast to the much earlier work on heading period by Morinaga and Nagamatsu 1942, in which strains of wild paddy barnyard grass, of unspecified taxonomic status, were collected from various districts of Japan, making interpretation of their results much more difficult.

The complexities inherent in the taxonomy of *Echinochloa* make it clear that the material used in biological studies should be kept for posterity in the form of good herbarium specimens and mature seeds so that the findings can be related to particular taxa. If not the reader must have confidence that those doing the studies are thoroughly familiar with the taxa in question.

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## TAXONOMIC AFFILIATIONS AND NOMENCLATURE STATUS OF WEEDY RICE ACCESSIONS IN MALAYSIA - SOME DESCRIPTIVE ANALYSIS

A. B. Mislamah<sup>1</sup>, B. B. Baki<sup>2</sup>, J. Abdul Munir<sup>3</sup>, M. Z. Abdullah<sup>4</sup>, and D. Tan<sup>5</sup>

<sup>1</sup>Dept of Agriculture, Kuala Lumpur, Malaysia,

<sup>2</sup>Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia,

<sup>3</sup>Statistical Unit, MARDI, Serdang, Selangor, Malaysia,

<sup>4</sup>MARDI Rice Research Station, Bertam, Penang, Malaysia, and Dept. of Agriculture, Kangar, Perlis, Malaysia.

**Abstract:** Weedy rices (*Oryza sativa* L.) otherwise known locally as padi angin, are a scourge in the Malaysian rice granaries since the late 1980's. To ascertain their taxonomic status and species affiliation of the prevailing weedy rice accessions *vis-à-vis* the commercial and wild rices, a series of field surveys was conducted in the rice granaries of Peninsular Malaysia in 1997-1999. More than 250 samples of weedy rice accessions were collected. These collections were compared with the germplasm collections in the National Rice Germplasm Bertam, Penang, Malaysia and the published materials in the literature. Twenty-five morphologically different weedy rice accessions were identified. These accessions vary in their phenology and growth habit producing 2-13 tillers genet<sup>-1</sup> *vis-à-vis* 12-13 tillers genet<sup>-1</sup> produced by the commercial variety MR 84. While MR 84 is *ca.* 67 cm in height, weedy rice accessions are taller with plant heights ranging from 95 to 170 cm. Weedy rice accessions display erect or semi-erect flag-leaf (10°–50°), open or closed panicles of variable lengths (11.7cm – 45.0cm) and 5 – 10 rachis panicle<sup>-1</sup>. The average grain size ranges from 0.8 to 1.0cm in length. These grains display a range of colours, *viz*: light- and golden- yellow, straw coloured or pigmented. The grains are either awnless or with awns of variable lengths and with white- or red- coloured pericarp. We believed that these weedy rice accessions are not related to their wild species prevalent in Peninsular Malaysia, despite exhibiting some of the latter's morphological traits. Weedy rice accessions are in fact off-types, evolving over the years with commercial varieties as possible parents.

**Keywords:** *Oryza sativa* L. weedy rices, taxonomy

### INTRODUCTION

Each taxonomist had his own classification system based on the requirements of the International Code of Botanical Nomenclature. In the case of the genus *Oryza*, studies on its taxonomy had been dealt with as early as 1753 by Linneus followed by a host of workers, *viz*: Baillon (1894), Prodechi (1922), Roschevics (1931), Chavelier (1932), Chatterjee (1948), Sampath (1962), Tateoka (1963), Sharma & Shastri (1965 and 1972) and Oka (1988). Baillon (1894) started a more systematic treatment of the genus. Vaughan (Vaughan, 1989), *inter-alia*, reviewed some of Baillon's work. Sato (1998) gave a review on the origin and evolution of wild, weedy and cultivated rice. Lu (1999) gave an up-to-date treatment on the taxonomic historical perspective and current status of the genus *Oryza*.

The importance of the wild species for breeding programs further enlarged the gene pools of the genus *Oryza*. The Malaysian weedy rices, otherwise known locally as padi angin, were believed to be off-shoots of the genus *Oryza* and were classified under the

tribe Oryzeae, subfamily Oryzoides of the family Poaceae. This genus has two cultivated species *Oryza sativa* L. and *O. glaberrima* Steud and more than 20 wild species distributed throughout the tropics. Presently, four wild rice species had been reported to occur in Malaysia (Abdullah *et al.* 1991). *Oryza* spp. displayed wide diversity across the Americas, Africa and Asia, whilst gross differences in genome size had been observed in the gene order of the *Oryza* genomes and these genomes were remarkably small (Vaughan, 1989). In Malaysia, among the cultivated rice, the morphological traits were found to be relatively diverse (Abdullah *et al.* 1994). The finding by Abdullah *et al.* (1996) that Malaysian weedy rices were closely related to the cultivated varieties give strong indication that evolutionary forces are still operating in the present rice agro-ecosystems.

The characteristics that distinguished species within the genus *Oryza* include the attributes of the spikelets, lemma, glumes, culms and ligules. Some taxa in the genus *Oryza* had been described as consisting of complexes of closely related species although some were not related (Tateoka, 1962). The Malaysian weedy rices mimic cultivated commercial varieties and the former had invaded and infested the rice ecosystems. These weedy rices probably could have been evolved from the gene pools of the annual, perennial and wild relatives of *Oryza* spp. as they shared the same AA genome (Vaughan, *et al.*, 1999).

In wetland rice ecosystem, we observed that wild rices, especially *O. rufipogon* has turned into an important weed. Consequently, weedy rices in Malaysian rice ecosystems had broader gene pools from the wild and weedy sources. It follows that the prevailing weedy rices are a reservoir of the tall long-awned shattering, the tall long-awned pigmented shattering, the tall yellow shattering and the medium golden yellow shattering types with many intermediates. As such, weedy rices could not simply be classified under one taxonomic name but have to be identified within the particular ecosystem in which they occurred. Arguably, weedy rices in the Malaysian context are in fact a loose aggregation within the *Oryza sativa* complex. *Oryza* spp showed wide diversity among the heterogeneous populations in the rice ecosystem due to the genetic make up which possibly carried genes of the wild, weedy and off-types as reported by Abdullah (1996).

This paper is an attempt to look at the taxonomy and the nomenclature of weedy rices in the Malaysian rice ecosystems. The selected accessions below were described based on Tateoka's classification.

## MATERIALS AND METHODS

Random field surveys on weedy rices in the Malaysian rice granaries (Fig. 1) were conducted for 6 consecutive seasons in 1997–1999. About 250 samples of seemingly related but morphologically distinct accessions were collected from the granary areas in Peninsular Malaysia. Plant samples of the commercial rice (*Oryza sativa* var. MR84) and wild rice species such as *O. rufipogon*, *O. officinalis* and *O. ridleyi* were obtained from the National Germplasm Centre, MARDI Seberang Prai, Penang. These collections were augmented with collections from field sites in Lundu, Kuching and Samarahan districts, Sarawak while those of *O. meyeriana* were collected from field sites in Tenom, Sabah. The seeds of these samples were sown onto moist soils of the Java series in PVC pots measuring 55cm in diameter x 30cm in depth in insect-proof house in the Crop Protection and Quarantine Unit, Department of Agriculture, Kuala

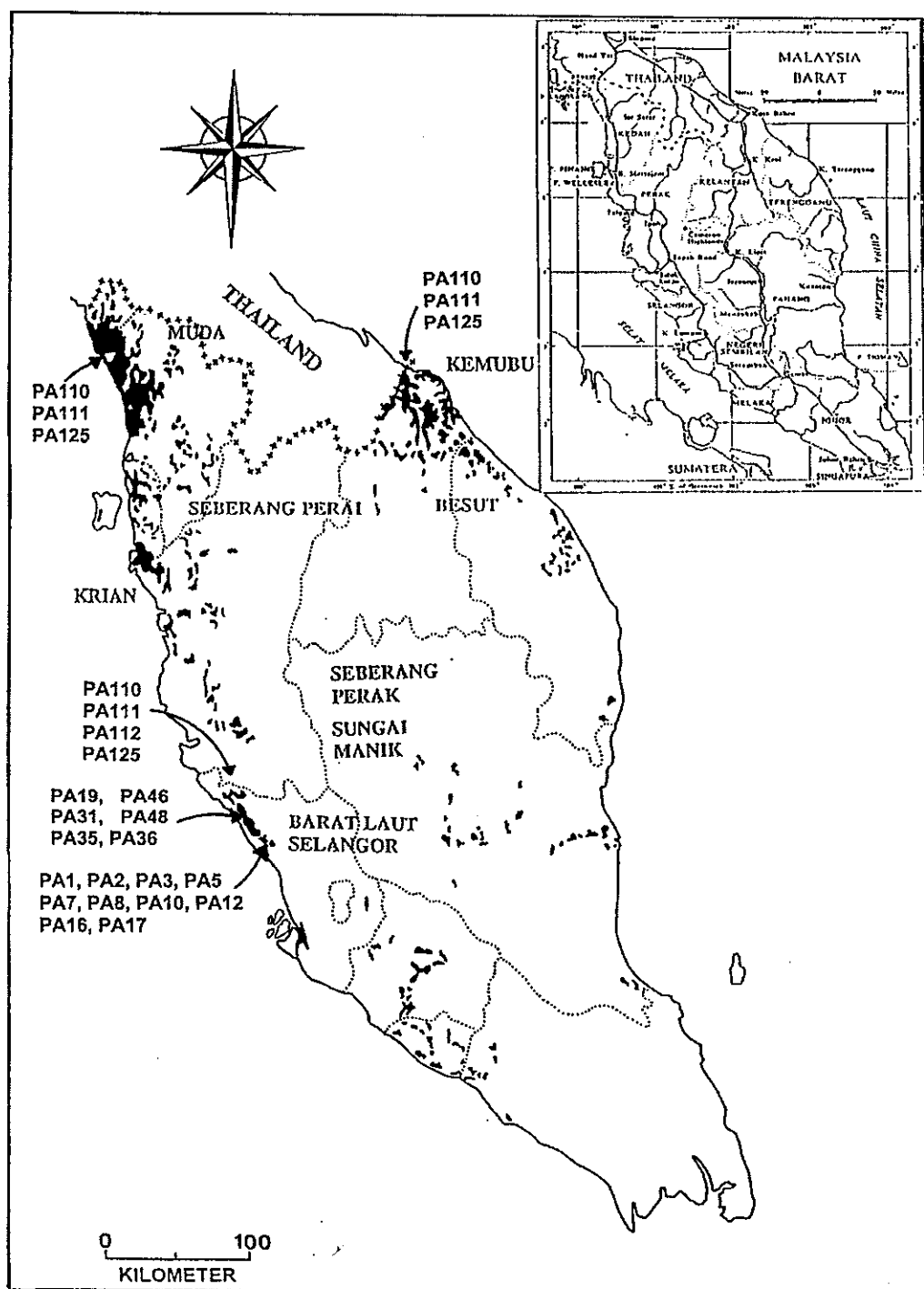


Fig. 1. The prevalence of dominant weedy rice accessions in the Malaysian rice granaries

Lumpur, Malaysia. Emerged seedlings were thinned leaving four uniform cohorts per pot per accession for the study. These seedlings were raised to maturity with standard crop care. Recording on the phenology, growth pattern and morphological differences were made including inflorescence traits and features, plant stature, culms, plant heights, panicle lengths, ligules' lengths, auricles and the percentage shattering of the rice plants. Plant development from the seedling, tillering, booting, panicle initiation

and grain ripening stages of each weedy rice accession were recorded while noting any prevailing peculiar characteristics.

## RESULTS AND DISCUSSION

From the total initial collections of 250 seemingly different samples, we believed that only 32 accessions were distinctly different from each other based on the phenology and morphological traits analysis. Our analysis took into consideration slight differences in any of the morphological characters from the samples collected in the fields. Such differences in either phenological or morphological traits would be treated as new weedy rice accession. Some of the weedy rice accessions had close affinities to the cultivated, the off-types and/or their wild relatives. Their nomenclature status could be considered under the family *Oryzaceae*, the tribe *Oryzoideae* and could be classified taxonomically as the *Oryza sativa* complex following Tateoka's classification (Takeoka, 1962) although workers like Sato (Sato, 1998) and his associates may tend to classify some of these accessions as *O. spontanea*.

Spikelets as a taxonomic key character would be suitable for distinct wild relatives of the two *Oryza* spp. viz. *O. rufipogon* and *O. offinalis* but proved unsuitable to display the association of the closely related species. It is often argued that the great morphological diversity within the genus *Oryza* itself is manifested in the enormous diversity of weedy rices in the Malaysian granary areas. Samples of selected weedy rice accessions displayed a range of diverse forms of all morphological traits of the genus *Oryza*. Marked variations prevailed among accessions in the form of spikelets' dimension, shapes, lengths, widths and grain sizes and shapes. The spikelet of weedy rice accession is a 1-flowered, typical of the genus *Oryza sativa* and is disarticulated above the glume. Weedy rices spikelets are bisexual, similar to those of cultivated varieties of MR84, MR167, MR 219 and MR165. The shapes of spikelets vary from roundish in wild rices, to oblong and oval in weedy rice accessions. The spikelets too are either long, intermediate or short awned. A range of awnless grain types do prevailed. The grains of all weedy rice accessions shatter at  $90 \pm 5$  days. Considerable variations in the leaf lengths, culms and panicle lengths or the pedicel length of the panicle and the flag leaf angles were recorded among the weedy rice accessions. The leaf length ranges from 17.0 cm to 45.0 cm long and are either erect, semi-erect drooping. They presumably have evolved from the cultivated varieties through the ecological and environmental processes. However, the alternate pattern of the leaf positioning on the plant remains the prominent features of the cultivated and weedy rice accessions alike. Considerable variations in the tillering capacity of weedy rices were observed from low and intermediate to dense types. Rooting occurred at the nodes in some weedy rice accession such as PA8. Panicles of weedy rices ranged from those of the open, open-pigmented, closed and closed-pigmented types. Most of weedy rices are of the tall types except in the Sekinchan rice granary areas. Weedy rices accessions showed non-uniform heights from the tall-, medium- to dwarf-types measuring 69 - 170cm. The most common and prevalent weedy rice accession inflicting considerable damage with 70% infestation in rice fields in most granaries in Malaysia was weedy rice accession PA7. This accession was also present with high population counts in Chenderong Balai of the Krian-Sungai Manik Irrigation Scheme, Perak, District IV of MADA, Kedah and Ketara Irrigation Scheme, Trengganu (Fig. 1). Weedy rice accessions with pigmented-grain and horizontal flag leaf features were also common

Table 1. Morphological traits of a commercial rice (*Oryza sativa* L. var. MR84), wild rice species and selected weedy rice accessions

<i>Oryza</i> spp.	Flag leaf Position	Annual/ Perennial	Plant Heights (cm)	Awn/ Awn-less	Panicle Close/ Open	Awn Colour	Stigma Colour	Lemma & Palea Colour	Spike -lets (cm)	No. of Tillers
<i>Oryza rufipogon</i>	Semi – erect	Perennial	Semi erect 150-200 Erect	Awn	Open	Reddish/ purple	Purple	Straw	20.3	4 - 10
<i>Oryza ridleyi</i>	Semi – erect	Perennial	92.5-150	Awn	Open	Reddish	Purple	Straw	9.0	2 - 3
<i>Oryza officinalis</i>	Erect	Perennial	Erect 200	Awn-less	Open	-	Yellow	Straw	29	5 - 7
MR 84	Erect (distinct V-Shaped)	Annual	Semi erect 69-150	Awn-less	Close	-	Yellow	Golden	29	13-14
PA 1	Horizontal	Annual	Horizontal 122	Awn-less	Close	-	Yellow	Pigmen- ted	25	6 - 7
PA 2	Erect	Annual	Erect and Decumbent 99.0	Awn-less	Close	-	Orange	Pigmen- ted	25.9	7 - 8
PA3	Horizontal & Erect	Annual	Decumbent 89.9	Awn	Open	Green	Orange	Pigmen- ted	26.1	4 - 5
PA 4	Horizontal	Annual	Decumbent 127	Awn-less	Close	-	Yellow	Straw	21.0	5 - 6
PA 5	Semi-erect (drooping)	Annual	Erect 137	Awn-less	Close	-	Yellow	Light yellow	31.3	6 - 7
PA 6	Horizontally drooping	Annual	Decumbent	Awn-less	Open	-	Yellow	Light Yellow	35.1	6 - 9
PA 7	Semi-erect	Annual	Semi-erect	Awn-less	Close	-	Yellow	Straw	23.8	10-14
PA 8	Erect	Annual	Semi-erect (drooping) 107	Awn-less	Close	-	Orange	Golden Yellow	22.5	5 - 7
PA 9	Horizontally drooping	Annual	Semi-erect Drooping	Awn-less	Close	-	Yellow	Straw	45.1	7 - 9
PA 10	Semi-erect	Annual	Semi-erect & Drooping at middle & lower culm	Awn-less	Close	-	Yellow	Straw	36.5	4 - 8
PA 12	Semi-erect	Annual	Erect 127	Awn-less	Close	-	Orange	Pigmen- ted	29.5	7 - 9
PA 16	Drooping (horizontal)	Annual	Decumbent 151	Awn-less	Open	-	Yellow & Straw	Golden Yellow	32.7	8 - 9
PA 17	Semi-erect (dominant V-Shaped)	Annual	Erect 131	Awn-less	Close	-	Yellow	Golden	22.5	3 - 10
PA 19	Decumbent	Annual	Decumbent 145	Awn-less	Close	-	Yellow	Yellow	30.7	6 - 9
PA 31	Semi - decumbent	Annual	Semi - Decumbent 145	Awn-less	Close	-	Yellow	Yellow	26.5	3 - 5
PA 35	Semi - erect & horizontal	Annual	Decumbent 133 cm	Awn-less	Close	-	Yellow	Golden	45.0	7 - 11
PA 38	Semi - rust	Annual	Semi – rust 163	Awn-less	Close	-	Yellow	Golden	49.9	11-15
PA 40	Semi-erect	Annual	Semi-erect 75	Awn-less	Close	Yellow	Yellow	Yellow	20.5	4 - 5
PA 49	Semi-erect & Horizontal	Annual	Erect 161	Awn-less	Close	-	Yellow	Yellow	37.6	7 - 9
PA 110	Semi-erect	Annual	Erect 136	Awn-less	Close	Green	Orange	Pigmen- ted	3.1	8 - 9
PA 111	Horizontally drooping	Annual	Decumbent	Awn-less	Close	-	Orange	Straw	20.9	8-10
PA 112	Horizontally drooping	Annual	Erect with horizontally drooping leaves 154	Awn-less	Open	-	Orange	Straw	20.9	2-10
PA 125	Semi-erect & decumbent	Annual	Semi – rust 140	Awn	Open	Green	Yellow & Black	Pigmen- ted	39.1	4 - 10

and wide spread in their infestation of the rice granaries and so were the accessions with pigmented grain and semi-erect drooping leaves. Weedy. Long-awned weedy rices were also prevalent in Sungei Besar of the Tanjung Karang granary areas, MADA, Seberang Perak and Ketara Irrigation Scheme, Trengganu. These weedy rices were present and apparently stable over many seasons.

Detailed morphological traits of weedy rice accessions, wild rice species and commercial rice (*Oryza sativa* var. MR84) is shown in Table1.

It would be an interesting adventure to try on the numerical analyses of weedy rice accessions prevailing in Malaysia. Such attempts may yield useful information on the possible relationships between the wild, weedy and cultivated varieties.

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## DIURNALLY FLUCTUATING TEMPERATURE STIMULATES SEED GERMINATION OF GOOSEGRASS (*Eleusine indica*)

R. K. Nishimoto

Department of Plant and Environmental Protection Sciences, University of Hawaii,  
USA, nroy@hawaii.edu

**Abstract:** The requirement of light and the effect of seed age on the fluctuating temperature and light stimulation of goosegrass (*Eleusine indica* (L.) Gaertn.) seed germination were examined. In addition, the effect of mediating influences, such as a known stimulant (potassium nitrate) and retardant (water stress) on the fluctuating temperature stimulation of goosegrass seed germination were also investigated. Goosegrass seed was about 1.5 years old in all experiments, except where seed age was a variable. At constant 20 °C, goosegrass seed germination was about 25% or less, and with 16 cycles or more of diurnally fluctuating temperature of 20 °C for 16 h and 35 °C for 8 h, germination increased to more than 95%. Light stimulated goosegrass seed germination with diurnally fluctuating temperatures, but not at constant 20 °C. As seed germination approached 100% with many cycles of diurnally fluctuating temperature, there was little difference with the presence or absence of light. As seed age increased, more goosegrass seed germination occurred with the same degree of stimuli (diurnally fluctuating temperature  $\pm$  light). Goosegrass seed germination increased slightly with the addition of potassium nitrate, and decreased with increasing water stress created by increasing levels of polyethylene glycol. Diurnally fluctuating temperature stimulated germination with the presence of potassium nitrate or with various levels of polyethylene glycol. Goosegrass seed germination was strongly stimulated by diurnally fluctuating temperature under all conditions tested, such as the presence or absence of light, various seed ages, potassium nitrate stimulant, and at a wide range of water stress levels.

**Key words:** Light, seed age, potassium nitrate, water stress, polyethylene glycol.

### INTRODUCTION

Goosegrass is a serious weed of cultivated crops and fine turf areas such as golf courses. It is widely distributed in the tropics as well as in temperate areas. In temperate areas, its germination is often predicted by soil temperature increases in the spring; in the southeast United States, goosegrass seed germination occurred when soil temperatures reach 16 to 18 °C (McCarty, 1994). However, in the tropics, soil temperatures are typically always higher than the minimum temperature that initiates germination in temperate areas. An understanding of the key factors that trigger seed germination would enable more effective management of goosegrass, particularly in the tropics.

Diurnally fluctuating temperature was shown to be an important promoter of goosegrass seed germination (Toole and Toole, 1940; Fulwider and Engel 1959; Nishimoto and McCarty 1997). Diurnally alternating temperatures stimulates seed germination of many plant species (Thompson et al. 1977). Fluctuating temperature was proposed as a major signal for seed germination of weeds, such as johnsongrass (*Sorghum halepense* (L.) Pers.) Benech Arnold et al. 1990), and for sprouting of purple



nutsedge (*Cyperus rotundus* L.) tubers (Miles et al. 1996; Sun and Nishimoto, 1999).

While a small population of about 5 to 20% of goosegrass seed always germinated readily at constant temperature, germination of the remainder of the seed population was shown to be strongly related to the number of diurnally fluctuating temperature cycles, and to the duration of the high temperature cycle (Nishimoto and McCarty, 1997). When a sufficient number of diurnally fluctuating temperature cycles were provided, 100% germination was achieved. When only a few fluctuating temperature cycles were provided and followed by constant temperature, only a small fraction of the seed population germinated. Once seeds were exposed to constant temperature, no further germination occurred. If the ungerminated seeds were returned to a temperature regime with a sufficient number of diurnally fluctuating cycles at a later date, 100% seed germination was achieved. (Nishimoto and McCarty, 1997). Thus, seed germination appeared to be strongly regulated by diurnally fluctuating temperature regimes. However, in all of these studies, seeds were also exposed to light to obtain a time-course assessment of germination (Nishimoto and McCarty, 1997). There is a need to clarify whether the diurnally fluctuating temperature stimuli can function without the presence of light. In addition, if fluctuating temperature is critical, the fluctuating temperature stimuli should function in the presence of a stimulant, such as potassium nitrate (Toole and Toole, 1940), or retardant, such as water stress, as well as on goosegrass seed of different ages. The objectives of this research were to determine the effect of the presence or absence of light, seed age, potassium nitrate, and water stress on goosegrass seed germination when mediated by diurnally fluctuating temperature cycles.

## MATERIALS AND METHODS

Goosegrass seed were collected at the Waimanalo Research Station, University of Hawaii. Goosegrass seed was about 1.5 years old in all experiments, except where seed age was a variable. For seed age experiments, seeds were aged in the laboratory at  $23 \pm 2$  °C for 0, 4, 8, and 16 weeks, then stored in a  $-20$  °C freezer until used. Seeds were germinated in 9-cm plastic petri dishes on one piece of Whatman No. 3 filter paper with 7.5 ml deionized water per dish. Three or four replicates of 50 seeds per dish were used. Germination was determined at 28 days by observing protrusion of the radicle.

Incubators with temperature and light control were used to impose constant or fluctuating temperature regimes. In the fluctuating temperature regimes that changed from 20 °C to 35 °C or 35 °C to 20 °C, about 20 min were required for the air temperature within the incubator to reach the set temperature, but petri dishes required 60 min. Light was provided at  $8$  to  $16 \text{ } \mu\text{mol m}^{-2}\text{s}^{-1}$  during 8 h of the 35 °C temperature phase or for 8 h daily in the constant 20 °C regime. Dark treatments were imposed by double wrapping the petri dishes with aluminum foil.

Four experiments were conducted. The first compared goosegrass seed germination in light and darkness at constant 20 °C, as well as four, eight, twelve, sixteen, twenty, and twenty-four cycles of 20 °C for 16 h and 35 °C for 8 h, followed by constant 20 °C. The second experiment provided eight cycles of 20 °C for 16 h and 35 °C for 8 h with or without light to seeds that were aged in the laboratory for 0, 4, 8, and 16 weeks. In the third experiment, seeds were exposed to constant 20 °C in light or seven cycles of 20 °C for 16 h and 35 °C for 8 h, followed by constant 20 °C in light with  $\text{KNO}_3$  at 0, 20, 40, and 80 mM. In the final experiment, seeds were exposed to constant 20 °C in light, as

well as eight or sixteen cycles of 20 °C for 16 h and 35 °C for 8 h, followed by constant 20 °C in light with water potential levels of 0, -0.33, -1, -2.5, -5, -6.25, -7.5, -10 and -15 MPa. Water potential levels were created with different concentrations of polyethylene glycol in water as described by Michel (1983).

Each experiment was conducted twice. Data were pooled from the two trials, and differences among means were determined by the *t* test. Germination was regressed on different variables, such as number of diurnally fluctuating cycles in light or dark, seed age, and potassium nitrate concentration.

## RESULTS AND DISCUSSION

Goosegrass seed germinated more than 95% when exposed to 16 cycles of fluctuating temperature regime of 20 °C for 16 h and 35 °C plus light for 8 h; only 25% germination occurred at constant 20 °C plus light (Figure 1). These results were similar to a previous study (Nishimoto and McCarty, 1997). Light stimulated seed germination when exposed to four to twenty cycles of 20 °C for 16 h and 35 °C for 8 h, but not when exposed to constant 20 °C. At sufficient numbers of diurnally fluctuating temperature (24 cycles) in darkness, germination approached 100% and differences between the light and dark cycles were not detected. Thus, light was not an absolute requirement for germination.

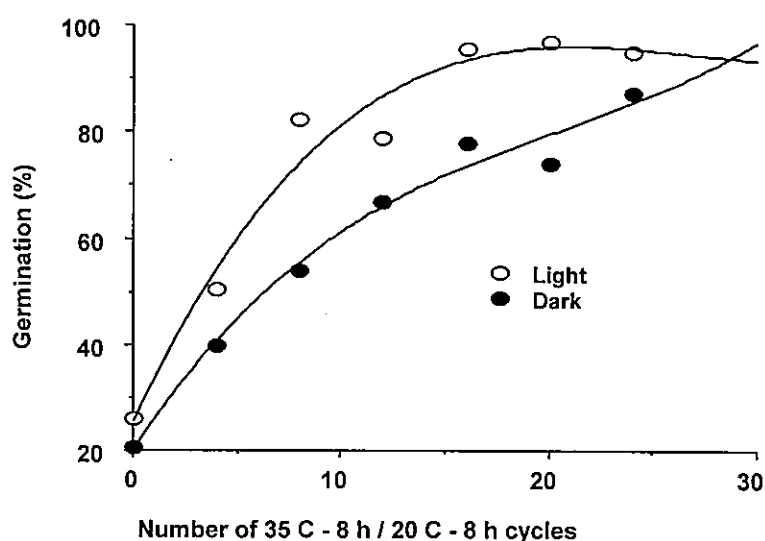


Figure 1. Goosegrass seed germination in response to constant 20 °C and increasing numbers of cycles of 35 °C for 8 h and 20 °C for 16 h followed by constant 20 °C in light and in dark. Percent germination between light and dark for four to twenty cycles of diurnally fluctuating temperatures were significantly different by the *t* test at *P* = 0.05. No significant differences in germination were detected for constant 20 °C and for 24 cycles of diurnally fluctuating temperature. Germination of goosegrass in light was described by  $y = 25.39 + 8.45x - 0.33x^2 + 4.04e-3x^3$  ( $R^2 = 0.97$ ). Germination of goosegrass in dark was described by  $y = 20.17 + 5.98x - 0.22x^2 + 3.65e-3x^3$  ( $R^2 = 0.98$ ).

In a previous paper, we suggested that within a goosegrass seed population, a small

fraction of the seed population germinated readily at constant temperature, another fraction germinated with a few cycles of diurnally fluctuating temperature, and other fractions required many cycles of diurnally fluctuating temperature for germination to occur (Nishimoto and McCarty, 1997). In that context, it appeared that light was not required for germination of the fraction of the seed population requiring none or a few cycles of diurnally fluctuating temperature. However, light stimulated germination for that fraction of the seed population that were inherently more difficult to germinate, which required many cycles of diurnally fluctuating temperature.

Goosegrass seed age affected the degree to which germination occurred (Figure 2). Younger goosegrass seed was inherently more difficult to germinate, but responded positively to increasing cycles of diurnally fluctuating temperature in darkness and in light (Figure 2). When comparing the extent of stimulation by light in Figure 1 for seeds that were 1.5 years old and in Figure 2 where seeds were  $\leq 16$  weeks old, it appeared that light had a stronger stimulatory role with young seeds, or that older seeds were able to germinate better in the dark. For example, at eight cycles of diurnally fluctuating temperature, light enhanced germination from 55% in dark to 84% in light with 1.5 year old seed (Figure 1), and 11% in dark to 75% in light with 8 week old seed (Figure 2). Thus, goosegrass seed germination occurred more readily as seeds aged.

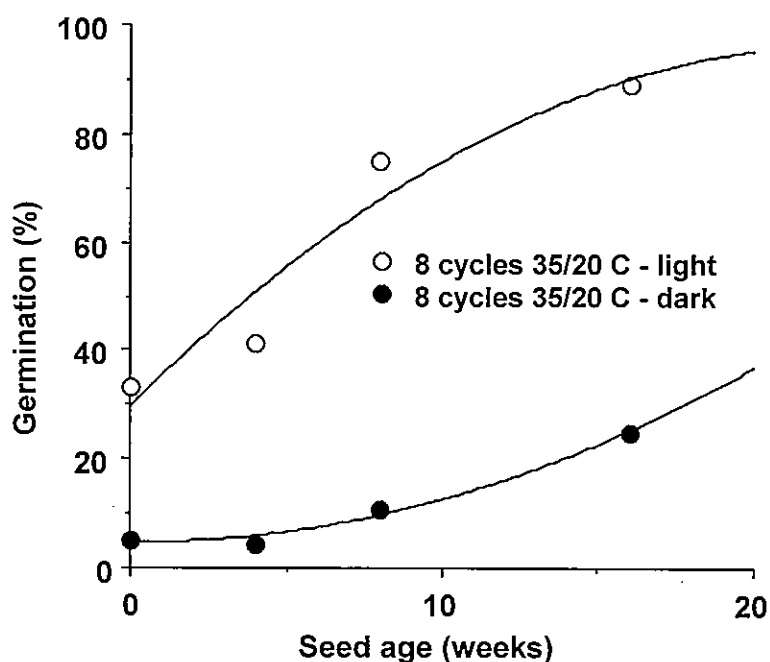


Figure 2. Goosegrass seed germination in response to seed age when exposed to eight cycles of 35 C for 8h and 20 C for 16 h followed by constant 20 C in light and in dark. Germination of goosegrass in light was described by  $y = 29.67 + 5.79x - 0.13x^2$  ( $R^2 = 0.93$ ). Germination of goosegrass in dark was described by  $y = 4.78 - 3.23e-2x + 8.18e-2x^2$  ( $R^2 = 0.98$ ).

Potassium nitrate provided a slight, but significant increase in germination at

constant 20 C, as well as with seven cycles of diurnally fluctuating temperature (Figure 3). However, potassium nitrate at 80 mM did not stimulate germination at constant 20 C. Germination greatly increased with diurnally fluctuating temperature with or without potassium nitrate.

As expected, increasing water stress depressed goosegrass seed germination at constant 20 C or with diurnally fluctuating temperature regimes (Figure 4). However, increasing numbers of diurnally fluctuating temperature with light increased seed germination at all levels of water stress, except at a water potential of -10 MPa or higher, where little to no seed germination occurred. Water stress had a greater effect on seed germination at constant 20 C than at the diurnally fluctuating temperature cycles.

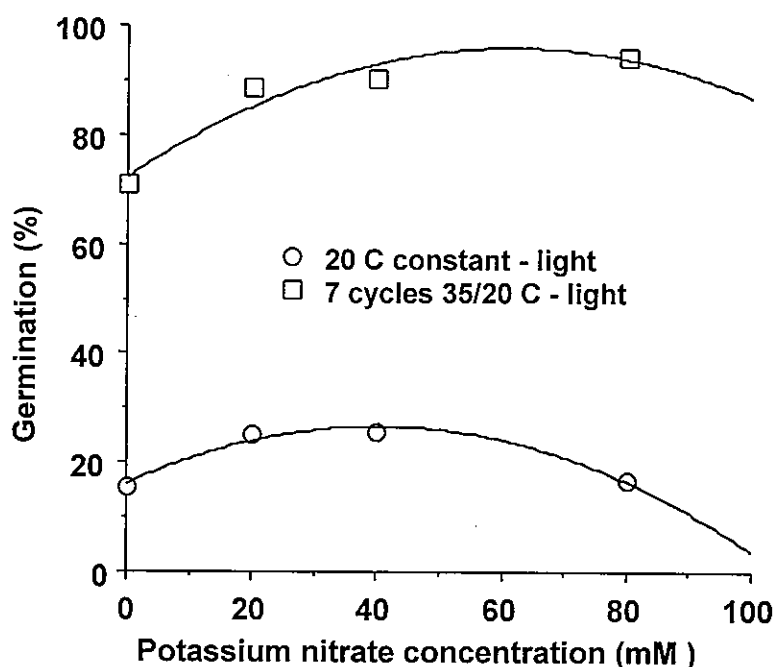
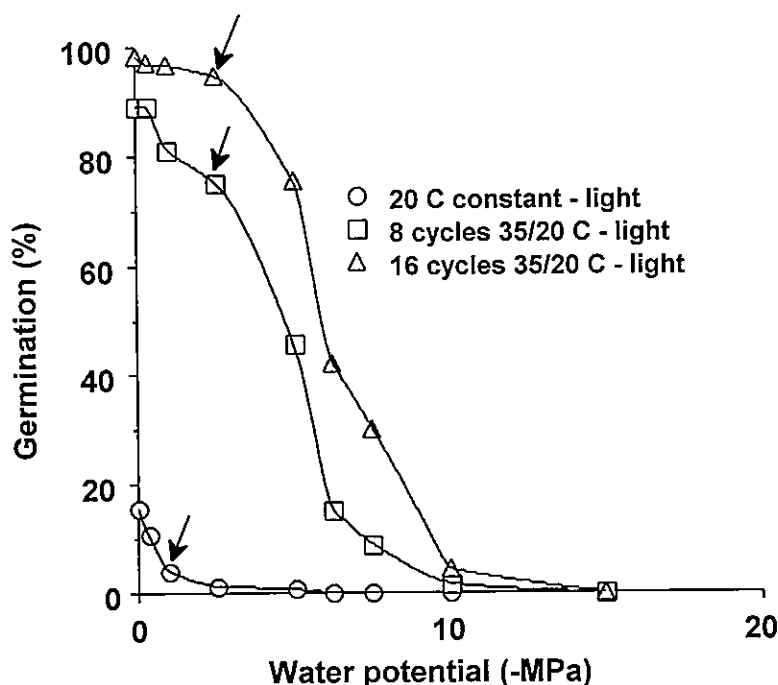


Figure 3. Goosegrass seed germination in response to potassium nitrate concentration when exposed to constant 20 C or seven cycles of 35 C for 8h and 20 C for 16 h followed by constant 20 C with light. Germination of goosegrass at constant 20 C with light was described as  $y = 16.19 + 0.52x - 6.44e-3x^2$  ( $R^2 = 0.97$ ). Germination of goosegrass at seven cycles of fluctuating temperature with light was  $y = 72.34 + 0.76x - 6.18e-3x^2$  ( $R^2 = 0.93$ ).

Diurnally fluctuating temperature stimulated germination under various conditions, such as the presence of potassium nitrate stimulant, a wide range of water stress levels, and at various seed ages. Light appeared to have a promotive role, and it was not an absolute requirement. Light stimulated germination under some conditions; its role appeared to be greatest with young seeds. The strong relationship with diurnally fluctuating temperature and seed germination suggests that goosegrass germination can be predicted by the magnitude of the diurnal temperature shift (Nishimoto and McCarty, 1997) and numbers of diurnally fluctuating temperature cycles. These results corresponds with our observations on goosegrass emergence on golf courses;

emergence typically occurs on bare ground and scalped areas, where maximum diurnal fluctuating temperatures and light exposure to goosegrass seed would be expected. In addition to the removal of turf cover, there are other factors that contribute to maximizing the diurnal amplitude of fluctuating temperature, such as intensity of radiant energy and soil depth of the seed. This enhanced understanding of goosegrass seed germination should enable the development of more effective management strategies for goosegrass.

Figure 4. Goosegrass seed germination in response to water potential when exposed to



constant 20 C, eight or sixteen cycles of 35 C for 8 h and 20 C for 16 h followed by constant 20 C with light. The arrows at each curve signify when germination was significantly lower than the control by the t test ( $P = 0.05$ ).

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## INFLUENCE OF SUBMERGING ON EMERGENCE AND GROWTH OF *LEPTOCHLOA CHINENSIS*

P. Poolkumlung<sup>1</sup>, P. Zaprung<sup>1</sup>, K. Yanagisawa<sup>2</sup>, M. Yokoyama and K. Kondo<sup>3</sup>

<sup>1</sup> T.J.C. Chemical Co., Ltd., R & D Department, Bangkok, Thailand

<sup>2</sup> Kumiai Chemical Industry Co., Ltd., Life Science Research Institute, Tohoku Research Center,  
Miyagi, Japan

<sup>3</sup> Kumiai Chemical Industry Co., Ltd., Overseas Department, Tokyo, Japan.  
kum01151@nifty.ne.jp

**Abstract:** In direct seeded rice cultivation in Asian counties, *Leptochloa chinensis* is becoming more and more serious problem weed in recent years, and now it became one of the dominant weeds in the central Thailand. In view of the fact that *Leptochloa chinensis* generally grows vividly under shallow-flooded condition and seldom grows in deep-flooded transplanted rice fields, some greenhouse trials were carried out in Thailand and Japan to clarify the effect of flooding on the growth of the weed. The results of the trials showed that *Leptochloa chinensis* grows normally until 2 leaf stages when the seed was immersed in the following day of sowing, but gradually growth was suppressed and died. In the trials, the plants were submerged under different water conditions at 2, 3, 4, 5 and 6 leaf stages, respectively, when leaves and stems were completely covered with water, all the plants died regardless of their leaf stages. When the plants were not completely covered with water, their growth was inferior to that of non-flooded *Leptochloa chinensis* but did not die. Bubbles on the surface of the leaves was observed soon after the weed being submerged then the leaves turned yellow to brown from the lower part and died in 7 to 10 days. Submersion period needed for killing the weed was over 7 days in Shizuoka, Japan (mean temperature during the trial was 23.1°C). Our results suggest that *Leptochloa chinensis* can be controlled by water management, namely deep flooded condition.

**Keywords:** *Leptochloa chinensis*, emergence, growth, submersion

## INTRODUCTION

Direct-seeded rice (*Oryza sativa* L.) cultivation is one of the most common and economic ways of producing rice in the world. In wet-seeded rice cultivation in Thailand, *Leptochloa chinensis* is becoming more and more serious problem weed in recent years, and now it became one of dominant weeds especially in the central Thailand. It is considered that the change of dominant weeds is largely attributed to low efficacy against the weed of some rice herbicides which have been widely used for many years in this country, and to rice growers who prefer not to keep their rice fields flooded condition in early leaf stage of rice in order to prevent the damage of rice seedlings from golden apple snail (*Pomacea canaliculata*).

It is generally said that *Leptochloa chinensis* grows vividly under shallow-flooded condition and seldom grows in deep-flooded transplanted rice field. Therefore, some greenhouse trials were carried out in Thailand and Japan in order to clarify the effect of flooding timings, submersion period and water depth on the growth of *Leptochloa chinensis*.

## MATERIALS AND METHODS

**Trial-1** Effect of flooding timings and water depth on growth of rice, *Echinochloa crus*

**Preparation of pots** After dry paddy soil (clay loam soil) was put into each plastic pot, the pots were flooded and puddled (wet-seeded rice). Pre-germinated rice seeds (rice variety: Suphanburi No. 1/ Indica type variety) and seeds of *Echinochloa crus-galli* (ECHCG) and *Leptochloa chinensis* (LEFCH) were sown in separate rows on the soil surface of each pot after draining water. The pots were then placed in a greenhouse and the plants were grown until their target leaf stages.

**Flooding timings and water management** The pots were flooded at 0 (following day of sowing), 2 and 4 leaf stages of *Leptochloa chinensis* at three different water depth (0, 2.5 and 5 cm), and the water level was kept during the whole trial period. The trial was conducted with 3 replications.

**Evaluation method:** Leaf stage and plant height of each plant were measured at 10, 20 and 30 days after flooding.

### **Trial-2 Effect of flooding timings and water depth on growth of *Leptochloa chinensis* (greenhouse trial in Shizuoka, Japan)**

**Preparation of pots** *Leptochloa chinensis* was grown until its target leaf stages for testing in separate plastic pots containing clay loam soil in a greenhouse.

**Flooding timings and water management** The pots were flooded at 2, 3, 4, 5 and 6 leaf stages of the weed at three different water depth (1, 3 and 5 cm), and the water level was kept during the whole trial period.

**Evaluation method** Visual observation was conducted at 10, 20 and 30 days after flooding (DAF) in accordance with the 0 to 100 rating system from 0 = no effect to 100 = complete kill.

### **Trial-3 Effect of flooding period on growth of *Leptochloa chinensis* (greenhouse trial in Shizuoka, Japan)**

**Flooding timings and water management** At 3.3 to 4.1 leaf stages of *Leptochloa chinensis*, the experimental pots were flooded to cover the whole plants or a half of the plant height with water. The water level was kept for 0, 3, 5, 7 and 10 days after flooding, and then water in the pots was released completely.

**Evaluation method** Visual observation was conducted in every week after flooding in accordance with the same evaluation scale.

The experimental methods were the same as the previous trial other than flooding period, water depth, leaf stage of *Leptochloa chinensis* at flooding and evaluation timings.

## **RESULTS AND DISCUSSION**

Table 1 shows the result of the trial conducted in Thailand to clarify the effect of flooding timings and water depth on the growth of rice, *Echinochloa chinensis* and *Leptochloa chinensis*. Leaf stage and plant height of each plant at 30 days after flooding are shown in the table. The trial result indicated that leaf stages of rice were not affected by flooding when flooded at pre-emergence of the plant. However, when flooded at 2 and 4 leaf stages, the progress of leaf stage delayed slightly compared with non-flooded rice. On the other hand, plant height of rice was not suppressed by flooding at 2.5 and 5 cm in water depth in all flooding timings. In the case of *Echinochloa crus-galli*, the growth was not inhibited by flooding regardless of flooding timings and water depth tested in this trial. On the other hand, the growth of *Leptochloa chinensis* was suppressed remarkably by flooding at 2.5 and 5 cm deep when test pots were flooded at pre-



emergence, 2 and 4 leaf stages of the plant. The growth suppression was stronger in 5 cm deep than 2.5 cm deep in all flooding timings.

Table 1. Effect of flooding timings and water depth on the growth of rice, *Echinochloa crus-galli* and *Leptochloa chinensis* (greenhouse trial in Suphanburi, Thailand)

Timing of Flooding	Plant height at flooding (cm) <sup>1)</sup>	Water depth (cm)	Leaf stage <sup>2)</sup> (30 DAF <sup>3)</sup> )			Plant height <sup>2)</sup> (30 DAF <sup>3)</sup> )		
			Rice	ECHCG	LEFCH	Rice	ECHCG	LEFCH
0 leaf stage (pre-emergence)	0 (wet soil)	0	100 (8.6) <sup>4)</sup>	100 (6.1)	100 (5.1)	100 (35.1)	100 (41.4)	100 (15.1)
Emergence of LEFCH		2.5	101	100	71	102	94	15
		5.0	116	98	33	108	101	6
2.0 leaf Stages of LEFCH	0.5	0	100 (9.0)	100 (6.2)	100 (5.5)	100 (35.2)	100 (41.5)	100 (14.8)
		2.5	76	102	51	108	111	20
		5.0	72	98	51	107	120	9
4.0 leaf Stages of LEFCH	3.0	0	100 (10.6)	100 (7.1)	100 (7.0)	100 (39.2)	100 (44.3)	100 (17.5)
		2.5	85	99	40	110	119	34
		5.0	87	100	19	108	117	9

1) Plant height of *Leptochloa chinensis* at flooding; 2) Proportion to non-flooded pot, average of 3 replications (10 plants/ pot); 3) DAF = days after flooding; 4) Average leaf stage and plant height (cm) of 30 plants in non-flooded pots; ECHCG = *Echinochloa crus-galli*, LEFCH = *Leptochloa chinensis*

Table 2. Effect of flooding timings and water depth on the growth of *Leptochloa chinensis* (greenhouse trial in Shizuoka, Japan)

Timing of Flooding	Water depth (cm)	Mean % weed control <sup>1)</sup>			Appearance of LEFCH at flooding
		10 DAF <sup>2)</sup>	20 DAF	30 DAF	
2.0 LS <sup>3)</sup>	1.0	86	90	88	Tip of the plant was above water surface
	3.0	96	100	100	The plant was covered with water completely
	5.0	98	100	100	The plant was covered with water completely
3.0 LS	1.0	66	50	40	Some leaves were above water surface
	3.0	98	98	96	The plant was covered with water completely
	5.0	98	100	100	The plant was covered with water completely
4.0 LS	1.0	50	44	50	Some leaves were above water surface
	3.0	76	82	86	Tip of the plant was above water surface
	5.0	80	100	100	The plant was covered with water completely
5.0 LS	1.0	6	8	4	Most part of the leaves and stem was above water surface
	3.0	10	20	10	Most part of the leaves and stem was above water surface
	5.0	20	30	20	Some leaves were above water surface
6.0 LS	1.0	14	10	6	Most part of the leaves and stem was above water surface
	3.0	16	10	10	Most part of the leaves and stem was above water surface
	5.0	30	24	14	Most part of the leaves and stem was above water surface

Evaluation scale: 0 = no effect – 100 = complete kill; 1) Average of 3 replications; 2) DAF = days after flooding; 3) Leaf stages (LS) of *Leptochloa chinensis* at flooding.

When *Leptochloa chinensis* was submerged in the following day of sowing, the weed grew

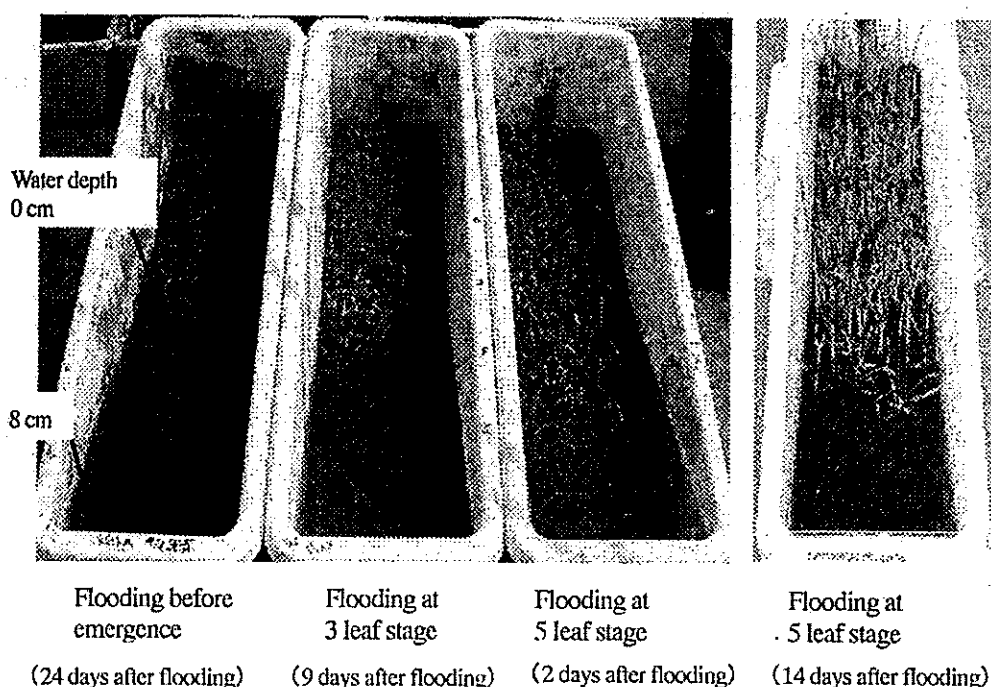


Fig. Effect of water depth on emergence and growth of *Leptochloa chinensis* when flooded at 0, 3 and 5 leaf stages of the weed

- 1) Plastic pots were placed slanted at a 30° angle just before flooding to set up different water depth (0 to 8 cm) and the water depth was kept during whole test period
- 2) Maximum plant height of *Leptochloa chinensis* at flooding: 2 cm (3 leaf stages) and 6 cm (5 leaf stages)

*Leptochloa chinensis* was submerged at 2 and 4 leaf stages, bubbles on the surface of the leaves was observed soon after the weed was completely covered with water, and then the leaves turned yellow to brown from the lower part and almost all the plants died in 7 to 10 days after flooding.

A greenhouse trial was conducted in Japan to clarify the relationship between the growth of *Leptochloa chinensis* and flooding more precisely. Table 2 shows the effect of flooding timings and water depth on the growth of the weed. When the leaves and stem were covered with water completely, all the plants died regardless of their leaf stages at flooding. On the other hand, when the tips of the leaves were above water surface, their growth was inferior to that of non-flooded *Leptochloa chinensis* but did not die. When some leaves were above water surface, 20 to 40% of their growth was suppressed at 30 DAF. In the case that most part of the leaves and stems were above water surface, the growth was affected slightly (4 to 14% suppression at 30 DAF).

From the above result, it was clarified that *Leptochloa chinensis* can not grow under prolonged submerged condition.

Table 3 shows the result of the trial conducted in Japan to clarify the effect of flooding period on the growth of *Leptochloa chinensis*. When the weed was covered with water completely, the growth was not affected by flooding for 3 days, moderately suppressed but did not die for 5 days. However, the growth stopped and then died for over 7 days. When *Leptochloa chinensis* was covered with water up to a half of plant height, the growth was not affected.

From the above result, submersion period needed for killing the weed was considered to be over 7 days under this trial conditions (mean temperature during the trial was 23.1°C).

In conclusion, our results suggest that *Leptochloa chinensis* can be practically controlled by water management, namely by keeping submerged condition in their early leaf stages for 7 to 10 days. Further studies will be needed to clarify the relationship between water temperature and submersion period required to control the weed.

Table 3. Effect of flooding period on the growth of *Leptochloa chinensis* (greenhouse trial in Shizuoka, Japan)

Water depth during flooding period	Flooding period (days)	Mean % weed control <sup>1)</sup>	
		14 DAF <sup>2)</sup>	28 DAF
Submersion	0 (non-flooding)	0	0
	3	10	0
	5	72	66
	7	100	100
	10	100	100
A half of plant height	0 (non-flooding)	0	0
	3	0	0
	5	0	0
	7	0	0
	10	20	0

Evaluation scale: 0 = no effect – 100 = complete kill; 1) Average of 3 replications; 2) DAF = days after flooding. Leaf stages of *Leptochloa chinensis* at flooding: 3.1 to 4.1 leaf stages (plant height: 2 to 3 cm); Test period: March 3 to April 28 (mean temperature during the trial: 23.1°C)

## ECOLOGY OF INVASIVE WEEDS: IMPACT AND MANAGEMENT OF THE EXOTIC WEEDS, GORSE (*ULEX EUROPAEUS*) AND SCOTCH BROOM (*CYTISUS SCOPARIUS*) IN BRITISH COLUMBIA, CANADA

R. Prasad<sup>1</sup>, and S. Kushwaha<sup>2</sup>

<sup>1</sup>Pacific Forestry Centre, 506 West Burnside Road, Victoria, B.C., Canada, V8Z 1M5

rprasad@pfc.forestry.ca

<sup>2</sup>50 Meadow St., Amherst, Massachusetts, USA. 01002

**Abstract:** Many exotic pests have made rapid incursions beyond their natural range of potential dispersal and have caused a negative economic, ecological or social impact. Gorse (*Ulex europaeus*) and Scotch broom (*Cytisus scoparius*) are two such leguminous weeds that arrived from overseas and have established themselves in the western landscapes of USA and Canada. In Canada, they are localized in agricultural, industrial, urban, right-of-way, rangeland and forestry landscapes of British Columbia and have impacted negatively on the native ecosystems. Experiments carried out in some forest districts on Vancouver Island suggest, that both species are nuisance and suppress the growth of young Douglas-fir seedlings. Control tactics involves mechanical removal, use of chemical herbicides or potential deployment of new biological control measures. An integrated approach using a bioherbicide, a herbicide and mulching has been tested and some treatments were found to show promise against gorse.

**Keywords:** bioherbicide, Douglas-fir, exotic weeds, gorse, Scotch broom

### INTRODUCTION

Many organisms such as crop plants, selected ornamentals, game animals and livestock were introduced into North America for various beneficial purposes but some of these exotics have invaded and expanded their range into the new environment beyond usefulness. Thus, they have facilitated ecosystem changes and displaced native organisms through habitat alteration, predation, parasitism or simply by competition for space, light, food or nutrition. Exotics that appear to cause the greatest share of forest damage are the invasive insects and pathogens. However, some introduced flora (woody species, ornamentals and weeds) are also proving to be destructive and highly competitive with native forest vegetation for space light, nutrients and water (Prasad 2000). Two such invasive species are Scotch broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*) that pose serious threats to sustainability of the Canadian forest ecosystem, the Garry oak - *Arbutus* ecosystem (*Quercus garryana*-*Arbutus menziesii*) on southeastern Vancouver Island and the southern Gulf Islands of British Columbia (Peterson and Prasad 1998; Clements, Peterson and Prasad 2001). A successful bio-invasion includes three elements; introduction, establishment and spread. To this can be added the fourth element of impact of invasion. Clearly, not all introductions become pests but when they do, their impacts are devastating.

Scotch broom was introduced to Vancouver Island in 1850 by Walter Grant as seeds planted near Sooke, B.C. (Prasad 2000) and gorse is recorded to be thriving in Victoria, B.C. since 1915 (Henry 1915; Clements, Peterson and Prasad 2001). After nearly one and a half centuries, Scotch broom and gorse have expanded their range, occupying roadsides, riparian right-of-ways and other disturbed areas along coastal B.C.

Broom is also found in some isolated patches along Kootenay Lake and Castlegar areas in the interior of the province. Both species have several characteristics which promote their invasiveness and displacement of native plant species: (a) reduced leaves and active stem photosynthesis during unfavorable periods; (b) nitrogen fixation capability; (c) profuse seed production and longevity of seed banks; (d) rapid vertical growth and intense spatial competition; (e) adaptability to varying ecological niches and (f) lack of natural enemies (parasite-predator complex).

Gorse has expanded its range along the coasts of British Columbia and evolved pockets of infestations from the southern tip of Vancouver Island to the Queen Charlotte Island, including some of the mainland. One of these coastal sites is at the Rocky Point near Victoria and is owned by the Department of Defense, Govt. of Canada. Colonisation by gorse of naval and military grounds is interfering with military exercises and training as well as creating a fire hazard. Various control measures (Clements, Peterson and Prasad 2001) have been described but none was found to be completely satisfactory. Therefore, a field experiment was conducted to determine the effects of a bioherbicide agent (*Chondrostereum purpureum*), a triclopyr herbicide (Release), a plastic mulch and manual cutting on resprouting behavior of gorse.

## MATERIALS AND METHODS

Three sites were selected for experimental use at the Rocky point and only healthy gorse plants (3 m high, 5 cm diameter) were chosen. Gorse has been growing there for 10-20 years and has formed dense colonies. Two of the sites were old fields that are being invaded by Scotch broom and gorse. The other site was an old right-of way which is invaded by gorse alone. The soils on all sites were brunisol with a sandy clay to silty clay in texture. The gorse was dense enough to shade out all other vegetation underneath and was 2-3 metres tall with an average stem diameter (at ten centimetre above ground) of 5 cms. At each site, a randomised block layout of 6 plots containing all treatments (control, control treated with a blank formulation, the bioherbicide formulation, the herbicide formulation, the mulches and uncut gorse stems) was laid out. Each plot size was 3x3 m and contained 15 stems of cut or uncut gorse plants and was randomly selected to receive all treatments. Only erect, healthy, vigorously growing stems were chosen, tagged and measured for the initial height and diameter growth. Thus, there were 45 variates of each treatment spread over 3 blocks. The six types of treatment were: cut and treated with sterile water; cut and treated with a blank formulation (without Cp); cut and treated with Cp formulation; cut and treated with triclopyr herbicide; cut and covered with the plastic mulch and uncut stems. To minimise drying out of cut surfaces of the stems, the treatment solutions were applied within one minute to the cut ends of the stem with a squeeze bottle.

*Chondrostereum purpureum* (Cp) was prepared and applied according to a procedure described by Prasad and Kushwaha (see this Proceedings), triclopyr herbicide was obtained from a local store (Garden Store, Victoria) and applied at 480 gm/l. All treatment solutions were delivered through the squeeze bottle at the rate of ca. 3 ml/ cut stem. For mulching, a commercial plastic sheet (black, 2 cm thick and 3x3 m in size) obtained from a local Garden Store, Victoria, was cut and fitted to each plot in such a way as to completely cover all the cut stems. The plastic sheet was fastened to the ground with 10 cm staples at each corner and in the middle of the plot. Few heavy stone pieces were also placed over the mulch to prevent being blown away. All stems

were cut at 25 cm above the soil level with a brush saw and all treatments were applied immediately, on a rain free day, during August. The response (number and height of sprouts) was measured after one year. Data were analysed statistically by the least significant difference(LSD)method .

## RESULTS AND DISCUSSION

Table 1 presents the effects of treatments on the sprouting behavior of gorse cut-stems after one year. As can be seen, there was profuse resprouting in the untreated control or control treated with the blank formulation but no regrowth occurred in plots treated with the triclopyr herbicide. Thus, there was a 100% inhibition of resprouting in these plots and the herbicide formulation seemed to be highly efficacious. Plots treated with Cp showed variable response - in some treated stems, slow, diseased or mutilated regrowth occurred whereas in others, many other cut-stems resprouted normally and fully. This is not surprising since the bioherbicide mycelia require time to penetrate, establish, and infect; it is possible the effects will be more visible or pronounced in subsequent years. Bioherbicides (unlike chemical herbicides) are living organisms and influenced by the environmental conditions (drought, temperature, rainfall etc.) for their effectiveness. This is why several adjuvants were added to their formulation to enhance efficacy (Prasad and Kushwaha 1999).

Table 1. Influence of various treatments on resprouting of gorse stems after one year.

Treatment	Sprouts (#)	Sprout Height (cm)
Uncut stumps	10.8	7.9
Cut stumps	15.1	15.8
Cut stumps + blank	16.7	16.1
Cut stumps + mulching	10.9	6.8
Cut stumps + herbicide (Triclopyr)	0.0	0.0
Cut stumps + bioherbicide (Cp)	16.8	14.5
L.S.D. (0.05)	1.9	1.2

Mulching treatments were only partially effective because the cut-stems underneath the covered plastic sheet, produced several etiolated and at times few longer sprouts. Many of these were twisted, curled and brittle. Even though, the black plastic (mulch) prevented sunlight filtering through it, rainfall and accumulated snow on top of the mulch, allowed seepage of moisture and thus causing profuse production of etiolated regrowth. How long these etiolated sprouts would survive, remains to be ascertained but it is safe to assume that all would eventually die/decay in the subsequent years. It is of interest to note that some resprouting occurred in the uncut plots, possibly due to damage caused to the apical growth of gorse by snow, wind and wildlife or due to increased light after cutting the neighboring plants. Besides production of large number of seeds, gorse can regenerate by resprouting after cutting or damage and this may explain why damaged, prostrate or buried stems regenerated.

In conclusion, these one-year data demonstrate that an integrated approach to control of gorse by application of mulches and herbicide might hold promise. Mulching a large area with uneven terrain is not economical but mulching may have some limited application on small areas where other methods can not be employed. The tactics is to suppress all new growth so that flowering and formation of new seeds/resprouts can be

arrested. It is also evident that cutting without any treatment does not seem to work under these conditions even though some workers (A. Robinson. personal communication) have reported a decline in regrowth after mechanical cutting and chipping of gorse plants. There is growing opposition by the public to curtail further use of chemical pesticides in sensitive (aquatic) areas and alternatives such as (mulching) or use of bio agents to contain further incursion of these exotic weeds, is required.

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## WHY DOES *CYPERUS FLACCIDUS* R. BR. INFEST THE RICE FIELD IN JAPAN?

R. Sago, A. Atawong, R. Matsuura, and T. Matsuda

College of Agriculture, Ibaraki University, 4668-1 Ami, Inashiki, Ibaraki, Japan 300-0331. sago@ipc.ibaraki.ac.jp

**Abstract:** Recently, it was reported that *C. flaccidus* R. Br. infested the paddy rice field and caused the loss of rice yield. These prompted us to clarify the germination characteristic, ecology and mechanism of the distribution of *C. flaccidus*. We reported that the germination rate was highest at 20°C and light was necessary to the seed germination. The diurnal fluctuations in temperature caused the higher germination rate. The germination rate of the seed stored in the water at 5°C for 2 to 4 weeks was accelerated in comparison with the outdoor dried storage. Though the germination rate improved from November to March by 5°C under water storage, the germination rate did not improve after April by this process.

Seasonal variation in emergence was investigated. *C. flaccidus* emerged mainly in May outdoors under the flooding. Under drainage, the emerged rate improved from April to September. These seemed to relate with the amount of rainfall. Eleven herbicides were applied to *C. flaccidus*. Its sensitivity to sulphonylurea and pyrazol herbicides was low. From the observation in the field, *C. flaccidus* was found to grow in the gap of canopy. According to the results obtained here, the germination characteristic of *C. flaccidus* may be the adaptive ability into the gap of canopy. Moreover, sulphonylurea herbicide, which is widely used in Japan, has no effect on *C. flaccidus*. These may be the reason why does *C. flaccidus* infest the rice field in Japan.

**Key words:** *Cyperus flaccidus*, dormancy, germination, sulphonylureas, chloroacetamides

## INTRODUCTION

*Cyperus difformis* L. has been noticed as a main paddy field weed of Japan for a long time (Kasahara 1972). And also *Cyperus flaccidus* R. Br. has been observed to grow in the stream bank, swamp in the flatland and paddy field (Kitamura 1967). Recently it was reported that *C. flaccidus* has infested the paddy rice field and caused the loss of rice yield (Takahashi 1996).

We reported that seed emergence of *C. flaccidus* varied according to the amplitudes of fluctuation and in the presence or absence of light. The emergence rate was the highest at 20°C within the range of 15°C to 30°C. In addition, light was necessary to the seed germination at 20, 25 and 30°C under the constant temperature. Gibberellin (GA3) at 100 ppm promoted the seed emergence, when the seeds were dried and stored outdoor. Furthermore, as a result of dealing with 100ppm GA at the same condition, the tendency that the emergence rate was the highest at 20°C did not change. The diurnal fluctuations in temperature also accelerated the seed emergence. The higher mean temperature and the bigger in the difference temperature caused the higher emerged rate (Sago 2001).

In order to describe the reason why does *C. flaccidus* infest the rice field in Japan,



we tried to determine the seed dormancy in outdoor condition and the sensitivity to herbicide, which are used in Japan.

## MATERIALS AND METHODS

### Source of seed

Mature seeds of *C. flaccidus* R. Br. and *C. difformis* were collected from the rice paddy field at Ibaraki Prefecture in the fall of 1996 and 1997. Seeds were cleaned to remove the chaff and then stored outdoor under dry condition until used.

### Seasonal variation in emergence

Seeds were sowed in to 1/2000 a wagner pot which filled with the volcanic soil. The soil surface was then covered by the sterilized soil at 5-10 mm depth. The flooding and drainage condition were performed in order to represent the condition of rice field. The seeds were sowed in January of each year under flooding or drainage condition. Then water under flooding condition was removed from the pot in order to perform the drainage condition after July. Sampling was done every week by counting the germinated seeds in each pot. After sampling, the germinated seeds were then removed from the pot. This experiment has been investigated for 3 years since 1997 to 1999 at Experimental Farm of Ibaraki University. This experiment was done in duplicate.

### Sensitivity to herbicide

Pot experiments were conducted in 1998 at Experimental farm of Ibaraki University. The soil, which was used in this experiment, was light brown volcanic from Ibaraki prefecture. Seeds were sowed in a plastic pot (9 cm diameter, 13.5cm depth). Herbicides, which were used in this experiment, were molinate, pretilachlor, mefenacet, pyrazolate, bensulfuron-methyl, pyrazosulfuron, imazosulfuron, cinosulfuron, cyhalofop-butyl, 2-4-D (Na) and bentazon. Herbicides were applied at pre-emergence and early post-emergence when *C. flaccidus* was grown at 1.5 leaf stage. The application rates were 50 and 100% of the recommendation rate. After 4 weeks of herbicides application, the remaining plants were harvested from each pot for measure the fresh weight and the number of plants. Each treatment was replicated for three times.

## RESULTS AND DISCUSSION

### Seasonal variation in emergence

Seasonal variation in emergence was investigated from 1997 to 1999. The results showed that *C. flaccidus* emerged mainly in May outdoors under flooding. After water was removed for establish the drainage condition, the emerged rate improved in August (Fig. 1). The result obtained from *C. flaccidus* was different from the emergence characteristic of *C. difformis*. Under flooding, the emerged rate of *C. difformis* was improved from April to June. After condition was changed to drainage, the emerged rate was lower than 4% (Fig. 2). Under continual drainage, the seed of *C. flaccidus* and *C. difformis* were emerged from May to August and mainly emerged in June (Fig. 2 and Fig. 3). These seemed to relate with the opportunity and amount of rainfall (Fig. 4). Then the emerged rate significantly decreased in poor rainfall term.

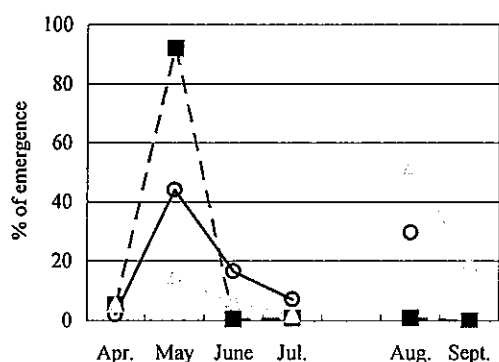


Fig. 1 Seasonal variation in emergence of *C. flaccidus* under flooding condition until July and drained off from Aug. to Sept.

—○— 1997    -■- 1998    ···· 1999

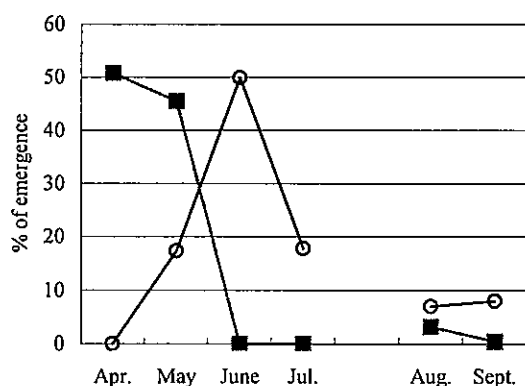


Fig. 2 Seasonal variation in emergence of *C. difformis* under flooding and drainage condition until July, 1999 and drained off from Aug. to Sept.

—○— drainage    —■— flooding

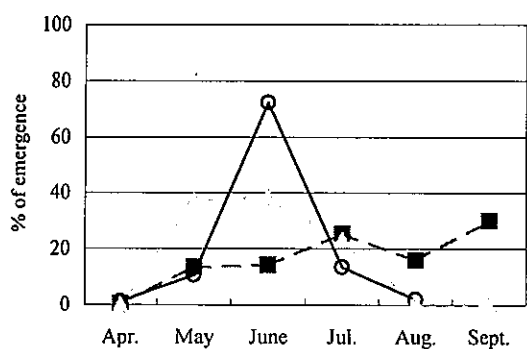


Fig. 3 Seasonal variation in emergence of *C. flaccidus* under drainage condition

—○— 1997    -■- 1998    ···· 1999

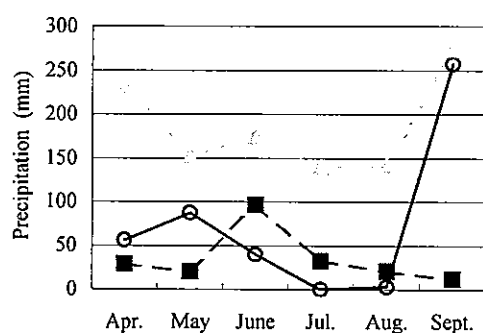


Fig. 4 Precipitation during 1997 to 1999 of growing seasons

—○— 1997    -■- 1998    ···· 1999

### Sensitivity to herbicide

Eleven herbicides were applied to *C. flaccidus*. A pre-emergence application of 2,4-D (Na), bentazon, thiocarbamate herbicide; molinate, chloroacetamides herbicide pretilachlor and mefenacet, controlled *C. flaccidus* effectively. Moreover, an early post-emergence application of 2, 4-D (Na) and molinate controlled *C. flaccidus* more than 90%. However, the sensitivity of *C. flaccidus* to sulphonylureas herbicide; bensulfuron-methyl, pyrazosulfuron-ethyl, imazosulfuron-ethyl and cinosulfuron, including pyrazole herbicide; pyrazolate and cyhalofop-butyl was low.

### Discussion

We reported that the germination rate of the seeds of *C. flaccidus* stored in water at 5°C for 2 to 4 weeks was accelerated its emergence in comparison with the outdoor dried storage. Though, its germination rate improved from November to March at 5°C, under water storage, the germination rate did not improve after April by this process.

Although, low temperature and underwater storage are effective to break an innate dormancy of *C. flaccidus* and *C. difformis* (Sago 2001). The seed of *C. difformis* enter to an induced dormancy by high temperature in summer (Chisaka 1977a, 1977b). The emerged rate of *C. flaccidus* which was observed in summer, indicated that its seed does not enter to an induced dormancy but an enforced dormancy. Not only the suitable temperature which was about 20°C, but also the fluctuation temperature, light and suitable water condition, are necessary for the emergence of *C. flaccidus*. Furthermore, *C. flaccidus* was found to grow in the gap of rice canopy in the field and the characteristics of germination were suitable for the exploitation of gap in the canopy (Thompson 1977). Moreover, the seeds of *C. flaccidus* can emerge after entering to the suitable condition in summer when the applied herbicides were degraded. Therefore, it is one reason of the difficulty to control *C. flaccidus* due to its physiological mechanism. Moreover, sulphonylurea herbicide, which is widely used in Japan, has no effect on *C. flaccidus*. These may be the reason why does *C. flaccidus* infest the rice field in Japan.

Table 1. Effect of herbicides on *C. flaccidus*

Herbicide	Dose ga.i./ha	Preemergence(% of cont.)		Early-postemergence(% of cont.)	
		No. of Plants	Fresh Weight	No. of Plants	Fresh Weight
Molinate	1500	25.1	7.8	100	31.3
	3000	6.7	t	100	7.8
Pretilachlor	300	9.1	t	100	7.8
	600	6.7	t	66.7	t
Mefenacet	600	8.9	t	35.3	t
	1200	4.9	t	35.3	t
Pylazolate	1500	100	36.7	97.3	68.8
	3000	100	85.9	39.3	37.5
Bensulfuron-methyl	27	100	33.6	78.7	78.1
	54	22.0	7.8	72.0	33.6
Pyrazosulfuron-ethyl	10.5	100	31.3	100	78.1
	21	100	39.1	92.7	39.1
Imazosulfuron	45	98.0	18.0	81.3	39.1
	90	71.3	15.6	80.7	46.9
Cinosulfuron	9	88.7	5.5	100	36.7
	18	88.7	5.5	80.0	18.0
Cyhalofop-butyl	90	100	72.7	-	-
	180	100	75.8	-	-
2,4-D	22.5	4.5	t	40.7	21.1
	45	2.9	t	26.0	5.5
Bentazon	1500	0	0	0	0
	3000	0	0	0	0

Note: - means no data, t means less than 5%

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## STUDY ON WEED SHIFT IN RUBBER-BASED INTERCROPPING SYSTEMS

H. Suryaningtyas<sup>1</sup>, G. Wibawa<sup>1</sup>, Y. K. Leksana<sup>2</sup>, and E. S. Saragih<sup>2</sup>

<sup>1</sup>Indonesian Rubber Research Institute, Sembawa Research Station, PO. Box 1127  
Palembang 3001, Indonesia. irri-sbw@mdp.co.id

<sup>2</sup>PT. Monagro Kimia, Jl. MH. Thamrin 57, Jakarta 10350, Indonesia

**Abstract:** Two experiments were conducted at Sembawa Research Station in South Sumatra, Indonesia, to study the effect of various herbicide treatments for general weed control in maize-rubber intercropping systems, and in the planting strip of rubber monoculture systems on weed shift. Results of the two experiments revealed that weed vegetation in rubber plantings might be changed or maintained depending on the weed control methods. In young immature rubber planting with the dominant weed species of *Ottlochloa nodosa*, sequential spraying of glyphosate or its mixtures with metsulfuron methyl tended to decrease the proportion of the grass weed, with the emergence of broadleaf of *Borreria alata*. Whereas paraquat herbicide, and manual weeding method, seemed to maintain the original weed flora composition. Regarding the effectiveness of herbicide on weed control, glyphosate at the lowest dose of 1.08 kg a.e. ha<sup>-1</sup> provided longer lasting weed control as compared to that of paraquat at 0.56 kg a.e. ha<sup>-1</sup> and/or manual weeding, in which satisfactory weed control could be obtained for up to 30 days.

**Key words:** Weed shift, glyphosate, metsulfuron methyl, paraquat, rubber, maize, intercropping systems.

## INTRODUCTION

Weeds management is an important agronomic input in rubber cultivation as a considerable amount of production cost is allocated for weeding. Weeding contributed to 50-70% of the total cost of rubber production during the immature phase, and between 20 and 30% in the mature period (Tjitrosoedirdjo *et al.*, 1984). The high cost of weeding is due to a large number of weeding rounds (24-30 rounds) using various means of weed control (manual or chemical method using herbicides) for circle and/or strip weeding during the immature phase of the crop (Chee, 1989).

Good weed management is very important to minimize or prevent direct and/or indirect economic losses as have been reported by many workers (Suryaningtyas and Terry, 1993; Suryaningtyas *et al.*, 1997; Wibawa *et al.*, 1997; Teoh *et al.*, 1989; Amypalupy, 1996; Suryaningtyas and Kuswanhadi, 1996; Bagnall-Oakeley *et al.*, 1997). In good weed management practice, knowledge of weed succession resulting from continuous usage of particular herbicides is of importance. This allows the selection of appropriate herbicides for sequential spraying to prevent noxious weeds from being dominant. In addition, the choice of effective herbicide is also important, otherwise it could lead to dominance of weed species tolerant to the particular herbicide (Faiz, 1989). This paper discusses some early results of long-term experiments conducted to study the effect of various herbicide treatments on weed shift in maize-rubber intercropping and rubber monoculture systems.

## MATERIALS AND METHODS

Two experiments were established at the experimental garden of Sembawa Research

Station, in South Sumatra, Indonesia, from October 2000 for four years. The experiments were carried out under five-month-old immature rubber planting of clone RRIC 100. Vegetation dominating the experimental sites were *Ottlochloa nodosa*, and creeping legume cover crops (LCC) which was present at very low population.

The first experiment was laid out in maize-rubber intercropping systems. Rubber plant was planted at a double-row spacing of (6.0m x 2.0m) x 14.0m on May 2000, whereas maize was sown in the rubber inter-rows at a spacing of 75cm x 20cm on October 2000. Maize seeds (one seed per hole) were sown using a mechanical equipment of *No-Till Planter* to obtain 4 and 12 rows of maize at the rubber inter-rows of 6.0m and 14.0m wide, respectively. For optimum growth and yield, maize crop was maintained using a standard recommendation, except for weeding, which was done according to treatments. The second experiment was laid out at the planting strip of rubber monoculture system. The plant spacing was 7.0m x 3.0m, and the width of planting strip was 4.0m, i.e. 2.0m wide at each side of rubber planting rows.

Treatments of the first and second experiments (Tables 1 and 2, respectively) were arranged in randomized complete block design, with three replicates.

Table 1. Herbicide treatments in maize-rubber intercropping systems (Experiment 1).

No.	Herbicide treatments	Rates (a.e. or a.i./ha)
1.	Glyphosate (Roundup 480 AS)	1.08 kg
2.	Glyphosate (Roundup 480 AS)	1.62 kg
3.	Glyphosate (Polaris 200/8 AS)	1.08 kg
4.	Glyphosate (Roundup 480 AS) + metsulfuron methyl (Ally 20WDG)	0.54 kg + 75 g
5.	Paraquat (Gramoxone)	0.56 kg
6.	Manual weeding (farmer practice)	-

Table 2. Herbicide treatments at the planting strip of rubber monoculture system (Experiment 2).

No.	Herbicide treatments	Rates (a.e. or a.i./ha)
1.	Glyphosate (Roundup 480 AS)	1.08 kg
2.	Glyphosate (Roundup 480 AS)	1.62 kg
3.	Glyphosate (Roundup 480 AS) + metsulfuron methyl (Ally 20WDG)	0.54 kg + 75 g
4.	Paraquat (Gramoxone)	0.56 kg
5.	Manual weeding (farmer practice)	-

In all experiments, initial herbicide spraying was done using a SOLO knapsack sprayer fitted with an ICI red polijet nozzle giving a spray swath of 2.0m wide, and delivering a spray of 500 l ha<sup>-1</sup>. Subsequent spraying, particularly after maize emergence in the first experiment, was done using a similar nozzle type, but with narrower spray swath (25cm wide). In addition, a conical shield was also attached to the edge of spray lance. This was done to prevent or minimize crop injury due to herbicide drift while spraying the weeds growing amongst maize crop.

Observations were made of maize growth and yield, and weed development.

Percentage of weed control was visually assessed at periodic intervals. Re-spray of herbicide was carried out when individual treatment recorded percentage of weed control of approximately less than 40%. Percentage of weed composition by species before treatment and at periodic intervals were recorded. This current paper presents the effects of treatments on weed aspects only, as observations on maize growth is still on going.

## RESULTS AND DISCUSSION

### Effect various herbicide treatments on weed growth and weed flora composition in maize-rubber intercropping systems

Before experiment, the dominant weeds in the experimental site, which was previously an old rubber planting area, were *Ottlochloa nodosa*, and creeping legume cover crops (LCC). As presented in Table 3, sequential spraying of the herbicide treatments has caused changes in weed composition by 12 weeks after initial application (WAA). Glyphosate obviously decreased the grass weed *Ottlochloa nodosa*, with emergence of high percentage of broadleaf weed of *Borreria alata*. This was not the case with paraquat treatment showing high proportion of *Ottochola nodosa* and low percentage of broadleaf weeds.

Regarding the effectiveness of herbicide treatments on weed control, glyphosate as a single application and/or in tank-mixture with metsulfuron methyl, provided a longer lasting weed control as compared to that of paraquat and/or manual weeding. Percentage of weed cover could be maintained at low level (less than 30% for grasses, and 16% for broadleaves and legumes) for up to 60 days, even if at the lowest herbicide dose of 1.08 kg a.e. ha<sup>-1</sup>. In contrast, weed coverage at paraquat and manual weeding treatments have reached 80% and 32%, respectively, by 60 days after herbicide application (Table 4).

Table 3. Effects of various herbicide treatments on weed succession in maize-rubber intercropping systems

Treatments	No. spray rounds	% Weed Composition by Species *							
		Pre-treatment		Post-treatment at 12 WAA					
		Grasses	Legume	Grasses				Broadleaves	Legume
		ON	LCC	ON	AC	PC	DA	BA	LCC
Roundup 1.08 kg a.e./ha	2	94.3	5.0	4.3	2.7	2.7	0	20.0	1.3
Roundup 1.62 kg a.e./ha	2	94.3	5.7	3.0	3.7	1.7	0	10.0	1.0
Polaris 1.08 kg a.e./ha	2	92.7	6.7	28.3	1.7	1.7	0	2.0	2.0
Roundup + Ally (0.54 kg+75 g)/ha	2	91.0	5.0	16.7	2.0	2.0	1.3	3.0	5.7
Gramoxone 0.56 kg a.i./ha	3	83.3	7.7	36.7	6.7	28.3	0	4.0	8.3
Manual weeding	3	86.7	7.0	0	5.6	29.9	19.9	10.0	10.0

\*ON=*Ottlochloa nodosa*; AC=*Axonopus compressus*; PC=*Paspalum conjugatum*; DA=*Digitalaria adscendens*; BA=*Borreria alata*; LCC=Legume cover crops.

### Effect various herbicide treatments on weed growth and weed flora composition in the planting strip of rubber monoculture systems.

Along the the planting strip of young immature rubber dominated by *Ottlochloa nodosa*, sequential spraying of glyphosate alone and/or its tank-mixture with metsulfuron methyl, showed a clear decrease in *Ottlochloa nodosa*, meanwhile broadleaf weed of *Borreria alata* emerged at higher proportion as compared to before herbicide treatment. This was different for paraquat herbicide, in which there was not an obvious shift of weed flora by 12 weeks after initial herbicide application (Table 5)

Table 4. Effect of various herbicide treatments on weed growth in maize-rubber intercropping systems.

Herbicide Treatments *	Percentage of weed cover					
	Grasses *			Broadleaves + Legumes **		
	10 DAA	30 DAA	60 DAA	10 DAA	30 DAA	60 DAA
Roundup 1.08 kg a.e./ha	85	15	8	5	0	16
Roundup 1.62 kg a.e./ha	80	10	7	5	0	11
Polaris 1.08 kg a.e./ha	85	20	29	5	0	4
Roundup + Ally (0.54 kg+75 g)/ha	85	25	15	5	0	7
Gramoxone 0.56 kg a.i./ha	0	80	-	0	2	-
Manual weeding	0	32	-	0	2	-

\*Grass weeds consisted of *Ottlochloa nodosa*, *Axonopus compressus*, *Paspalum conjugatum*, and *Digitaria adscendens*; whereas broadleaves and legume consisted of *Borreria alata*, and legume cover crops, respectively.

Table 5. Effect of various herbicide treatments on weed composition at the planting strip of rubber monoculture systems.

Treatments *	No. spray rounds	% Weed Composition by Species							
		Pre-treatment		Post-treatment at 12 WAA					
		Grass ON	Legume LCC	Grasses				Broadleaf BA	Legume LCC
Roundup 1.08 kg a.e./ha	2	90.0	4.0	1.0	6.7	21.5	6.3	23.4	4.2
Roundup 1.62 kg a.e./ha	2	90.0	4.0	0.3	1.0	34.9	6.1	41.7	1.7
Polaris 1.08 kg a.e./ha	2	88.3	4.0	2.0	10.0	13.3	10.0	11.7	0.7
Roundup + Ally (0.54 kg+75 g)/ha	2	86.7	3.0	41.7	21.7	25.0	0	0	0
Gramoxone 0.56 kg a.i./ha	3	83.3	2.0	35.0	1.7	11.7	0.7	15.0	5.3
Manual weeding									

\*ON=*Ottlochloa nodosa*; AC=*Axonopus compressus*; PC=*Paspalum conjugatum*; DA=*Digitaria adscendens*; BA=*Borreria alata*; LCC=Legume cover crops.

With respect to the duration of weed control, paraquat herbicide provided quick kill of weeds, but gave shorter period weed suppression, in which similar to that of manual weeding treatment. However, this was not true for a single application of glyphosate and/or its mixture with metsulfuron methyl, in which by 60 DAA the percentage of weed coverage could be maintained at not more than 53% and 44%, for grasses, and broadleaf weed and legumes, respectively (Table 6).

Table 6. Effect of various herbicide treatments on weed growth at the planting strip of rubber monoculture systems.

Herbicide Treatments *	Percentage of weed cover							
	Grasses **				Broadleaves + Legumes **			
	10 DAA	30 DAA	60 DAA	90 DAA	10 DAA	30 DAA	60 DAA	90 DAA
Roundup 1.08 kg a.e./ha	85	10	23	38	4	1	16	27
Roundup 1.62 kg a.e./ha	80	5	12	43	4	0	26	44
Polaris 1.08 kg a.e./ha	85	15	17	35	4	0	5	13
Roundup + Ally (0.54 kg+75 g)/ha	0	40	89	-	0	0	0	-
Gramoxone 0.56 kg a.i./ha	0	35	-	-	0	5	-	-
Manual weeding								

Grass weeds consisted of *Ottlochloa nodosa*, *Axonopus compressus*, *Paspalum conjugatum*, and



*Digitaria adscendens*; whereas broadleaves and legume consisted of *Borreria alata*, and legume cover crops, respectively.

Results of the two experiments clearly indicated that weed vegetation in rubber plantings might be changed or maintained depending on the weed control methods. In the very young immature rubber planting with the dominant weed species of *Ottlochloa nodosa*, a common vegetation in newly replanted rubber area, sequential spraying of glyphosate or its mixtures with metsulfuron methyl seemed to decrease the proportion of the grasses weed. This was particularly obvious at the experiment conducted in the planting strip of young-immature-rubber monoculture systems. Whereas paraquat herbicide, and manual weeding method, tended to maintain the weed flora composition. Similar findings have also been reported by Chee (1989) and Hee et.al. (1994) that glyphosate provided lower percentage cover of *Ottlochloa nodosa* in weed control in immature rubber than that of paraquat herbicide.

The shift of weed flora, might closely linked with different mode of action of the herbicides. Glyphosate is a systemic herbicide which is absorbed by plant leaves and translocated to other parts of plant, including roots, stolons and underground rhizomes, thereby damaging the whole plant system. Therefore it might be able to eradicate soft grass weed like *O. nodosa*. and inevitably resulting in exposure of the soil surface to full sun light. This condition gives the opportunity for weed seeds of annual broadleaves to germinate and eventually dominate the area. Whereas paraquat is a contact herbicide, which kills only the plant parts which are in direct contact with the herbicide, without causing damage on other plant parts such as roots and/or under ground systems. Therefore the vegetation can rapidly regenerate without giving the chance to other weeds seeds in the vicinity to germinate, establish and compete with the regenerating weed species

With regard to the emergence of noxious weeds, the current study revealed that sequential spraying of glyphosate and/or its tank-mixtures with metsulfuron methyl for weed control have not resulted in the emergence and dominance of relatively more noxious weed species. After sequential application of glyphosate, the vegetation appearing to increase in its population was *Borreria alata*, which is not regarded as a noxious weed in rubber plantation (Nasution, 1989). Slightly different with glyphosate, paraquat still tended to maintain population of the original weed flora.

In good weed management, the succession of weed species and the cost-effectiveness are two aspects which are very important to be considered, as this would relate to the proper choice of herbicides. The use of paraquat often maintained the original weed flora, but gave only a relatively short period of weed suppression (for up to 30 days). Whereas sequential spraying of glyphosate or its mixture with metsulfuron methyl tended to cause reduction in soft grasses with emergence of many broadleaf weeds. However, the duration of weed control provided by this herbicide was relatively longer (for up to 60 days) than that of paraquat. In the current experiments, further observations would be carried out to study the changes in weed flora in relation to their effect on crop yield, and possible difficulty in the choice of herbicides.

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## THE WEED, ENCOURAGED WEED AND CROP CONTINUUM IN *ARTHRAUXON HISPIDUS* (THUNB.) MAKINO (GRAMINEAE) OF VOLCANIC HACHIO ISLAND IN THE PACIFIC OCEAN

S. Umemoto

Subtropical Plant Institute, Graduate School of Agriculture, Kyoto University,  
Wakayama 649-3632, Japan. umeane@pearl.ocn.ne.jp

**Abstract:** *Arthraxon hispidus* (Thunb.) Makino is a heteromorphic and globally widespread annual species of Gramineae family. This species complex occurs commonly as a troublesome weed in the paddy levee, ditch side and other humid places of the Japan Archipelago. In the volcanic small island of Hachijo of the Idzu Islands of Japan, dwellers have regarded *A. hispidus* not only as an agrestal and ruderal weed but also utilized as the one and only dying plant necessary for the traditional golden yellow pigmentation of special silk since the Edo period. A few decades ago, three professional local dyers initiated to transplant the seedlings of *A. hispidus* for stable production into upland field because the paddy sides of rice field for the habitat of the species were rapidly abandoned and remarkably decreased by political and socio-economic problems. Field surveys and morphological analysis showed that the present populations of *A. hispidus* cultivated in the upland fields of Hachijo Island have developed such a domestication syndrome as characterized by the synchronous stem branching, late heading of spike, shorter period to heading, uniform plant maturation and less seed shattering, with the larger spike, spikelet, leaf and stem. The origin, history and evolution of the weed, encouraged weed and crop continuum of *A. hispidus* populations of Hachijo Island is discussed with special reference to the sustainable management and utilization of vegetation including weeds in an isolated and limited resource system.

**Key words:** *Arthraxon hispidus*, encouraged weed, dyeing, cultivation, vegetation management.

### INTRODUCTION

*Arthraxon hispidus* (Thunb.) Makino, jin-cai in Chinese, is a very variable annual species of Gramineae family. This species is commonly distributed in rather wet places as a weed of Japan. In Hachijo Island, a remote volcanic island of the Idzu Islands in the Pacific Ocean, 300km south of Tokyo, It is not only a weed but also traditionally utilized as dyeing plant resources for silk products. Recently, rapid changes in socio-economic situations have invited a kind of domestication of the species in the island.

The purpose of this paper is to show the habitat and morphological variation and to show the present and historical contents of cultivation and utilization of *A. hispidus* in Hachijo Island, in order to discuss the origin, history and evolution of the weed, encouraged weed and crop continuum of the species with reference to sustainable vegetation management in an isolated and limited ecosystem.

### MATERIALS AND METHODS

Field surveys, *in situ* morphological analysis and interviews were conducted in Hachijo Island of Idzu Islands in the Pacific Ocean, 300km south of Honshu Island, for

elucidating the morphological and habitat variation of *A. hispidus* and the history and practices of the cultivation and utilization of the species. In making field surveys, 27 populations within the island were randomly selected and investigated to estimate their habitat conditions, population size and morphological traits. When having interviews to the dwellers and cultivators of the island, six transplanted populations of *A. hispidus* were recorded to understand the relationship between the variation pattern and the modes of cultivation and utilization. Historical literatures of the Edo period deposited by one professional dyer and at the Hachijo town museum were also examined to estimate the status of *A. hispidus* in the past. These investigations were carried out in 1997 to 1999.

## RESULTS AND DISCUSSION

### Habitat and morphological variation

20 populations of *A. hispidus* investigated in the island were recognized as weeds in such habitats: roadside, road slope, *Zoysia* turf, garden hedge, farm road, vacant place near the road, paddy levee, aquaduct side, road in the park, ornamental field and levee in the upland field. On the other hand, seven populations were recognized as crops in upland field. Weed populations were consisted of five to 50 individuals and most of them were successful in setting seeds. Cultivated populations included more than one thousand individuals and almost of them were harvested before heading.

The morphology of plants of weed populations is rather procumbent, more stem-branching and small (approximately 30cm at most) while that of crop populations is very tall (up to 150cm), rather erecting and less stem-branching. The leaf of crop populations is apparently larger than that of weed populations. The spikelet of the plants of weed populations is awned (5mm in length) and the matured seeds of spikelets showed easy-to-shattering when touching. The spikelets of plants of crop populations also have awns (5mm in length) but showed less seed-shattering.

### Cultivation and utilization of *A. hispidus*

According to three professional dyers of the island, seedlings raised from seeds collected in the last year's cultivation field or directly collecting seedlings from the cultivation field were manually transplanted into completely weeded upland fields on April to May. Spacing among seedlings was about 40 to 80cm. During the growing seasons, no fertilizers and agro-chemicals were treated because such treatments must decrease dyeing quality. All the plants including stems and leaves were harvested by sickle strictly before heading, on September to October, because heading decreased the ability to dye silk textiles. Seeds scattered by matured plants in non-harvested parts of fields played an important role of seed bank for the next year's seedling. Harvested plant materials were transported to the dyeing factories and naturally dried. Plant materials from *A. hispidus* were boiled to obtain the stock for dyeing. Repeated dyeing of silk by the stock, using *A. hispidus* with natural water gushed out from accumulated ash layers of an extinct volcano of the island, squeezed out an amazing and valuable golden pigmentation. The ashes made from the warm temperate evergreen broad-leaved forest tree species covering the extinct volcano were also used to strengthen the pigmentation. Used plant materials were re-transported back to the upland fields and utilized as the organic fertilizer.

Descriptions of the cultivation system in detail are noted in Umemoto (1999).

### Historical records of cultivation and utilization of *A. hispidus*

Based on the note by Kondo (1749?) in Hachijo Island, *A. hispidus* was cultivated in upland fields in the middle Edo period. Seeds collected were sowed in the last cultivated field of *Angelica* as daily vegetables and the stems and leaves were harvested before heading. Plant materials from *A. hispidus* and ashes from forest trees such as *Camellia japonica* L. and *Cleyera ochracea* DC. were used for the silk yellow golden dyeing. His description thought to be just the same as that of the present time.

According to Mrs. Yamashita, some places of Hachijo Island had cultivation of *A. hispidus* for the silk dyeing in 1920's and 1930's which correspond to the late Taisho and the early Showa eras.

In 1950's and 1960's, *A. hispidus* was not only a weed of agricultural fields but also a resource plant for the silk dyeing. Plants collected from paddy levees and upland fields were mainly utilized. However, it was gradually becoming difficult to ensure biomass necessary for dyeing, because the cultivation areas of rice field came to decrease and cultivation of ornamental palm species was widespread, as the High Economic Growth Policy progressing. As a result, a few professional dyers managed and initiated to cultivate *A. hispidus* in the field for keeping the traditional skill of dyeing and their lives in Hachijo Island.

### Weed, encouraged weed and crop continuum in *A. hispidus*

In Hachijo Island, at least 250 years history of the cultivation and utilization of *A. hispidus* can be estimated by historical records. During the history, dwellers and professional dyers have treated *A. hispidus* as one of multi-purpose plant resources of the island. Sometime they regarded the species as a weed or as a crop and sometimes regarded as both. The status of *A. hispidus* has gone to and fro along the weed and crop continuum. However, *A. hispidus* has been inconsistently the pride for dyers.

A genecological experiment using three crop populations and 11 weed populations by Ishigami *et al.* (2000, 2001) demonstrated that the morphological and physiological differences between the weedy and cultivated *A. hispidus* have genetic basis. It clearly showed a domestication syndrome as characterized by the synchronous stem branching, late heading of spike, shorter period to heading, uniform plant maturation and less seed shattering, with the larger spike, spikelet, leaf and stem.

Ishigami *et al.* (2001) pointed out that a few weed populations comprehended less shattering individuals despite of non-cultivation conditions of the habitats. It is firstly supposed that this is due to some gene flows of the present populations between the weed and crop and due to the genetic relict, which has continued from the start of cultivation. It is secondly supposed that a satisfactory term of collecting of *A. hispidus* from the weed vegetation covering agricultural fields and the cyclic phenotypic disturbance as a result have invited the situation of 'encouraged weed' (Harlan, 1992).

It is therefore proper that the case of *A. hispidus* in Hachijo Island is listed as an example of 'weed, encouraged weed and crop continuum'.

### Establishment of the management and utilization of the continuum

In Hachijo Island, the silk textile using *A. hispidus* as dye material for golden staining is called 'ki-hachijo', which means yellow and location name. This dyeing system is the most original, judging from historical evidence. It requires the dried leaves and stems of *A. hispidus* as well as the ashes of *Camellia japonica* L. and *Cleyera ochracea* DC., which occur in the evergreen broad-leaved forest of the island.

The area of Hachijo Island is only 66.33 square km and is so small that plant resources are quite limited. Therefore, the dwellers and dyers must have efforts to utilize biota completely and in sustainable manners. *A. hispidus*, whatever as a weed or as an encouraged weed or as a crop, is very suitable to the ecology of the island. The aptitude of a given resource managing system has been from the environmental and ethical viewpoints checked for more than 250 years in the weed, encouraged weed and crop continuum in *A. hispidus*. Ashes made from *Camellia japonica* L. and *Cleyera ochracea* DC. are also traditionally assessed from the environmental and ethical viewpoints because the trees must be adequately cut down enough to supply materials and enough to re-grow in holy forests. All the plant materials for 'ki-hachijo' dyeing are just the results of the stable and long term management and utilization of anthropologically affected vegetation. This situation is a good example of proper 'vegetation tuning' (Umemoto, 2001). In this island, economy, ecology and ethics are in symbiosis by sustaining the resource- and variation- rich vegetation dependent on cyclic disturbance (Umemoto, 2000) or by proper vegetation tuning.

### Hypotheses on the origin of utilization of *A. hispidus*

At the present, two possible hypotheses, both of which explain the origin of the dyeing system using *A. hispidus* in Hachijo Island, can be proposed: the endemic hypothesis and the dispersal hypothesis (Umemoto, 2000). In the scenario of the endemic hypothesis, the dyeing system and the selection of *A. hispidus* as indispensable dyeing materials should be indigenous to the culture of Hachijo Island. In this case, the past talented dwellers developed dyeing techniques in limited ecological and cultural resources. In a scenario the dispersal hypothesis assumes, dye techniques with *A. hispidus* were brought about from the China Continent. A Chinese classic book, Bencao-jing-ji-zhu, based on the edition by People and Health Publishing, Beijing, states plant materials harvested from *A. hispidus* on September to October (by the lunar calendar?) can stain golden yellow and this was used in west Yizhou (around Sichuan Province at present?). A part of trans-immigrant people from there by boats had reached to Hachijo Island along and across the Kuroshio warm current and handed down the whole dyeing techniques to the dwellers of Hachijo Island. In the endemic hypothesis, golden yellow symbolizes the color of the sun above the Pacific Ocean while in the dispersal hypothesis, golden yellow is equivalent to the color of the Chinese emperor of the China Continent.

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## QUANTITATIVE ANALYSIS OF WEED COMMUNITIES IN THE WHEAT FIELDS OF JIANGSU PROVINCE

K. J. Wang<sup>1</sup>, S. Qiang<sup>1</sup>, and D. Y. Zhang<sup>2</sup>

<sup>1</sup> Weed Research Laboratory, Nanjing Agricultural University, 210095, China  
wrl@njau.edu.cn

<sup>2</sup> Plant Protection Station, Jiangsu Province, 210011, China

**Abstract:** The outputs of the principal component analysis (PCA) were ecologically interpreted, after the overall weed infestation indexes of all weed populations occurred in 150 sampling sites amounted to 147.2hm<sup>2</sup> wheat fields in Jiangsu Province were assessed by visual scoring the level of weed infestation to wheat in seven scales. The results showed that the main factors determined the weed communities and infestation in the wheat fields were soil moisture and other ecological factors determined by latitude. According to the result of PCA, 150 sampling sites could be divided into four groups in wheat fields all over the province. The southern dry-land group rotated with dry crops in hilly region of the south of Jiangsu Province had such dominants as *Galium aparine* var. *tenerum*, *Veronica persica* and *Avena fatua* while the dominant weeds of the northern dry-land group in the north of Jiangsu Province were *Galium aparine* var. *tenerum*, *Veronica persica*, *Descurainia sophia* and *Lithospermum arvense*. *Sclerochloa kengiana* and *Polypogon fugax* were the two main weeds occurred in the group of rotated with rice in the north of Jiangsu Province. The rice group rotated with wheat in the south of Jiangsu Province had three dominant weeds as follows: *Alopecurus japonicus*, *Alopecurus aequalis* and *Beckmannia syzigachne*. The suggestion for the integrated weed management was also put forward according to weed distribution pattern.

**Key words:** Jiangsu Province, weed communities, principal component analysis (PCA), ecological interpretation, integrated weed management, wheat fields.

### INTRODUCTION

About 111 species of weeds occurred in the wheat fields in Jiangsu Province, Weed communities vary with different ecological environments. Tillage, herbicides and other human factors also influence the structure of weed communities and their distribution patterns. The quantitative research on the structure and distribution of the weed communities is of great importance to the integrated weed management in this region. The related studies were only conducted in the summer crop fields of Anhui Province neighbor to Jiangsu Province (Qiáng & Li 1990; Qiang et al. 1994, Qiang & Liu 1996) and in the cotton fields of Jiangsu Province (Qiang & Hu 1999). Former studies on the weed community distribution in wheat fields in Jiangsu conducted by others only gave rough descriptions that could not elucidate the relationship between weed communities and ecological conditions clearly (Xue & Li, 1987). The quantitative study of weed community in the wheat fields of the province has not done before. In this article, principal component analysis (PCA) was applied to study the weed communities in the wheat fields in Jiangsu Province and to give quantitatively ecological explanation so that it may provide scientific basis for integrated management of wheat weeds.

Jiangsu Province lies in the warm temperate-subtropic and semi-humid zone in east



of China. Huaihe River is the dividing line of warm temperate and subtropical climate. In this region, annual average temperature is 13-16°C and rain capacity is about 800-1000mm per annum with major peaks in summer, more in south-east and less in north-west. Jiangsu Province is one of the main grain, cotton and oil product bases in China. The main crops in this region are rice, wheat, cotton, rape and so on. Tillage changes with natural condition. Rice rotated with wheat or rape is popularized in the well-irrigated regions, and dry crops rotated with wheat are common in dry regions.

## MATERIALS AND MATHODS

The surveys of weed community were conducted in May 1999, 2000 respectively when the wheat was in the stage of flowering-booting. The investigated covered about 50 counties and cities in the main agricultural regions of Jiangsu Province. 150 sampling sites amounted to 147.2hm<sup>2</sup> wheat fields were randomly surveyed according to the different natural conditions, tillage, rotations, history of use of herbicides and other factors. 10 approximately uniform units (wheat fields) were sampled as just one sampling site. Then the level of the infestation of each weed species in every unit to wheat was recorded down in seven scales by visual score. The overall value of all weed populations occurred in wheat fields were calculated as following formula (Qiang, et al.1989, 1990, 1994).

Overall value =  $\sum$  (number of the scale of the weed in a unit  $\times$  value of the scale) / 50

40 species of weed whose frequency over 10% were taken as analysis variables (Table.1). All the data of the 40 species in 150 sampling sites were arranged a data matrix. The data matrix was analyzed by SPSS 10.0 after the data matrix transformed by three-time successive square. The two-dimensional scatter plot of PCA ordinations for the 40 weed species and 150 sampling sites in wheat fields of Jiangsu Province were obtained from results of PCA. (Ren et al. 1994; Xu et al. 1983) All the sampling sites could be divided into four groups according to the result of ordinations. The dominants of weed community were determined by calculating the overall weed infestation index (OWII) and frequency of each species of weed in each group(Qiang, et al.1990).

OWII =  $\sum$  (number of the scale of the weed in a unit  $\times$  value of the scale) / (total number of units of the group  $\times$  value of the highest scale)

## RESULTS AND DISCUSSION

The results of the investigation showed that 111 species of weeds occurred in the wheat fields in Jiangsu Province subjected to 21 families. Most of them are annual weed. *A. aequalis*, *A. japonicus*, *B. syzigachne*, *M. aquaticum* and *L. apogonoides* were the typical weeds occurred in wheat field rotated with rice in the south of Yangze River region while *S. kengiana* and *P. fugax* were the main weeds in wheat fields rotated with rice of the north of Jiangsu. *A. futua*, *G. aparine* var. *tenerum*, *V. sativa* often infested to wheat fields in the hill region in south region. *D. sophia* and *L. arvense* were the typical weed of warm-temperate zone in the north of Huaihe River region. The structure of weed communities had some changes compared to those of ten years before. The harmfulness of some weeds in this region such as *B. syzigachne*, *P. annua* and *G. carolinianum* became more serious and these weeds show some degree of resistance to

common herbicides which should worth the attention of weed researchers.

Table 1. Loading of the previous three principal components of 40 weed species in wheat fields of Jiangsu Province

Weed species	1st-principal component	2st-principal component	3st-principal component
1 <i>Alopecurus aequalis</i>	0.848	-0.01236	-0.08124
2 <i>Alopecurus japonicus</i>	0.872	-0.112	-0.170
3 <i>Beckmannia syzigachne</i>	0.893	0.07116	-0.0105
4 <i>Avena fatua</i>	-0.09698	0.344	-0.404
5 <i>Sclerochloa kengiana</i>	-0.241	-0.828	-0.02707
6 <i>Polypogon fugax</i>	0.07346	-0.790	0.08218
7 <i>Poa annua</i>	0.527	0.09676	0.220
8 <i>Geranium carolinianum</i>	0.483	0.305	-0.446
9 <i>Galium aparine</i> var. <i>tenerum</i>	-0.465	0.449	-0.04327
10 <i>Vicia sativa</i>	0.150	0.589	-0.296
11 <i>Vicia cracca</i>	-0.162	0.341	-0.397
12 <i>Veronica persica</i>	-0.368	0.715	0.03086
13 <i>Rumex dentatus</i>	0.437	0.05287	0.293
14 <i>Polygonum lapathifolium</i> var. <i>salicifolium</i>	0.268	0.233	0.203
15 <i>Polygonum aviculare</i>	-0.363	0.344	0.002394
16 <i>Phleum paniculatum</i>	-0.303	0.05276	0.590
17 <i>Ixeris dentata</i>	-0.341	0.220	0.620
18 <i>Descurainia sophia</i>	-0.577	0.180	-0.226
19 <i>Lithospermum arvense</i>	-0.513	0.240	-0.265
20 <i>Salvia plebeia</i>	0.579	0.170	0.053
21 <i>Kalimeris indica</i>	0.522	0.146	0.217
22 <i>Conyza bonariensis</i>	0.623	0.480	-0.136
23 <i>Cerastium glomeratum</i>	-0.01777	0.377	0.639
24 <i>Malachium aquaticum</i>	0.573	-0.06198	0.256
25 <i>Mazus japonicus</i>	0.47	-0.01918	0.09175
26 <i>Veronica anagallis-aquatica</i>	0.374	-0.144	0.173
27 <i>Euphorbia helioscopia</i>	-0.134	0.279	0.197
28 <i>Calystegia hederacea</i>	-0.412	0.260	0.05741
29 <i>Chenopodium album</i>	-0.529	0.215	0.426
30 <i>Plantago asiatica</i>	0.363	0.225	0.209
31 <i>Alternanthera philoxeroides</i>	0.585	0.132	0.004262
32 <i>Capsella bursa-pastoris</i>	-0.272	-0.102	0.231
33 <i>Rorippa indica</i>	0.32	0.0386	0.05618
34 <i>Trigonotis peduncularis</i>	0.322	0.200	-0.117
35 <i>Bothriospermum tenellum</i>	0.441	0.05867	0.161
36 <i>Lapsana apogonoides</i>	0.863	0.129	0.02232
37 <i>Ixeris polycephala</i>	0.811	0.282	0.09634
38 <i>Hemistepta lyrata</i>	0.542	0.07498	-0.07271
39 <i>Cephalanoplos segetum</i>	-0.533	0.504	0.145
40 <i>Gnaphalium affine</i>	0.506	0.04404	0.155
Eigen value	9.797	4.162	2.682
Cumulative information (%)	24.493	34.898	41.602

In Table 1, the loading of the previous three principal components, eigen value and cumulative information of 40 weed species in wheat fields of Jiangsu Province were listed in. From which the following two-dimensional scatter plots were obtained (Figure1; Figure2). According to distribution feature of the scatter plots of the Figure 1 and Figure 2, all the scatter plots (weed species) in Figure 1 could be divided into four groups (I, II, III, IV), plots in Figure2 could be divided into four groups (A, B, C, D). In Figure 1, group I includes the weeds as follows: *A. aequalis*, *A. japonicus*, *B. syzigachne*, *P. annua*, *G. carolinianum*, *R. dentatus*, *P. lapathifolium* var. *salicifolium*, *S. plebeia*, *K. indica*, *C. bonariensis*, *M. aquaticum*, *M. japonicus*, *V. anagallis-aquatica*, *P. asiatica*, *A. philoxeroides*, *T. peduncularis*, *B. tenellum*, *L. apogonoides*, *I. polycephala*, *G. affine*; group IV includes *S. kengiana*, *P. fugax*. The species of weeds in group I, IV are hygrophytes, of which. *A. aequalis*, *A. japonicus*, *B. syzigachne*, *L. apogonoides* et al. often infested in wheat fields rotated with rice in the south of Jiangsu Province while *S. kengiana*, *P. fugax* were the main weeds in wheat fields rotated with rice in the north of Jiangsu province. Group II includes *A. futua*, *V. sativa*, *V. cracca*, *C. glomeratum*, *E. helioscopia*, which all are xerophilous. Weed species in group III are *G. aparine* var. *tenerum*, *P. aviculare*, *P. panioulatum*, *I. dentata*, *D. sophia*, *L. arvense*, *C. hederacea*, *C. album*, *C. segetum*, most of which usually found in wheat fields rotated with dry crops. Those species are typical warm temperate weeds in north of Huaihe River.

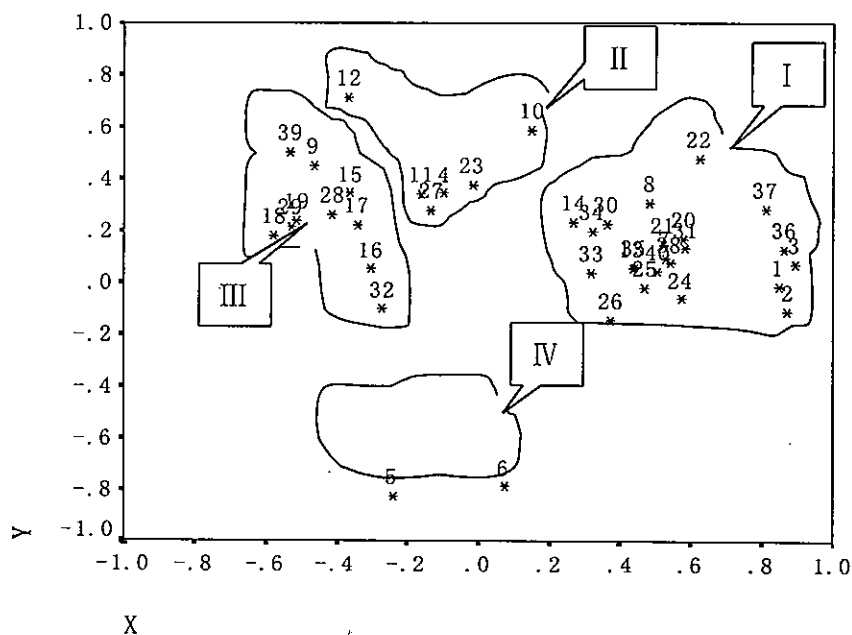


Figure 1. A two-dimensional scatter plots of PCA ordination for the 40 weed species in wheat fields in Jiangsu Province

The total sampling sites in Figure 2 could be divided into four groups (A, B, C and D). Group A composed by 68 sampling sites which includes 1-41, 43-49, 56, 57, 58, 60, 61, 62, 64, 67-74, 76, 77, 78, 80, 81. All of them were from the wheat fields rotated with rice in the south of Jiangsu Province. The soil in those fields kept high moisture over the most of year and the pH of soil was under 7.0. So hygrophilous weed species such as *A. aequalis*, *A. japonicus*, *B. syzigachne*, *L. apogonoides*, *M. aquaticum* grew well in this condition. Grass weeds like *A. aequalis*, *A. japonicus*, *B. syzigachne* were dominant.

*L. apogonoides* and *M. aquaticum* subdominants in some regions surrounding Taihu Lake.

Group B includes 13 sampling sites as follows: 42, 50, 51, 52, 53, 54, 55, 59, 63, 65, 66, 75, 79. They were sampled from the wheat fields rotated with dry crops in the hilly region in the south of Jiangsu Province. *Galium aparine* var. *tenerum*, *Veronica persica* and *Avena fatua* were suitable to the dry environment and became dominants.

Group C was consisted of 27 sampling sites, which were 95, 96, 97, 98, 99, 100, 101, 102, 103, 105, 119, 120, 122, 123, 124, 127, 128, 129, 130, 131, 133, 135, 138, 139, 143, 148, 149. All of them were the sampling sites of the wheat fields rotated with dry crops of Subei Plain in the north. In this region, there were the high pH and saline soil, low moisture. So the xerophilous and salt-resistant temperate weeds like *G. aparine* var. *tenerum*, *V. persica*, *D. sophia* and *L. arvense* usually grow well.

Group D contains 42 sampling sites as follows: 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 104, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 121, 125, 126, 132, 134, 136, 137, 140, 141, 142, 144, 145, 146, 147, 150. They were sampled from the wheat fields rotated with rice in the north of Jiangsu Province, where the pH of the soil was usually over 7.0 and the humidity of soil high. *S. kengiana* and *P. fugax* are comparatively resistant to higher pH and soil moisture environment, so that both of them were the main weeds in the fields.

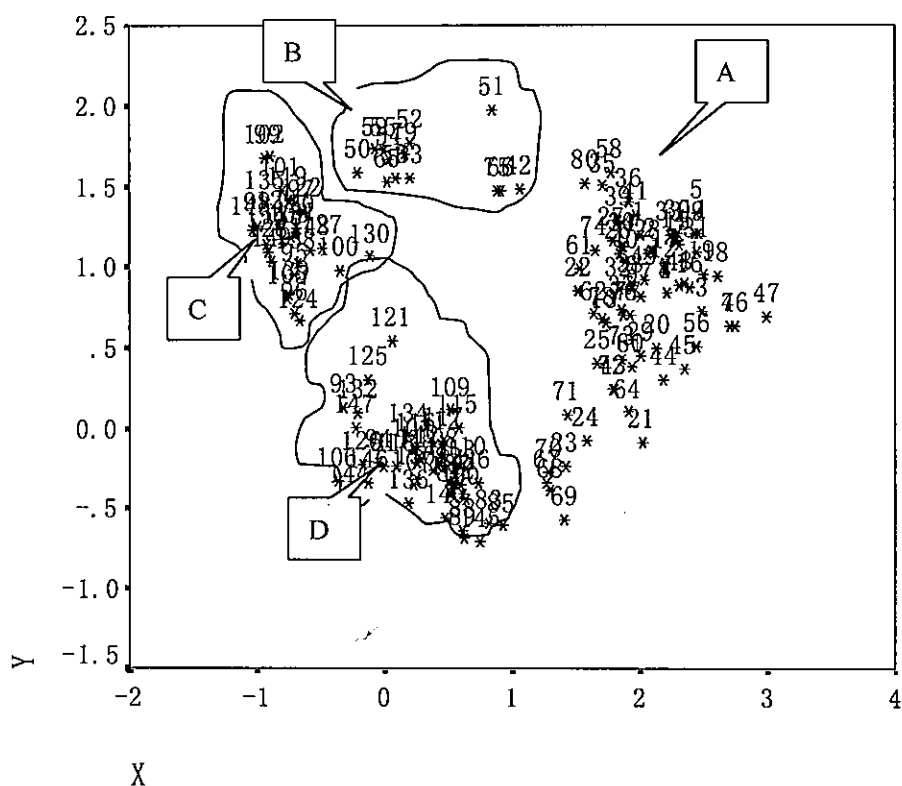


Figure 2. A two-dimensional scatter plots of PCA ordination for the 150 sampling sites of wheat fields in Jiangsu Province

Through the above analysis and comparison of the quantitative characteristics of main weed communities listed in Table 2, we may conclude that the structure and

distribution of weed communities comprehensively reflected the humidity and pH of soil, climate related with latitude, tillage, application of herbicides and other human factors. Among the above many factors, the most important two factors were the humidity of soil and latitude. The humidity of the soil was the first factor, through which tillage and topography influence on the weed communities indirectly. The moisture of soil did not only affect the growing weeds, but also the weed seed bank in the soil where was the source weed occurrence in the coming years because of a huge number of weed seeds. The seeds of xerophilous weed usually can not survive through dormancy period in the soil with high moisture while the viability of the hygrophilous weed seeds decrease drastically in the coming year in the dry soil. (David 1997; Joseph. 1989). The second main factor was latitude. The weed flora changed gradually from the subtropical zone in the south to the warm-temperate zone in the north with the increase of the latitude. The climate determined by the latitude has marked influence on the plant communities. The pH and type of soil affected the weed communities also have some relations with the latitude. The typical temperate weeds like *D. sophia* and *L. arvense* grew well in the north but could not survive in the south. *A. aequalis*, *A. japonicus*, *B. syzigachne* were scarcely found in the north. In Figure 2, Group A, B are the sites in low latitude region in the south of Jiangsu Province, where in the sub-tropic zone the annual rainfall is more than it in the high latitude region in north, which was beneficial to hygrophyte growth. In the hillside fields in the south, it is difficult to irrigate and keep the rainfall resulted in the dry field environment, so the xerophytes like *A. futua*, *V. sativa*, *G. aparine* var. *tenerum* could grow well. The sites of Group C and D were from the high latitude region in the north of Jiangsu Province in warm-temperate zone with less annual rainfall and higher saline soil compared to the south, in which weed species were different. The xerophilous, salt-resistant and temperate weeds often found in fields instead of those types of hygrophilous weeds, so weeds like *G. aparine* var. *tenerum*, *V. persica*, *D. sophia* and *L. arvense* usually grew well in wheat fields rotated with dry crops while *S. kengiana*, *P. fugax* do seriously harm to wheat rotated with rice in the north of Jiangsu Province.

Considering the tendency towards convergence between the different weed communities and environment in the wheat fields, the integrated weed management mainly based on herbicide combined with other control methods may be feasible. Post-emergence herbicides such as MPCA, Dicamba, Starane and Express et al. may be applied to the wheat fields rotated with dry crops where dominants are mainly broad-leaf weeds like *G. aparine* var. *tenerum*; *V. sativa*; *V. persica*; *D. sophia* and *L. arvense*. Puma super can be combined to those fields infested seriously by *A. futua*. The soil-applied herbicides like isoproturon, acetochlor+chlorosulfuron and post-emergence herbicides puma super, diclofop-methyl may be applied to the wheat fields rotated with rice infested badly by grassy weeds like *A. aequalis*, *A. japonicus*, *B. syzigachne*, *S. kengiana*, *P. fugax*. Those fields also damaged seriously by *L. apogonoides* and *M. aquaticum* et al can be treated with above herbicides plus MPCA, Dicamba, Starane and Express et al. Other non-chemical methods of ecological, agricultural, biological, cultivate measurements also should be adapted in the wheat weed management with herbicides, for example, farmers can decrease the weeds damage to wheat by changing the rotation between rice and other crops that may worse the weed growth and the seed reserve conditions. The study on the weed communities and suggestions for weed management in wheat fields may provide useful reference to the farmers and herbicide factories.

Table 2. The quantitative characteristics of main weed communities in wheat fields in Jiangsu Province

Weed species	Group A		Group B		Group C		Goup D	
	OWII	F(%)	OWII	F(%)	OWII	F(%)	OWII	F(%)
1 <i>Alopecurus aequalis</i>	24.63	99.68	2.66	22.86	0.059	1.48	0.97	17.5
2 <i>Alopecurus japonicus</i>	30.21	99.84	3.76	25.48	0.022	1.11	2.29	34.7
3 <i>Beckmannia syzigachne</i>	26.35	95.81	1.24	12.86	0	0	0.68	0.095
4 <i>Avena futua</i>	0.37	8.23	26.49	87.14	1.911	20.4	2.18	24.75
5 <i>Sclerochloa kengiana</i>	0.95	6.45	0	0	0.119	4.44	28.4	97.25
6 <i>Polypogon fugax</i>	3.73	31.94	0	0	0.237	5.93	12.5	88.5
7 <i>Geranium carolinianum</i>	1.91	42.25	4.11	47.80	0.356	10.4	3.95	12.75
8 <i>Galium aparine</i> var. <i>tenerum</i>	1.0	23.87	39.57	99.29	10.5	77.4	1.63	35.0
9 <i>Vicia sativa</i>	2.49	40.65	35.80	97.14	1.022	24.4	3.15	11.75
10 <i>Veronica persica</i>	0.37	14.83	23.97	97.87	10.31	70.4	0.16	3.0
11 <i>Descurainia sophia</i>	0	0	0	0	11.87	59.6	0.15	0.75
12 <i>Lithospermum arvense</i>	0	0	0	0	9.044	48.1	0.98	11.5
13 <i>Cerastium glomeratum</i>	0.32	11.61	4.48	35.00	8.311	39.3	0.98	11.5
14 <i>Malachium aquaticum</i>	4.21	34.67	0.08	1.43	0.015	0.74	0.35	8.25
15 <i>Lapsana apogonoides</i>	8.61	65.64	0	0	0	0	0.20	4.75
16 <i>Cephalanoplos segetum</i>	0.07	3.54	2.55	33.57	4.948	43.7	0.06	3.25

### ACKNOWLEDGEMENTS

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## EFFECT OF CHEMICAL HERBICIDE ON SHIFT OF WEED COMMUNITY IN PADDY FIELD

J. L. Wu<sup>1</sup> and H. C. Zhou<sup>2</sup>

<sup>1</sup>Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, China

<sup>2</sup>Agricultural Production Means Incorporation in Yizhen City, Jiangsu, China

**Abstract:** The mixture of acetochlor and bensulfuron is highly effective against most of annual and perennial weeds. The density of dominant damage weeds such as *Echinochloa varinialis*, *Sagittaria pygmaea*, *Eclipta prostrata*, *Ammania baccifera*, *Juncellus serotinus*, *Potamogeton distinctus* and *Lindernia prostrata* in transplanted paddy decreased markedly and the components of the weed community changed because of continuous application of the mixture herbicide. The five-year experimental results demonstrated that application of the herbicide once every year for five years compared to four, three, two and one year without application treatments, the difference between total weed density, coverage and biomass were all significant. The application treatment for five times compared to one-application, the total weed density increased by 108.5%; the coverage by 111.5%; the biomass by 109.9% respectively.

**Key words:** Paddy weed, shift of weed community, herbicide

Paddy weed community component and its shift mainly determined by the weed seed bank, tillage and cultivation mode (Yu et al. 1995), light, water, manure and other ecological environmental conditions (Derksen 1993; Chesson 1986). At the same time, it is closely related with the continuous application of single kind of selective herbicide (Feng et al. 1990). In order to confirm the effect of chemical herbicide on weed community shift and rice yield, a selective herbicide YB (the mixture of acetachlor and bensulfuron, highly effectively against the most annual and perennial monocotyledon and dicotyledon weeds in paddy) was applied to transplanted paddy in succession for years. The research results were summed up as the followings.

### MATERIALS AND METHODS

#### Test site location

The test site was located at Xingcheng town, Yizheng City, Jiangsu province, belonging to single and double rice cropping sub-region on the middle and lower valley of the Yangtze River, with continuous rice-wheat double cropping rotation system. The soil type at the site was applying clay sandy loam with moderately sands in clay, no plow pan and good permeability. The plowed soil layer was about 18 cm in depth with 1.5% organic matter and a pH of 7.5.

The test field was 0.083 ha in area. The dominant weeds were annual weeds such as *Echinochloa crusgalli* (L.) Beauv., *Lindernia procumbens* (Krock) Tang, *Eclipta prostrata* L., *Rotala indica* (Willd.) Kochne, *Aeschynomene indica* L., *Juncellus serotinus* (Kottb) Clarke, *Ludwigia prostrata* Roxb, *Potamogeton distinctus* A. Benn, *Cyperus difformis* L. There were some *Alternanthera philoxeroides* (Mart.) Griseb, *Paspalum distichum* L., *Fimbristylis miliacea* (L.) Vahl, *Cyperus iria* L. on the field ridge.



## Experimental treatments

Five treatments were designed: (1) no application of herbicide from 1996-1999, (2) application of herbicide only in 1996, (3) application from 1996-1997 and no from 1998-1999, (4) application of herbicide only in 1999, (5) continuous application from 1996-1999. The herbicide used in the research was 14% YiBian, produced by Rudong Pesticide Factory, Jiangsu Province, at the dosage of 84g(ai) per ha. All treatments were completely randomized block with two replicates. Each plot was 83m<sup>2</sup>. Each plot was separated with ridge. Irrigation and drainage were done at intervals. The rice was Wuyugeng 3, a conventional variety in local rice production. The rice seedlings were above 30 days old when being transplanted. Five days after transplanting, 14% YiBian was mixed with soil and applied by hand to the paddy field in which 3-4cm depth of water was keeping for 5-7 days. Then, the conventional management was applied to the experimental field.

## Parameters collected

**Weed investigation** Five samples were taken from each plot. The sample size was 0.5m × 0.4m. Twenty and sixty days after transplantation, numbers of weed plants, relative height, shady coverage as well as frequency were investigated. After the second investigation, all weeds were rooted out and their biomass was weighed. The control efficiency was calculated by measuring the number of weed plants and fresh weight above ground.

**Yield and yield components** Taking five samples from each plot and then four hills rice from each sample, the number of spikes per hill, length of spike, grains per spike, 1000-seed weight were measured. In addition, rice plants were sampled from two 5m<sup>2</sup> area of each plot. After being individually harvested and threshed, actual yield, estimated yield and the effect on yield increase were calculated.

## RESULTS AND DISCUSSIONS

### Shift of weed community

Eleven species of common weeds, belonging to 9 families, were found in the experimental site. The annual weeds were *Echinochloa crusgalli*, *C. difformis*, *Lindernia procumbens*, *Rotala indica*, *Ammannia baccifera*, *Ludwigia prostrata*, *Eclipta prostrata*, *Monochoria vaginalis*, while the perennial weeds were *Sagittaria pygmaea*, *Juncellus serotinus* as well as *Potamogeton distinctus*. The experimental results showed that there were marked differences in weed density and occurrence between treatments of continuous application of herbicide and that of no application for four years. Numbers of plants per square meters were 26.5 and 255.5, coverage 10.5% and 126.0%, biomass 107.0g and 945.5g respectively.

**The shift of annual weed** The research results showed in treatment (a) weed quantity increased drastically, especially *E. crusgalli*, *C. difformis*, *L. procumbens*, *A. baccifera* as well as *R. indica*. Because these weeds bore small seeds and high seed setting percentage, their seed accumulated rapidly in soil and ultimately led to the severe occurrence. The density of *E. crusgalli* had the quickest increment. For four years without herbicide application, there were 41.5 plants per square meters; and without application for three, two and one years, weed density was 34.5, 25.0 and 22.5 plants per square meters respectively. However, in the treatments with continuous application for four years, weed density only 4.5 plants. From 1996 to 1999, after having

applied herbicide three years except 1999, *M. vaginalis* did not occur again, but without application for successive two, three and four years, its density were 2.2, 5.5 and 7.7 plants per square meters respectively, which was a tendency to go up gradually. *E. prostrata* and *L. prostrata* had a low seed setting percentage and because they mainly propagated by water and application of manure, their occurrence were comparatively stable. In addition, *A. indica*, *Scirpus juncoides* also had the developing tendency to go up.

The results for 60 days after transplantation suggested that the density of eight species of annual weeds in treatment I, II, III, IV and V were 196.0, 180.5, 141.5, 102.0 and 18.5 plants per square meters respectively. The treatments IV, III, II, I compared to the treatment V, the weed density increased by 9.6, 8.8, 6.6, and 5.5 times, respectively. According to above results, we can predict that if one year without chemical herbicide application, annual weeds such as *E. crus-galli*, *C. difformis*, *L. procumbens* as well as *E. prostrata* will increased by 4.5 times (Figure 1).

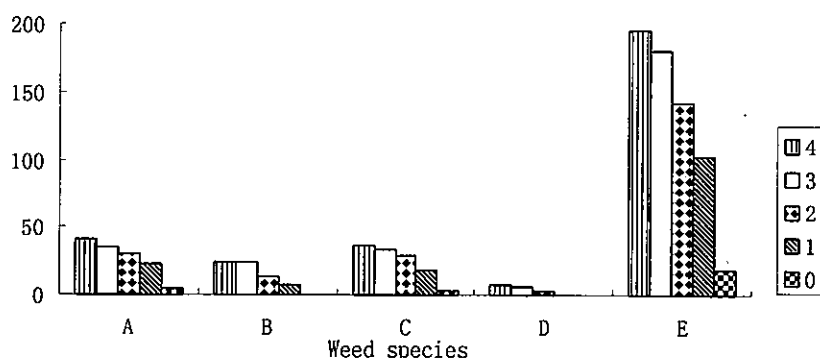


Figure 1. Annual weeds shift as affected by applying the mixture of acetochlor and bensulfuron for different years continuously. Note: 4,3,2,1,0 stand for difference years by applying mixture of Acetochlor and Bensulfuron continuously. A is *Echinochloa crus-galli*; B is *Cyperus difformis*; C is *Eclipta prostrata*; D is *Monchoria vaginalis*; E is total weeds.

**Shift of perennial weed** The results showed that as a result without chemical application for many years, the density of perennial weeds as *P. distinctus*, *J. serotinus* and *S. pygmaea* were in the tendency of increase. *P. distinctus* never emerge after three years successive application and even no herbicide in the fourth year. But only one year no application, the density of *J. serotinus* had a two-fold increase. Compared to the four year's application treatment, the weed density of those without application for one, two, three and four years increased by 4, 5, 4, 6.4 and 7.4 times. *S. pygmaea* had the similar developing tendency as *J. serotinus*, its density increased by 2.1, 3.8, 4.3 and 4.6 times, respectively, compared to the four applications treatment.

The results for sixty days after transplantation indicated the density of three species of perennial weed in five treatments I, II, III, IV, V were 59.5, 50.6, 43.0, 21.5 and 8.0 plants per square meters respectively. Compared to treatment V, the density of treatment I, II, III and IV increased by 1.7, 4.4, 5.3 and 6.4 times (Figure 2).

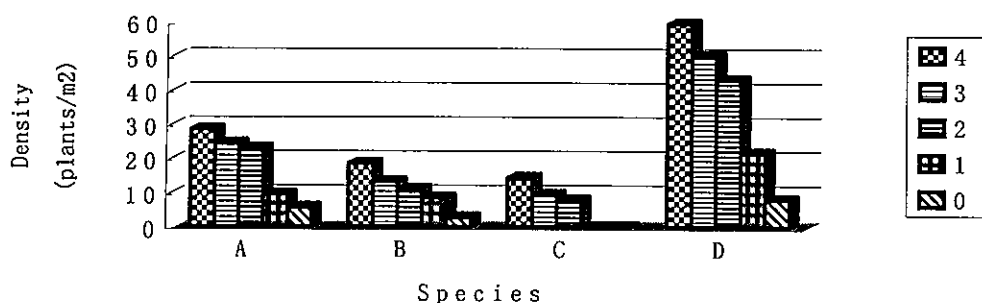


Figure 2. Perennial weed shift as affected by continuously applying the mixture of acetochlor and bensulfuron. Note: 4, 3, 2, 1, 0 stand for different years by applying mixture of acetochlor and bensulfuron continuously. A is *Sagittaria pygmaea*; B is *Juncellus serotinus*; C is *Potamogeton distinctus*; D is total weeds

### Weed developing tendency

The five-year experimental results demonstrated that application of the herbicide once every year for five years compared to four, three, two and one years without application treatments, the difference between total weed density, coverage and biomass were all significant. The five-time application treatment compared one-application, the total weed density were 368.0 and 176.5 plants per square meters, an increase of 108.5%; the coverage were 139.6% and 66.0%, an increase of 111.5%; the biomass were 5326g and 2547g per square meters, an increase of 109.9%, respectively.

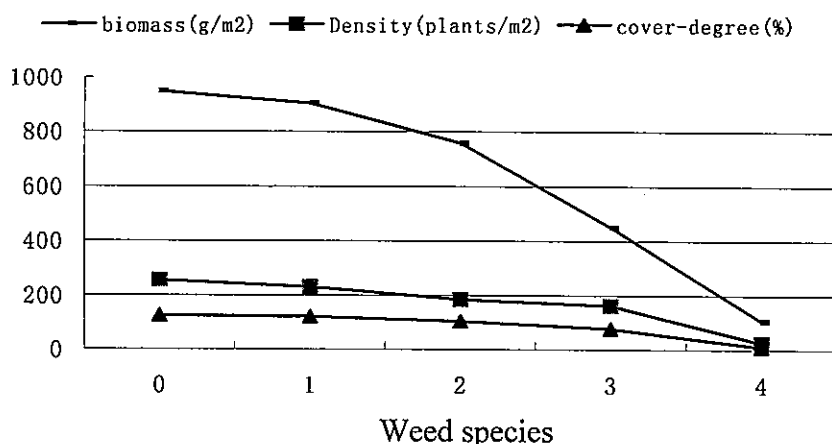


Figure 3. Weed biomass was affected by applying the mixture of Acetochlor and Bensulfuron for different years continuously

### The effect on rice yield

The yield of the treatments applied the mixture every year was all between 8542.5—8959.5kg per ha. Compared to no application treatment, increased by 13.9—27.4%. The result in 1997 suggested that compared to no application treatments for one or two years, the yield of the treatments applied the chemical herbicide for consecutive two years increased by 13.9% and 17.8% respectively. No application only for one year compared to 2-year no application increased only by 3.4%. In 1998 and 1999, the

experimental results had the similar tendency, which without application for one year, the yield decreased by 25.1% and 27.4%; while with application for two, three or four years, the yield decreased by 28.9%, 30.7%, 35.4%, 3.7% and 37.9% respectively. Treatment III compared to treatment IV, the yield decrease by 2.6%; treatment II to III and IV, the yield only by 2.3% and 4.1%; treatment I to II, III and IV, the yield by 3.2%, 5.5% and 8.2% respectively (Figure 4). Due to the large number of seeds in transplantation paddy fields, only one year without application (or no other weed control measurements), weed damaged heavily and the yield decreased by 27.4%. Without application for two, three and four years in succession, the yield decreased by 30.7%, 33.7% and 37.9% respectively. In the treatment applied chemical herbicide for consecutive four years except the fifth year compared to applied for three year except the fourth and the fifth year, the yield increased by 7.2%. The chief factor resulted in the yield decrease was that weed competed manure, light and water with rice, inhibited the normal development of rice and decreased grains of per spike. The number of fertile spike and 1000-grain wt were also decreased. The result indicated that there were severe competition between rice and weeds. At the same time, there was mutual ecological competition among weeds.

The mixture of acetochlor and bensulfuron had a high effectiveness against annual weeds, especially for *E. crus-galli*, *C. difformis*, *L. procumbens*, *E. prostrata*, *M. vaginalis*, *L. prostrata*, *A. baccifera* and *R. indica*. It was also effective for perennial

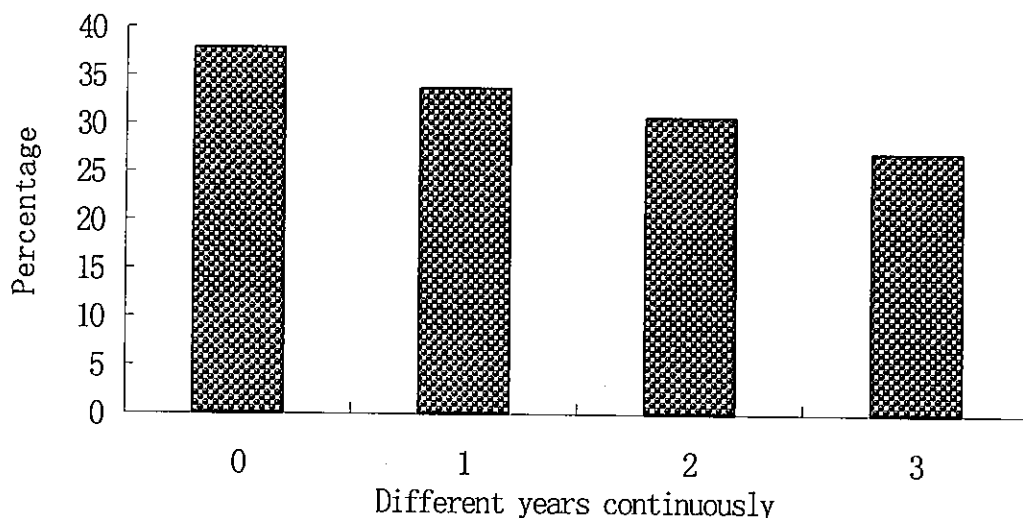


Figure 4. The rice yield increasing rate by applying the mixture of Acetochlor and Bensulfuron for 4 years continuously compared to 0, 1, 2, 3 years respectively

weed such as *P. distinctus*. *M. vaginalis* did not emerge again after applying the mixture for successive years. But when applied at the dosage of 600g ai per ha, the herbicide had a lower control percentage for *S. pygmaea* and *J. serotinus* in extension scale.

There were a large number of weed seeds in weed seed bank. From 1996 to 1998, with three-year's application in succession except in 1997, the total weed density reached 126.5 plants per square meters. This results was due to the gradually accumulation of weed seed in paddy soil. So it was necessary to control the paddy weed every year. If no

control measurements for only one year, the weed would caused a sever damage.

Acetochlor plus bensulfuron could control annual and perennial weed effectively and increased yield significantly. By effective weed control, the seed grain per spike was increased. This was the chief yield-contributing factor. Treatment IV compared to treatment V, the yield decreased by 27.4%; treatment III to IV, yield by 2.6%; treatment II to IV, yield by 4.9% .This result suggested that the paddy weed must be controlled every year unless the rice yield will be decreased significantly .

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## THE EFFECT OF GLUME ON SEED GERMINATION OF *SORGHUM HALEPENSE* (L.) PERS.

L. P. Yin and Z. F. Yan

Shanghai Entry-Exit Inspection and Quarantine Bureau, 200135, 1208 Mingsheng Road, Pudong New District, Shanghai

**Abstract:** Seeds of *Sorghum halepense* (L.) harvested after 7 months were highly dormant. In order to study the glume effect on the seeds germination, the following treatments were conducted: (1) seeds of *S. halepense* (L.) Pers., (2) seeds removed of glumes, (3) seeds removed of glumes plus glumes, (4) seeds treated with concentrated sulfuric acid for 15 min., then rinsed with water for half an hour. Seeds removed the glumes or treated with concentrated sulfuric acid 15 min. could be germinated. The germination rate was about 75-77.5%. When seeds removed of glume and glume were germinated in same culture dish, the germination rate was only about 0.5-1.5%. It suggested that an inhibitor in their glumes caused the dormancy of the seeds.

### INTRODUCTION

*Sorghum halepense* (L.) Pers. (Johnsongrass), a kind of dangerous weed is list on Pest list of Plant Quarantine of People's Republic of China. Its seeds in grain or other cargoes from oversea were often found or detected. However, it was difficult to test the germination rate of the seeds because it was uncertain sometimes. A better understanding of dormancy character would benefit to both weed quarantine and other biologists.

Harrington (1916) first reported that freshly harvested johnsongrass seeds could be germinated if the seed coats was removed or were treated with concentrated sulfuric acid for 2 min. Harrington (1917) recognized that the germination increasing caused by scarification did not result from increasing of water uptake. This implies that embryo would not be dormant but the enveloping structures restrained germination. Some researchers (Anderson 1968; Harrington 1923) agreed that glumes on the seeds inhibited germination, however others (Sistachs 1985; Taylorson 1969) showed that the removal of glumes had no effect on germination. The germination of the seeds was affected by dormant character and outer conditions. Our studies focused on the effect of glume on seed germination of *Sorghum halepense*.

### MATERIALS AND METHODS

Seeds of *Sorghum halepense* found or detected in Soybean imported from USA and Argentina in 1998. They were planted separately in isolated place and harvested their seeds in October 1998.

#### Methods

Experiment was conducted in April 1999. Four treatments as follow:

- (1) seeds of *S. halepense* (L.) Pers.
- (2) seeds removed of glumes of *S. halepense* (L.) Pers.
- (3) seeds removed of glumes plus glumes
- (4) seeds treated with concentrated sulfuric for 15 min, then rinsed with water for half an hour.

Germination was conducted in culture dish. 100 seeds collected from USA and 10 granules from Argentina in each treatment. Each treatment replicated twice. Germination rate was calculated when radicle and embryo bud grew out seed.

All treatments were put in the culture box and germinated in total darkness at constant temperature of 20°C.

## RESULTS AND DISCUSSION

Some seeds began to germinate after two days. The germination rates of each treatment were recorded and counted after 14 days. The results were put in Table 1 and Table 2:

Table 1. The seed germination rates of *Sorghum halepense* under different treatments(%) (USA).

Treatment	(1)seeds	(2)seeds removed of glume	(3)glume + seeds removed of glume	(4) treated with concentrated sulfuric
The rate of germination in repeat 1	0	80	2	73
The rate of germination in repeat 2	0	75	1	71
Average	0	77.5	1.5	72

From Table 1, it is concluded that the seed of from USA harvested after 7 months could not germinate, but the germination rate increased to about 77.5 percent when its glumes were removed. Then in treatment of seeds removed of glumes plus glumes, there was only 1.5 percent germination rate. In treatment of seeds treated with concentrated sulfuric acid for 15 min, the germination rate was 72%.

The average germination rate of seeds from Argentina was 0%, 75%, 0.5%, 70% in different treatment, similar as that from USA.

Usually, four main factors caused dormancy of seeds. Firstly, some seed embryo is undeveloped or hypogenesis. Secondly, some seed has hard seed coat which hinders embryo to absorb water and oxygen and also obstructs radicle and embryo bud to shoot out seed coat. Thirdly, there is a inhibitor or dormin in seed coat, which control the activities of enzyme and make seed dormancy temporarily. And fourthly, some seed is mature and will be germinated by a process of after ripening. In our experiment, seed of

Table 2. The seed germination rate of *Sorghum halepense* with different treatment(%) (Argentina)

Treatment	(1)seeds	(2)seeds removed of glume	(3)glume + seeds removed of glume	(4) treated with concentrated sulfuric
The rate of germination in repeat 1	0	80	1	70
The rate of germination in repeat 2	0	70	0	70
Average	0	75	0.5	70

*S. halepense* harvested after 7 months could not germinate. It showed that seed of *S. halepense* harvested after 7 months had high dormancy. Seeds removed of glume and seeds treated with concentrated sulfuric acid could germinate. This implied that the embryo was developed or not dormant and its germination needed not a processing of after ripening. When we put the glume into petri dish with seed removed of glume, the rate of germination was very lower. It showed there would be an inhibitor or dormin in the glume. The rate of germination of seeds treated with concentrated sulfuric acid was higher, because seeds glumes was broken by concentrated sulfuric acid and this inhibitor substance was cleared away by water or broken too.

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## WILD RICE BIOLOGICAL CHARACTERISTICS AND INTEGRATED CONTROL

W. M. Chen

Changzhou Agriculture Bureau, Changzhou, 213001, China

**Abstract:** Wild rice is a kind of dangerous weed in paddy field in many countries, which seriously inhibited rice growth and caused rice yield loss more than 50%. The dormancy, germination, reproduction, blooming, seeding, biological characteristics of wild rice varieties *Oryza rufipogon*, *O. barthii*, *O. punctata*, and *O. longistaminata* were observed for two years at Mubalali Farm in Tanzania. Germination rate of *O. rufipogon* seeds in 0-4cm depth of soil was more than 98% and under 4cm depth of soil very low. Compared with rice, wild rice had the characteristics such as rate of high seed reproduction, short growth period, quick ear coming, blooming, maturing and seed falling. *O. longistaminata* seeds had more than one-year dormant period with very low germination rate. *O. longistaminata* reproduced vegetatively on roots and stems mainly and its root system can reach more than 40cm depth when high soil moisture exists. Integrated control methods for wild rice are water flood crop and dry land crop rotation, germination induction, combined with chemical control. Encouraged germination of annual wild rice seeds, killing of wild rice seedling at 3-4 leaf stage by Gramaxone, integrated with direct seeding under non-tillage gave 96% control of *O. rufipogon* and other two species. The perennial wild rice *O. longistaminata* with buds induced at 5-6 leaf stages was controlled with Glyphosate, and combined with zero-tillage cowing, which had than 90% control result.

**Key words:** Biological characteristics, integrated control, wild rice

### INTRODUCTION

Wild rice is a troublesome weed of rice field in many countries. Dry seedbed preparation of rice field followed by direct seeding and delayed flooding result in heavy infestation of wild rice in Tanzania and other African countries. The author studied the wild rice problem in Mubalali Farm of Tanzania in 1986-1988 to search for efficient methods of control.

The Mubalali Farm is situated on the Ursangu plain of East African plateau (1040m above sea level) where annual mean temperature is 22.9°C, the rainy season there(Nov. to next April, also the growing period of rice) receives a precipitation of about 650mm. The area under rice is about 3000 ha and the soils are sandy loam in texture. Major rice varieties were IR8 and the native varieties-kibibi and klenbelo. Infestation of wild rice had caused the yield of rice to drop from 7-7.5t/ha to 3.5t/ha.

### MATERIALS AND METHODS

Habitat and infestation of wild rice were surveyed, morphological characteristics studied and the main species differentiated in Mubalali and other four farms.

Distribution of seeds of *O. rufipogon* in different depths of soil was investigated in

field (3 quadrats of 1/3 m<sup>2</sup> each, 4 soil layers). Dormancy period of seeds of *O. rufipongon* and *O. longistaminata* was studied in pot experiments (100 seeds sown in each pot, 4 replicates); germination ability of seeds of *O. rufipongon* in different depths of soil observed by pot experiment (100 seeds sown in each pot, 3 replicates) and field investigation. The tillering ability, flowering and seed maturation characteristics of *O. rufipongon* was studied in pot experiments (2 plants in each pot, 4 replicates).

Field trials were conducted to find out effective herbicides, application methods and integrated cultural measures to control *O. longistaminata* and *O. rufipongon*.

## RESULTS AND DISCUSSION

### Morphological characteristics, habitat and weediness of the four wild rice species

***O. Longistaminata*** Perennial, rhizomes may penetrate to 40-45 cm deep. Sexual reproduction of minor. Plant height 150-250 cm, auricle length up to 5cm, panicle length 35-45cm long, sterility very high. Length of awn 8-10cm, caryopsis purple brown, grain size 9-9.5mm × 2.8-3mm. Often occurred in clusters or patches, infestation heavy in low and damp fields with high water table level; patch density reached 80-450 seedling/m<sup>2</sup>, coverage reached 20-85%, causing 50-100% yield reduction.

***O. rufipongon*** Annual, the dominant wild rice species in Tanzania, plant height 120-150cm, panicle length 25-30cm, sterility low, high seed productivity. Awn 6-8cm long, pericarp purplish black, aleurone layer pink red, grain size 5-6mm × 1.8-2mm. May branch and form panicles from nodes, regeneration strong. Infestation serious, density reached 800-5000 seedlings/m<sup>2</sup>.

***O. barthi*** Annual, plant height 110-130cm, panicle length 25cm long, Awn 12-21cm long, pink or red in color, pericarp yellowish brown, grain size 9.5-11mm × 2.8-3mm. Sporadic occurrence with a tendency to spread.

***O. Punctata*** Annual, plant height 50-80cm, panicle length 15-18cm. Awn short or absent, pericarp brownish yellow, grain small, size 4-4.5mm × 1.5-2mm. Infestation low.

### Main biological characteristics of wild rice

**Distribution of seeds of *O. rufipongon* in cultivated soil layers** The weediness of wild rice is closely related to the amount and distribution of seeds in the cultivated soil layers. Field investigation showed that, in a soil sample of 1m<sup>2</sup>(area) × 15cm(depth), the number of wild rice seeds ranged from ten to thirty thousands. After harvest and before disk harrowing, 84.4% seeds remain in the 0-3cm surface layer of soil. Disk cultivation of soil helped seeds to move downward to the 3-15cm soil layer. Accumulation of vast amount of wild rice seeds in deeper layers of cultivated soil made infestation serious and control difficult.

**Flowering and maturing** The four wild rice species rapidly passed through flowering and starch transport stage. The growth duration of *O. rufipongon* lasted about 130 days, 30-40 days shorter than IR8. The period from start of inflorescence to seed maturation lasted only 14-15 days and starch transport stage only 10 days. Wild rice had strong reproductive ability and the habit of grain shattering readily before fully ripe. A single *O. rufipongon* plant produced 86 tillers, 38 panicles and over one thousand seeds in pot experiment.

**Seed dormancy** The seeds of wild rice species have strong and long term dormancy,

the length of dormancy period is different for different species. The seed dormancy period of *O. rufipogon* lasted 74/76-170 days; germination started from 74 and 76 days respectively in 2 experiments; reached the height of germination after 130 days; germination percentage was 88 and 89% respectively after 170 days. Seeds of *O. longistaminata* did not germinate within the year after ripening, length of dormancy period over one year.

Table1. Distribution of seeds of *O. rufipogon* in soil

Investigation time			0-3cm	3-6cm	6-10cm	10-15cm	Total
After harvest	Seeds/m <sup>2</sup>		8748	593	541	486	10368
Before tillage	%		84.4	5.7	5.2	4.7	100
After tillage	Seeds/m <sup>2</sup>		2490	2393	2694	1285	8862
Before sowing	%		28.1	27.0	30.4	14.5	100

**Germination of seeds** Pot experiment result showed that 98% seeds germinated within the 0-4cm soil layer, only 0.8% seeds germinated in the 4-15cm soil layer. Field investigation further proved that seeds of *O. rufipogon* germinated almost only in the 0-4cm layer, very few seeds germinated below the 4cm depth.

## Control of wild rice

### Control of perennial *O. longistaminata*

**Cultural control:** Field survey revealed that *O. longistaminata* infested seriously low and damp fields where the water table level was at about 0.5m; in fields with water table level below 1m, propagation and spread of rhizomes was restricted. The *O. longistaminata* is a hydrophilous plant, improve drainage system and lower the water table level to below 1m is an effective measure to control the spread of the wild rice.

**Chemical control:** The field was irrigated 40-50 days before sowing and soil kept moist for 30-35 days to induce regrowth of rhizomes and shoots. 2.5-3kg ai/ha glyphosate was applied when the shoots reached 5-6 leaf stage and food reserves in rhizomes exhausted (rice stubble burned off after harvest to facilitate spraying). The shoots and rhizomes were killed after 10-15 days and 20-30 days respectively. The field were cultivated and sown 35-40 days after herbicide application. Field investigation showed that after 2 successive years of control, the coverage of perennial wild rice was reduced from 80-90% to about 5%, the effect of control was over 90%.

### Control of annual wild rice

Seedbed was finely prepared by disking and tine harrowing about one month before sowing and irrigated 2 to 3 times to keep the surface soil moist for 25-30 days to stimulate germination of wild rice seeds. When seedlings reached 3-4 leaf stage (95% of seeds in the 0-4cm soil layer had germinated) 1-1.2kg ai/ha Gramaxone + 0.7-1kg ai/ha Ronstar tank mixture was sprayed. Rice seeds were direct seeded to a depth of 1-2cm by drilling machine under zero-tillage to avoid turning up of wild rice seeds from the deeper soil layers. Field was irrigated after sowing to promote germination of rice seeds and flood-irrigation established from 3.5 leaf stage of rice to check emergence of wild rice and other weeds. Encouraged early germination of wild rice seeds, killing of weed

seedlings by herbicides before planting, integrated with direct seeding under non-tillage gave 96% control of *O. rufipongon* and other two wild rice species. The control of barnyard grass and broad leaved weeds also exceeded 90%.

Rotation of rice with maize or grain sorghum integrated with application of atrazine or primagram also gave effective control of annual wild rice.

Wild rice species were differentiated with the help of reference material issued by the wild Rice Conference held by the Tanzania Ministry of Food and Agriculture in 1986. Wirjahardja (1981) described *O. rufipongon* as a perennial. According to Documenta (CEIBA GEIGY 1980) and Baker (1981) the dominant wild rice in Tanzania may be an annual variety of *O. rufipongon* called red rice.

The higher the percentage of stimulated germination of wild rice seeds or buds, the higher will be the effect of control. To increase the percentage of germination, the key point is to keep the soil moist for *O. longistaminta* and to improve the quality of seedbed preparation for annual wild rice species.

### LITERATURE CITED

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layer. The greatest amount of leaf area in all three weed species was absorbed in 50-75cm layer (Figure 3).

Wheat crops was significantly taller when competing with *Rapistrum rugosum* compared to control. This was because of lower light penetration through the canopy and undistruction of ouxin , internodes were longer and thus taller wheat crop was observed . The study of interaction effects between weed species and densities on wheat height , also indicated that turnip weed was the only weed species that significantly increased the wheat height (Figure 4) .

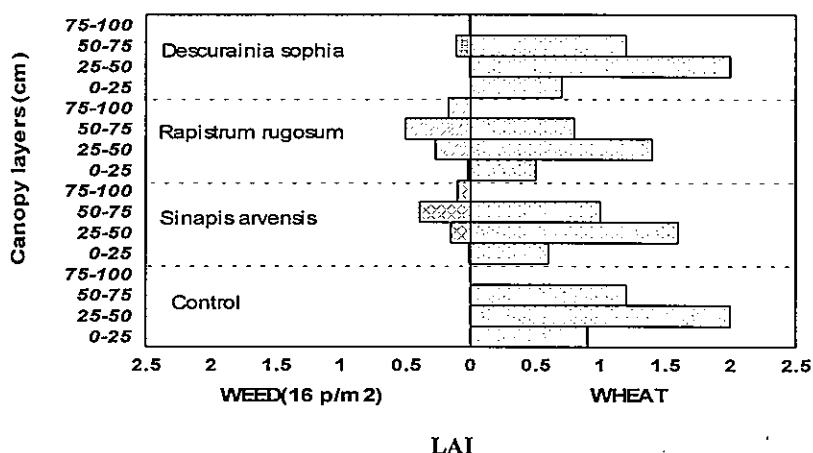


Figure 3. Leaf Area Distribution in Canopy Layers for Weed Species and Wheat

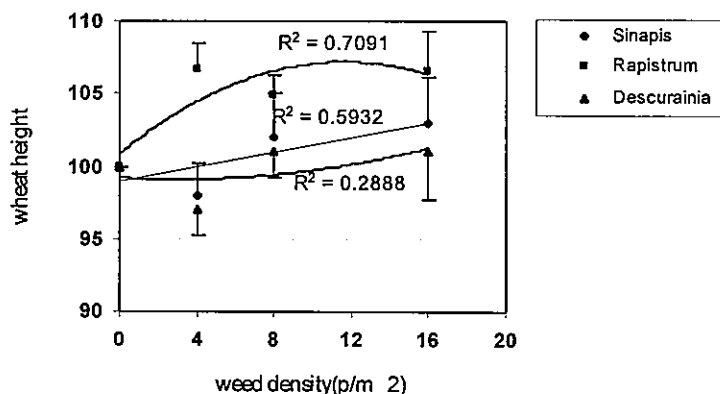


Figure 4. Effect of Weed Density and Weed Species on Wheat Height

The results of this experiment showed that there was a direct relationship between light penetration through the canopy with leaf area and its distribution within the canopy. It was also observed taller plants possessed higher leaf area in the upper canopy layers enabling it to be more successful in competition. The rate of light extinction was enhanced more in mixed canopy compared to pure wheat canopy (Figure 5a, 5b, 5c, 5d).

## **Competition and Allelopathy**

## EFFECT OF WEED PERSISTENCE ON GROWTH OF SUGAR BEET (*BETA VULGARIS*)

A. Awad<sup>1</sup>, A. M. Abd El-Wahab<sup>1</sup>, G.M. Yakout<sup>1</sup>, and N. A. Abo El-Kheir<sup>2</sup>

<sup>1</sup>Agronomy Department, Faculty of Agriculture - Suez Canal University Ismailia Governorate, Egypt.

<sup>2</sup>Institute of Efficient Productivity, Zagazig University Sharkia Governorate, Egypt.

**Abstract:** Two field experiments were carried out at Sharkia Governorate, Egypt during 1989/1990 and 1990/1991 seasons to study the response of growth sugar beet Raspoly cv. to persistence of weed and some weed control treatments. The study included 14 treatments; 4 treatments as hand weeding for 6, 9, 12 and 15 weeks from sowing sugar beet, 4 treatments as hand weeding after sowing with 6, 9, 12 and 15 weeks till harvesting, one treatment as hand weeding during all the growing season, two chemical weed control treatments by using pyramin + TCA as a mixture and Goltix, one treatment as hand hoeing, intercropping fenu-greek plants with sugar beet and the unweeded treatment. At 140 days from sowing, the experimental survey showed that sugar beet plants were infested by *Beta vulgaris* (wild chard), *Rumex dentatus*; L., *Polypogon monspeliensis*; L; *Sonchus aleracens*; L, *Lepidium sativum*; L, *Cynodon dactylon*; L and *Melilotus indica*; L. The number of chard plants were the highest species infested sugar beet plants. The reduction in number of broad and narrow leaved weeds, total number of weeds, fresh and dry weight of weeds as a result of applying hand weeding for 6, 9, 12 and 15 weeks from sowing or after 6, 9, 12 and 15 weeks till harvesting, hand weeding for all growing season and hand hoeing was more efficient than using the herbicides pyramin + TCA or Goltix. Intercropping fenugreek plants with sugar beet plants resulted in significant reduction in weed characters than using herbicides but less reduction than of hand weeding and hand hoeing treatments. The vegetative growth characters of sugar beet plants were measured at 150 days from planting. Number of leaves, fresh weight of leaves increased consistently as the period of keeping sugar beet plants free from weeds. Application of pyramin + TCA or Goltix was not efficient concerning the effect on vegetative growth characters of sugar beet plants.

**Key words:** Weed persistence, sugar beet

## INTRODUCTION

In Egypt sugarcane was the only source of sucrose sugar till 1982. Thereafter, the local sugar production from sugarcane became less than the consumption because of the over increases in population as well as to the increase in sugar consumption per capita. To solve this problem, introducing and growing sugar beet in the Northern areas of the country is being given big efforts from the government as well as from research institutions. In 1994, beet sugar represented about 10% from the total production of sugar in Egypt. Nowadays, a great plan to increase the areas under sugar beet is undertaken due to the ability of the crop to be cultivated on the new reclaimed lands as well as to its high water use efficiency as winter crop comparing with sugar cane.

Weeds are considered one of the main problems in sugar beet production. The competitive effect of weeds in sugar beet fields depends on their densities, species and

period of their presence to compete with the crop through the growth season (Weatherspoon and Schweizer; 1969, Hewson and Roberts; 1973, Dawson; 1977, Kropff et al; 1987 and 1992). In this respect, Zimdahl and Fertig (1967) mentioned that broad leaved weeds in sugar beet fields were more competitive than annual grasses. To eliminate weed competition to beet crop, it was recommended to keep the fields free of weeds for 4 weeks from beet emergence (Hewson and Roberts; 1973, Scott et al; 1979 and Wilcockson and Scott; 1981). However, Ivashchenko (1990) recommended that weeding should continued for 60-80 days after emergence. On the otherhand, Hewson and Roberts (1973) reported that the presence of weeds during the first 4 weeks after the emergence of red beet did not affect the crop provided that it was kept subsequently clean.

The efficiency of herbicides on weed control in sugar beet were studied by many researchers (Bejnar, 1973; Osman, 1978; Kolbe, 1984; Yashuk and Shabelnyl, 1984; Garnett, 1986; Ivashchenko, 1990 and Ferrero, 1993).

The influences of time of weed removal, application of other weed control treatments and use of herbicides on growth of sugar beet were studied by Scott et al (1979), Aly (1985) and Rost (1991).

## MATERIALS AND METHODS

Two field experiments were carried out at Shiba village, Zagazig district, Sharkia Governorate, Egypt during two successive seasons 1989/1990 and 1990/1991. To study the influence of density and persistence of weeds on growth of sugar beet (*Beta vulgaris*, L.) raspoly variety. This study includes 14 treatments as follow:

1-4 Hand weeding for 6, 9, 12 and 15 weeks from planting of sugar beet.

5-8 Hand weeding after 6, 9, 12 and 15 weeks from planting of sugar beet till harvesting.

9 Hand hoeing three times after 4, 8 and 12 weeks from sowing.

10 Hand weeding throughout the growing season.

11 Chemical weed control by using as a mixture from pyramin (5-aimno-4-chloro-2 phenyl- 3 (2H) pyridazinone + TCA (Sodium trichloroacetate).

12 Chemical weed control by using Goltix (4-amino-3- methyl-6-phenyl- 1, 2, 4-triazin-5- (4H)- one).

13 Planting fenugreek (*Trigonella foenum-graecum*) with sugar beet plants as a smother crop.

14 Unweeded treatment (control).

The rate of application of each herbicide was 2 kg pyramin, 4 kg TCA and 2 kg Goltex/fad. Water was used as dissolvent in rate of 200 L/fad. The three herbicides were used as pre-emergence. The 14 treatments were arranged in Randomized Complete Blocks design with 4 replications. The plot area was 12 m<sup>2</sup> and consisted of 6 rows, each row 4 m in length and 0.5 m in width. The proceeding crop in the two seasons was maize. Sugar beet was sown on Oct. 17<sup>th</sup> 1989 and 30<sup>th</sup> 1990 in the two seasons, respectively.

Fenugreek seeds were broadcasted after sowing sugar beet seeds and before irrigation. The other agronomic practices were followed as recommended. At 140 days from planting the weeds were hand pulled from 2 m<sup>2</sup> in each plot to determine weed characters, namely number and fresh weights of each total, broad and narrow leaved weeds. To study vegetative growth characters of sugar beet plant 5 guarded plants were



taken randomly at 150 days to determine the vegetative growth characters such as number of leaves/plant fresh and dry weight of leaves, petioles root and total plant, leaf area index (LAI).

The obtained data were statistically analyzed according to Sendecor and Cochran (1967) by using "MSTAT" computer program. The differences among averages of treatments followed by different letters were statistically significant, while the averages followed by the same letter or letters were differ insignificantly.

## RESULTS AND DISCUSSION

### Weed characteristics

#### Number and weed types

It was evident from field survey that the remaining weed species at 140 days from planting were *Beta vulgaris* (chard plants var. *cicla*), *Rumex dentatus*, L.; *Polypogon monspeliensis*, L.; *sonchus oleraceus*, L.; *Medicago hispida*, Gaertn.; *Portulaca oleraceae*, L.; *Lepidium Sativum*, L.; *Cynodon dactylon*, L. and *Melilotus indica*, L. However, chard plants (*Beta vulgaris* var *cicla*) was the highest prevailing species in both seasons. It represent 83.2% and 71.2% from the total number of weeds per faddan in first and second season, respectively.

At 140 days from sowing, the number of broad and narrow leaved weeds and total number of weeds were reduced as a result of applying different weed control treatments comparing with the unweeded treatment in both seasons except the treatment of hand weeding for 6 weeks from planting. The number of narrow leaved weeds was increased and reached to 106.3 and 165.3% in first and second season, respectively and also the treatment of hand weeding for 9 weeks from planting in the second season, whereas percentage of increments in narrow leaved weeds reached to 124.7% compared with the unweeded treatment (Table 1).

The treatments of hand weeding after 6,9, 12 and 15 weeks from planting (Treatments 5, 6, 7 and 8) and hand weeding for all growing season (Tr.12) were more efficient in reducing the number of weeds (Table 1). Using the mixture from pyramin +TCA (Tr.9) was more efficient in controlling the weeds compared with Goltix herbicide (Tr.10) in the two seasons. At the same time, applying the herbicides reduced the number of narrow-leaved weeds more than the other treatments in both seasons (Table 1).

For the effect of the intercropping fenugreek plants with sugar beet plants, the data in Table 1 show that the number of both types of weeds and their total number were reduced whereas the percentage of total number of weeds represent 28.69% and 61.12% for first and second season, respectively in comparison with the unweeded check (Tr.14). So, it could be concluded that the efficiency of herbicides or intercropping fenugreek plants with sugar beet plants in controlling the existence weeds were less than the treatments of hand weeding either for 6, 9, 12 and 15 week or after 6, 9, 12 and 15 weeks or hand hoeing 3 times and hand weeding for all season (Table 1).

These results are in agreement with those reported by Ivashchenko (1990), and Ferrero (1993).

#### Fresh weight of broad leaves weeds

The fresh weight of broad leaved weeds at 140 days from planting of sugar beet were decreased significantly as a result of applying different treatments of weed control under this investigation in both seasons (Table 2). The highest reduction was attained by

applying the treatments of hand weeding after 6, 9, 12 and 15 weeks from planting sugar beet as well as with hand weeding through all growing season and the differences among these five treatments were not significant. The fresh weight of broad leaved weeds significantly was reduced by using a mixture from two herbicides pyramine + TCA or Goltix and sowing fenugreek with sugar beet but these reductions were less than of the other treatments. The percentages of reduction in fresh weight of broad-leaved weeds in the first season were 32.59%, 29.58% and 52.17%, respectively and 12.79%, 7.66% and 47.71% in second season, respectively. The wild beet represented the main weed species growing with sugar beet plants (Table 1). Both of sugar beet plant and wild beet followed the same species and that referred to the less efficiency of herbicides in controlling broad-leaved weeds. This could interpret the high fresh weight of remained weeds in plots treated with herbicides.

Table 1. Effect of weeding treatments on number of weeds surviving after 140 days from sowing in 1989/90 and 1990/91 seasons (thousands/fad.).

Treatments	1989/1990 season			1990/1991 season		
	No. of broad leaves	No. of narrow leaves	Total no.	No. of broad leaves	No. of narrow leaves	Total no.
HW for 6 WFP	115.4	85.7	201.1	155.6	158.7	314.3
HW for 9 WFP	64.5	44.5	109.0	94.2	119.7	213.9
HW for 12 WFP	44.1	28.6	72.7	63.5	76.2	139.7
HW for 15 WFP	37.2	19.7	56.9	44.1	18.1	62.2
HW after 6 WFP	15.0	11.7	26.7	11.8	8.4	20.2
HW after 9 WFP	11.7	13.8	25.5	11.0	6.3	17.3
HW after 12 WFP	12.9	11.7	24.6	8.1	6.3	14.4
HW after 15 WFP	17.6	10.4	28.0	5.5	7.6	13.1
Pyramin + TCA	207.4	4.2	211.6	461.3	3.4	464.7
Goltix	258.6	9.2	267.8	488.4	4.2	492.6
HH (3 times)	76.8	26.4	103.2	50.9	24.3	75.2
HW for all season	13.4	12.5	25.9	7.3	6.3	13.6
Fenugreek + sugar beet plants	239.1	31.9	271.0	352.8	11.7	364.5
Unweeded (control)	863.9	80.6	944.5	500.3	96.0	596.3

HW-hand weeding; WFP: weeks from planting; HH-hand hoeing.

Combining fenugreek with sugar beet significantly reduced fresh weight of broad-leaved weeds. The weight of broad leaved weeds represents 47.82 and 52.29% comparing to the unweeded control. This significant reduction in weight of broad leaved weeds could be attributed to the high competition between fenugreek plants and weeds. These results are in harmony with those reported by Sullivan et al., (1965), Bejnar (1973), Siwicki and Kriese (1980); Yashuk and Shabel'nyl (1984), Garnett (1986) and Ivashchenko (1990).

#### **Fresh weight of narrow leaved weeds**

Table 2 indicates that fresh weight of narrow leaved weed decreased significantly with applying different weed control treatments. The highest reductions in the first season resulted with applying the herbicides Pyramin + TCA or Goltix which gave nearly full elimination for these weeds at this age (140 days). In the second season that

was obtained with hand weeding for 15 weeks from planting, hand weeding after 6, 9, 12, 15 weeks from planting till harvesting, hand weeding throughout the season or combining fenugreek as smother crop with sugar beet crop and the differences among these treatments were not significant. These results are in accordance with the findings of Satarov et al (1981), Kolbe (1984), Yashuk and Shable'nyl (1984).

Table 2. Effect of weeding treatments on fresh and dry weight of weeds after 140 days after sowing in 1989/90 and 1990/91 seasons (kg/fad.).

Treatments	1989/90 season			1990/91 season		
	Broad leaves	Narrow leaves	Total weeds	Broad leaves	Narrow leaves	Total weeds
HW for 6 WFP	4728.8e	398.7 b	5127.5 e	5150.0e	725.0 b	5875.0 e
HW for 9 WFP	1449.8g	135.3 c	1585.1 g	3315.0f	560.0 c	3875.0 f
HW for 12 WFP	405.0h	34.4 e	439.4 h	422.0h	265.0 d	1687.0 h
HW for 15 WFP	166.3i	21.0 gh	186.3 i	630.0i	127.5 e	757.5 i
HW after 6 WFP	46.2j	25.8 f	72.0 j	4.3j	11.3 f	15.6 j
HW after 9 WFP	44.9j	33.6 e	77.5 j	4.8j	8.0 f	12.8 j
HW after 12 WFP	30.9j	28.0 f	57.9 j	9.8j	8.0 f	17.8 j
HW after 15 WFP	37.5j	20.2 gh	57.7 j	7.0j	10.5 f	17.5 j
Pyramin + TCA	13020.0c	13.4 i	13033.4 c	1360.0c	3.0 f	1363.0 c
Goltix	13602.0b	9.5 j	13611.5 b	14400.0b	4.0 f	14404.0 b
HH (3 times)	2392.3f	123.2 d	2512.3 f	1585.0g	215.0 d	1800.0 g
HW for all season	34.5j	8.4 h	52.9 j	5.8j	5.0 f	10.8 j
Fenugreek + sugar beet plants	9237.5d	22.0 g	9259.3 d	8155.0d	16.5 f	8171.5 d
Unweeded control	19315.0 a	424.9 a	19739.9 a	15595.0a	900.0 a	16495.0 a

HW-hand weeding; WFP: weeks from planting; HH-hand hoeing.

### Fresh weight of total weeds

The data in (Table 2) show that, at 140 days from sowing sugar beet, the highest reduction in total fresh weight of weeds at both seasons was attained when hand weeding took place after 6, 9, 12 and 15 weeks from planting until harvesting as well as by hand weeding throughout the growing season which gave nearly complete weed control. Meanwhile, data also show that the total fresh weight of weeds at 140 days from planting decreased as the period of hand weeding after planting had been prolonged up to 15 weeks. These results could be explained on the light of the decrease in total number of remained weeds as the period of hand weeding after planting was increased.

The two herbicidal treatments, pyramin + TCA mixture and Goltix gave little but significant reductions in this respect at both seasons. This reduction may be attributed to the prevailing of the wild beet (*Beta vulgaris*) which is resistant to the studied herbicides.

Combining fenu-greek with sugar beet plants depressed the growth of weeds and reduced their total fresh weight by about 53.09% and 50.46% after 140 days in both seasons, respectively. This could be attributed to the competition of fenu-greek plants to weeds. These results are in accordance with those obtained by Luijendijk (1965), Bejnar (1973), Siwicki and Kriese (1980), Satarov et al., (1981); Wilcockson and Scott (1981); Yashuk and Shabel'nyl (1984), Kvasov (1988), Osman et al. (1989), Derylo (1991) and Cioni (1992).

### Sugar beet plant growth characteristics

The averages of the studied vegetative growth characters, namely, number of leaves/plant, fresh and dry weights of leaves, petioles, root and total weight/plant as well as LAI at 150 days from planting are given in Tables 3 and 4 for two seasons, respectively.

Table 3. Effect of weeding treatments on some growth characteristics of sugar beet plant at 150 days from sowing in 1989/90 season.

Treatments	Number of leaves per plant	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)	Fresh weight of petioles (g/plant)	Dry weight of petioles (g/plant)
HW for 6 WFP	34.94 e	375.5 d	43.57 e	223.78 d	24.61 d
HW for 9 WFP	36.66 d	570.8 c	65.76 d	373.03 c	41.03 c
HW for 12 WFP	38.31 c	683.1 b	79.03 c	422.80 b	46.50 b
HW for 15 WFP	43.02 a	746.2 a	86.38 b	459.75 a	50.57 a
HW after 6 WFP	37.43 cd	661.6 b	75.93 c	451.23 a	49.63 a
HW after 9 WFP	31.33 f	390.9 d	45.44 e	228.08 d	25.09 d
HW after 12 WFP	26.73 g	244.4 e	27.99 f	153.70 e	16.91 e
HW after 15 WFP	23.10 hi	199.6 f	23.50 g	115.23 f	12.67 f
Pyramin + TCA	24.47 h	189.7 f	22.08 g	108.65 fg	11.95 fg
Goltix	20.77 j	169.5 fg	19.78 gh	93.45 fg	10.28 fg
HH (3 times)	40.60 b	771.1 a	88.75 ab	462.50 a	50.90 a
HW for all season	44.23 a	780.0 a	90.47 a	470.00 a	51.70 a
Fenugreek + sugar beet plants	22.77 i	183.9 f	21.41 g	104.63 fg	11.51 fg
Unweeded control	20.14 j	148.9 g	17.27 h	88.17 g	9.70 g

Table 3. Continued.

Treatments	LAI	Root fresh weight (g/plant)	Root dry weight (g/plant)	Total fresh weight (g/plant)	Total dry weight (g/plant)
HW for 6 WFP	3.35 e	333.3 g	71.99 g	708.8 g	115.93 g
HW for 9 WFP	4.37 d	466.6 e	100.61 e	1037.4 e	166.56 e
HW for 12 WFP	5.17 c	641.6 d	138.57 d	1324.7 d	217.68 d
HW for 15 WFP	5.69 b	1025.0 b	221.38 b	1771.2 b	307.75 b
HW after 6 WFP	4.65 d	816.1 c	176.27 c	1477.6 c	252.18 c
HW after 9 WFP	3.40 e	379.9 f	82.07 f	770.9 f	127.48 f
HW after 12 WFP	2.12 f	221.1 h	47.76 h	465.5 h	75.98 i
HW after 15 WFP	1.98 fg	191.6 i	41.39 i	390.3 i	64.63 j
Pyramin + TCA	1.89 fg	173.5 ij	37.47 ij	363.2 ij	59.58 h
Goltix	1.78 gh	152.9 jk	33.02 jk	322.4 jk	52.78 k
HH (3 times)	6.02 ab	1028.5 b	221.80 b	1807.2 b	310.48 b
HW for all season	6.16 a	1102.2 a	238.07 a	1882.2 a	328.55 a
Fenugreek + sugar beet plants	1.85 fgh	145.9 kl	31.52 kl	329.8 j	52.90 k
Unweeded control	1.51 h	124.5 l	26.89 l	273.3 k	44.15 l

HW-hand weeding; WFP: weeks from planting; HH-hand hoeing.

It is evident that as the period of keeping sugar beet plants free of weeds was prolonged up to 15 weeks from planting, the averages of the studied growth characteristics increased consistently and significantly and that was true for all traits in the two seasons except number of leaves in the second season did not differ significantly with keeping the crop free from weeds for 9, 12 or 15 weeks. However, comparing with the

weeding check (hand weeding throughout the growth season), hand weeding for 15 weeks from planting resulted plants with significantly less vegetative growth as expressed by total dry weight per plant (6.33% and 9.32% in both seasons, respectively). This could be attributed to the competition of the new emerged weeds at

Table 4. Effect of weeding treatments on some growth characteristics of sugar beet plant at 150 days from sowing in 1990/91 season.

Treatments	Number of leaves per plant	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)	Fresh weight of petioles (g/plant)	Dry weight of petioles (g/plant)	LAI
HW for 6 WFP	37.50 bc	373.8 e	43.34 f	225.00 e	24.75 e	3.28 f
HW for 9 WFP	41.25 ab	525.0 c	60.49 d	343.75 c	37.83 c	3.97 e
HW for 12 WFP	42.38 a	680.0 b	78.64 g	423.75 b	46.61 b	5.09 c
HW for 15 WFP	42.63 a	735.0 a	85.07 b	453.75 ab	49.91 ab	5.59 b
HW after 6 WFP	41.00 ab	650.0 b	74.59 c	443.75 ab	48.81 ab	4.31 d
HW after 9 WFP	35.25 c	449.0 d	52.17 e	264.00 d	29.04 d	3.87 e
HW after 12 WFP	29.33 d	231.3 f	26.76 g	143.75 f	15.82 f	2.02 g
HW after 15 WFP	28.00 de	170.0 g	19.73 h	95.00 g	10.45 g	1.99 g
Pyramin + TCA	26.88 de	156.3 g	18.18 h	90.00 g	9.89 g	1.65 h
Goltix	24.13 ef	149.8 g	17.43 hi	86.25 g	9.48 g	1.58 h
HH (3 times)	44.15 a	766.3 a	88.88 ab	460.00 a	50.60 a	6.09 a
HW for all season	43.00 a	773.3 a	89.68 a	465.25 a	51.17 a	6.12 a
Fenugreek+sugar beet plants	22.38 f	115.5 h	18.34 h	91.25 g	10.06 g	1.76 g
Unweeded (control)	27.15 de	175.5 g	13.42 i	67.75 g	7.45 g	1.26 i

Table 4. Continued.

Treatments	Root fresh weight (g/plant)	Root dry weight (g/plant)	Total fresh weight (g/plant)	Total dry weight (g/plant)
HW for 6 WFP	328.8 f	71.01 f	702.5 g	114.35 g
HW for 9 WFP	427.5 e	92.37 e	952.5 e	152.86 f
HW for 12 WFP	643.8 d	139.05 d	1323.8 d	217.69 e
HW for 15 WFP	1012.5 b	218.71 b	1747.5 b	303.77 c
HW after 6 WFP	800.0 c	172.80 c	1450.0 c	247.39 d
HW after 9 WFP	432.5 e	93.42 e	881.5 f	145.59 f
HW after 12 WFP	212.5 g	45.90 g	443.8 h	72.66 h
HW after 15 WFP	167.5 h	36.18 h	337.5 i	55.91 i
Pyramin + TCA	145.0 i	31.37 i	301.3 ij	49.55 ij
Goltix	122.0 j	26.35 j	271.8 j	43.78 j
HH (3 times)	1031.3 b	222.75 b	1797.5 b	311.63 b
HW for all season	1093.0 a	236.09 g	1866.3 a	325.77 a
Fenugreek + sugar beet plants	121.3 j	26.20 j	278.8 j	44.54 j
Unweeded (control)	97.8 k	21.20 k	213.3 k	34.54 k

late of the season. On the other hand, data in Tables 4 and 5 indicate also that number of leaves, fresh and dry weight of leaves, petioles and root/plant, LAI and total fresh and dry weight of beet plant dropped sharply and significantly as the period of weed competition to the crop after planting was prolonged from 4 up to 15 weeks after planting. Comparing with hand weeding throughout the season, total dry weight/plant decreased by 23.24%, 61.99%, 76.87% and 80.33% as weeds were allowed to compete with the crop for 6, 9, 12 and 15 weeks after planting, respectively. Meanwhile, total dry weight of plant under these four treatments overweighed that of the unweeded control by 458.25%, 188.74%, 72.10% and 46.39%, respectively. It is worthy to mention that the entire competition from weeds to beet plants throughout the growth season (the unweeded control) severely affected the all studied characteristics. Comparing with the weedy check, decrements attained 54.6% and 36.9% in number of leaves/plant, 80.95 and 85.0% in leaves dry weight/plant 75.5% and 79.4% in LAI and 86.6% and 89.4% in total dry weight/plant in the two seasons, respectively. Hand hoeing 3 times early in the season did not differ significantly with the treatment of keeping beet plants free from weeds for 15 weeks concerning the effect on most of the studied vegetative growth characteristics. Furthermore, hand hoeing resulted plants slightly less but significant in their fresh and dry weight comparing with the weedy check. The decreases attained 3.98% and 3.68% in fresh weight/plant and 5.50% and 4.35% in total dry weight/plant in the two seasons, respectively. The two herbicidal treatments Pyramine + TCA and Goltix did not give satisfactory results in this respect. They did not differ significantly from each other as well as from the treatment of keeping weeds to compete the crop for 15 weeks after planting. Moreover both treatments resulted plants surpassed slightly although significantly those of the unweeded control in the fresh and dry weight/plants (Tables 3 and 4). This could be explained on the light of poor weed control resulted by both herbicidal treatments due to the dominance of the wild beet (chard) which is tolerant to the studied herbicides. Also, it is evident from the same tables, that combining fenugreek with sugar beet did not enhance the growth of beet plants due to the poor weed control with applying such treatment.

These results could be interpreted by the competition of weeds to beet plants as well as the duration of this competition. They are in agreement with the results obtained by Scott et al (1979) and Aly (1985).

Finally, it could be concluded that under the condition of this study, the minimum effects of weeds competition on the growth of beet plants could be attained by keeping beet plants free from weeds after 15 weeks from planting till harvesting as well as hand hoeing 3 times.

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## MEASURING CROP COMPETITIVENESS AND IDENTIFYING ASSOCIATED TRAITS IN CULTIVAR FIELD TRIALS

B.P. Caton<sup>1</sup>, T.C. Foin<sup>2</sup>, J.E. Hill<sup>1</sup>, and A.M. Mortimer<sup>1</sup>

<sup>1</sup> International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Philippines.  
b.caton@cgiar.org

<sup>2</sup> Agronomy and Range Science, University of California, Davis, CA, 95616

**Abstract:** Despite many years of research, very few crops have been bred for weed competitiveness. In most studies, crop varieties have been compared in competition with weeds in field trials. Objectives are usually to (1) assess differences in competitive ability between cultivars, and (2) find traits that confer competitiveness. Using published results for two major crops, rice and wheat, we evaluated whether those objectives were consistently met. For the first objective, differences in competitiveness were found in every study, but measures varied. Competition may be measured as either "crop tolerance" (CT), the response of the crop to weeds, or "weed suppression" (WS), the crop effect on weeds. Linear regressions of measures of CT and WS gave all possible responses: positive, negative, and neutral. Those results support the following ideas. First, yield and competitiveness are not always negatively correlated. Second, and more importantly, CT and WS responses are inseparable, and both are needed to identify the most competitive cultivars. For the second objective, in each study some traits were reported to be associated with competitiveness. However, most were state variables like crop drymass, which reflect competitive success, rather than parameters that affect growth and competition, such as root-shoot ratios or height extension rates. Cultivar trials seem useful for objective 1, especially if both CT and WS are measured, but are less appropriate for objective 2 without careful planning and a high labor capacity. To meet objective 2, using separate studies to quantify differences in cultivars and identify important traits may be a simple improvement in methodology. For trait identification, testing hypotheses about isolated trait effects will be best.

**Key words:** breeding, crop tolerance, rice, weed suppression, weed competition, wheat

### INTRODUCTION

The importance of crop interference with weed growth as a weed management tool has been known for decades, and variation in competitive ability has been found for many crops (Berkowitz 1988; Callaway 1992). Despite this, breeding for crop competitiveness against weeds is rare, while breeding for resistance to other pests, such as diseases and insects, is very common (Callaway 1992). This is due in part to farmers' ability to control weeds, compared to other pests, even if by hand removal. It may also be due the relatively high efficacy of most herbicide, tillage and rotation-based weed management systems, and the lack of understanding about which traits confer competitiveness (Pester et al. 1999). Moreover, this research is difficult, because competition is dynamic (Goldberg 1997) and outcomes vary with changes in management, environment, and season (e.g., Cousens and Mokhtari 1998) that may not be easily controlled.

Researchers have predominantly studied crop competitiveness by growing cultivars (here, this includes both varieties and lines) with weeds in field trials. Such trials usually have two objectives: (1) to assess whether varieties differ in competitiveness, and (2) to

identify important competitiveness traits.

The first of those requires competitive ability to be estimated from crop or weed growth data (or both) (Goldberg 1997). The crop response, or 'tolerance' (CT), indicates ability to maintain shoot or seed drymass (DM) in competition. Weed growth, or 'suppression' (WS), indicates ability to reduce weed shoot or seed DM. Apparently, no measurement standard exists: CT and WS were both measured in only 16 of 40 studies on rice, maize, soybean, and wheat (compiled from Callaway 1992), and were about equally split in other trials (CT = 13 and WS = 11). Callaway (1992) noted that the choice of CT or WS or both was rarely justified. Pester et al. (1999) called this a disagreement over which response should be improved. This may be overstated, but arguments for and against each have been made (e.g., Callaway and Forcella 1992; Jordan 1993), based on the idea that a tradeoff exists between CT and WS (e.g., Jennings and Aquino 1968).

In general terms, crop traits conferring increased competitiveness must (1) increase crop resource capture, (2) decrease weed resource capture, and (3) have neutral or positive effects on crop resource use. For identifying such traits (objective two), crop responses are usually measured and correlation or path analysis is used to estimate contributions to competitiveness. Hypothesis testing for particular trait effects, such as comparisons of isogenic lines (e.g., Seefeldt et al. 1999) has rarely been used.

After almost forty years of research (Callaway 1992), most efforts to improve crop competitiveness have been unsuccessful. The objective here was to assess whether the design in field trials has been appropriate for the experiment objectives. We collected published data sets from cultivar field trials for two major crops, rice and wheat. Using these, we evaluated how CT and WS responses have been used to quantify competitiveness, and how well relevant traits have been identified in these studies.

## MATERIALS AND METHODS

Published data sets with five or more cultivars and both crop and weed DMs were identified. Eight studies were used here, four each for rice and wheat. We used only data from treatments with the greatest weed abundance. For Ogg and Seefeldt (1999), data was interpolated from figures. For results to be comparable, we calculated CT and WS responses relative to mean crop and weed DMs ( $X_i$  where  $i$  denotes crop or weed). Responses by cultivar ( $j$ ) were fractional DMs of crops ( $F_C$ ) and weeds ( $F_W$ ) as follows:

$$F_{Cj} = DM_{Cj} / X_C \quad (1)$$

$$F_{Wj} = X_W / DM_{Wj} \quad (2)$$

so that values greater than 1 indicated better performance than the mean. Linear regressions of  $F_C$  on  $F_W$  were done for each year or season of data.

## RESULTS AND DISCUSSION

### Measuring cultivar competitiveness

Competitiveness differences were reported in every study, but methods varied for determining which cultivars were best. For example, Wicks et al. (1986) ranked cultivars based on visual estimates of percent weed control at harvest from one of two years, while Garrity et al. (1992) used two-year mean weed DM. None used a formal ranking based on both crop and weed data.

Moreover, we found no single, simple relationship between CT and WS (Figures 1 and 2). Positive, negative, and non-significant regressions all occurred. In rice trials, regressions often were significant and positive (Figure 1). The exceptions were nonsignificant regressions in 1993 from Fofana et al. (1995) (Figure 1b), and in 1995 from Fofana and Rauper (2000) (Figure 1c). In wheat trials, positive regressions were found for Balyan et al. (1991), Wicks et al. (1986), and Ogg and Seefeldt (1999) in 1993 (Figure 2acd). Data from Challaiah et al. (1986) gave a negative regression (Figure 2b), and the regression for Ogg and Seefeldt (1999) in 1994 was nonsignificant (Figure 2c).

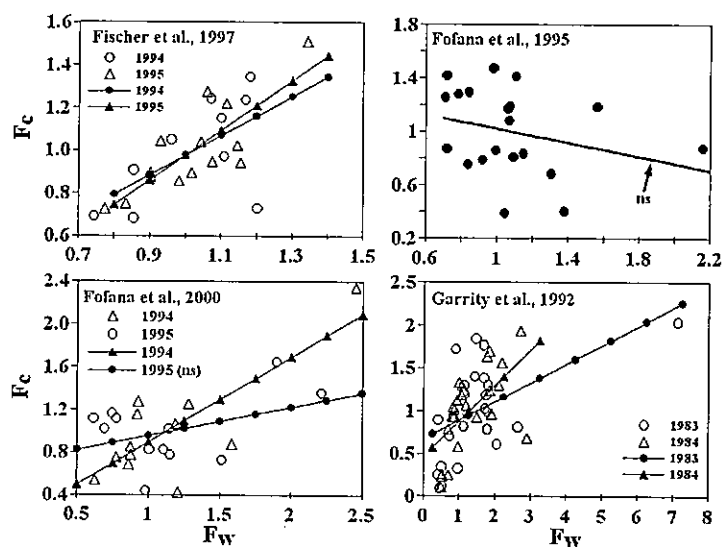


Figure 1. Competitiveness of cultivars for rice-weed competition by year, with observed data and linear regressions (lines) of weed suppression ( $F_w$ ) and crop tolerance ( $F_c$ ). See text for mathematical definitions of  $F_w$  and  $F_c$ .

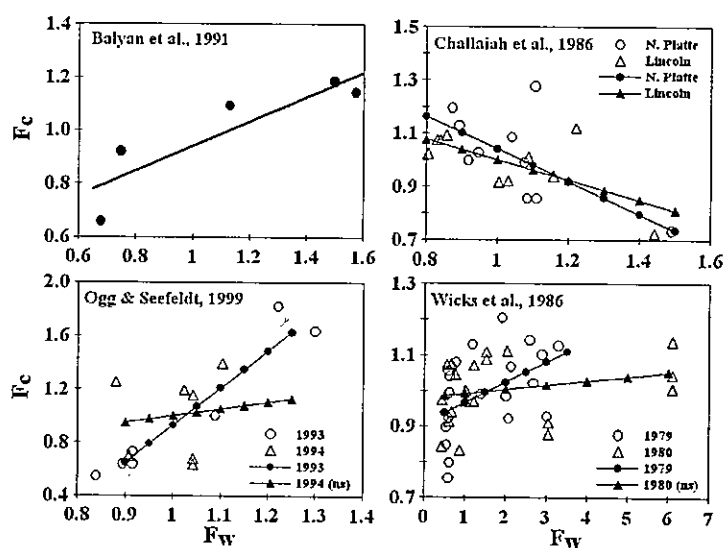


Figure 2. Cultivar competitiveness in wheat-weed competition, with observed data and linear regressions of weed suppression ( $F_w$ ) and crop tolerance ( $F_c$ ) by year of study.

An understanding of competitive interactions—increased crop growth feeds back to decreased weed growth and vice versa—is sufficient to argue that CT and WS responses are dynamic and inseparable. Crop-weed simulation studies also indicate that outcomes depend on interacting factors (e.g., Bastiaans et al. 1997; Caton et al. 1999, 2001). The results above provide empirical evidence of this. For example, cultivar differences in maximum wheat height (Challaiah et al. 1986) gave a negative regression, because taller crop plants often have lower harvest indexes and increased lodging. In contrast, differences in early season height growth rates of wheat (Ogg and Seefeldt 1999) gave a positive regression, probably because that trait did not adversely affect wheat resource use. Lastly, a neutral relationship may result from trait improvements that affect either resource capture by the crop or the weed, but not both. An example is increased crop duration (Smith 1974), which may not always decrease weed resource capture. They may also be caused by a lack of consistent trait differences, e.g. the broad range of rice genotypes used in 1993 by Fofana et al. (1995).

It has therefore been a mistake for weed scientists to focus solely on either CT or WS. Both must be measured to identify the most competitive cultivars. In figure 3, cultivar (2) is much superior to both (1) and (3), but both CT and WS are needed to clearly distinguish (2) from the others. In theory, the most competitive cultivars will be found toward the upper right of the CT vs. WS plot. This is at least more objective than the methods used to rank cultivars in the reviewed studies.

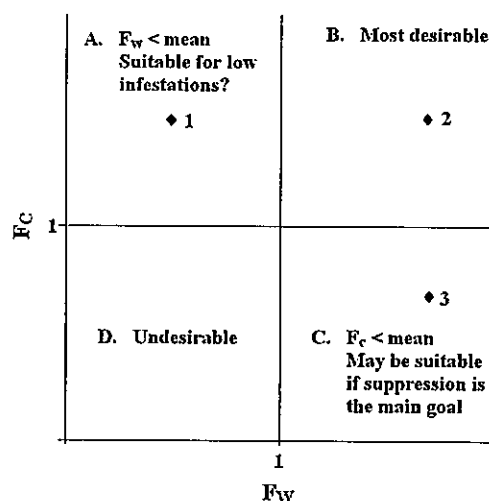


Figure 3. General differences in cultivars for weed suppression ( $F_w$ ) and crop tolerance ( $F_c$ ). Only cultivars in quadrant (B) were better than average for both responses. Example cultivar (2) is much more competitive than (1) and (3).

It also follows that tradeoffs between CT and WS are not inherent. If all three goals for trait selection are met (see introduction), both CT and WS will improve. If they are not all met, only one response will improve. A failure to understand this probably led to conclusions that rice yields and weed suppression were inversely related (e.g., Jennings and Aquino 1968), which recent studies have refuted (e.g., Fischer et al. 1997; Gibson et al. 2000; Johnson et al. 1998). Unfortunately, that mistake hindered research on rice competitiveness (e.g., Nantasomsaran and Moody 1995) and perhaps other crops too.

As defined here, the limits of  $F_C$  and  $F_W$  are  $0 < F_i < \infty$ , but the upper limit is unlikely to be higher than about 10, or a ten-fold increase or reduction in DMs. In practice, large reductions in weed DM seem more likely than large increases in crop DM, so maximum  $F_W$  values may be higher than those for  $F_C$  (e.g., Figures 1d and 2d). An alternative formulation might be based on one or more standard cultivars, which would allow absolute levels of improvement in competitiveness to be estimated.

Together,  $F_C$  and  $F_W$  may be used to quantify total crop competitiveness. Callaway and Forcella (1992) proposed the following index for this:

$$C_1 = (a \times F_C) \times (b \times F_W) \quad (3)$$

where  $C_1$  estimates total crop competitiveness, and  $a$  and  $b$  are weighting coefficients. We know of no reason to weight  $F_C$  and  $F_W$  differently, since the values of  $a$  and  $b$  would likely be set arbitrarily, but it might be useful to include fractional weed-free crop yield ( $F_{YWF}$ ) (relative to mean weed-free yield) as follows:

$$C_2 = F_C \times F_W \times F_{YWF} \quad (4)$$

$C_2$  gives additional information about the risk of low yields if weeds are not present. In our tests (not shown), cultivar rankings were not different when based on shoot DMs instead of seed DMs, and LAIs or other state variables could be substituted for DMs.

The use of  $F_C$ ,  $F_W$ , and indices arises because competitive outcomes are measured as two separate responses. It may be difficult to capture that complexity in a single number, but the important point is that both responses matter. Using strict mathematical definitions may also help scientists avoid misinterpretation or perceived bias. For example, some authors have equated competitive ability with 'tolerance' (e.g., Callaway 1992; Callaway and Forcella 1992; Cousens and Mokhtari 1998). That usage may be confused with other forms of crop tolerance, such as drought or submergence tolerance, and terminology that is more precise should be used instead.

### Identifying competitiveness traits in cultivar trials

**Rice** The character most often reported as positively correlated with WS was LAI (Fischer et al. 1997; Fofana and Rauper 2000; Garrity et al. 1992). Other traits identified were tiller density, light interception (Fischer et al. 1997), root length density ( $\text{m m}^{-3}$ ) (Fofana and Rauper 2000), and plant height (Garrity et al. 1992).

**Wheat** Traits associated with WS were early height and DM (Balyan et al. 1991), height gain rate (Ogg and Seefeldt 1999), maximum height, tiller density, and light interception (Wicks et al. 1986), and maximum height (Challaiah et al. 1986).

With a few exceptions, measured 'traits' were the effects rather than the causes of competition. For example, DMs and LAIs indicate successful capture and utilization of resources, but not why. They result from traits like specific leaf area and shoot-root ratios, which were often unmeasured. In modeling terms, state variables were measured instead of parameters, so the causative factors remain unknown. Hence, only three traits identified above seem useful: early height (Balyan et al. 1991), height gain rate (Ogg and Seefeldt 1999), and root length density (Fofana and Rauper 2000). (Maximum height and tiller density were not included because the former negatively affects crop resource use, and it is unclear how tiller density affects resource capture and use.) Thus, the success rate for identifying competitiveness traits in these trials was extremely low.

Problems inherent to competition field studies, such as high interplot variation (Wicks et al. 1986), contributed to this lack of success. Another was seasonal or annual variation in management, environment, or biological factors (Fofana and Rauper 2000; Ogg and Seefeldt 1999; Wicks et al. 1986). A tradeoff also exists between numbers of cultivars and replications, and study size may limit the number of traits that can be reliably measured. Still, the choice of improper response variables was most important.

To identify important traits in these trials (objective 2), researchers tried to do two things at once: (1) quantify differences in cultivars for certain traits, and (2) determine which of those traits were important (in cultivars that might differ in multiple ways). A simple improvement in methodology may be to separate these two activities. The first may best be done in competitive growth analyses on several representative varieties. Using controlled environment studies may be important to reduce variation, while considering genotype-by-environment interactions if necessary.

The second activity is more difficult and more likely involves hypothesis testing. Ideally, isogenic lines, which differ by only a single trait, would be compared, but this may be impossible in most programs. However, researchers can use growth analysis results to compare cultivars that differ in only a few traits. Growth analysis results can also be used to parameterize models, for tests of whether known differences account for observed outcomes. One might also determine for which resources crops and weeds compete, and then test the effects of related traits. One advantage of that is that such understanding is more easily extrapolated to new cropping situations.

Crops can also be selected for other traits to improve weed management. For example, submergence tolerant rice varieties may improve weed suppression by water management. Hence, comparing alternative breeding objectives may be important.

## Conclusions

Weed scientists can benefit by applying a better understanding of the dynamics of crop-weed competition (Berkowitz 1988; Cousens 1991; Goldberg 1997), and more informed experimentation to the study of crop competitiveness. It may be better to use separate experiments to quantify differences in cultivar growth in competition, and test the effects of traits on competition (perhaps contiguous to growth analyses). Cultivar field trials may become important only after improved genotypes have been developed, for large-scale evaluations. Such trials could focus on practical, bioeconomic impacts of improvements in competitiveness. Field trials may still be useful for the simpler objectives of ranking released cultivars, or identifying cultivars with different competitive abilities for more detailed studies (e.g., Fofana et al. 1995).

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## CANOPY LEAF AREA DISTRIBUTION AND LIGHT ABSORPTION PROPERTIES OF THREE CRUCIFEROUS WEEDS COMPETING WITH WINTER WHEAT (*TRITICUM AESTIVUM*)

A. Hosseinnia<sup>1</sup> and H. Rahimian<sup>2</sup>

<sup>1</sup>Weed Research Department, Plant Pest And Disease Research Institute,  
Tehran, Iran. [ahosseinnia@yahoo.com](mailto:ahosseinnia@yahoo.com)

<sup>2</sup>Department of Agronomy, College of Agriculture, Ferdowsi University  
of Mashhad, Iran.

**Abstract:** Turnip weed, wild mustard and tansy mustard are three cruciferous weeds that reduce winter wheat yield in Iran. An experiment was conducted to evaluate the competitive ability of these weeds in wheat crop. The experimental design was a complete randomized block with factorial arrangement of treatments and four replications. Treatments were the above three weed species and four weed densities (0, 4, 8, 16 plants per m<sup>2</sup>). Wheat density was fixed at 450 plants per m<sup>2</sup>. Results showed that turnip weed was taller, had higher leaf area compared to the other two weeds. Tansy mustard had the least light absorption and the least leaf area. Leaf area distribution and light absorption properties of weed and crop plant indicated that weeds tend to keep their leaf area in the upper canopy profiles that wheat.

**Key words:** Interference, light extinction, leaf dispersion.

### INTRODUCTION

Weeds compete with wheat crop for light, nutrients, space and water with wheat. Weed infestation may cause up to 50 percent in wheat yield loss (Gill and Davidson 2000). Cruciferous weeds are among the important weeds in wheat fields (Cudney 1991). Kropff indicates that the leaf area is a good index to describe the competition between two species, since it integrates both the weed density and its time of emergence (Kropff 1988).

Proportion light absorption for each species in a mixed canopy depends on leaf area index of each species, plant height and light absorption features of leaves.

Leaves of the first organs that compete for light. A plant which places its leaves in the top of the canopy would have a greater competitive ability (Zimdahl 1993).

Barbure and Bridges have defined the main parameters for evaluating the plants competition for light such as: maximum PAR extinction by weeds, time of weed leaf placement in the top of the crop canopy, time of maximum light extinction and finally the distance of weed effectiveness.

Cudney et al (1991) observed that by having higher height related to wheat, wild oat will cause lower light penetration through the canopy.

### MATERIALS AND METHODS

Wheat and weed seeds were planted at the same time in October. Wheat at 450 seeds per m<sup>2</sup> was planted in 20 cm rows. Weed seeds were planted between wheat rows.

The experimental design was complete randomized blocks with factorial arrangement of the 12 treatments. Treatments were weed species turnip weed



(*Rapistrum rugosum*), wild mustard (*Sinapis arvensis*), and tansy mustard (*Descurainia sophia*) and weed density (0, 4, 8, and 16 plants per m<sup>2</sup>). Plots were 6m\*3m.

During the growing season, the radiation intensities of the canopy profiles were measured using a tube solarimeter. Stratified leaf area of crop and weeds were measured in 25cm layers. Means were compared by Duncan multiple rang test ( $\alpha < 0.05$ ).

## RESULTS AND DISCUSSION

*Rapistrum rugosum* and *Sinapis arvensis* caused significant reduction in wheat leaf area (Figure 1).

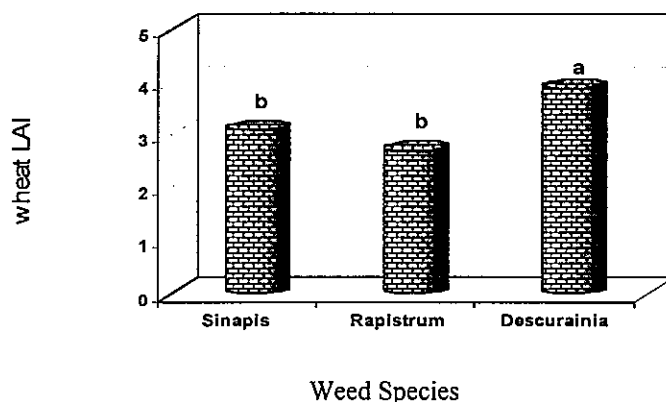


Figure 1. Wheat LAI in Weed Species

The study of interaction effects of weed species and densities on leaf area of weeds and wheat indicated that *Rapistrum rugosum* caused the highest reduction of wheat leaf area (Figure 2). Wheat leaf area distribution profile at canopy closure was different from weed, indicating that when there was no competition between crop and weeds, the greatest amount of leaf area was in the 25-50cm layer. There was no leaf in 75-100cm

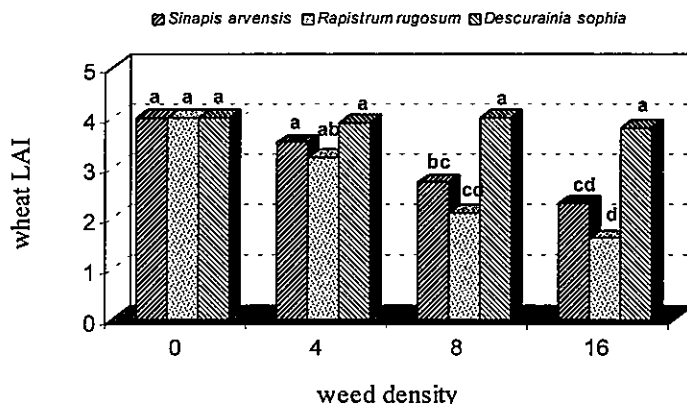


Figure 2. Wheat LAI in Weed Densities.

layer. The greatest amount of leaf area in all three weed species was absorbed in 50-75cm layer (Figure 3).

Wheat crops was significantly taller when competing with *Rapistrum rugosum* compared to control. This was because of lower light penetration through the canopy and undistruction of ouxin , internodes were longer and thus taller wheat crop was observed . The study of interaction effects between weed species and densities on wheat height , also indicated that turnip weed was the only weed species that significantly increased the wheat height (Figure 4) .

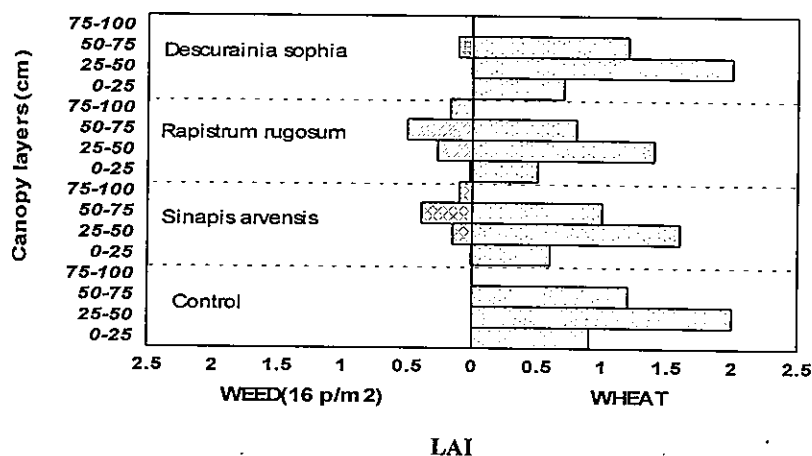


Figure 3. Leaf Area Distribution in Canopy Layers for Weed Species and Wheat

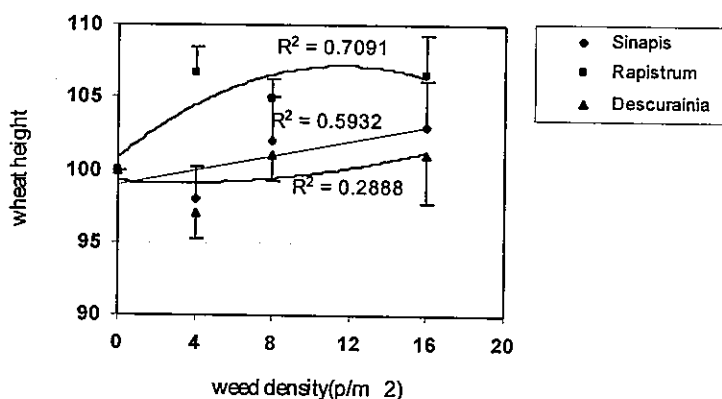


Figure 4. Effect of Weed Density and Weed Species on Wheat Height

The results of this experiment showed that there was a direct relationship between light penetration through the canopy with leaf area and its distribution within the canopy. It was also observed taller plants possessed higher leaf area in the upper canopy layers enabling it to be more successful in competition. The rate of light extinction was enhanced more in mixed canopy compared to pure wheat canopy (Figure 5a, 5b, 5c, 5d).

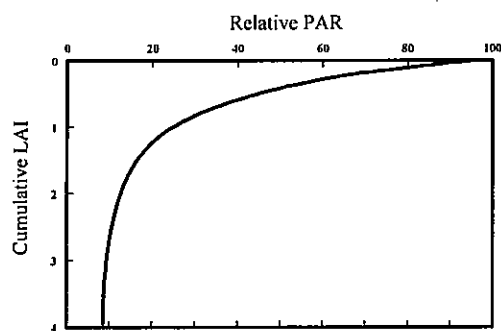
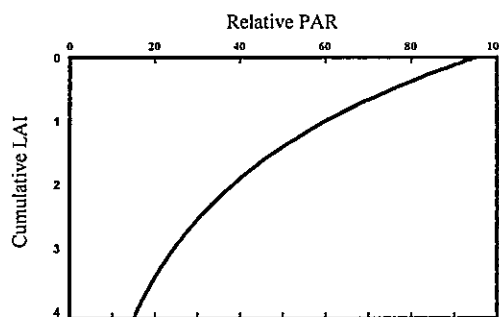
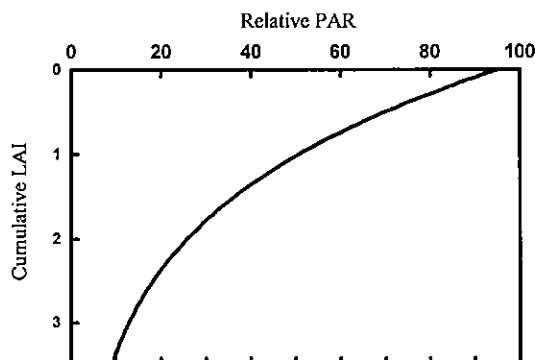
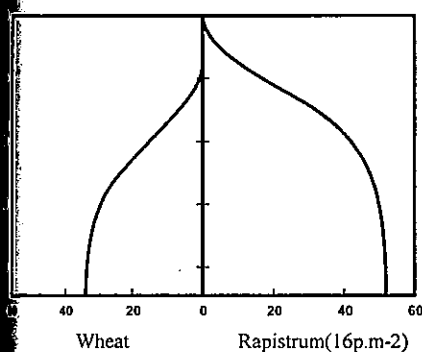


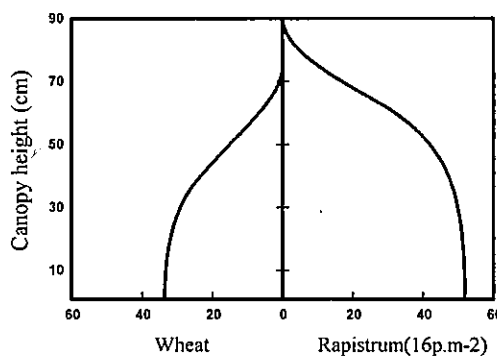
Figure 5a -Light extinction over canopy depth in control

Figure 5b- Light extinction over mixed canopy depth (wheat & *Descurainia sophia*)Figure 5c- Light extinction over mixed canopy depth (Wheat & *Rapistrum rugosum*)

Light absorption study in weed and wheat canopies indicated that highest proportion of light was absorbed by turnip weed, wild mustard and tansy mustard, respectively. In the mixed canopy of the wheat and turnip weed (16 plants per m<sup>2</sup>), turnip weed absorbed more than 55 percent of the total light. The highest light absorption occurred in 40 cm canopy height. Wheat absorbed more than 45 percent of total light and most of the light absorption was occurred in upper 60 cm layer of mix canopy (Figure 6a, 6b).



Cumulative absorbed PAR



Cumulative absorbed PAR

Figure 6a-Simulated absorbed PAR over canopy height (Wheat & *Rapistrum rugosum*)Figure 6b-Simulated absorbed PAR over canopy height (Wheat & *Rapistrum rugosum*)

## ACKNOWLEDGEMENTS

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## EFFECTS OF THREE CRUCIFEROUS WEEDS ON YIELD AND YIELD COMPONENTS OF WINTER WHEAT (*TRITICUM AESTIVUM*)

H. Rahimian<sup>1</sup>, A. Hosseinnia<sup>2</sup> and A. Ghanbari<sup>3</sup>

<sup>1</sup>Department of Agronomy, Colledge of Agriculture , Ferdowsi University of Mashhad , Iran .

<sup>2</sup>Weed Reasearch Department , Plannt Pest And Disease Reasearch Institute , Tehran , Iran

<sup>3</sup>Department of Agronomy, Colledge of Agriculture , Ferdowsi University of Mashhad , Iran .

**Abstract:** In order to evaluate the damage and competitive ability of three broadleaf weeds (turnip weed: *Rapistrum rugosum*, wild mustard: *Sinapis arvensis*, tansy mustard: *Descurainia sophia*) with winter wheat (*Triticum aestivum*, L.CV.: C-73-5) an experiment was conducted in 2000 in Iran. The experimental design was complete block with factorial arrangement of treatments and four replications. Treatments included above three weed species and four weed densities (0,4,8 and 16 plants per m<sup>2</sup>). Wheat density was fixed at 450 plant per m<sup>2</sup>. Results showed that turnip weed emerged earlier, were taller, had higher leaf area and competition ability than other two weeds. It was observed that relative growth rate (RGR) of turnip weed and wild mustard was greater than wheat. Turnip weed had the greatest leaf area index (LAI), crop growth rate (CGR) and accumulation rate compared to wild mustard and tany mustard. Turnip weed caused greatest wheat yield reduction in 16 plant per m<sup>2</sup> density. Among wheat yield components, number of spike per m<sup>2</sup> and grains per spike decreased by increasing of weed species densities. The greatest reduction was observed in turnip weed. Wheat infertile spikletS decreased by increasin weed densities, specially in competition with turnip weed, but average wheat seed weight remained unchanged.

**Key words:** Interference, competition, weed density.

### INTRODUCTION

Successful weed management for reduction of their damage is on of the determinates of high yield (Parsons and Cuthberston 1992 ). Average yield loss of wheat due to weeds has been reported to be 14-20 percent. Weeds of brassicaceae family including *Sinapis arvensis*, *Rapistrum rugosum* and *Descurainia sophia* are of most important broadleaf weeds of wheat fields in Iran (Parsons and Cuthberston 1992). Blackshaw and Dekker (1988) showed that *Sinapis arvensis* when grows with oilseed rape produces more biomass compare to when it grows alone. Streiberg reported that percent yield loss due to various weeds in crop plants may have 50 fold difference. Donald et al reported that wheat grain yield was reduced by increasing *Circium arvense* density. Canada thistle reduced number of spikes and seed per spike, but had a little effect on seed weight (Donald and Mohammad Khan 1996). In canada, *Sinapis arvensis* reduced wheat yield 36 and 40 percent in dry and wet years, respectively. The results showed that water in not an important factor in wheat competition with *Sinapis arvensis* (Burrows and Olson 1995).

## MATERIALS AND METHODS

Wheat and weeds seeds were planted at same time in october. Wheat at 450 seeds per m<sup>2</sup> was planted in 20cm rows. Weed seeds were planted between wheat rows. The experimental design was complete randomized blocks with factorial arrangement of the 12 treatments. Treatments were weed species (turnip weed: *Rapistrum rugosum*, wild mustard: *Sinapis arvensis*, tansy mustard: *Descurainia sophia*) and weed density (0, 4, 8 and 16 plants per m<sup>2</sup>). Plots were 6m\*3m.

Wheat and weed phenological stages were recorded at each sampling time. A fixed 30cm\*30cm quadrat was used for phenology study during the growing season. Destructive sampling were performed every two weeks to determine growth trend during the growing season from 0.3 m<sup>2</sup> of each plot. 1/2 of each plot was harvested at wheat maturity and wheat yield and yield components were measured. Means were compared by Duncan multiple range test ( $\alpha < 0.05$ ).

## RESULTS AND DISCUSSION

Results showed that wheat RGR reduction was more severe in *R. rugosum* and *S. arvensis* than *D. sophia* indicating high competition of these weeds compared by *D. sophia*. Wheat RGR was less than that of weeds and this may be one reason for better competition of weeds against wheat (Figure 1)

Weed density affected wheat tillering. Tiller number was reduced with increasing weed density. This reduction was not significant among weed densities, but was significant compared to control. Evaluation of interaction of weed species and density revealed that highest wheat tiller occurred in competition with *R. rugosum* (Figure 2). This interaction indicated that spike number per unit area decreased by increasing weed density and was statistically significant at high densities

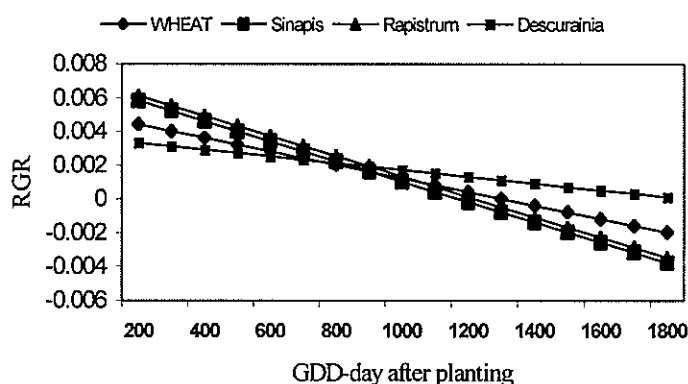


Figure 1. RGR of weed species and wheat

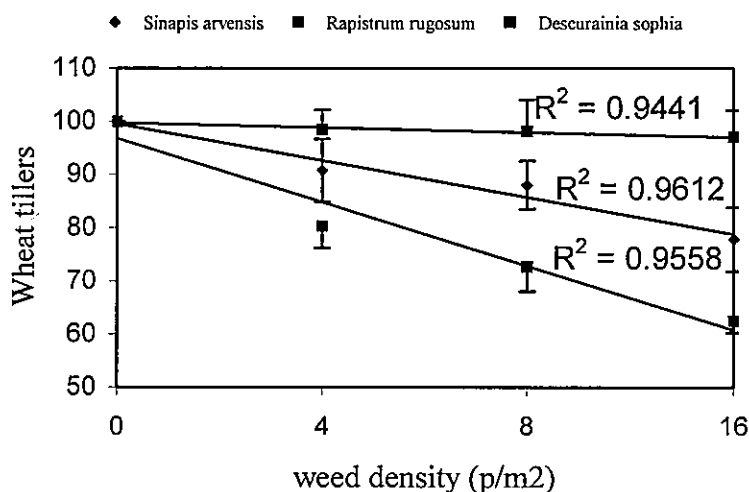


Figure 2. Effect of weed species and weed density on wheat tiller (percent to control).

of *R. rugosum* and *S. arvensis* as compared with control (Figure 3).

The greatest reduction in seed number per spike was observed in competition with *R. rugosum*, *S. arvensis* and *D. sophia*, respectively (Figure 4). All three weed species also decreased the number of wheat infertile spiklets. The least number of infertile spiklets was observed with *D. sophia* treatments (Figure 5).

Weed species at all densities decreased wheat yield. The highest yield reduction was observed in wheat competition with *R. rugosum* (Figure 6). Other researches also reported the difference of various weeds in crop yield losses (Donald and Mohammadkhan 1996; Streibig et al. 1989).

Significant interaction of weed density and species on wheat yield loss was observed. Wheat grain yield reduction was linear in all four densities *S. arvensis* and *D. sophia*. This indicates that higher densities should have been selected to obtain a normal hyperbolic curve, however increasing *R. rugosum* density reduced wheat

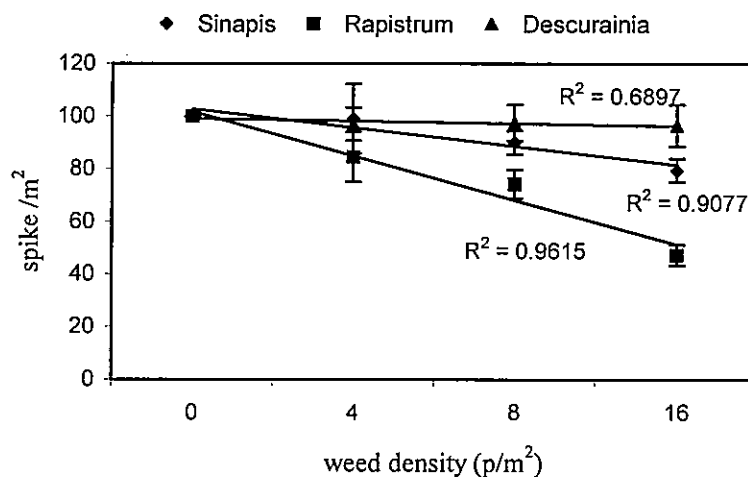


Figure 3. Effect of weed density and weed species on spike number per m² (percent to control).

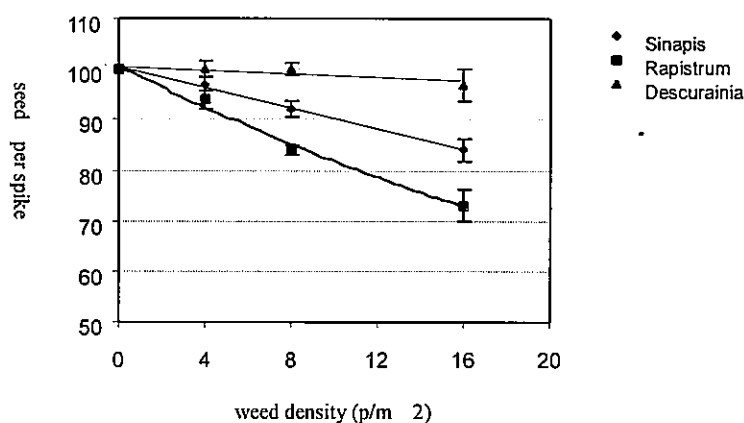


Figure 4-Effect of weed species and weed density on seed number per spike (percent to control).

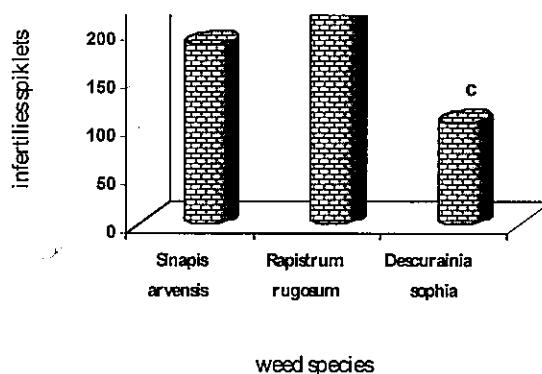


Figure 5-effect of weed species on infertile spiklets (percent to control).

grain yield hyperbolically. The greatest wheat yield loss was observed in *R. rugosum* densities (Figure 7).

Effect of interaction of weed density and species on wheat HI showed that only different densities of *R. rugosum* reduced HI in comparison with control and it was not significant difference in various *R. rugosum* densities (Figure 8).

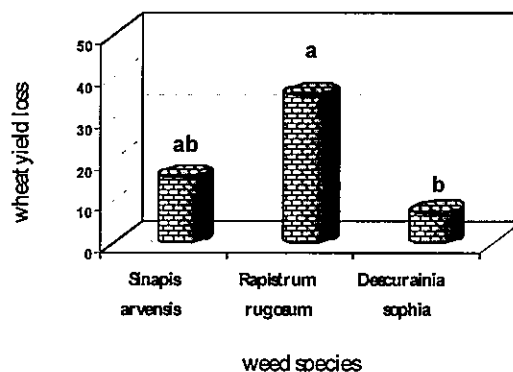


Figure 6- Effect of weed species on wheat yield loss (percent to control).

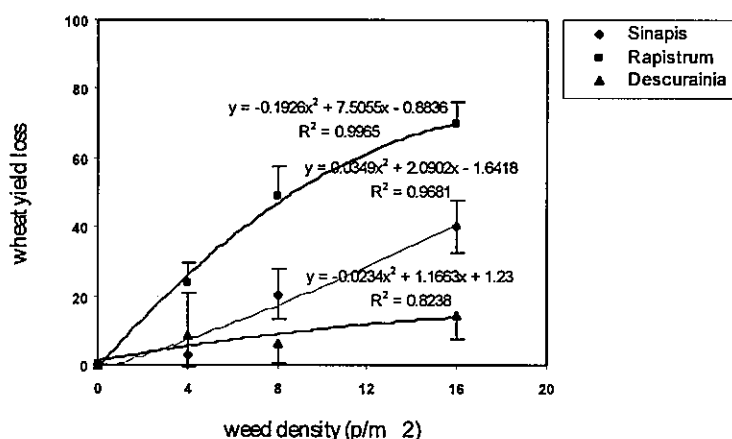


Figure 7- Effect of weed species and weed density on wheat yield loss (percent to control).

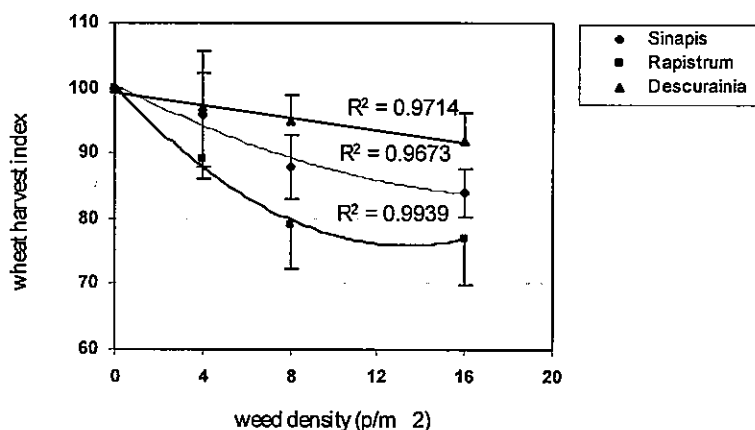


Figure 8- Effect of weed species and weed density on wheat harvest index (percent to control).



## ACKNOWLEDGEMENTS

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## EFFECT OF WEED COMPETITION ON GROWTH AND YIELD OF DIRECT-SEEDED KDML 105 RICE UNDER DROUGHT CONDITION.

C. Wongwattana<sup>1</sup>, P. Romyen<sup>2</sup>, and N. Chansena<sup>2</sup>

<sup>1</sup>Department of Biology, Srinakharinwirot Univ., Bangkok 10110, Thailand.  
chalermc@psm.swu.ac.th

<sup>2</sup>Ubonratchatani Rice Research Center, Ubonratchatani 34000, Thailand.

**Abstract:** Weeds, kinds and density, and their effect on growth and yield of direct seeded (dibbled) KDML 105 rice were investigated under drought condition in upland paddy field at Ubonratchatani Rice Research Center. Growth and yield of rice were compared among 3 weed conditions; Weed-free, Unweeded, and Hand weeding (once at 1 month after rice emergence). The major weeds found in this area were *Digitaria adscendens*, *Lindernia anagallis*, *Ludwigia hyssopifolia*, *Mitracarpus villosus*, *Fimbristylis miliacea* and *Cyperus haspan*. Comparing on weed density at 1 month after rice emergence and at harvesting, number of weeds in unweeded treatment were 1,519.4 and 1,415.9 plants/m<sup>2</sup>, respectively, and 1,267.2 and 514.7 plants/m<sup>2</sup>, respectively, in hand weeding treatment. Hand weeding reduced weeds density to about 36% of those in unweeded control, and grassy weeds were most affected. Rice plant in unweeded treatment died due to drought and weeds competition. Hand weeding, in this study, increased rice yield to about 38% of that in weed-free treatment. However, the rice yields were very low in both weed-free and hand weeding conditions because of the drought

**Key words :** weeds density, direct-seeded rice, KDML 105, drought

### INTRODUCTION

Rice in Northeast Thailand is mainly rainfed rice and the yield in this area is very low when compare to the other areas (Panichapat *et al.*, 1987). The three major causes for low yield and production instability are erratic rainfall with long interspells, low soil fertility and poor water holding capacity of soil (Anonymous, 1980). For the production management, the farmer can do only at low level by their own family labors. The use of high technology for rice production are not available due to their economic problems (Panichapat *et al.*, 1987).

Many low level technologies for rice production in Northeast rainfed areas were studied in order to solve the problems. Growing rice by direct seeding; dibbling, drilling, broadcasting etc.; are recommended in the drought-prone areas which frequently lag of water for transplanting (Panichapat *et al.*, 1987). In direct seeding, the weed problems are more severe than in transplanting (Anonymous, 1980 and Anonymous, 1991). Weeds decrease crop yield by many ways, including the competition in growth factors; CO<sub>2</sub>, light, nutrient, water etc. (Aldrich, 1984 and Patterson, 1985). In drought condition, water is a limiting factor for growth, competition of water by crop and weeds are so severe. Generally, 1-3 % of water absorbed from soil by plant root will be used in photosynthesis, the rest are loss from plant by transpiration (Aldrich, 1984). The higher density of crop and weed, the more water loss from soil. The drought condition with weed competition may severely decrease yield of rice.

The propose of this study is to determine the effect of weeds and their density on direct-seeded KDML105 rice yield under drought condition.

### MATERIALS AND METHODS

Seeds of KDML 105 rice were planted by dibbling method at the spacing of 0.25 x 0.25 meters and 5 seeds per hole, in the upland paddy field at Ubonratchatani Rice Research Center in the middle of July, 1991. Plot size was 8.5 x 10.5 square meters. The experiment was designed as Randomized Complete Block with 3 replications and 3 treatments; Un-weeded, Weed-free and Hand Weeding (once, one month after rice emergence). The data collected in this study were

1. Amount of rainfall, maximum and minimum temperatures of that area.
2. Number of weeds, and fresh and dry weight of individual weed per one square meter. These data was the everage from two points of 1 square meter in each plot of the treatments of Un-weeded and Hand Weeding , at one month after rice emergence and at harvesting.
3. Plant height and tiller number of rice, average from 10 plants per plot, at harvesting.
4. Rice yield, average from 2 sub-plot of 2 x 5 square meter of each plot

### RESULTS AND DISCUSSIONS

This experiment was conducted in upland paddy field in Ubonratchatani Rice Research Center. Soil component in this area is mainly sand which have low water holding capacity. Rain water rapidly penetrated to the lower soil, only small amount of water retained on soil surface in short period after raining. The amount of rainfall here during the experiment was also low (Fig. 1). All data including high air temperature indicated the drought condition of this area during this experiment.

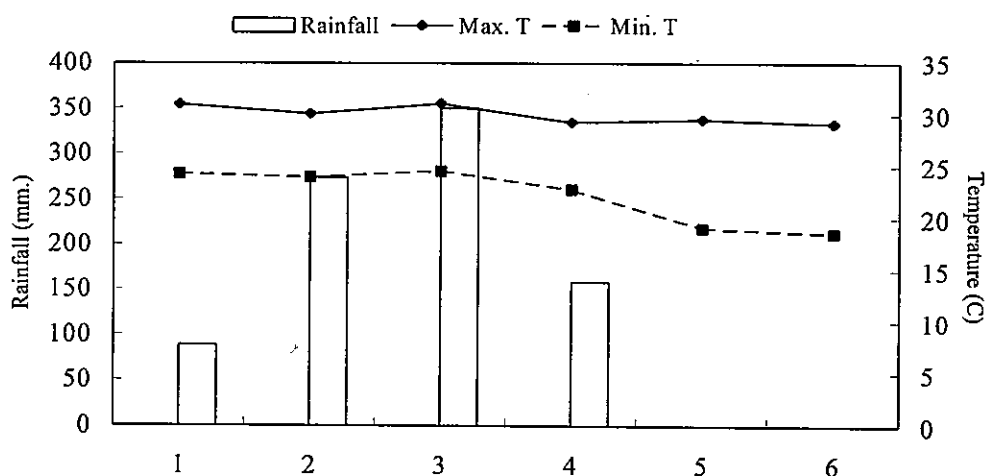


Figure 1. Amount of rain fall and the average maximum and minimum temperatures of each month during the experiment (from July to December), at Ubonratchatani Rice Research Center.

The major weeds found in this experiment were *Digitaria adscendens*, *Lindernia anagallis*, *Ludwigia hyssopifolia*, *Mitracarpus villosus*, *Fimbristylis miliacea*, *Cyperus*

*haspan* and *Bulbostylis barbata*. At one month after rice emergence (before weeding in Hand Weeding treatment), weeds density were high in both Un-weeded and Hand Weeding treatments (1,519.4 and 1,267.2 plants per square meter, respectively) (Table 1). At harvesting, weed density was still high in Un-weeded treatment but was remarkably decreased in Hand Weeding treatment (Table 1 and 2). Data of fresh and dry weight of weeds were also indicated in the same direction of density. This may be resulted from drought that prevented re-growth, and germination and growth of the new weeds. Hand Weeding, once at one month after rice emergence under drought condition could suppress weed population till harvesting, especially grassy weeds (Fig. 2). Hand weeding in this study reduced weed population to about 36.4 % when compared to Un-weeded control. Grassy weeds was mostly affected by hand weeding and the next was narrowleaf weeds. The high population of broadleaf weed species at harvesting in Hand weeding treatment indicated the better regrowth of this weed including growth of the new plants than other kinds of weeds under that conditions (Fig. 2).

Table 1. Weeds density in Un-weeded treatments (T1) at one month after emergence (MAE) and at harvesting.

Weed	Scientific Name	1 MAE		Harvesting	
Groups		No. <sup>1</sup>	D.W. <sup>2</sup>	No. <sup>1</sup>	D.W. <sup>2</sup>
BL <sup>3</sup>	Hedyotis diffusa Willd.	4.7	0.3	14.7	0.5
	Lindernia anagallis (Burm.f.) Pennell	286.0	5.7	33.3	0.6
	Ludwigia hyssopifolia (G. Don) Excell	266.0	7.3	24.0	2.1
	Melochia corchorifolia Linn.	2.7	1.7	1.3	0.7
	Mitracarpus villosus (Sw.) DC.	120.0	7.7	50.0	14.8
	Sub total	679.4	22.7	123.3	18.7
G <sup>4</sup>	Dactyloctenium aegyptium (L.) P. B.	2.0	0.7	-	-
	Digitaria adscendens (H.B.K.) Henr.	386.0	49.3	1,149.3	123.0
	Eleusine indica (L.) Gaertn.	15.3	0.1	-	-
	Eragrostis elongata	-	-	16.7	3.7
	Ischaemum rugosum Salisb.	-	-	1.3	0.7
	Sacciolepis indica (L.) Chan	-	-	15.3	1.7
Sub total	403.3	50.1	1,182.6	129.1	
C <sup>5</sup>	Bulbostylis barbata (Rottb.) C.B.Clarke	172.7	9.3	-	-
	Cyperus haspan	95.3	6.0	28.0	1.0
	Fimbristylis dichotoma (L.) Valh.	-	-	30.0	1.9
	Fimbristylis miliacea (L.) Valh.	106.0	2.3	-	-
	Sub total	374.0	17.6	58.0	2.9
NL <sup>6</sup>	Cyanotis axillaris Roem. & Schult.	-	-	1.3	0.7
	Murdannia nudiflora (L.) Brenan	62.7	10.0	50.7	5.6
	Sub total	62.7	10.0	52.0	6.3
Total		1,519.4	100.4	1,415.9	157.0

<sup>1</sup>Number of weeds per square meter; <sup>2</sup>Dry weight of weeds (g.)/m<sup>2</sup>; <sup>3</sup>Broadleaf weeds; <sup>4</sup>Grassy weeds;

<sup>5</sup>Cyperus weeds; <sup>6</sup>Narrowleaf weeds.

Rice growth and yield are shown in Table 3. Rice plants died from drought and weed problems in Un-weeded treatment. Although hand weeding remarkably decreased weed population at harvesting, the rice yield was not so much increased. The yield was only 38 % of that of Weed-free control. However, rice yield in Weed-free condition was

also very low (763.1 kilograms per hecta). Rice plant in Weed-free treatment were also higher and had higher tiller number than in Hand Weeding treatment (Table 3).

Table 2. Weeds density in Hand weeding treatments (T3) at one month after emergence (MAE) and at harvesting.

Weed Groups	Scientific Name	1 MAE		Harvesting	
		No. <sup>1</sup>	D.W. <sup>2</sup>	No. <sup>1</sup>	D.W. <sup>2</sup>
BL <sup>3</sup>	<i>Hedyotis diffusa</i> Willd.	43.2	1.7	64.7	2.2
	<i>Lindernia anagallis</i> (Burm.f.) Pennell	303.5	9.2	228.0	6.6
	<i>Lindernia ciliata</i> Pennell	-	-	26.0	1.4
	<i>Ludwigia hyssopifolia</i> (G. Don) Excell	139.7	4.0	36.0	1.5
	<i>Melochia corchorifolia</i> Linn.	2.0	0.2	-	-
	<i>Mitracarpus villosus</i> (Sw.) DC.	80.0	7.0	52.0	5.7
	<i>Mollugo pentaphylla</i> L.	5.3	1.2	-	-
	<i>Tridax procumbens</i> Linn.	1.3	0.3	-	-
	Sub total	575.0	23.6	406.7	17.4
G <sup>4</sup>	<i>Cynodon dactylon</i> (L.) Pers.	-	-	6.7	0.1
	<i>Dactyloctenium aegyptium</i> (L.) P.B.	7.3	1.7	1.3	0.5
	<i>Digitaria adscendens</i> (H.B.K.) Henr.	309.8	57.5	26.7	4.7
	<i>Echinochloa colona</i> L.	0.7	0.7	-	-
	<i>Eragrostis elongata</i>	-	-	3.3	0.9
	<i>Sacciolepis indica</i> (L.) Chan	-	-	2.0	0.1
	Sub total	317.8	59.9	40.0	6.3
C <sup>5</sup>	<i>Bulbostylis barbata</i> (Rottb.) C.B. Clarke	93.3	12.8	-	-
	<i>Cyperus haspan</i>	165.7	11.8	36.7	1.3
	<i>Cyperus iria</i> Linn.	7.3	2.0	5.3	0.1
	<i>Cyperus rotundus</i> Linn.	7.3	0.3	-	-
	<i>Fimbristylis dichotoma</i> (L.) Vahl.	-	-	6.7	0.3
	<i>Fimbristylis miliacea</i> (L.) Vahl.	50.8	5.3	8.0	0.4
	Sub total	324.4	32.2	56.7	2.1
NL <sup>6</sup>	<i>Cyanotis axillaris</i> Roem. & Schult.	20.0	2.0	-	-
	<i>Murdannia nudiflora</i> (L.) Brenan	30.0	7.8	11.3	0.8
	Sub total	50.0	9.8	11.3	0.8
	Total	1,267.2	125.5	514.7	26.6

<sup>1</sup>Number of weeds per square meter; <sup>2</sup>Dry weight of weeds (g.) per square meter; <sup>3</sup>Broadleaf weeds;

<sup>4</sup>Grassy weeds; <sup>5</sup>Cyperus weeds; <sup>6</sup> Narrowleaf weeds.

These results indicated that drought severely affected growth and yield of rice, as can be seen that rice yield with out weed (Weed-free,T2) was also very low. However, weeds was also an important problem in rice production. Under drought condition, weeds may increased yield lost much more by competing on water with rice and sometimes rice yield may be decreased to zero. Under this condition, hand weeding (once at one month after rice emergence) might suppress weed growth and population till harvesting. However, the growth of the rice plant was already inhibited by drought and weeds competition in early stage after germination. After hand weeding, although

the weed density was decreased, growth of rice plant was still suppressed from drought. So that the rice yield in the Hand Weeding treatment was only slightly increased

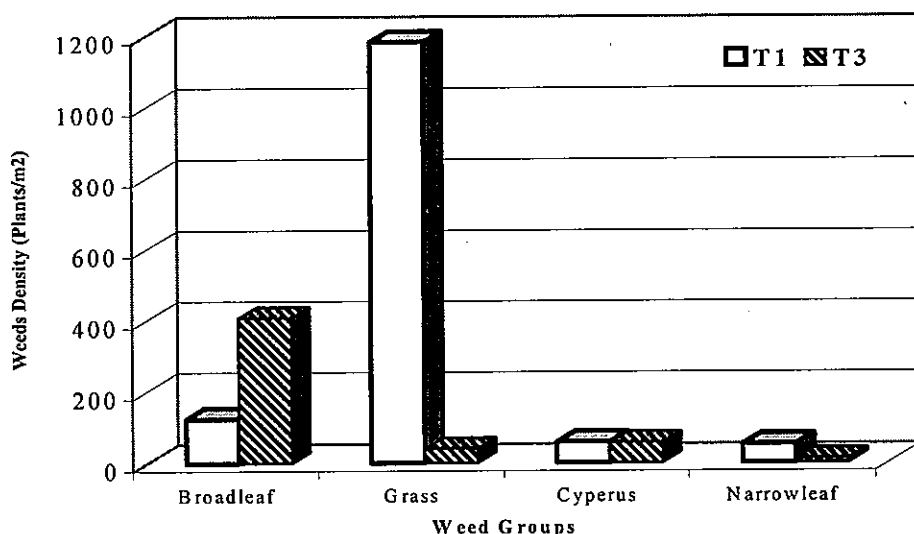


Figure 2. Weeds density (plants/m<sup>2</sup>) at harvesting, comparing between the Un-weeded (T1) and Hand weeding T3) treatments.

Table 3. Yield and yield components of direct-seeded KDML 105 rice under drought condition at Ubonratchathani Rice Research Center.

Treatments	Grain Yields <sup>1</sup> (Kg/ha)	Plant Height <sup>2</sup> (cm)	Tiller No. <sup>2</sup> per Plant
Un-weeded	0.0 a <sup>3</sup>	0.0 a	0.0 a
Weed-free	763.1 a	79.5 b	10.1 b
Hand weeding	286.3 a	65.2 b	7.8 b

<sup>1</sup>Grain yields at 14 % moisture content; <sup>2</sup>Averaged from 30 samples; <sup>3</sup>Numbers in the same column followed by the same letter are not significantly different by DMRT at 0.5%.

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## THE INTRA- AND INTER-SPECIES COMPETITION OF *DIGITARIA SANGUINALIS* (L) SCOP AND *ECLIPTA PROSTRA* L.

J. R. Wu<sup>1</sup>, C. J. Chen<sup>2</sup>, J. M. Shen<sup>3</sup>, C. H. Li<sup>2</sup>, and C. Wu<sup>2</sup>

<sup>1</sup>Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences,  
Nanjing 210014, China

<sup>2</sup>Institute of Agricultural Sciences in the Coast Area of Jiangsu 224002, China

<sup>3</sup>Institute of Agricultural Sciences in Nantong Area of Jiangsu 226000, China

**Abstract:** The experiment studied the intra- and inter-species competition of *Digitaria sanguinalis* (L) Scop. and *Eclipta prostra* L. at elongation stage. The results showed that the increasing pattern of biomass and branches of *D. Sanguinalis* (L) Scop changed from exponential models in pure culture to hyperbola models depending on increasing inoculated weed densities. The patterns of biomass and height of *E. prostra* L changed from hyperbola models in pure culture to exponential models in mixed culture. It meant that *E. prostra* L was more competitive than *D. sanguinalis* (L) Scop at elongation stage.

**Key words:** competition *Digitaria sanguinalis* (L) Scop. *Eclipta prostra* L

### INTRODUCTION

*D. sanguinalis* (L) Scop. is the dominant weed species in cotton fields. The rate of broad-leaf weed species such as *Eclipta prostra* L is increasing gradually in recent years with the use of grass-specified herbicides widely such as haloxyfop. The experiments were conducted to determine whether *E. prostra* take the place of *D. sanguinalis* and become the most important weed in cotton in the absent conditions of competitive ecology.

### MATERIALS AND METHOD

The experiment was conducted in the Agricultural Institute of Along Yangzi River Area in Jiangsu Province. Weed seeds of both *D. sanguinalis* and *E. prostra* were sown with different densities on July 18, 1999 in experimental fields planted with cotton the year before.

#### Establishment of growth model for pure population

Six density level 20, 40, 60, 80, 100 and 120 plants /m<sup>2</sup> were designed for *E. prostra*. Six density levels were also arranged for *D. sanguinalis*(L) Scop., which were 30, 60, 90, 120, 150 and 180 plants/m<sup>2</sup>.

The blocks for 2.25m<sup>2</sup>(1.5m×1.5m) of each were randomly designed. Each treatment repeated for three times. Weed densities were set on the July 28 for the first time and were reset ten days latter when most plants reached three leave stage.

#### Establishment of growth model for mixture population of *E. prostra* and *D. sanguinalis*.

7 treatments on the basis of weed densities for mixture population of *E. prostra* and *D. sanguinalis* were designed, namely 10×130, 30×110, 50×90, 70×70, 90×50, 110×30, 130×10 respectively. The plants were set as the pure population.

### Parameters collected

The fresh weight, branch (*D. sanguinalis* (L) Scop. only) and plant height of the two weeds sampled from 0.11m<sup>2</sup> per block were surveyed at elongation stage. Also weed seeds per ear, seed weights were measured on the Sep 30, 1999

## RESULTS AND DISCUSSION

### The intraspecific competition of *D. sanguinalis* and its model

From Table 1, it seemed that the increasing rate of weed biomass decreased gradually depending on the increasing of weed densities. Weed branches and heights of *D. sanguinalis* in 1 m<sup>2</sup> increased gradually too. It meant that the necessity of *D. sanguinalis* to growing resource from surrounding where it was limited increased with the increasing of weed densities. So intraspecific competition occurred. *D. sanguinalis* developed deeper and wider both under and above ground. Regarding weed density ( $D_D$ ) as independent variable, weed fresh weight ( $W_D$ ), weed branches ( $B_D$ ) in 1 m<sup>2</sup> field and weed height as dependent variables, we got the fittest growing competition models as follows:

$$W_D = 31.7363e^{0.09509D} \quad R=0.9459 \quad (1)$$

$$B_D = 14.8598D_D e^{-0.035D} \quad R=0.9219 \quad (2)$$

$$H_D = D_D / (0.06295 + 0.0178D_D) \quad R=0.9497 \quad (3)$$

The models showed that elongation stage was a critical stage for to spread to limited spare. Both *D. sanguinalis* weight and branches in 1 m<sup>2</sup> field developed in exponential increasing patterns, while mean plant heights developed in hyperbola pattern (formula③). Formula③ could be changed to below:

$$1/H = 0.1781 + 0.06259/D \quad (4)$$

It showed clearly that  $D \nearrow$ ,  $1/D \searrow$ ,  $1/H \searrow$ ,  $H \nearrow$ , namely, with the increase of inoculated weed densities, area shared by per weed decreased, and weed height increased (the biggest theoretical height valued 56.15cm). It meant the competition of *D. sanguinalis* to limited surrounding factors increased.

Table 1. The growth habits of *E. prostra* and *D. sanguinalis* at elongation stage in different densities under pure culture.

	Weed species											
	<i>D. sanguinalis</i>						<i>E. prostra</i>					
Inoculated weed densities (plants/m <sup>2</sup> )	30	60	90	120	150	180	20	40	60	80	100	120
Fresh weight (gram/m <sup>2</sup> )	312	558	939	1347	1488	1590	613	600	723	1017	870	1035
Branches/m <sup>2</sup>	399	656	1026	1170	1059	1410	20	40	60	100	102	102
Height (cm)	285	363	345	372	369	495	297	288	258	225	234	239

### The intraspecific competition of *E. prostra* and its model

The table 1 showed that fresh weight of *E. prostra* in 1 m<sup>2</sup> field increased and branches sustained unchanged and weed height decreased depending on weed density increasing. Possibly the efficient factors were limited and the amount absorbed by each plant decreased with weed density increasing. It could not satisfy the necessity of weed growing.



Regarding weed density of *E. prostra* ( $D_E$ ) as independent variable, weed fresh weight ( $W_E$ ) and weed branches ( $B_E$ ) in 1 m<sup>2</sup> field and mean weed height ( $H_E$ ) as dependent variables, we got the most suitable growing competition models as below:

$$W_E = D_E / (0.03163 + 0.00672 D_E) \quad (5)$$

$$1/W_E = 0.00672 + 0.03163/D_E \quad (6)$$

$$H_E = D_E / (-0.03559 + 0.04195 D_E) \quad (7)$$

$$1/H_E = 0.04195 - 0.03559/D_E \quad (8)$$

What showed from the models were that the biomass of *E. prostra* in 1 m<sup>2</sup> field increased in the pattern of hyperbola and mean height decreased in the pattern of hyperbola with the increasing of weed density. So we concluded that while the biomass in species competition increased with the increasing of *E. prostra* density, the *E. prostra* population competed mainly in horizontal direct, and the weight of single plant increased too. It was very different from that in species competition of grassy *D. sanguinalis* which established mainly in the mode of branch increasing.

#### The interspecies competition of *D. sanguinalis* and *E. prostra* and its model

##### The effect of interspecies competition on the biomass, branches and height of *D. sanguinalis*

When growing in mixture with *E. prostra*, the biomass of *D. sanguinalis* in 1 m<sup>2</sup> field increased in the pattern of hyperbola with the increasing of weed density while increased in elongation pattern when growing in pure culture.

The increasing patterns of *D. sanguinalis* branch were the same as that of weed fresh weight in different culture. Under same inoculated weed density, the branches of *D. sanguinalis* in m<sup>2</sup> field under mixed culture were less than that under pure culture. The weed height under mixed culture increased in hyperbola pattern, the same as that in pure culture.

The relationship among biomass ( $W_D$ ), branches ( $B_D$ ), mean height ( $H_D$ ) and weed density inoculated ( $D_D$ ) per 1 m<sup>2</sup> field could be modeled as below:

$$W_D = 24.7062 + 2.5830 D_D - 0.9152 H_D \quad (9)$$

##### The effect of interspecies competition on the biomass, branches and height of *E. prostra*

When growing in mixture with *D. sanguinalis* in 1 m<sup>2</sup> field the increasing patterns of the biomass and mean height of *E. prostra* were both of elongation model which were quite different from that in pure culture when they were both of hyperbola models. The biomass in mixed culture were less than that in pure culture at lower weed density level and were more when the weed densities exceeded a certain level. It meant that to compete with *D. sanguinalis*, *E. prostra* had to develop faster than in pure culture. As to height, something was different too. The mean plant height of *D. sanguinalis* in mixed culture condition increased with weed density when it was lower than 52 plant/m<sup>2</sup> and decreased when higher than 52 plant/m<sup>2</sup>. But in pure culture, it decreased with increasing weed density.

The growing components of *D. sanguinalis* in mixed culture could be modeled as follows:

$$\begin{aligned}
 W_D &= 21156.49 - 1362.02 D_D + 1.0504 B_D - 1361.26 D_E + 0.1331 W_E & R &= 0.9988 & \textcircled{10} \\
 W_E &= -4175.446 + 278 D_D + 0.7220 W_D - 1.6411 B_D + 278 D_E & R &= 0.9944 & (11)
 \end{aligned}$$

It appeared through correlation analysis that the biomass of *D. sanguinalis*(L) Scop influenced by a many factors filed according to their importance as:

$$B_D (0.9603) > D_D (0.8863) > H_D (0.6306) > H_E (0.1286) > W_E (-0.8416) > D_E (-0.8836).$$

It showed that the biomass of *D. sanguinalis* in 1 m<sup>2</sup> field depending mainly on weed branches, inoculated weed density, plant height was greatly checked by the biomass and inoculated density of *E. prostrata*.

The effects of interspecies competition between *D. sanguinalis* and *E. prostrata* at elongation stage in mixed culture on the growing of both weeds were great. The increasing pattern of biomass and branches of *D. sanguinalis* changed from exponential models in pure culture to hyperbola models depending on increasing inoculated weed density. The biomass and height patterns of *E. prostrata* changed from hyperbola models in pure culture to exponential models in mixed culture. It meant that *E. prostrata* was more competitive than *D. sanguinalis* at elongation stage.

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## ALLELOPATHIC POTENTIAL OF WINTER CEREAL STRAWS

P. An and H. Shibayama

Marine and Highland Bioscience Center, Saga University, Karatsu, 847-0021 Japan

**Abstract:** The leachates of winter cereal straws wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) of different part of organs were used in a bioassay test with lettuce (*Lactuca sativa* L.). The inhibitory effects of the straw leachates on lettuce seedling growth differed greatly with the each part of straws. The leachates from leaf blade of straws had the most inhibitory effects on the lettuce radicle growth and the effectiveness of leachates were not significantly different by leaf position. The inhibitory effects of the straw leachates in distilled water on barnyardgrass and rice seedling growth related to the hours after immersing straws in distilled water. The most inhibitory effect was founded after immersing straws in distilled water for 24 hours. Straw extract of each part of winter cereals were analyzed using high-performance liquid chromatography (HPLC). The results showed that the extracts from each part of winter cereal straws contained 16 phenolic compounds in which *para*- coumaric acid and ferulic acid were big amount. The amount of phenolic compounds in internode was more than in leaf blade of winter cereal straws.

**Key words:** Winter cereal, straw, allelopathy, inhibitory activity, extract, phenolic compounds

### INTRODUCTION

Recently, it is concerned to take advantage of allelopathic potential of winter cereal straws in the development of integrated weed management (IWM) strategies in crop production. The detrimental interference of winter cereal straw on germination and seedling growth of weed species has been demonstrated in the field. It was shown by various investigators that some of the chemical compounds contained in certain plant residues had phytotoxic properties. Some of these substances have also been identified and were shown to be various types of phenolic acid (Tang *et al.*, 1978). The objectives of this study were (1) to test the phytotoxicity of various parts straw of winter cereals on lettuce seedling growth, (2) to isolate and determine the phenolic acids content of each part straw extract of winter cereals and (3) to determine whether or not these phenolic acids could be related to the inhibitory activity on lettuce seedling growth.

### MATERIALS AND METHODS

#### Bioassay

Wheat (Chikugoizumi) and barley (Miharugorudo) plants were grown at Kyushu National Agricultural Experiment Station in Chikugo, Fukuoka Prefecture, in 1999, and their straws were harvested at full maturity. The part of rachis, internode (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, from top to down), node, leaf blade (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) and leaf sheath (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) of straws were collected and dried at 60°C for 24 hours, chopped into pieces about 1.5cm long. The bioassays were carried out in culture dishes, 50 mg of each material was put into each dish, and 5 of autoclaved agar (0.5%, 45°C) was added to each dish. The agar was gelatinized for 30~60min, and another 5 of agar was added to each dish. After

gelatinization, lettuce (Great Lakes 366) was used as test plant, and 5 seeds were placed on each dish. After incubation at 25°C in the dark for 3 days, the inhibitory effect of elongation growth was observed by measuring the length of hypocotyl and radicle (Fujii *et al.*, 1991).

### Phenolic Acid Contents in Straw extracts

Five grams of powdered straw of each part were put into 50 MCW (Methanol: Chloroform: Distilled water, 2:1:0.8) and centrifuged at 2000rpm for 15min. The resulting filtrate concentrated into small volumes under reduced pressure at 40°C. The concentrated filtrate was extracted with 50 ethyl acetate in a separatory funnel for 30min. The upper fraction (ethyl acetate phase) was collected, and the aqueous fraction was extracted three times. The ethyl acetate fractions were combined, and the final aqueous fraction was discarded. The ethyl acetate fraction was rotary evaporated at 40°C to dryness, weighed and dissolved in methanol at a 1mg/concentration. The presence of phenolic acid in the winter cereal straw extracts were determined by high-performance liquid chromatography. Gallic acid, coumarin, protocatechuic acid, catechin, hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, salicylic acid, *o*-coumaric acid, cinnamic acid, and *p*-chlorobenzoic acid as the chemical standards used for HPLC in this study. Table 1 shows the phenolic compounds used for HPLC and their retention time (Rt). Phenolic compounds in the extracts samples were identified by comparison of retention times. The concentrations were determined by comparison with calibration curves established with reference mixtures of standard compounds based on peak area (Fujii *et al.*, 1991).

## RESULTS AND DISCUSSION

The leachates of barley and wheat straws of different part of organs exhibited different bioactivity (Figure 1). It showed that the radicle length of lettuce seedlings was most inhibited by each leaf blade of winter cereals, but the effectiveness of leachates from 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> leaf blade of winter cereal were not significantly different. When compared to the control, radicle growth was reduced 39.8, 40.9 and 34.8% by the leachates of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> leaf blade of wheat, and 31.9, 32.5 and 48.5% by the leachates of 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> leaf blade of barley, respectively. And radicle length was more inhibited than hypocotyl length of lettuce seedlings (data not shown). The inhibitory order of leachate of wheat and barley straws of different part of plant were in order leaf blade > node > rachis > leaf sheath > internode. The inhibitory effect of node, rachis and leaf sheath leachates were somewhat lower but leachates from internode exhibited lowest. The results showed the level of toxicity of leachate from leaf blade and internode on lettuce radicle growth were significantly different at the 0.05 level.

The straw extracts of all part of wheat and barley used in this study contained gallic acid, catechin, hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, *para*-coumaric acid, ferulic acid, sinapic acid, and salicylic acid. Among the standards used in this study, a number of compounds were not identified in the certain parts of cereal straws extracts. *O*-coumaric acid, cinnamic acid and *p*-chlorobenzoic acid were not identified in the leaf blade of wheat, coumarin, protocatechuic acid, cinnamic acid and *p*-chlorobenzoic acid were not identified in the internode of wheat. Benzoic acid and *p*-chlorobenzoic acid were not found in the leaf blade extracts of barley, and coumarin, protocatechuic acid, cinnamic acid and *p*-chlorobenzoic acid were not found in the

internode extracts of barley.

The cereal straws of different part in this experiment differed in content of phenolic compounds. The internode contained the highest level the total content of phenolic compounds, and it was twice as much that contained in the leaf blade. Among the identified acids *p*-coumaric acid and ferulic acid were quantitatively predominant. Four phenolic compounds extracted in this experiment showed a significant difference between leaf blade and internode. These were *p*-coumaric acid, ferulic acid, coumarin and catechin. The content of *para*-coumaric acid and ferulic acid were higher in extracts from internode than leaf blade of wheat and barley. The similar results were noticed for sinapic acid, salicylic acid and *o*-coumaric acid.

Coumarin was not found in the extracts of rachis, internode and node, but was identified in the extract of leaf blade and leaf sheath both wheat and barley. Catechin was detected at a higher content in leaf blade extract than in other parts of wheat and barley. The results strongly suggest that coumarin and catechin are among the phenolic compounds responsible for the allelopathic potential of winter cereal straw.

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## ALLELOPATHY AS A TOOL FOR SUSTAINABLE WEED MANAGEMENT

D. R. Batish, H. P. Singh, and R. K. Kohli

Department of Botany, Panjab University, Chandigarh 160 014, India  
daizybatish@yahoo.com

**Abstract:** Modern agriculture is commercial and target-oriented and hence in order to achieve enhanced production, large amount of synthetic herbicides and weedicides are used to control weeds. However, the sustainability of the agricultural land is fast deteriorating because of the residual effect of the synthetic chemicals coupled with shift in weed populations and increasing resistance to herbicides. Thus, in order to maintain the sustainability of the agro-ecosystems it is essential to explore the eco-friendly means of alternative weed management. Allelopathy is one of the promising alternatives which is being manipulated for getting the benefits of weed management through various strategies. Use of allelopathic cover / smother / rotational / companion or green manure crops is one such strategy. A number of crops such as buckwheat, foxtail millet, rye, sorghum, clover, sunflower, and cruciferous plants are commonly used as cover/smother/rotational or companion crops. They are not only useful in controlling weeds but also provide biomass. Another strategy of using allelopathy is the use of wild accessions of the modern day cultivars that have lost the allelopathic trait because of the selection pressure favouring high yields. They are being screened and the allelopathic traits from them are being incorporated in the high yielding crop cultivars so as to achieve the target of suppressing weeds selectively. Finally, natural phytotoxic chemicals from allelopathic plants can be exploited as novel agrochemicals for controlling the harmful weeds. Such chemicals not only provide the desired results but also pose no threat to environment and have more target sites in contrast to commercial herbicides. All these strategies and potentials of using allelopathy for weed management are discussed.

**Key words:** Allelochemicals, cover crops, eco-friendly weed control, green manure crops, smother crops, sustainable agriculture.

### INTRODUCTION

Plants produce a diversity of secondary metabolites which not only play an important physiological function for the plant itself, but also result in plant-plant interactions upon release from the donor plant. These interactions are complex and diverse in nature and provide the donor plant a number of selective advantages. One of such interactions is *allelopathy* which literally means *mutual sufferings* and was first coined by Molisch in 1937. Since the inception of this term, this science gained momentum only during the last 3 decades owing to the advancement in methodology, sophisticated instrument and its applied values. Rice (1984) described allelopathy as any direct or indirect positive or negative effect of one plant on the other (including microbes) through the release of chemicals into the environment. These chemicals are called as allelochemicals and are of late catching the attention of scientists for their potential to suppress weeds in an environmentally safe manner. Allelochemicals show a great diversity of chemical nature and have also been named differently. In plants they are synthesized as secondary metabolites and are found localised and sequestered in

specialized structures. Unlike the earlier beliefs that they are metabolic wastes, their significance is now well known. Within the plant they provide resistance to plants against pathogens, insects and other pests and also increase the reproductive fitness of the plants. Upon release through leachate, volatilization, root exudation or death and decay of plant parts, they bring about allelopathic interactions either directly or indirectly by undergoing changes in chemical nature by the action of microbes. The phenomenon of allelopathy has a great potential in improving crop productivity, genetic diversity, maintenance of ecosystems stability and eco-friendly means of pest and weed management. Out of these, its role in weed management is more important as worldwide weeds are estimated to cause enormous crop losses. Allelopathy, particularly of crops, can play an important role in weed management, if suitably managed and applied (Weston 1996; Kohli et al. 1998; Wu et al. 1999). Some of the strategies by which allelopathy can be exploited for weed management are use of allelopathic cover and smother crops, use of rotational and companion crops, green manure crops, potential of crop residues, screening of wild relatives and transfer of allelopathic traits into modern cultivars, and direct use of allelochemicals as herbicides.

### USE OF ALLELOPATHIC COVER AND SMOTHER CROPS

Cover crops are generally grown during the fallow period between two crops with a view of reducing soil erosion, improving nutrient status, conserving moisture, and controlling weeds vis-à-vis providing living biomass (Foley 1999). On the other hand crops which are either grown as cash crop or in rotation to main crop and kill the weeds by shading due to their quick growth are called as smother crops (Foley 1999). Cover and smother crops suppress the weeds by competing for nutrients, light and water, and releasing allelochemicals (Barnes and Putnam 1983). If suitably managed and manipulated the allelopathic cover and smother crops can considerably reduce the weed incidence and provide a natural and non-herbicidal method of weed control (Barnes and Putnam 1983; Swanton and Murphy 1996). Some of the important cover and smother crops are mustard *Brassica* spp. (Oleszek 1987), buckwheat *Fagopyrum esculentum* (Eskelsen and Crabtree 1995), sunflower *Helianthus annuus* (Leather 1987), rye *Secale cereale* (Putnam et al. 1983), foxtail millet *Setaria italica* (Schreiber 1992), sorghum *Sorghum* spp. (Weston, 1996), clover *Trifolium* (Dyck et al. 1995), and wheat *Triticum* spp. (Jordan et al. 1999).

### USE OF ROTATIONAL AND COMPANION CROPS

Crop rotation is one of the traditional agricultural practices when some crops particularly legumes are grown in rotation with main crop in order to overcome the problem of soil sickness. Likewise, companion cropping was also used in the past to maintain the fertility of soil. In addition the weed incidence is greatly reduced under these practices. Unfortunately with the advent of synthetic chemicals and fertilizers these practices declined, but have now resurfaced because of declining soil quality of agroecosystems (Schreiber, 1992). Kalantri (1981) and Crookston et al. (1991) reported an increase in yield of corn when grown in rotation with soybean compared to corn alone.

### GREEN MANURE CROPS

Some crop plants improve the soil through the addition of organic matter and nitrogen (in legumes) besides controlling weeds and are known as Green manures, e.g. legumes such as *Mucuna* spp. and *Trifolium* spp., cruciferous plants and *Ipomoea*

species. A number of cruciferous plants like *Brassica hirta*, *B. juncea*, *B. napus*, *B. campestris*, and *Lepidium sativum* are excellent green manure and known to check the weeds and pests. These are known to release glucosinolates which undergo enzymatic hydrolysis to form isothiocyanates and the later are potent weed suppressants (Al-Khatib and Boydston, 1999; Vaughn, 1999). Velvetbean (*Mucuna pruriens* var. *utilis*) when used as green manure effectively controls the weeds owing to the presence of L-DOPA (Fujii, 1999). Residues of green manure red clover suppress the growth and establishment of wild mustard by the release of allelochemicals (Ohno and Doolan, 2001).

### POTENTIAL OF CROP RESIDUES

Crop residues are the whole plants or their parts left in the field for decomposition after the crop harvest. These upon decomposition not only enhance nitrogen cycling and conserve soil moisture but also suppress weeds due to the release of allelochemicals. Their presence on the soil surface as mulch reduces the incidence of weeds and can also reduce the reliance on herbicides (Weston 1996). Barnes and Putnam (1983) reported that residues of cover crops viz. rye, wheat, and barley reduced the density and biomass of broad-leaved weeds and these were later found to contain allelochemicals such as  $\beta$ -phenyl lactic acid and  $\beta$ -hydroxybutyric acid, and DIBOA and BOA (Shilling et al. 1985). Residues from a number of crops like rye, barley, wheat, clover and triticale have been reported to reduce the growth of weeds (William et al. 1998; Ohno and Doolan 2001).

### SCREENING OF WILD RELATIVES AND TRANSFER OF ALLELOPATHIC TRAITS IN MODERN CULTIVARS

Several wild accessions of modern day crop plants such as oat, cucumber, soybean, mustard, and rice are found to possess allelopathic traits that impart them resistance against weeds and pests (Dilday et al. 1998; Kohli et al. 1998; Olofsdotter et al. 1995). During the process of cultivation and selection of high yielding varieties these characters and traits gradually got eliminated from the modern day cultivars. However, these can be incorporated in the modern day cultivars through several modern techniques as a plant protection strategy. Not only so, even the different cultivars differ in their allelopathic behaviour and genotypes with more allelopathic activity can be selected for IWM programmes (Wu et al. 1999). Dilday et al. (1998) observed that out of the 16,000 rice accessions from different countries many exhibited allelopathic suppression of ducksalad and redstem. The genes responsible for synthesis of allelochemicals in these cultivars can be transferred by DNA recombinant technology and conventional breeding programmes. Genetic approaches like PCRs, RAPD, RFLPs and NIL should, therefore, be explored for crop improvement and development of molecular markers to aid in breeding cover crops so as to bring about weed suppression (Foley 1999).

### DIRECT USE OF ALLELOCHEMICALS AS HERBICIDES

The overuse of the synthetic chemicals for the control of pests is posing a serious threat to the environment. It is strongly realized that the natural plant products, being



biodegradable are eco-friendly and can be relied upon more to enhance the crop productivity in a sustainable way. Allelochemicals from the higher plants and microbes can, therefore, prove to be ideal agrochemicals (Dayan et al. 1999; Duke et al. 2000; Kohli et al., 1999; Macias et al. 1997; Rice 1995). They are normally broad-spectrum molecules that are bio-efficacious, economical and environmentally safe and have greater shelf life with a wide range of storage condition and ease of application.

Some of the noteworthy and promising allelochemicals from higher plants with known weed suppressing ability are aianthone, artemisinin, isothiocyanates, cineole, DIBOA and BOA, L-DOPA, parthenin and sorgoleone (Fujii 1999; Kohli et al. 1999; Nimbal et al. 1996; Romagni et al. 2000). Not only the higher plants, even the allelochemicals from fungi, bacteria and actinomycetes too provide an excellent source of natural herbicide products. A number of phytotoxins with potential herbicidal activity have been isolated from *Streptomyces* spp., *Alternaria* spp., *Actinomyce*, *Paecilomyces*, *Pseudomonas* spp., and *Ustilaginoidea* spp., etc. (Cutler 1999; Hoagland 1999; Duke et al. 1996). Although a number of compounds from microbes with herbicidal activity have been isolated and patented, yet only two bialaphos and phosphinothricin have been successfully commercialized. Even some of the allelochemicals like cineole, benzoxazinones, quinolinic acid and leptospermones from higher plants either as such or their derivatives have been marketed by companies in Germany, USA and Japan (Dayan et al. 1999). Though such commercialized products represent a small fraction, yet they offer novel herbicide target sites (Duke et al. 2000).

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## **SIMPLE BIOASSAY METHODS FOR RICE ALLOELOPATHY**

K. U. Kim, D. H. Shin, I. J. Lee, H. Y. Kim, and J. H. Lee

Dept. of Agronomy, College of Agriculture, Kyungpook National University, Taegu  
702-701, Republic of Korea. Kukim@knu.ac.kr

**Abstract:** This research was conducted for evaluating a simple bioassay method for rice allelopathy. Water extract, relay seeding and agar medium method were tested to obtain reliable data applicable to different conditions for screening rice allelopathic potential. Relay seeding method was simple and easy to carry out, but it had some problems in discriminating a specific allelopathic line because all tested cultivars exerted to some extent allelopathic effects. Agar medium test was not simply easy to handle. Water extraction from seeds gave relatively reliable information, and thus, water extraction directly from seeds seemed to be a simple and reliable method for evaluating rice allelopathic potential.

**Key words:** Water extract, relay seeding, agar medium test, allelopathic potential

### **INTRODUCTION**

Bioassays are a necessary tool in allelopathy research, enabling qualification of biological responses in living organisms. Bioassays also frequently detect biological activity of chemicals in concentrations beyond of the limit of detection (Olofsdottir et al. 1994). Thus, bioassays are needed both in initial screening for allelopathic potential and in the extraction and purification of allelopathic substances. For screening rice allelopathic potential, several methods such as stairstep method (Bonner 1950; Liu and Lovett 1993a), hydroponic culture test (Einhelig 1985), relay seeding technique (Navarez and Olofsdottir 1996), agar medium selection (Fujii 1992; Wu et al. 1999), cluster analysis using HPLC (Mattice et al. 1999), and water extract method (Kim et al. 1999 and 2000) have been developed and tested for bioassays. Each screening method has its own advantages, but what we need is a reliable and universal method which gives a similar result in both the lab and the field.

Much works have been done on rice allelopathy, since Dilday and his group in Arkansas (USA) found allelopathic potential in rice accessions (Dilday et al. 1989, 1991, 1994), several others have reported allelopathic activity in rice, particularly, genetics of rice allelopathy (Bach-Jensen et al. 1999) and autotoxicity (Duke et al. 1999), but there needs to be a more simple, easy and precise evaluating method which can be applicable to any condition. The main purpose of this paper was to evaluate the most simple method for screening rice allelopathic potential.

### **MATERIALS AND METHODS**

#### **Water extraction from seed**

Rice seeds from 7 cultivars such as Dongjinbyeo, IAC 165, Kouketsumochi, Sathi, Musashikogane, PI 312777 and Tang gan were grinded in a grinder (Ika-Werka, Germany). One hundred ml of distilled water was added to 10 grams of grinded rice sample. Water extraction was done for 48-h at  $26 \pm 2^\circ\text{C}$ . Water extraction was filtered through Whatman no. 2 paper. Dilution was made to make 1% and 5% solution. Ten ml

of 1%, 5% and 10% water extract was treated on a petri dish containing 20 barnyardgrass seeds with 3 replications. At day 10, germination and growth of barnyardgrass were determined.

#### **Relay seeding method**

Seeds of barnyardgrass were relay-seeded to 7-day-old seedlings of the same cultivars as above and grown together for 10 days on a petri dish inside a transparent germination box. The grass petri dish (9.0 cm, pyrex) was lined with a bridge filter paper strip (4×23 cm, Whatman no. 1) for supplying water from germination box (11.5 cm high, 13×13 cm bottom), which initially contained 50 ml of distilled water. This technique was adapted from the procedure recommended by Navarez and Olofsdotter (1996). The petri dish contained 20 sterilized rice seeds in sodium hypochloride sown in rows and was covered with 4.0 g white, fine of powered perlite to restrain arching of rice roots. The germination box was covered with a transparent plastic lid. Additional amount of distilled water was added later to maintain adequate moisture.

The set-up was placed in a temperature maintained room at  $26 \pm 2^\circ\text{C}$  with 4000 lux of light intensity. At day 7, 30 sterilized seeds of barnyardgrass were sown evenly on filter paper supported by perlite in one row parallel to the rice rows. Barnyardgrass seedlings were harvested at day 17 for allelopathic potential.

Germinated seeds of barnyardgrass were counted and recorded at Day 17. At harvest time, each seedling was pulled gently. Rice seedlings were removed first and carefully separated from barnyardgrass to avoid damage. Shoot and root lengths of each seedling were measured using a ruler. The length was measured from the junction of shoot and root to their corresponding tip.

#### **Agar medium method**

Three percent agar medium was prepared and 100 ml of this medium poured in a 500 ml beaker. After some time, 30 dehulled rice seeds of the same cultivars as above were sown on agar medium in 2 rows and 20 barnyardgrass seeds were sown in between rice rows. After covering with a transparent plastic held in place by a rubber band, they were placed in a temperature maintained room at  $26 \pm 2^\circ\text{C}$ . At day 17, shoot and root lengths of barnyardgrass with dry weight were determined.

### **RESULTS AND DISCUSSION**

#### **Water extraction from seed**

Figures 1 and 2 indicate the effect of water extract made directly from seeds of 7 cultivars on the growth of barnyardgrass. There is a very clear difference among rice cultivars tested. All levels of water extract from Kouketsumochi, potential allelopathic cultivar showed very high inhibitory effects on barnyardgrass root growth, but 10% of water extract from Dongjinbyeo showed rather stimulatory than inhibitory effect in both barnyardgrass shoot and root growth. Almost no growth of barnyardgrass root was observed in 10% water extract from Kouketsumochi and Musashikogane. However, barnyardgrass root grew normal at all levels of water extract from Dongjinbyeo. Response of barnyardgrass root growth can be used as a screening method for rice allelopathic potential by us of water extraction from seed (Figures 1 and 2). Water extraction from seed is a very simple and easy method to screen rice allelopathic

potential, strongly suggesting that rice seeds of each cultivars may possess different levels of alleopathic potential.

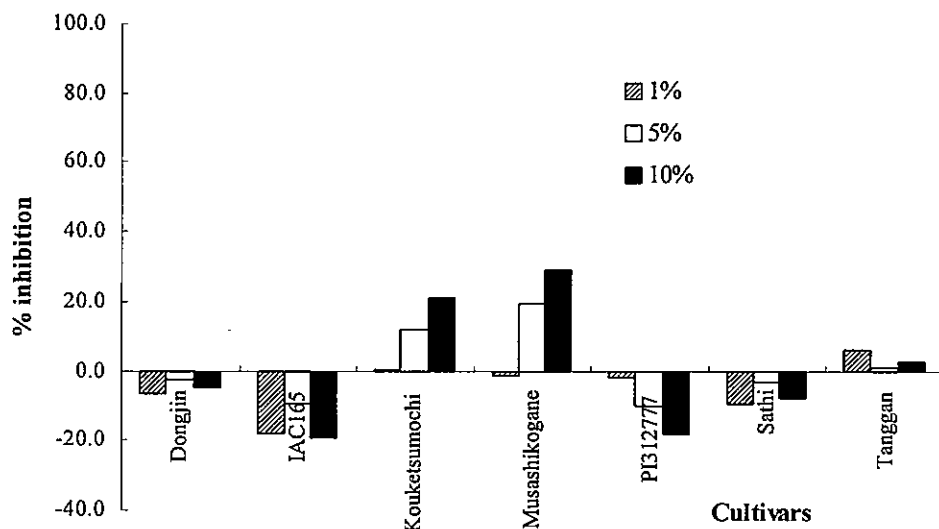


Figure 1. Effect of water extract from seed on barnyardgrass shoot growth.

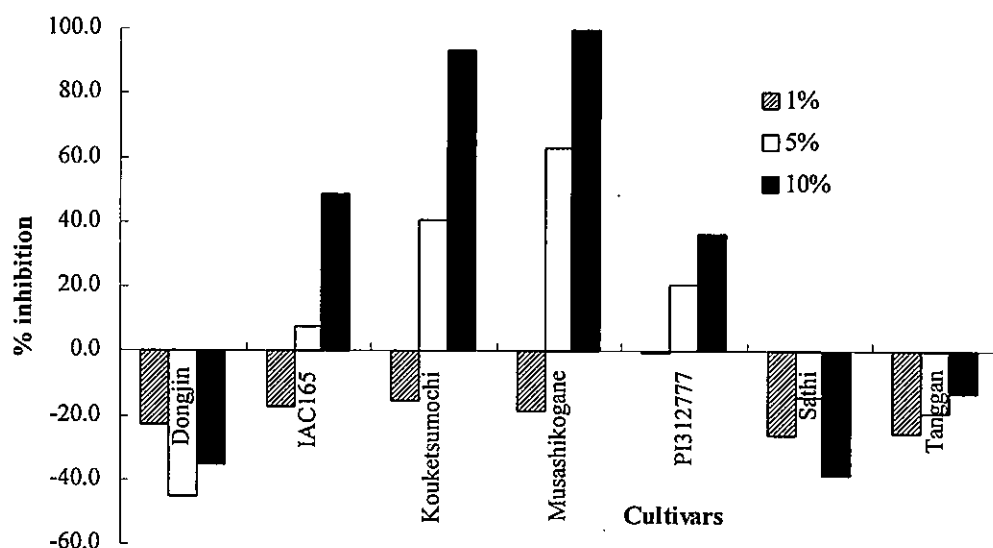


Figure 2. Effect of water extract from seed on barnyardgrass root growth

### Relay seeding method

Figure 3 shows the effect of potential alleopathic cultivars on the growth of barnyardgrass determined by relay seeding method. All the cultivars showed various inhibitory effects on shoot and root growth of barnyardgrass ranging from 2% to 27%, including Dongjinbyeo which was classified as a nonalleopathic rice in water extract bioassay. All the cultivars tested except for Dongjinbyeo were internationally known potential alleopathic accessions. One of disadvantages of this method was difficulty to distinguish alleopathic and nonalleopathic cultivars since all the rice cultivars exhibited to some extent alleopathic effect. However, it may be possible for

categorizing into high and low allelopathic groups.

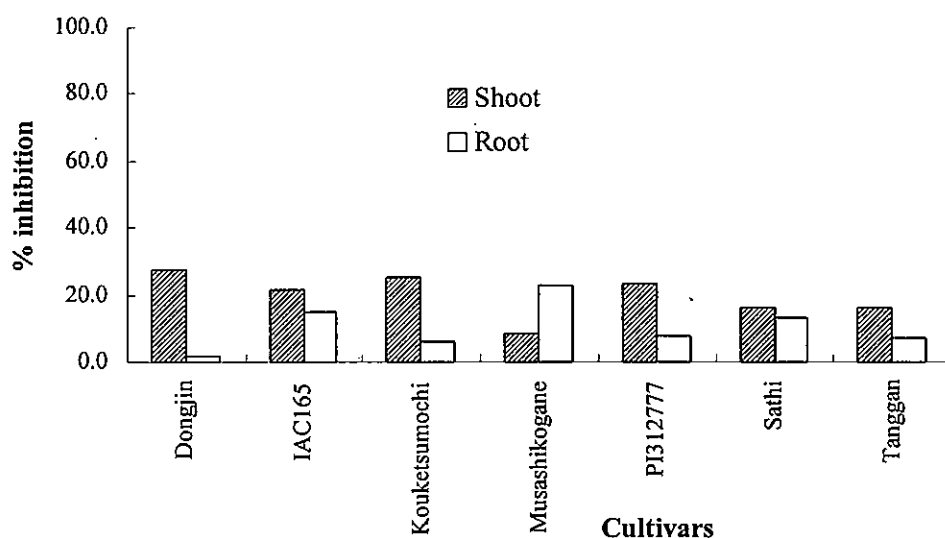


Figure 3. Effect potential allelopathic cultivars on the growth of barnyardgrass assayed by relay seeding method.

#### Agar medium method

Figure 4 shows the effect of potential allelopathic cultivars on the growth of barnyardgrass. All the cultivars tested showed inhibitory effects on the growth of barnyardgrass root ranging from 5% to 25%. It is hard to explain a relatively high inhibitory effect of Dongjinbyeo on barnyardgrass root growth, about 21%. This suggests that agar medium assay may not be an appropriate method for evaluating rice allelopathic potential.

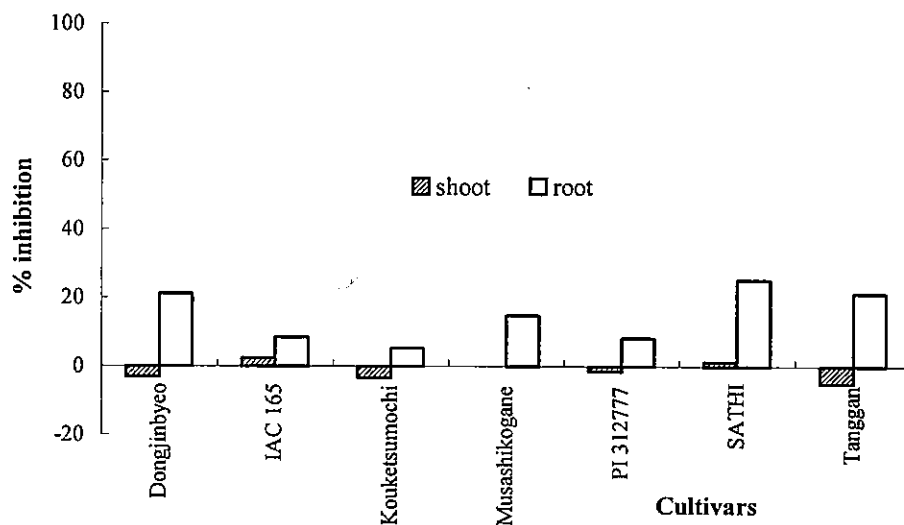


Figure 4. Allelopathic potential of some selected rice cultivars on barnyardgrass growth tested in agar medium (3%).

Several screening methods were employed for evaluating rice allelopathic potential. Among them, relay seeding technique was a very simple, rapid and inexpensive method as suggested by Navarez and Olofsdotter (1996). However, this method has a problem in categorizing cultivars or accessions into allelopathic or nonallelopathic rice because all the tested samples showed some response. Agar medium test is also a quite simple and easy method like relay seeding technique. The problem of this method was the difficulty in maintaining moisture inside a container covered with transparent plastic and thus it was hard for rice plants to grow normally. Another problem might have been the use of dehulled rice seeds to eliminate contamination because the rice hulls might have had allelopathic properties. Direct field screening might be the best method for screening allelopathic potential, but it has difficulty in dealing with a large number of germplasm. The screened cultivars or accessions allelopathically active in one method were not always active in other screening methods employed. For instance, Dongjinbyeon, an improved variety, was evaluated as nonallelopathic in the water extraction method, but it showed inhibitory effects in both the relay seeding technique and the agar medium test. Therefore, what we need is a reliable and universal method which gives consistent results when tested by all the available screening methods.

For this purpose, the water extraction method made from seeds was conducted as an alternative screening method, particularly, exhibiting very clear differences among potentially allelopathic cultivars, which were internationally tested. This suggests that seeds of every cultivar or accession already possesses allelopathic activity, and if so, it is possible to screen allelopathic potentials directly from seeds. It is well known that most allelochemicals are released during germination and early growth (Dekker & Meggitt 1983). Recently, Mattice et al. (1999) proposed cluster analysis using HPLC as a simple assay method evaluating allelopathic potential within 10 days after germination using only 10 mg tissue per ml of methanol. This method can be done in a relatively short period of time in a small amount of space and screened samples can be used for direct field evaluation. Further study on discriminative ability of water extraction method from seeds will shed more light on establishing a reliable and universal screening method for evaluating allelopathic potential.

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## EVALUATION OF ALLELOPATHIC ACTIVITY OF RICE PLANTS THROUGH HYDROPONIC CULTURE

S.Y. Kim<sup>1</sup>, A.V. Madrid<sup>2</sup>, M. Olofsdotter<sup>2</sup>, S.T. Park<sup>1</sup> and H.Y. Kim<sup>1</sup>

<sup>1</sup>Rice Division, National Yeongnam Agricultural Experiment Station, 1085 Milyang, Korea  
kimsy3@rda.go.kr

<sup>2</sup>Agronomy, Plant Physiology and Agroecology Division, International Rice Research  
Institute P.O. Box 933, 1099 Manila, Philippines

**Abstract:** To investigate the differential allelopathic activity of rice plants, the inhibitory activity of water extract of the shoot and root of three rice cultivars, Taichung natine I(TN1) and IAC165(allelopathic rice) and AUS196(non-allelopathic rice), grown in hydroponic culture was evaluated. The release of germination inhibitors by allelopathic rice plants into hydroponic solution was also determined with freshly collected solution and XAD-4 resin desorbate. The degree of the inhibition was quantified in terms of root growth in *Echinocloa colona*, *E. crus-galli*, *E. crus-galli* var. *oryzicola*, *Triantema portulacastrum* and *Lactuca sativa*. The allelopathic activity of rice plants was species specific, and depended on source and concentration. Root length of all test species was inhibited by the different concentrations of shoot extract of allelopathic and non-allelopathic rices. However, of the three cultivars, TN1 showed higher inhibition than the IAC165 and AUS196 in all test species. Water extracts of shoot and roots significantly inhibited root growth in *E. crus-galli* but the shoot extract gave the greater inhibitory effect on *E. crus-galli* than the root extract. Root exudate of TN1 inhibited root elongation of *E. crus-galli* from 2 weeks after transplanting (WAT) and the inhibition was prolonged for 4 WAT. The results confirmed the previous finding of laboratory bioassay that the TN1 had allelopathic activity and produced allelochemicals that inhibit growth of weed species.

**Key words:** allelopathy, rice, weed control

### INTRODUCTION

Recently, research on rice allelopathy in weed control is accelerating as an alternative weed control strategy against chemical weed control method. Many scientists have been screening allelopathic rice cultivars both in laboratory and field and they found that several cultivars or lines showed promise in inhibiting the growth of certain weed species. Dilday group screened 10,000 accessions of rice and they found 3.5% to show allelopathic potential against duckweed (*Wolffia*)(Dilday et al. 1991). In Philippines, 11-21 cultivars out of 111 rice cultivars suppressed root growth of *E. crus-galli* by more than 50% (Olofsdotter and Navarez 1996). In Korea, 20-40 cultivars out of 110 rice cultivars showed above 50% inhibition of *E. crus-galli* seedling growth in field and water extract experiments (Kim et al. 2000). However, the results of the allelopathic activity of the rice cultivars in the laboratory and field are sometimes varied depending on the screen methods and experimental places because in the field, competition and allelopathic effect could not be differentiated (Rice 1974). In addition, allelochemical production is affected by biotic and abiotic factors (Putnam 1985). Such bias can be avoided through confirming the allelopathic activity of water extract and germination inhibitor released from the root of allelopathic rice plants.

Without validation of allelopathic activity, the results remain obscure. Therefore, the objective of this study was to evaluate the allelopathic activity of laboratory-screened allelopathic and non-allelopathic rice cultivars through hydroponic culture.

## MATERIALS AND METHODS

### General procedures

Experiments were conducted in laboratory and greenhouse of the International Rice Research Institute from January to March, 1999. Three rice cultivars such as TN1 and IAC 165 (allelopathic), and AUS 196(non-allelopathic) were selected for this experiment based on previous rice allelopathy study (Olofsdotter and Navarez 1996). Rice plants were grown in hydroponic culture inside the greenhouse. For hydroponic culture, rice seeds were surface-sterilized with NaOCl for 20 minutes, rinsed with distilled water and germinated in petri dishes under temperature-controlled germination room. Seedlings grown for 12 days in laboratory were wrapped with foam and transplanted into a styrofoam float. The styrofoam float with seedlings was placed into a 24 liters plastic pail containing hydroponic culture solution prepared according to method (Yoshida et al. 1976). Nine seedlings with a 7cm distance between two seedling were grown in the pails. The pail was wrapped with aluminum foil to inhibit algae growth and put in a bench with a cool water to keep overheat away.

The culture solution was prepared using tap-water and changed every 2 or 3 days and pH was maintained at 5.5 throughout the whole experiment. The culture solution was kept at 50% until three days after transplanting, then changed to full strength. pH and electro conductivity were measured every day to investigate any change in culture solution.

Water samples were collected at 1, 2, 3, and 4 weeks after rice transplanting to investigate release of allelochemical through root. The culture solution was transferred to brown glass bottle after completely stirring and kept at 5°C until it was used.

### Collection and preparation of samples

At 30 days after transplanting, rice plants were harvested and separated to shoot and root. The samples were dried in an oven at 70°C for 5 days and then ground in an Wiley Mill using 20-mesh screen. Ground sample were stored in bottles at room temperature until they were used.

### Plant species response to shoot water extract

Ground rice shoot including leaf was soaked in water for 3 days at the ratio of 0, 1, 2, and 4%(w/v). Twenty five seeds of *E. crus-galli*, *E. colona*, *E. crus-galli* var. *oryzicola*, *T. portulacastrum*, and *L. sativa* were placed in petri dish (5.5cm in diameter) lined with one Whatman # 1 filter paper moistened with 3ml of the extract. The germination was conducted in germination room at  $28 \pm 1^\circ\text{C}$  in the presence of light for 12 hr. Root lengths were determined at 6 days after treatment. Each treatment was replicated four times in a completely randomized block design and experiment was repeated twice.

### Phytotoxicity of plant parts to *E. crus-galli*

To determine the part of rice plant that gives greatest inhibition, dried shoot and root were separately ground and water extract of each plant part at 0, 1, 2, 4, 6 and 8%(w/v) were prepared as above. Root lengths were determined at 7 days after treatment. The germination test was conducted as in the preceding section. To

investigate effect of the extract solution, pH and osmotic potentials were measured. For the measurement of pH, 8% concentration was used. Osmotic potential was measured using a freezing point osmometer (Precision Systems, Inc. Model 5004) and expressed in MPa.

#### **Determination of germination inhibitor in root exudate**

To investigate allelochemical released from root of rice plant, the hydroponic culture solution was collected at 1, 2, 3, and 4 weeks after rice transplanting and used for fresh solution and XAD-4 resin adsorbate study. For fresh solution, twelve ml per petridish (9cm in diameter) was used for bioassay as indicated in the above.

For recovery of root exudate, a column (5 x 20 cm) was packed with 190g of 20- to 60-mesh Amberlite XAD-4 resin (Aldrich Chemical). The resin was thoroughly washed with three volumes of methanol followed by at least of 10 volumes of distilled water. Three liter of culture solution was loaded on the column and then eluted with 4-bed volume of methanol. The eluent was dried over  $(\text{NH}_4)_2\text{SO}_4$  and was evaporated to dryness with rotary evaporator at 55°C. The residue was resuspended with 12ml of distilled water and the germination test was conducted as in the preceding section. A blank control with fresh prepared culture solution was used for comparison.

### **RESULTS AND DISCUSSION**

#### **Plant species response to shoot water extract**

The effect of aqueous extracts of rice shoot on the root growth of weed species is shown in Table 1. The root elongation decreased as the extract concentration increased. Of the test species, *L. sativa* showed the greatest inhibition followed by *E. colona*. *T. portulacastrum* showed least inhibition in root elongation. Although root inhibition of all test species was similar at 4% concentration, inhibition was more marked in TN1 at concentration of 1-2%.

This reveals that an example of species-specific growth-regulating effect of allelochemicals. Mersie and Singh (1987) demonstrated that inhibitory action of parthenium varied with test species. Similar differences in response among test species were reported by Kim (1992). The result demonstrates that rice shoot extract contains allelopathic substances that inhibit root growth of weed species.

#### **Phytotoxicity of plant parts to *E. crus-galli***

*E. crus-galli* is selected as a test species because it is most troublesome weed in the world. The water extract of shoots and roots significantly reduced root growth in *E. crus-galli* but the effect depended on rice plant parts and extract concentration (Figure 1). The shoot extract of three rice cultivars had greater inhibitory effect than root extract regardless of concentrations. At the lowest concentration (1%), the shoot extract of TN1, IAC165 and AUS196 reduced root growth of *E. crus-galli* 6 to 47% but the root extract reduced only less than 3%. As the extract concentration increased, root growth of *E. crus-galli* was inhibited by two extracts but the inhibition was greater in the shoot extract than in the root extract. Root growth of *E. crus-galli* was 98-100% inhibited at 4% concentration of shoot extract but it was inhibited only 91-94% even at 8% root extract.

At the highest concentration, the root length was not significantly different by rice cultivars. The greater root growth inhibition in shoot extracts suggest that the shoot contain proportionally higher amount of the allelopathic compounds than the root do.

Guenzi et al.(1967) reported that allelochemicals occur in different parts of sorghum plants including leaf, stem, root and seed, but the stem extract has the greatest inhibitory effect on wheat seedlings. Barnes and Putnam(1983) also reported that shoot extract of rye was more toxic than the root extract to test species. The results suggest that different concentrations of allelopathic compounds are present in different parts of rice plants as shown by the activity of the extracts of shoots and roots.

Table 1. Effect of shoot extract of rice cultivars on the root length of different plant species.

Concentration (%, w/v)	Root length(mm)		
	TN1	IAC165	AUS196
<i>Echinocloa crus-galli</i>			
0	53	53	53
1	31	51	54
2	9	28	19
4	1	1	1
LSD(0.05)	6.8	5.5	6.9
<i>Echinocloa crus-galli</i> var. <i>oryzicola</i>			
0	49	49	49
1	18	48	43
2	5	22	20
4	3	1	1
LSD(0.05)	3.9	5.2	4.7
<i>Echinocloa colona</i>			
0	35	35	35
1	10	15	17
2	2	7	7
4	0	0	0
LSD(0.05)	5.2	5.6	5.7
<i>Triantema portulacastrum</i>			
0	21	21	21
1	14	28	28
2	5	20	21
4	0	1	1
LSD(0.05)	2.7	4.6	3.1
<i>Lactuca sativa</i>			
0	38	38	38
1	5	21	18
2	0	0	1
4	0	0	0
LSD(0.05)	7.0	6.2	5.4

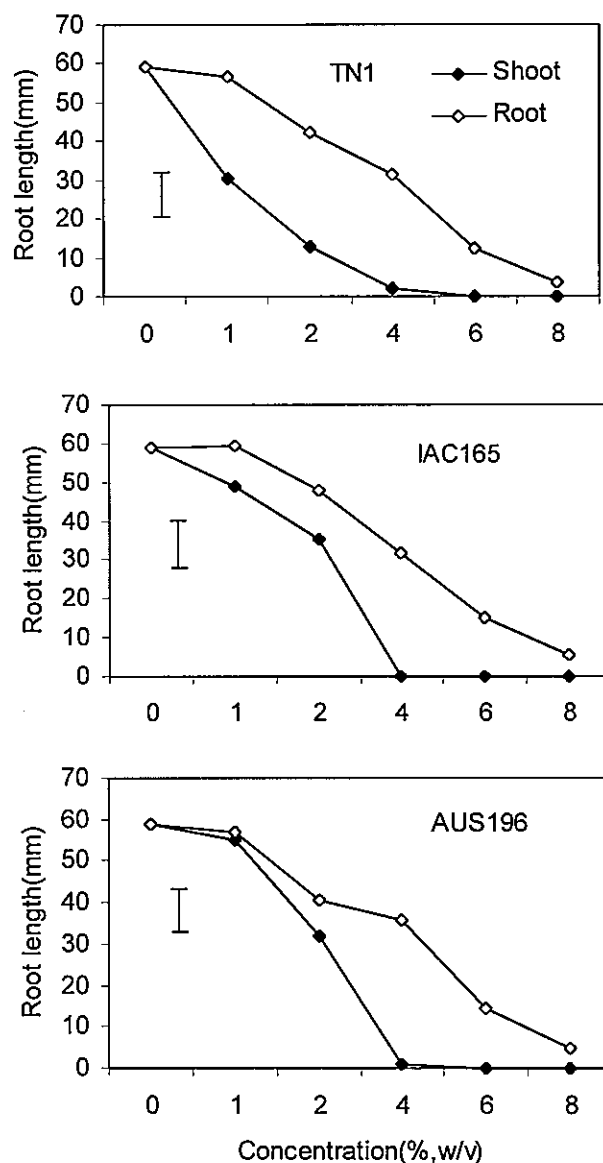


Figure 1. Effect of shoot and root water extracts on the root growth of *Echinocloa crus-galli*.

In studies concerning the effects of the plant extracts on germination and growth of sensitive indicator species, osmotic potential of the solution can be a problem. Therefore, it is important to test for osmotic effects. Osmotic potential of water extracts of shoot and roots ranged between -0.05 and -0.52 Mpa (Table 2). There were no significant differences in osmotic potential among various concentrations of three rice cultivars although TN1 had slightly higher osmotic potential in the both shoot and root extract. Significant differences in root growth depending extract concentrations of rice cultivars were evident while pH of the solution was not. This suggests that inhibition resulted from the phytotoxins presents in the extracts and not from osmotic stress.

#### Determination of germination inhibitor in root exudate

Release of germination inhibitor by rice roots is shown in Figure 2. Root growth of *E. crus-galli* seedling was not inhibited by freshly collected solution of three rice

cultivars for 4WAT(Figure 2A) but it was significantly differed by XAD-4 resin desorbate of three rice cultivars(Figure 2B). This indicates that concentration of inhibitors in fresh solution is too diluted to detect and needs to be concentrated. Of the three rice cultivars, only desorbate of TN1 inhibited root growth of *E. crus-galli* but those of AUS196 and IAC 165 did not. Desorbate of XAD-4 resin in TN1 significantly inhibited from 2WAT and inhibition was prolonged up to 4WAT. At 4WAT, root growth of *E. crus-galli* was inhibited 55% in desorbate of TN1 as compared with AUS 196. This indicates that TN1 releases large quantities of allelochemicals from root as early as 2 WAT and the release was lasted for 4WAT while IAC 165 and AUS 196 produced negligible amounts of germination inhibitors. This result showed similar trend to the previous bioassay results of water extracts.

Table 2. Osmotic potentials and pH of water extract of shoot and root of rice plants.

Extract concentration (%, w/v)	Osmotic potential(-MPa )					
	Shoot extract			Root extract		
	TN1	IAC165	AUS196	TN1	IAC165	AUS196
1	0.07	0.07	0.07	0.07	0.06	0.05
2	0.14	0.13	0.13	0.13	0.11	0.10
4	0.26	0.25	0.25	0.23	0.20	0.18
6	0.39	0.37	0.37	0.33	0.29	0.26
8	0.52	0.48	0.49	0.44	0.39	0.35
pH*	6.1	6.5	6.4	7.2	7.2	7.3

\*8% concentration of water extract

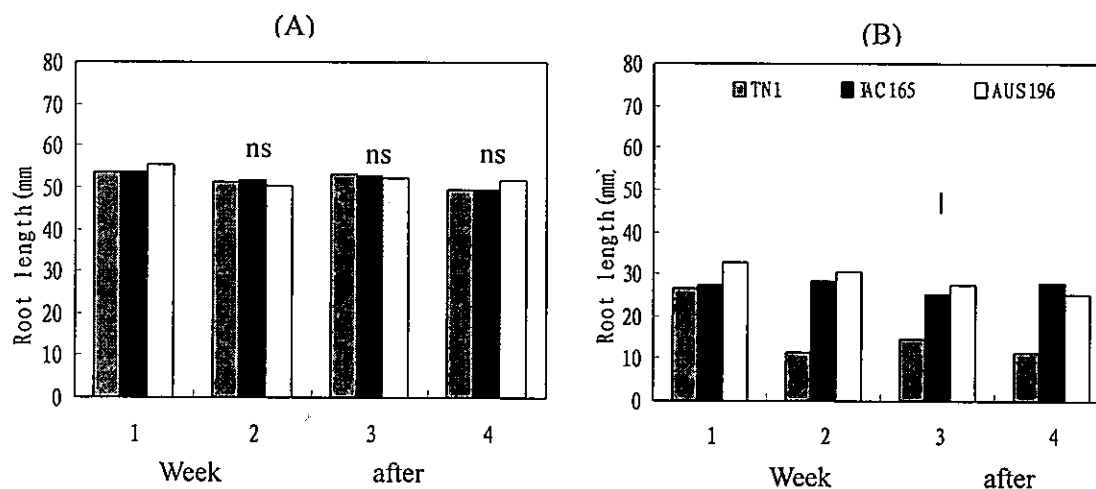


Figure 2. Effect of fresh hydroponic solution(A) and XAD-4 adsorbate(B) on the root growth of *Echinocloa crus-galli*. ns: not significant

On the other hand, pH and electro conductivity of the culture solution in the three rice cultivars for 30 DAT were similar (Table 3). This indicates that inhibition resulted from the inhibitors presents in the solution and not from pH change. The results confirmed the previous finding at laboratory bioassay that the TN1 had allelopathic activity and produced allelochemicals that inhibit growth of weed species.

However, both shoot water extract and XAD-4 desorbate of IAC165 inhibited root growth of *E. crus-galli* similar to the non-allelopathic rice of AUS196. The effect of shoot water extract of IAC165 at 45 DAT showed similar trend to that of 30 DAT (data not presented). This result is deviated from that of the laboratory screening using relay seeding technique. Therefore, further pot or field experiments are necessary to confirm the variation between the hydroponic culture and laboratory bioassay of IAC165 because allelochemical production of the cultivar in hydroponic culture may be different from that in soil.

Table 3. Average pH and electro conductivity of the hydroponic solution grown rice over 30 days.

Cultivar	pH	Electro conductivity (ds/m)
TN 1	5.60 ± 0.1	1.56 ± 0.04
IAC 165	5.60 ± 0.1	1.56 ± 0.04
AUS 196	5.60 ± 0.1	1.58 ± 0.03

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## TOWARDS THE PRODUCTION OF ALLELOPATHIC RICE CROPS IN CAMBODIA

S. Pheng<sup>1</sup>, S.W. Adkins<sup>1</sup>, G.C. Jahn<sup>2</sup>, M. Olofsdotter<sup>3</sup>, and H.J. Nesbitt<sup>4</sup>

<sup>1</sup>School of Land and Food, University of Queensland, St. Lucia, Qld.4072, Australia.  
s800661@student.uq.edu.au

<sup>2</sup>Entomology and Plant Pathology Division (EPPD). International Rice Research Institute (IRRI). IRRI, DAPO Box 7777, The Philippines.

<sup>3</sup>Weed Science, Department of Agricultural Science, KVL, Agrovej 10, 2630 taastrup, Denmark.

<sup>4</sup>Cambodia-IRRI-Australia Project (CIAP). P.O. Box 01, Phnom Penh, Cambodia.

**Abstract:** Following on from laboratory, then pot studies, a field experiment was conducted in Cambodia during the 1999 wet season to determine the weed-suppressing ability of six rice (*Oryza sativa* L.) cvs on two important weed species viz. barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) and small umbrella sedge (*Cyperus difformis* (L.)). The effect of the soil-incorporated residues from these rice cvs was also studied on the establishment of *E. crus-galli* and rice (cv. Santepheap 3 (ST-3)) in the following season. Variation in certain growth parameters, which are thought to be among those responsible for the competitive traits of rice could not explain the weed growth suppressing ability of the cvs. Therefore, it was thought that the previously identified allelopathic trait (and/or unknown competitive traits) were among those factors that were responsible for the growth suppressive ability of the cvs on the weeds. The soil-incorporated residues produced from six rice cvs (viz. CAR-3, CAR-4, CAR-8, Neang Kong, Neang Khat and Pa Thaut) were found to be suppressive towards the early growth and establishment of *E. crus-galli* whereas the residues of 5 cvs (viz. CAR-3, CAR-4, CAR-8, Neang Kong and Neang Khat) were inhibitory to rice (cv. ST-3). Rice production in Cambodia would benefit from the development of an allelopathic rice crop cv., however, the residues must not inhibit the growth of subsequent crop. The use of allelopathic rice cvs. that do not have a toxic residue may provide farmers with an affordable weed suppressing technique that is environmentally benign. Perhaps, Cambodian farmers have unknowingly applied this approach to rice production for many years, since the allelopathic trait was detected in some Cambodian traditional rice cultivars and some popular CAR lines that had been derived from some Cambodian traditional cultivars.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the most important crop in Cambodia. It dominates Cambodian agriculture in terms of its cultivated area, the volume of the harvested product, and the number of people involved in its production. However, the national average yield of about 1.45 t ha<sup>-1</sup> is still one of the lowest in Asia (Javier, 1997). This low productivity results from a number of unhelpful abiotic factors including poor soil and unpredictable rainfall. In addition, biotic constraints such as pest plagues, are always a major threat to production in any tropical countries.

Of the 1,868,900 ha of land devoted to rice production in Cambodia, 86% is planted to rainfed lowland rice (Javier, 1997). In these areas, water management and other cultural practices are not fully effective in controlling weeds and farmers tend to

rely heavily on in-crop hand-weeding. This practice is time-consuming, laborious and sometimes impractical for weed species with seedlings that look very much like rice seedlings. Herbicides can be used to control these in-crop weeds and is a labour-saving option, but are too expensive for most Cambodian farmers to use.

Recent studies on allelopathy *viz.* the production of chemicals by one plant that can influence the growth of another plant (Rice, 1974; Rice 1984), have revealed that this phenomenon may have a significant role in many agricultural systems (Rizvi *et al.*, 1992, Rice, 1995; Olofsdotter, 1998; Narwal *et al.*, 1998;). As with some other crops, the existence of allelopathic activity in rice has been investigated for its potential role in weed management. It has been proposed that with some rice cvs known to have the ability to suppress weed growth (Dilday *et al.*, 1998; Hassan *et al.*, 1998; Olofsdotter *et al.*, 1995, 1997) the planting of allelopathic rice cvs could reduce the use of synthetic herbicides. This would create a control approach that is already in the crop and provide the farmer with an affordable alternative to hand-weeding (Olofsdotter, 1998).

This present field experiment follows on from our earlier work (Pheng *et al.*, 1999) undertaken at the laboratory and glasshouse level. The aims of this present field experiment, conducted in Cambodia during the 1999 wet season, were: (1) to determine the weed suppressing ability of six rice cvs on *E. crus-galli* (L.) P. Beauv. and *C. difformis* (L.) and (2) to assess the effect of post-harvest crop residuals from these rice cvs on the establishment of *E. crus-galli* and rice (cv. ST-3).

## MATERIALS AND METHODS

### Site

The experiment was conducted at the Cambodian Agricultural Research and Development Institute (CARDI), Phnom Penh, Cambodia. The soil at the experimental site was a sandy, light textured soil overlaying a loamy- or clay-textured subsoil. This soil type is a very common type for this part of the world and is estimated to occupy about 25 to 30% of the Cambodian rice growing area (Oberthur, 1997).

### Climate

Situated in the tropics, Cambodia has two distinct seasons, wet and dry. The wet season begins in June and ends in November while November to June it is the dry season. The experiment was conducted from August 1999 to February 2000, covering 4 months of the wet season as well as the first 2 months of the dry season. The annual rainfall of 1999 was 1371 mm. Other climatic components (e.g., temperature, humidity, wind, and daylight) varied little from the mean values previously reported for this region of Cambodia (Nesbitt, 1997).

### Materials

A total of nine treatments (*viz.* CAR-3, CAR-4, CAR-8, Neang Kong, Neang Khat, Pa Thaut, TN-1, ST-3, and a weed monoculture) were introduced into this field trial. Six of the eight rice treatments (*viz.* CAR-3, CAR-4, CAR-8, Neang Kong, Neang Khat, and Pa Thaut) were selected based on their inhibitory effects in a previous screening experiment (Pheng *et al.*, 1999). The other two rice cvs, Taichung Native 1 (TN-1) and ST-3, were also used as they represented allelopathic and non-allelopathic rice controls, respectively (Olofsdotter and Navarez, 1996, Pheng *et al.*, 1999). The weed monoculture treatment involved two weed species; *E. crus-galli* (BG) or *C. difformis*

(SUS). With two weed species, the experiment was divided into two sets, BG was used as the test plant for Set 1 whereas SUS was the test plant for Set 2. The cvs of rice were obtained from the Cambodia-IRRI-Australia Project (CIAP) whereas seed of both weed species were hand collected at maturity from two provinces in Cambodia; Kandal and Takeo.

### **Trial design**

The experiment employed a randomized complete block design with four replications. The plot size for each treatment was 2.0 x 5.2 m forming a 10.4 m<sup>2</sup> plot area. To avoid the movement of weed seed and to ease the management of water, each plot was separated by small levees and surrounded by irrigation canals.

### **Fertilization**

Each plot received fertilizer at a rate of 50 kg ha<sup>-1</sup> of nitrogen, 23 kg ha<sup>-1</sup> of phosphorous (P), and 30 kg potassium (K). All P and K were applied before planting (basal application). Nitrogen was split into two applications, half applied before planting and half applied 45 days after seeding the rice.

### **Cultural method:**

The trial was undertaken in two phases. In the first phase (Experiment 1), the rice and the weeds were sown and grown together in mixed-culture and the effects of the growing rice plants tested on the growth of the two weed species. In the second phase (Experiment 2), the unharvested rice stubble (from Experiment 1) was chopped into pieces of *ca.* 5 cm length, then spreaded evenly onto the assigned plot and incorporated manually (hand-hoeing and hand-harrowing) into the soil and their residual effects on the early establishment of BG and rice (cv. ST-3) were observed.

### **Experiment 1**

Seeds of each rice cv was direct-seeded (distributed evenly) onto their assigned plots at a rate of 100 kg seed ha<sup>-1</sup>. Twenty-five days after seeding (DAS) the rice, BG and SUS were direct-seeded into half (2.5 x 2.0 m) of each plot at a rate of 7.5 kg ha<sup>-1</sup> and 3.75 kg ha<sup>-1</sup>, respectively. The weed free half of each plot was created to act as control to help assess the effect of weeds on the performance of the rice crop. Weed monoculture plots were also created at the same seeding rate as those of the mix-culture plots. Non-target weeds emerging in the trial were removed from each plot regularly by hand. Plant height and time to maturity of the rice crop was recorded at the maturity stage whereas the grain yield was assessed after drying and cleaning and the calculation was made after adjusting the grain moisture content to 14 %. Height of weeds was recorded when they were 25- and 55-day-old and their duration and dry biomass was assessed at harvest.

### **Experiment 2**

The unharvested plant parts of the rice in each plot were incorporated into the soil and their effects on the early establishment of BG and ST-3 were investigated. Since the phenology of rice cvs. used in this experiment was different (varied from 100 days for cv. TN-1 to 123 days for CAR-8), care was taken to keep rice stubble in each plot fresh by maintaining the water in the early-harvested plots. Weed monoculture plots were free from any plant materials. Seven days after rice straw incorporation, seeds of BG and

ST-3 were direct-seeded into Set 1, 2 at the rates of 7.5 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup>, respectively. By using the same data collection technique in Phase-1, shoot length of these newly established seedlings was assessed at 15 DAS whereas the dry matter was assessed at 30 DAS.

## RESULTS AND DISCUSSION

### Cultivar performance

Rice cvs. used in this trial were statistically different in their phenological timing (Fig. 1), plant height (Fig. 2), and grain yield (Fig. 3). The cv. TN-1, the allelopathic rice control, had the earliest growth maturation time (100 days) with shortest stems (54 cm) and lowest yields (*ca.* 2.3 t ha<sup>-1</sup>). This was true under weed and weed-free conditions. The cv. ST-3, the non-allelopathic rice control, was moderately tall (91 cm), required 116 days to mature and yielded 2.5 t ha<sup>-1</sup> and 2.8 t ha<sup>-1</sup> under weedy and weed-free conditions, respectively. The average yields of the other six tested rice cvs in the weed-free plots ranged from 2.5 to 3.2 t ha<sup>-1</sup> whereas yields in the mix-culture plots were in a range of 2.4 to 3.1 t ha<sup>-1</sup>.

### Effect of rice on weed performance (Experiment 1)

BG responded differently to the different rice cvs (Table 1) with which it was grown. The maturation time was not affected but the final heights and dry weights were different depending on the cvs. they were grown with. The height of BG at 25 DAS in the plots with CAR-4 and Pa Thaut (*ca.* 10.8 and 11.2 cm, respectively) were significantly shorter than that seen in the plots of the non-allelopathic rice control cv. ST-3 (*ca.* 13.7 cm). This reduced height effect was maintained until the BG reached the flowering stage. In terms of dry weight, the weed suppressing ability of the six tested rice cvs were all significantly greater than that seen with the non-allelopathic and allelopathic rice controls (cvs ST-3 and TN-1), and the order of their growth suppressing ability was; CAR-3 > Pa Thaut (3051) > Neang Kong (3029) > CAR-8 > Neang Khat (3034) > CAR-4. The BG biomass in the ST-3 (non-allelopathic) plot was greater than that seen in the TN-1 (allelopathic) plot as expected and was much lower than that seen in the weed monoculture plot.

Rice cvs did not alter the maturation time of SUS but could reduce the final height and the biomass of the weed (Table 2). The inhibitory effects of Pa Thaut on plant height, when measured at 25 DAS, were statistically greater than those seen for ST-3. When SUS reached maturity, in the plot with ST-3 it was significantly taller than SUS in the plots with CAR-4, CAR-8, Neang Kong, Neang Khat, and Pa Thaut, but was shorter than those plants seen in the weed monoculture. At harvest, SUS grown with TN-1 and all tested cvs (except Pa Thaut) produced less shoot dry weight than SUS in the plots of ST-3. The mean dry weight of SUS in the monoculture plots (2069 kg ha<sup>-1</sup>) was 3 to 13 times greater than that seen when it was grown with the rice treatments (range of 154 to 667 kg ha<sup>-1</sup>).

### Effect of rice residues on the weed and crop performance (Experiment 2)

BG sown after the incorporation of rice straw residues produced shorter heights and lower tissue dry weights (Table 3) to non-residue control. BG in the plots that were treated with TN-1 (the allelopathic rice control) and ST-3 (the non-allelopathic rice control) residues were significantly taller than plants growing after the residues of other

rice cvs. For BG, the dry matter production in the residue-free plots was significantly greater than all residue treated plots. Among the plots treated with the residues of tested rice cultivars, the order of growth inhibition was Neang Khat > CAR-3 > CAR-4 > CAR-8 > Pa Thaut. There was no significant difference in dry weed biomass between the plots treated with ST-3 and TN-1 residues, however, BG dry matter in these plots were about 4 times smaller than the weed monoculture plots.

Rice residues also altered the seedling height and dry biomass of the rice, cv. ST-3 (Table 4). Residues of two rice cvs, namely Neang Khat and Pa Thaut, significantly reduced its height when compared to the height of this cv. growing on its own residues. The height of ST-3 seedlings in the residue-free plots was statistically taller than all other plots. In terms of dry biomass production, ST-3 in the residue-free plots produced dry matter 3.0 to 6.7 times greater than those in the residues treated plots. There was no statistical difference in rice biomass between plots treated with TN-1 and ST-3 residues however, the rice biomass in these plots were statistically greater than those plots treated with the residues of the tested rice cvs.

### Methodology and non-allelopathic interference factors

A general perception of using laboratory bioassays in the science of allelopathy is that they allow researchers to work with large amounts of test plant material in a short time, to eliminate certain interfering climatic factors, and to study only those effects due to allelopathy (Inderjit and Olofsdotter, 1998). However, even when this is done successfully those laboratory experiments must always be validated in the field (Inderjit and Olofsdotter, 1998). Demonstrating the allelopathic phenomenon in the fields is very difficult because of the complex involvement of other interference mechanisms such as those due to competition (Inderjit and Olofsdotter, 1998). In Experiment 1 of this present field experiment, allelopathy and competition will have almost certainly acted simultaneously, sequentially, or/and in combination. In addition, they may have acted together with other plant interference mechanisms such as those due to abiotic stresses (Inderjit, 1998) which are known to enhance the production of allelochemicals (Einhellig, 1995). In the present study the competition for water, or from other water-related stresses was removed and other plant stresses that could be caused by pesticides, pollutants, herbivores or pathogens were also kept to a minimum. The soils of the experimental site were potentially deficient in N, P, K, sulphur (S), magnesium (Mg), and boron (B) (Obertur *et al.*, 1997), however, an application of N, P, K fertilizer at the recommendation rate would have eliminated this possible nutrient stress. It is safe to assume that the growth response of the test plants in Experiment 1 of this present study was due chiefly to competition and allelopathy while inter-specific competition was not part of Experiment 2.

### Effect of rice on weed performance (Experiment 1)

The reductions in height attained and the dry weight of the weed plants suggest that there were growth suppressive abilities in all the various rice cvs studied. In this competitive situation, the final height attained by the weeds may have resulted from a shortage of assimilates brought about by aspects of competition and this may have been especially true for weeds emerging after the crop (Kropff, 1993). This is the situation in the present field experiment. It has been suggested that certain species of weed, grown with tall stature rice cvs, would not grow as tall as weeds grown with short stature rice cvs due to the differences in the competition for light (Ampong-Nyarko and De Datta,

1991a). In this present field experiment, TN-1 control plants were about two times shorter than ST-3 so in theory ST-3 should have been a better competitor than TN-1. However, there was no statistical difference in height of BG or SUS plants between the plots of ST-3 and TN-1 in Set 1 or 2. Such a response does not support the idea and therefore suggests another mode of growth suppression for TN-1, which may be allelopathy. Dilday *et al.* (1998), Kim and Shin (1998), Navarez and Olofsdotter (1996) also confirmed the allelopathic effects of TN-1 on a number of weed species. For the reason that all six tested rice cvs were taller than ST-3, the reduction of weed shoot length in these plots can be due to both, competition and allelopathy.

When competition is the only interference factor in a given rice environment, rice dry matter will be reduced for every 1 Kg of weeds produced in the same area (Ampong-Nyarko and De Datta, 1991b). From this reason, the competitive ability of a rice cv. in a given field can be defined the other way in term of weed dry matter production; the lesser weed biomass is produced, the better a cultivar competes with weeds. Because the total dry weights of the plant organs are obtained by integrating their daily growth weights over time (Kropff, 1993), it is more likely that a late duration rice cv. has more competitive advantage than the early one. The weed biomass data (Tables 1 and 2), with the exception of SUS cultured with Pa Thaut in Set 2, support the expectation that the six tested rice cvs. are more competitive than TN-1 because of their late maturation time. However, there are some arguments when attempting to compare the competitive ability of ST-3 to TN-1 or ST-3 to tested rice cvs. In theory, TN-1 should be a poorer competitor than ST-3 because of their shorter growth duration but in reality the mean dry matter of BG and SUS grown with TN-1 was lesser than those grown with ST-3. In a similar way, the duration of three rice cvs namely CAR-3, Neang Kong and Neang Khat was shorter than ST-3 but both weed species that were grown with these rice cvs. yielded significantly lower than those in ST-3 plots. This response suggests the interference factor other than competition, principally allelopathy since the cultural practices of this field study limited the occurrence of other interference mechanisms.

#### **Effects of rice residues on weed and crop performance (Experiment 2)**

During plant tissue decomposition, chemicals are released directly from the residues, or indirectly through the action of microbes, and these compounds can be stimulatory, have no effects, or be inhibitory on the growth of other plants depending on their nature and concentrations in the soil (Fischer, 1986). The degree of microbial activity is generally dependent on the soil environments such as water and nutrient concentration (Inderjit and Foy, 1999), thus the microbial activity in the residue treated plots should be equal because those plots received the same management practices. The key factor determining different effects among the residual treatments is, therefore, the different kinds and quantities of the chemicals released directly from the residues rather than the actions by microbes. For that reason, it is possible to conclude that the residues of all tested rice cvs. in Set 1 of Experiment 2, except Pa Thaut, had more inhibitory effects on the growth of BG than the residues of TN-1 and ST-3. For Experiment 2, the residues of all tested rice lines had more inhibitory effects on the growth of ST-3 than residues of TN-1 and ST-3. As it is expected that chemical release would be absent in the residue-free plots, it should be possible to assume a non-chemical stress condition for these plots on subsequent plant growth. Indeed, both weeds and rice performed better on those plots not treated with residues.

Table 1. Phenology and growth parameters of *Echinochloa crus-galli* (L.) P. Beauv. when grown together with one of the 8 cultivars of rice or on its own.

Treatment/s	Duration (days)	Height (cm) at 25 DAS	Height (cm) at 55 DAS	Dry weight (kg ha <sup>-1</sup> )	Suppression in dry weight (%)
CAR-3	106.5 ns	11.1 abc	25.7 ab	349.3 a	87
CAR-4	107.3 ns	10.8 a	24.8 a	531.5 c	80
CAR-8	107.7 ns	12.9 abc	27.2 ab	426.6 b	84
Neang Kong (3029)	107.8 ns	11.6 abc	25.4 ab	376.2 ab	86
Neang Khat (3034)	107.8 ns	13.1 bc	27.6 ab	518.2 c	80
Pa Thaut (3051)	107.3 ns	11.2 ab	24.9 a	355.6 a	86
TN-1 (allelopathic)	107.0 ns	13.4 c	26.9 ab	666.0 d	75
ST-3 (non- allelopathic)	107.8 ns	13.7 c	28.5 b	749.6 e	71
<i>E. crus-galli</i> (monoculture)	106.8 ns	19.1 d	58.2 c	2629.9 f	—

In a column, means followed by a common letter are not significantly different; "ns"- not significant different.

Table 2. Phenology and growth parameters of *Cyperus difformis* (L.) when grown together with one of the 8 cultivars of rice or on its own.

Treatment/s	Duration (days)	Height (cm) at 25 DAS	Height (cm) at 55 DAS	Dry weight (kg ha <sup>-1</sup> )	Suppression in dry weight (%)
CAR3	68.0 ns	10.5 ab	30.6 bcd	349.2 bc	83
CAR4	67.8 ns	10.4 ab	28.6 abc	350.6 bc	83
CAR8	67.5 ns	9.9 ab	28.4 abc	372.8 c	82
Neang Kong (3029)	68.0 ns	10.5 ab	29.3 abc	154.7 a	93
Neang Khat (3034)	68.5 ns	9.2 ab	27.5 ab	301.5 b	85
Pa Thaut (3051)	68.5 ns	8.8 a	26.2 a	633.7 de	69
TN-1 (allelopathic)	68.0 ns	10.8 ab	31.3 cd	602.8 d	71
ST-3 (non- allelopathic)	70.5 ns	11.3 b	33.4 d	667.0 e	68
<i>C. difformis</i> (monoculture)	69.3 ns	17.6 c	58.4 e	2069.9 f	—

In a column, means followed by a common letter are not significantly different; "ns"- not significant different.

Our present idea under test is that the residues produced by TN-1 (a previously identified allelopathic cultivar) will be more growth suppressing than ST-3 (a non-allelopathic cultivar) residues. However, the data showed that residues of both rice controls were both equally able to suppress growth of weeds. It is possible that the modes of production, release, and storage of allelochemicals may be different in one cv. to another even within the same species. For some diminutive and potentially allelopathic plants whose final size was too small compare to others within the same species, they have little room for competing with the other species sharing the same niche. Allelopathy must operate under such situations, but the chemicals are produced and immediately released into the environment rather than distributing into the plant

Table 3. Performance of *E. crus-galli* grown after rice straw incorporation.

Residues of	Height (cm) at 15 DAS	Dry biomass (kg ha <sup>-1</sup> )	Suppression in dry weight (%)
CAR3	5.3 ab	111.6 b	87
CAR4	4.8 a	132.9 b	85
CAR8	6.2 bcd	139.5 b	84
Neang Kong (3029)	5.1 a	59.6 a	93
Neang Khat (3034)	6.4 cd	112.7 b	87
Pa Thaut (3051)	5.6 abc	261.3 d	70
TN-1 (allelopathic)	6.9 d	217.9 c	75
ST-3 (non-allelopathic)	7.9 e	199.7 c	77
No residue	10.1 f	873.7 e	-

In a column, means followed by a common letter are not significantly different.

Table 4. Performance of ST-3 after crop residues.

Residues of	Height (cm) at 15 DAS	Dry biomass (kg ha <sup>-1</sup> )	Suppression in dry weight (%)
CAR3	9.5 bc	192.7 a	85
CAR4	10.4 c	314.3 c	75
CAR8	9.8 c	249.3 b	80
Neang Kong (3029)	9.5 bc	223.5 ab	82
Neang Khat (3034)	8.2 a	313.7 c	74
Pa Thaut (3051)	8.4 ab	186.1 a	85
TN-1	9.9 c	381.6 d	69
ST-3	10.7 c	420.4 d	66
No residue	12.9 d	1246.6 e	-

In a column, means followed by a common letter are not significantly different.

storage organs. The reason is that the production of allelochemicals might require energy from the plants and use resources that would otherwise be used for grain production (Olofsdotter, 1998). In such a case, a live plant species may be allelopathic but it is not necessary that its un-harvested plant parts are allelopathic too.

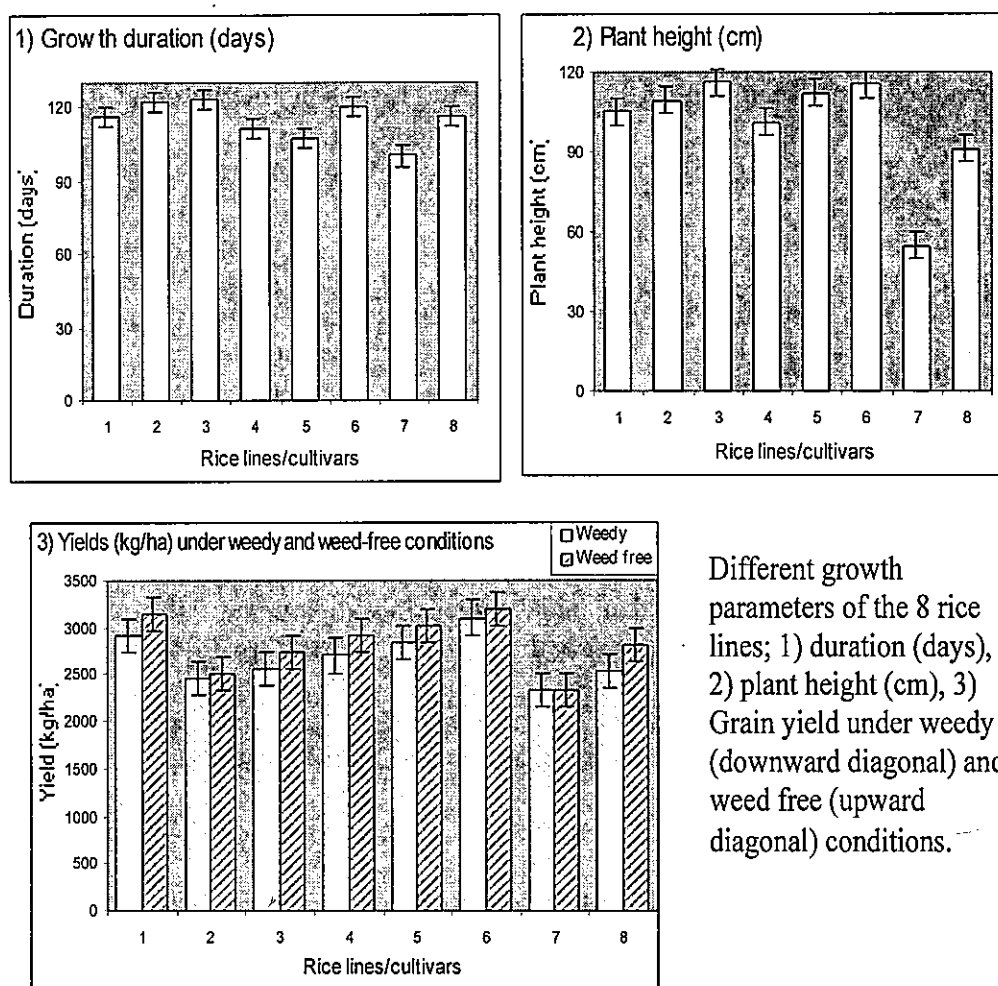
#### Is separating allelopathy from competition under field conditions possible?

Proof of allelochemical activity in field conditions is generally difficult to obtain, but the results of the present experiment have met with mixed success. In Experiment 1, some growth parameters (e.g., tall stature, long maturation time) which are thought to be among those responsible for the competitive traits of rice could not explain these cvs weed-suppressing ability and therefore suggests that allelopathy in combination with competitive ability of rice cultivars determines the final outcome of the crop-weed balance. In Experiment 2, however, an allelopathic activity was not detected in TN-1 residues despite the fact that live plants proved to be allelopathic. Nevertheless, it was evident that the residues of all six tested rice cvs (*viz.* CAR-3, CAR-4, CAR-8, Neang Kong, Neang Khat and Pa Thaut) suppressed the growth of BG whereas the first five cvs were inhibitory to rice (ST-3). This supports the previous statement that the growth reduction of BG grown with the six test lines resulted from the combined effects between allelopathy and competition. Similarly, the growth reduction of SUS that were grown with all tested lines, except Pa Thaut, can be due to both mechanisms.



### Applied aspects of allelopathic cultivar in rice production

Modern agriculture is being challenged to reduce environmental damage and health hazards from chemical inputs at the same time as maintaining a high level of production (Einhellig, 1995). Crop protection strategies based on allelopathy would be one way to help to achieve this ideal (Einhellig and Leather, 1988). The concept of using allelopathic rice for weed control started in late 1980s (Olofsdotter, 1998) and its potential for weed management in Cambodia has been discussed by Pheng et al. (1999). Rice production in Cambodia is based on non-irrigated rainfed systems. Thus water management and others cultural practices are much less effective for controlling weeds (Jahn et al., 1997; Pheng et al., 1999). In the present Cambodian subsistence farming systems, farmers rely heavily on hand-weeding despite this method is being time-consuming and laborious. Although herbicides can be used as a laborsaving option,



their costs and possible undesirable environmental effects make their use unlikely to many years to come. One possible economically sound solution is to develop rice cvs that can suppress the growth of weeds by means of allelopathy, this could be both with the crop or as a soil-incorporated stubble. A control method that is already "in the seed" provides farmers with a control method, which is cost effective, labour saving, and environmentally safe. Perhaps, Cambodian farmers have already applied this approach for many years since the phenomenon was found in some traditional rice cultivars and some popular CAR lines but it is just the matter late verification of applied allelopathy

in rice production in Cambodia.

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## STUDIES ON ALLELOPATHY OF RICE (*ORYZA SATIVA*) FOR BARNYARDGRASS CONTROL

L. Q. Yu, Z. H. Xu, and S. W. Huang  
China National Rice Research Institute, Hangzhou 310006, China  
wslab@public.fy.hz.zj.cn

**Abstract:** Allelopathic function of rice (*Oryza sativa* L.) for barnyardgrass (*Echinochloa crus-galli* (L.) Beauv. var. *mitis* (Pursh) Peterm) control was observed in the effect of 84 rice varieties on the growth of barnyardgrass in greenhouse during 1999~2000. Rice varieties of I-26, TJI43, RY503 and LY2186 provided excellent control of plant dry weight of barnyardgrass with 96%, 95%, 93% and 91% respectively compared with susceptible species of XSH63. The control of barnyardgrass plant density was given significantly in 42%, 35%, 30% and 30% from varieties of I-26, TJI43, RY503 and LY 2186. In these experiments we obtained good materials for developing the biotechnology research of rice allelopathy on the weed.

**Key words:** Allelopathic function, rice (*Oryza sativa* L.) varieties, barnyardgrass control

### INTRODUCTION

① Barnyardgrass reduced rice quantity and quality by competing on nutrition, solar energy and water resource. Rice competitive ability varied from different plant morphological type of cultivars. A rice with long-stalked-great-canopy had more powerful competition and less grain yield losses than that of middle-stalked-medium-canopy and short-stalked-small-canopy (Xu 2000; Yu 1999). Researches of rice allelopathy relative with ecological competition studies turned to be active in the past few years. Hassan (1994, 1996), determined that 30 varieties provided 50%~90% control of *Echinochloa crus-galli* in field after evaluating 1000 rice varieties in Egypt during 1993~1996. In IRRI, Olofsdotter (1996) offered their research results on rice allelopathy giving more than 50% barnyardgrass control for 11 cultivars in dry season and 21 cultivars in rainy season. Ill Min Chuny (2000) evaluated the allelopathic potentiality of Korea traditional 79 rice cultivars on *Echinochloa crus-galli* L. Beauv. var. *oryzicola* Ohwi and identified possible allelopathic compounds from selected rice cultivar in which Heugbalbyeon showed the greatest inhibition of 81% barnyardgrass dry weight. Compounds in Heugbalbyeon were detected by HPLC analysis which were o-coumaric acid, p-coumaric acid, salicylic acid, syringic acid and p-hydroxybenzoic acid. Liu Xiufen et al. (1998) screened and obtained 4-T-butylbenzoic acid from the upland soil rhizosphere in Shandong Province of China. Inhibiting rate on barnyardgrass germination were 21%, 47% and 65% in 100, 200 and 500 mg/L respectively. However, wheat and maize were also susceptible on this compound. In China, research on rice allelopathy started recently and information about it is less. The purpose of this experiment was to screen and determine the cultivars with allelopathic activity on barnyardgrass and to provide the scientific basis for the weed ecological control further.

### MATERIAL AND METHODS

A pot experiment was conducted in greenhouse of China National Rice Research Institute (CNRRI) during August to October, 2000. 84 cultivars provided by CNRRI for the screening experiment. Barnyardgrass (*Echinochloa cras-galli* (L.) Beauv. var. *mitis* (Pursh) Peterm.) seeds collected on October in 1999 and preserved in room temperature.

Each pot was added with 5kg sand clay soil of with pH7.1 and 3.4% organic matter. Fertilizers were applied as needed. Six grains of rice seed treated by disinfecting, presoaking and forced germination were sowed in the center of pot. Four rice plants were remained by thinning out poor germination seedling. 0.5g barnyardgrass seeds were sowed in each pot after one month of rice seeding. Data was collected after one month of barnyardgrass seeding.

Inhibition rate of barnyardgrass density and the weed dry weight were calculated as compare with the tested susceptible rice cultivars. All data were subjected to an analysis of variance. Treatment differences were separated into significant classes by using the Duncan's multiple range test.

## RESULTS AND DISCUSSION

A susceptible cultivar XSH63 with powerful tiller and semi-dwarf bio-type was found to be the weakest in competing with barnyardgrass among 84 cultivars.

I-26, the most resistance cultivar provided excellent control of the weed dry weight and better inhibition rate of the weed seedling density compared with XSH63 (Table 1). Barnyardgrass dry weight declined significantly resulted from 12 cultivars of J24, RY503, I-39, J21, I-44, ZS1, LY2186, XY1000, I- 27, I-28, TYD2 and I-45 showed 92% to 95% the weed control. It was concluded that rice cultivars could reduce the weed dry weight more effectively than control its seedling amount.

Compared with XSH63, I-26 could control the weed seedling number by 42% in 30d competitive period. and reduced 56% of the weed density compared with the check of barnyardgrass alone. I-26 showed allelopathic potential activity against barnyardgrass according to the inhibition rate of the weed dry weight and seedling density. This cultivar carries the gene for semidwarfism with 71cm plant height and 4 tillers per plant which were lower than XSH63 with 5.5 tillers per plant.

Research showed that allelopathy played a significant role in natural ecosystems and had the potential to become an important means in cultivated ecosystems and integrated weed management.

Whether I-26 exists allelopathic potential against barnyardgrass in field condition, experiment needed to further research in future.

Table 1. Rice allelopathy against barnyardgrass\*

Cultivar code name	Rice		Barnyardgrass				
	Stem number (no./plant)	Plant height (cm)	Plant height (cm)	Seedling number (no./pot)	Seedling reduced (%)	Plant dry weight (g/pot)	Control of plant dry weight (%)
BYG alone			36.1A	68.3 A	0	2.47A	0
XSH63	22.0 A	64.3	28.0B	52.0 A-F	0	2.54A	0
I-26	16.0 B-F	70.8	12.4L-P	30.3 G	42	0.11E	96

J24	15.3 B-H	73.6	13.4I-R	40.7C-G	22	0.13DE	95
RY503	11.3 G-Q	78.7	11.1P-R	36.3E-G	30	0.14DE	95
I-39	14.0 C-L	79.0	14.7G-R	38.0D-G	27	0.14DE	95
J21	11.3 G-Q	74.4	11.3O-R	38.3D-G	26	0.15DE	94
I-44	14.3 C-K	92.8	13.3I-R	41.7C-G	20	0.17DE	93
ZS1	15.7 B-G	76.1	12.3L-P	37.0E-G	29	0.17DE	93
LY2186	11.7 F-Q	83.2	11.7N-R	36.3E-G	30	0.17DE	93
XY1000	11.0 H-Q	81.5	10.5R	40.7C-G	22	0.18DE	93
I-27	10.3 J-S	76.4	13.6H-R	51.0A-G	2	0.18DE	93
I-28	13.3 C-N	76.0	12.7K-O	42.3C-G	19	0.19DE	92
TYD2	12.7 C-O	76.0	11.0P-R	41.7C-G	20	0.19DE	92
I-45	13.0 C-O	67.3	13.5I-R	51.3A-G	1	0.20DE	92
TN1	15.3 B-H	69.3	11.4O-R	46.7B-G	10	0.21C-E	92
I-40	9.3 M-T	78.6	10.7QR	48.7A-G	6	0.21C-E	92
TJI-42	10.7 I-R	108.6	12.8J-O	46.3B-G	11	0.21C-E	92
J18	15.0 B-I	80.9	11.5N-R	47.7A-G	8	0.21C-E	92
H21	16.7 B-D	70.6	12.4L-P	51.7A-F	1	0.21C-E	92
J32	9.7 L-S	89.4	11.9M-Q	51.0A-G	2	0.21C-E	92
XY1249	10.0 K-S	74	11.3O-R	49.3A-G	5	0.22C-E	91
J23	13.7 C-M	77.9	14.8G-R	43.7C-G	16	0.22C-E	91
TJI-43	6.0 ST	107.9	18.8C-G	33.7FG	35	0.22C-E	91
I-22	11.7 F-D	80.5	13.1I-R	49.3A-G	5	0.22C-E	91
I-55	12.7 C-O	71.9	13.5I-R	49.7A-G	4	0.23C-E	91
H33	11.3 G-Q	81.1	14.5G-R	42.0C-G	19	0.23C-E	91
YY97	12.0 E-P	75.9	13.0J-O	48.3A-G	7	0.23C-E	91
I-56	11.3 G-Q	79.4	14.1G-R	47.7A-G	8	0.24C-E	91
J31	14.3 C-K	72.3	12.8J-O	49.7A-G	4	0.24C-E	91
I-53	13.7 C-M	66.5	14.5G-R	49.0A-G	6	0.24C-E	91
J17	9.7 L-S	70.2	13.5I-R	50.7A-G	3	0.24C-E	91
I-49	11.3 G-Q	81.8	12.4L-P	55.3A-E	0	0.25C-E	90
J27	9.0 N-T	92.1	14.3G-R	44.0C-G	15	0.25C-E	90
H12	13.7 C-M	81.8	13.3I-R	47.7A-G	8	0.25C-E	90
I-23	17.0 BC	71.2	12.2M-Q	50.7A-G	3	0.25C-E	90
I-41	11.0 H-Q	78.5	13.6 I-R	55.7A-E	0	0.26C-E	90
I-36	17.0 BC	66.1	16.4 F-L	48.3A-G	7	0.26C-E	90
I-35	13.7 C-M	70.2	13.1 I-R	48.0A-G	8	0.26C-E	90
I-57	13.3 C-N	94.2	13.8H-R	55.0A-E	0	0.26C-E	90
J22	11.7 F-Q	96.6	14.9G-R	56.0A-E	0	0.27C-E	89
I-43	14.7 B-J	74.8	13.5I-R	54.3A-F	0	0.27C-E	89
FY964	14.7 B-J	73.7	12.9J-O	50.0A-G	4	0.27C-E	89
I-21	12.3 D-P	70.0	11.4O-R	50.7A-G	3	0.28C-E	89
SY963	19.0 AB	70.0	11.0P-R	56.0A-E	0	0.28C-E	89
I-45	12.7 C-O	73.1	15.0G-R	47.3A-G	9	0.28C-E	89
J25	12.3 D-P	65.4	13.0I-R	49.3A-G	5	0.29C-E	89
J15	14.3 C-K	57.6	15.0G-R	51.7A-F	1	0.29C-E	89
H19	17.0 BC	62.2	12.5K-O	44.0C-G	15	0.29C-E	89
SY46	13.7 C-M	62.8	11.8N-R	53.3A-F	0	0.30C-E	88
I-24	12.3 D-P	84.7	13.9G-R	56.0A-E	0	0.31C-E	88

I-32	11.7 F-Q	84.1	14.2G-R	55.3A-E	0	0.31C-E	88
I-29	14.0 C-L	66.6	15.2G-R	56.7A-E	0	0.31C-E	88
J19	17.0 BC	65.5	11.8N-R	61.0A-C	0	0.31C-E	88
J89	7.3 Q-T	64.3	15.5F-Q	40.0C-G	23	0.31C-E	88
IXRY	15.3 B-H	72.8	11.7N-R	55.3A-E	0	0.31C-E	88
IH98	12.7 C-O	72.1	15.8F-P	47.0B-G	9	0.32C-E	87
I-54	13.3 C-N	73.7	13.3I-R	54.3A-F	0	0.32C-E	87
J28	6.3 R-T	100.1	14.4G-R	46.0B-G	12	0.32C-E	87
I-25	10.0 K-S	81.9	14.4G-R	40.0C-G	23	0.33C-E	87
I-30	16.0 B-F	70.5	11.7N-R	52.7A-F	0	0.33C-E	87
J20	14.3 C-K	86.3	13.9G-R	49.7A-G	4	0.33C-E	87
M3132	15.0 B-I	62.5	17.2C-L	53.0A-F	0	0.34C-E	87
J16	9.7 L-S	81.2	13.9G-R	56.0A-E	0	0.34C-E	87
I-37	8.7 O-T	72.3	12.8J-O	48.7A-G	6	0.34C-E	87
H16	13.3 C-N	74.7	17.7C-H	52.7A-F	0	0.34C-E	87
HR97	8.7 O-T	76.6	17.4C-H	49.3A-G	5	0.34C-E	87
I-94	16.0 B-F	65.4	11.3O-R	66.7AB	0	0.35C-E	86
I-52	10.0 K-S	64.5	16.2F-O	57.0A-E	0	0.35C-E	86
I-38	16.7 B-D	80.9	15.5F-Q	53.0A-F	0	0.35C-E	86
HI	16.0 B-F	62.0	15.0G-R	52.3A-F	0	0.36C-E	86
TY97	14.3 C-K	71.9	12.1M-Q	53.3A-F	0	0.39C-E	85
YS4	10.7 I-R	92.4	13.4I-R	56.7A-E	0	0.39C-E	85
J29	10.0 K-S	96.0	18.5C-H	52.7A-F	0	0.40C-E	84
JZD	10.0 K-S	60.4	17.9C-H	52.7A-F	0	0.40C-E	84
MY128	11.7 F-Q	70.3	16.7E-K	60.3A-C	0	0.42C-E	84
H14	10.7 I-R	62.6	12.3L-P	58.3A-D	0	0.43C-E	84
9522	8.0 P-T	59.8	21.2C-E	42.0C-G	19	0.48C-E	83
H13	5.0 T	72.4	21.9CD	42.0C-G	19	0.50CD	80
J26	6.3 R-T	87.9	14.8G-R	49.7C-K	4	0.50CD	80
H20	10.3 J-S	72.8	21.9C	50.0A-G	4	0.50CD	80
J950	12.7 C-O	65.4	20.0C-F	46.0B-G	12	0.51CD	80
H22	13.7 C-M	97.4	14.0G-R	66.7AB	0	0.59C	77
H25	16.3 B-E	63.5	27.4B	56.0A-E	0	1.03B	59

\*Values within each column followed by the same letter are not significantly different at the 1% level by Duncan's multiple range test.

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## COMPARATIVE PHYTOTOXICITY OF CINEOLE AND CITRONELLOL AGAINST TWO WEED SPECIES

H. P. Singh\*, D. R. Batish, and R. K. Kohli

Department of Botany, Panjab University, Chandigarh 160 014, India,  
hpsingh\_01@yahoo.com

**Abstract:** Weeds - the unwanted plants, compete with agricultural crops for nutrition, water and space, thus causing enormous crop losses. Though a wide variety of synthetic herbicides are used for weed control, yet they have created more problems than solving as these synthetic chemicals are costly and remain in soil as persistent pollutants. Therefore, in order to achieve sustainable weed management alternative and eco-friendly means are being tried world over. Natural plant products being biodegradable, more target oriented and cost effective are being explored for the management of noxious weeds. In this context, a study was performed to explore the herbicidal potential of purified volatile monoterpenes viz. cineole and citronellol. These exerted a deleterious effect on the germination, growth, chlorophyll content and respiratory activity of two noxious agricultural weeds viz. *Avena fatua* and *Ageratum conyzoides* which have invaded the agricultural fields in the northwestern parts of India. Nearly 4 weeks after treatment the plants get wilted. Cineole was more effective than citronellol. Volatile terpenes, thus, have a potential to suppress the weeds and could be exploited as novel agrochemicals for future weed management programmes.

**Key words:** Allelopathy, *Ageratum conyzoides*, *Avena fatua*, essential oils, monoterpenes, weed suppression.

### INTRODUCTION

Weeds are essential component of agroecosystems interfering with crops leading to enormous crop losses. Modern day agriculture is based on excessive use of synthetic herbicides that has undoubtedly enhanced the much needed crop production yet have led to a number of toxicological and environmental repercussions. These may be reflected in the form of soil and water pollution consequently causing ill effects on human health (Macias, 2001). Therefore, currently the efforts are being made world over to search for safer and environmentally benign chemicals that have relatively shorter half-life. In this direction biologically active natural products such as allelochemicals are fast being tested for weed management. These compounds are derived from secondary metabolism and often have complex carbon skeleton, wide structural diversity, are environment friendly and have more modes of action (Dayan et al., 2000).

Natural compounds exhibit immense diversity and biological activity. Among them volatile terpenes, particularly cineole, have been shown to be promising with potential weed suppressing ability (Kohli et al., 1998; Romagni et al., 2000). These are commonly found as components of essential oils in a number of aromatic plants, e.g. *Salvia* spp. (Muller, 1965), *Eucalyptus* spp. (Singh et al., 1991), *Artemisia* spp. (Ahmad and Misra, 1994) and *Pinus* spp. (Singh et al., 1999). Besides, they exhibit considerable phytotoxicity, e.g. volatile terpenes of *Salvia leucophylla* are most effective inhibiting the growth of grasses (Muller et al., 1964); *Eucalyptus* volatile terpenes reduce growth of a number of plants (Kohli and Singh, 1991); cineoles (1,4- and 1,8-) reduce the

growth of weeds (Romagni et al., 2000). In spite of high phytotoxic levels, only a few studies have been conducted to explore their herbicidal potential against a wide range of weeds. A study was, therefore, undertaken to explore the comparative phytotoxic effect of two volatile monoterpenes viz. cineole and citronellol against *Avena fatua* and *Ageratum conyzoides* - two noxious agricultural weeds.

## MATERIALS AND METHODS

Seeds of *Avena fatua* were received as a gift from Prof. N.T. Yaduraja, weed scientist, department of agronomy, Indian agricultural research institute whereas those of *Ageratum conyzoides* were collected locally from wildy growing stands. Volatile monoterpenes cineole and citronellol were purchased from Sigma Inc., USA.

Germination bioassay in response to volatile terpenes was done following the method of Kohli and Singh (1991). Thoroughly washed, surface-sterilized seeds of both the weed species were soaked in distilled water for 18h. These were divided into seven groups of 50 each and placed on a Whatman no. 1 filter paper in an uncovered 6" diameter Petri dish. Petri dishes were placed in an environment chamber having  $25\pm 2^{\circ}\text{C}$  temperature, 16/8 light/dark photoperiod and  $70\pm 3\%$  humidity. Chambers were fumigated with cineole or citronellol to have a concentration of 10, 20, 30 nl/cc. For each treatment five replicates were maintained. A chamber without volatile terpenes served as control. After 15 days the number of seeds germinated, seedling length and dry weight of germinated seeds, chlorophyll content and cellular respiration values were measured.

Chlorophyll was extracted in dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). The amount was determined spectrophotometrically using the equation of Arnon (1949) and expressed in terms of dry weight as suggested by Daizy and Kohli (1991). Respiratory activity was determined using the method of Steponkus and Lanphear (1967). The data of per cent germination, seedling growth, chlorophyll content and respiratory activity was analyzed by one-way ANOVA followed by duncan's multiple range test.

## RESULTS AND DISCUSSION

Both cineole and citronellol reduced germination of *A. fatua* as well as *A. conyzoides* (Table 1). Reduction in germination was slightly more in case of *A. fatua* compared to *A. conyzoides*. However, cineole was more inhibitory / phytotoxic than citronellol (Table 1). Further, seedling length and seedling dry weight of both the test weed species were significantly reduced compared to control. Growth of *A. conyzoides* was more affected compared to *A. fatua* (Table 2). Inhibition of germination and growth of both the weedy species could be due to disruption of mitotic activity in the germinating seeds. Though, present study does not have supporting data in this direction yet references available in literature can strengthen this fact (Lehnen et al., 1990; Vaughan and Vaughn, 1990; Romagni et al., 2000). Several reasons have been put forward to find out the factors that disrupt mitosis such as disruption of microtubule organization or alternation of cell wall biosynthesis (Vaughan and Vaughn, 1990; Lehnen and Vaughn, 1992).

Table 1. Effect of Cineole and citronellol on germination of *A. fatua* and *A. conyzoides* (data presented as with respect to control).

Concentration ( $\mu$ l)	Per cent reduction in germination*	
	<i>A. fatua</i>	<i>A. conyzoides</i>
Cineole		
10	44.3 $\pm$ 1.2	47.6 $\pm$ 2.6
20	65.6 $\pm$ 1.5	62.8 $\pm$ 2.8
30	73.4 $\pm$ 3.7	71.4 $\pm$ 1.3
Cintronellol		
10	24.8 $\pm$ 1.3	19.0 $\pm$ 1.2
20	32.3 $\pm$ 2.1	28.6 $\pm$ 1.8
30	54.2 $\pm$ 2.5	47.3 $\pm$ 2.5

\* data presented is significant over control at 5% level applying *t*-test.

Table 2. Effect of Cineole and citronellol on the early seedling growth of *A. fatua* and *A. conyzoides* (data presented as with respect to control).

Concentration ( $\mu$ l)	Seedling length*		Seedling weight*	
	<i>A. fatua</i>	<i>A. conyzoides</i>	<i>A. fatua</i>	<i>A. conyzoides</i>
Cineole				
10	81.3 $\pm$ 4.5	62.7 $\pm$ 2.3	90.5 $\pm$ 4.8	85.7 $\pm$ 4.8
20	65.4 $\pm$ 3.5	41.8 $\pm$ 2.7	85.3 $\pm$ 3.9	42.8 $\pm$ 3.5
30	44.3 $\pm$ 3.9	38.6 $\pm$ 1.1	65.4 $\pm$ 2.7	14.3 $\pm$ 1.2
Cintronellol				
10	85.3 $\pm$ 3.7	60.0 $\pm$ 1.9	91.2 $\pm$ 5.7	71.4 $\pm$ 2.5
20	70.4 $\pm$ 4.2	44.5 $\pm$ 2.2	82.3 $\pm$ 2.5	52.3 $\pm$ 2.0
30	48.1 $\pm$ 2.4	39.1 $\pm$ 1.5	64.2 $\pm$ 2.1	14.1 $\pm$ 0.8

\* data presented is significant over control at 5% level applying *t*-test.

The content of chlorophyll extracted in DMSO was significantly less in treated weed species compared to control. More reduction was observed in *A. conyzoides* (Table 3). The decrease in chlorophyll suggests the diminishing photosynthesis efficiency in response to volatile terpenes. The mechanism as to how chlorophyll decreases in the target weed or whether it is due to decreased synthesis or enhanced degradation could not be known. Nevertheless, there are available references indicating reduced levels of chlorophyll pigment in response to allelopathy / allelochemicals (Kohli et al., 1998; Romagni et al., 2000; Saxena et al., 1991).

The study on cellular respiration which is an indirect method indicating rate at which O<sub>2</sub> molecule released through respiratory chain are trapped by 2,3,5-triphenyl tetrazolium chloride (TTC) leading to the formation of water insoluble red formazan (Steponkus and Lanphear, 1967). The intensity of this red coloured formazan extracted in alcohol is determined. The present study reveals a considerable and appreciable reduction in cellular respiration in both the weed species (Table 3).

Thus, from the present study it is clear that both cineole and citronellol have a tendency to reduce the growth and development of weed species and the cineole is more effective in controlling the weeds and can act as one of the lead molecules for future

weed management programmes. However, further studies are needed to determine their molecular site of action.

Table 3. Effect of cineole and citronellol on chlorophyll content and cellular respiration of *A. fatua* and *A. conyzoides* (data presented as with respect to control).

Concentration ( $\mu$ l)	Chlorophyll content*		Cellular Respiration*	
	<i>A. fatua</i>	<i>A. conyzoides</i>	<i>A. fatua</i>	<i>A. conyzoides</i>
Cineole				
10	85.5 $\pm$ 5.4	51.6 $\pm$ 2.4	70.3 $\pm$ 2.5	61.8 $\pm$ 2.8
20	60.5 $\pm$ 2.8	47.0 $\pm$ 1.2	55.6 $\pm$ 1.4	53.2 $\pm$ 3.2
30	51.8 $\pm$ 3.2	37.4 $\pm$ 3.7	24.9 $\pm$ 1.5	46.2 $\pm$ 4.3
Citronellol				
10	86.4 $\pm$ 4.8	48.4 $\pm$ 2.2	94.5 $\pm$ 1.9	90.5 $\pm$ 5.1
20	61.9 $\pm$ 4.3	45.2 $\pm$ 2.0	82.4 $\pm$ 3.4	79.1 $\pm$ 5.1
30	42.4 $\pm$ 3.2	38.5 $\pm$ 1.5	44.4 $\pm$ 1.8	51.3 $\pm$ 2.1

\* data presented is significant over control at 5% level applying *t*-test.

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# **Weed Problems and Management**

## AGRICULTURAL WEEDING IN CONJUNCTION WITH HERBICIDE APPLICATION IN RICE

Z. P. Zhang

Analytical and Testing Center, Chinese Academy of Agricultural Sciences, Beijing  
100081 China. zepu@sina.com

**Abstract:** Ten million hectares of rice fields and ten million tons of rice grains were lost by weeds annually in China. Agricultural weeding was used by farmers, but consumed many labors. Preventive weed control method for rice seedbed and paddy field can be practiced through agricultural weeding and herbicide application. Proper land preparation, high seeding rates, good water management, transplanting tall old rice seedlings, appropriate time and rate of fertilizer application, rotating rice with a dry land crops and double cropping rice with dry land crops are effective measures. Chemical weeding is most effective and becomes important, but continuous use of herbicides without integrating other methods induced weed shift, weed resistance to herbicides and environmental problems. Agricultural weeding in conjunction with herbicide application can be the strategy for rice weed control.

**Key words:** Weed, rice, integrated weed management

### INTRODUCTION

In the rice crop, barnyard grass (*Echinochloa crus-galli*), flatstalk bulrush (*Scirpus planiculmis*), arrowhead (*Sagittaria pygmaea*), distinct pondweed (*Potamogeton distinctus*) and knotgrass (*Paspalum distichum*) are the important weeds which causing heavy reduction of rice production, other weeds as late juncellus (*Juncellus serotinus*), Chinese sprangletop (*Leptochloa chinensis*), sheathed monochoria (*Monochoria vaginalis*) and alligator alternanthera (*Alternanthera philoxeroides*) infesting in rice fields are different depending upon the agricultural regions, the different soil types, cropping systems, types of cultural practices and the conventional weed control measures. More than ten million hectares of rice fields are infested with weeds resulting a reduction of ten million tons of rice grains annually.

### CHEMICAL CONTROL DEVELOPING AND PROBLEMS INDUCED

Before 1970's, in Chinese rice production regions, farming people used to adopted agricultural, mechanical and hand weeding for weed control could obtain high rice yields but had consumed too much farming labor input. Along with rural economic development, due to the shortage of rural labor in the developing area, more farming people want to increase labor productivity and to reduce labor intensity for improving agricultural production condition, chemical weed control becomes more attractive to them. Propanil, thiobencarb, butachlor, molinate, oxadiazon, bensulfuron-methyl, bentazon, MCPA and quinclorac are the wide used herbicides. The herbicide application area of paddy fields was steadily enlarged from several hundred thousand hectares before 1970's up to about 60 million hectares in 2000.

Chemical control has changed cultural practice in rice, cultivation is not needed after the herbicides applied the paddy fields and transplanted rice may be gradually

replaced by direct-seeded rice. In some areas, direct-seeded rice by air broadcasting is being adopted by the big state farms and direct drilling is also extended in rice growing regions.

Herbicides are highly effective to control dominant weed species, continuous use of the same herbicides on rice for a number of years has caused weed shift problems and induced weed resistance to herbicides. In some rice production areas, rice weed populations have been shifting from grass weeds to broadleaf weeds or from broadleaf weeds to grass weeds. As the example, the long-term use of thiobencarb, molinate or butachlor in transplanted rice results the dominant *Echinochloa* spp. population eliminated and the broadleaf weeds or sedges become dominant.

For overcoming these problems, using different kinds of herbicides alternatively and mixture of 2-3 herbicides in combinations by increasing the number of target weed species are being extended, for example, the combination of thiobencarb, butachlor or molinate with bensulfuron used by pre-emergence application for controlling grass weeds and both broadleaf weeds and cyperus weeds; propanil with bentazon or with MCPA by post-emergence application for controlling sheathed monochoria, water plantain (*Alisma orientale*), diffomed galingale (*Cyperus difformis*) and other broadleaf weeds or sedges; nowadays about 30 herbicides used in their combinations of two or more kinds of herbicides in rice fields for broadening weed spectrum or resistant weeds control have been being evaluated in China.

Although the chemical control in rice fields is developing in rice production, but depending upon herbicides to control weeds in rice only is not a most effective measure. there will bring more problems needed to be solved. In order to solve these problems, herbicide application integrated with agricultural weed control has be being attended.

### ACHIEVEMENT IN AGRICULTURAL WEED CONTROL

An integrated system of weed control in transplanted rice fields is being adopted by Chinese rice farming people.

The dominant weeds in the seedbed are barnyard grass and some other broadleaf weeds, barnyard grass begins to emerge within two weeks after sowing and grows with rice in the planting hills after transplanting, eliminating barnyard grass in rice seedling stage before transplanting for keeping planting hill weed free is very important.

In transplanted rice fields, weeds emerge in the period from one week to more than one month after transplanting, these weed population competes with rice at tillering stage and reducing the number of panicles causing the yield reduction, the later emerging weeds are not important in rice yield.

#### Preventive weed control

In rice seedbed and paddy field, through seed selection by use of high quality rice seeds uncontaminated with weed seeds, cleaning out weeds on field margins, irrigating weed seed free water, cleaning implements to avoid spreading weed seeds or propagules from field to field are the preventive weed control measures.

Cutting and burning rice stubble and residual matter after harvesting, to reduce weed seeds on the soil surface. In autumn or in winter time, deep plowing exposes the vegetative organs of the perennial weeds to winter cold resulting the drying and freezing of these underground organs. As an example, one autumn plowing to a depth of 20-30 cm reduced distinct pondweed about 60% compared with that check fields. Even if the



fields were plowed to a depth of 15-18 cm in comparison with a shallow tillage to a depth of 10 to 15 cm, the density of weeds was reduced significantly.

In the growing season, first tillage carried out about one month before sowing can remove perennial weeds; second tillage done at 1-3 weeks before sowing encourages germination of weeds, and a last tillage practiced within a week before sowing followed by leveling paddy fields can eliminate weed emergence.

Paddling the soil after land plowing may reduce the number of broadleaf weeds in transplanted rice fields. Paddy fields after one pass of harrowing remained more successive weeds in crop fields than that had more than two passes of harrowing, through soil paddling after more than two rounds of harrowing and raking, the buried small weed seedlings did not easily establish.

### **Dense planting**

High seeding rates at more than 1000 kg/ha are more popular in China and the rate as high as 2000 kg/ha is also used. The seeding rate increased from 500 kg/ha to 1500 Kg/ha results a significant reduction in broadleaf weed density.

### **Good water management**

In seedbed, before sowing for a few days, irrigating the seedbed to a water layer at a depth of more than 10 cm and after sowing, keep a water layer at the same depth until the rice seeds germinating, then draining out water allow the most seeds to emerge, the seedbeds are flooded again to a water layer at 10-20 cm in depth until weed seedlings died.

In rice fields, after rice transplanting, a deep water layer maintaining at a depth of 10-20 cm for a considerably long time as possible can reduce infestation of annual weeds such as barnyard grass and other grass weeds.

### **Transplanting tall old rice seedling**

Tall old rice seedlings at 5-6 leaf stage having more effective tillers and jointing earlier than younger ones, after translated in rice fields, the index of leaf area increased about 3 times more than the younger seedlings on two weeks before the peak of weed emergence. The ratio of leaf space between rice plants and weeds reached 10:1. The frequencies of occurrence and densities of weed plants for *Echinochloa* spp., *Eleocharis yokoscensis*, *Cyperus difformis*, *Scirpus planiculmis*, *Eclipta prostrata* decreased considerably.

### **Appropriate time and rate of fertilizer application**

Appropriate rates of nitrogen fertilizer applied after the weeds being removed may avoid the increase of perennial weed damages.

### **Cropping system**

Double cropping of rice with wheat, oil-seed rape, barley, broad beans or other winter cropping crops is an effective cropping system for reducing rice damage from perennial weeds.

Rotating rice with a dry land crop such as maize, cotton, sweet potatoes, sesame or soybeans can reduce infestation of water tolerant weeds in the rice crop due to the dry land cropping breaks the life cycle of water tolerant weeds.

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## **CONCLUSION**

Agricultural weeding in conjunction with herbicide application in rice fields can get satisfactory weeding results in rice growing.

## REDUCING *CYPERUS ROTUNDUS* TUBER POPULATIONS USING STALE-SEEDBED TECHNIQUES IN RICE-ONION CROPPING SYSTEMS

A.M. Baltazar<sup>1</sup>, E.C. Martin<sup>2</sup>, A.M. Mortimer<sup>3</sup>, M.C. Casimero<sup>2</sup>, S.R. Obien<sup>2</sup>,  
and S.K. De Datta<sup>4</sup>

<sup>1</sup>University of the Philippines Los Baños, Laguna, Philippines,  
amb@mudspring.uplb.edu.ph

<sup>2</sup>Philippine Rice Research Institute, Muñoz, Nueva Ecija, Philippines

<sup>3</sup>International Rice Research Institute, Los Baños, Laguna, Philippines

<sup>4</sup>Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

**Abstract:** Purple nutsedge (*Cyperus rotundus* L.) infestations are increasing rapidly in rainfed rice-onion systems, causing substantial yield losses. In onion, selective control of this weed with chemicals is difficult to achieve, while handweeding breaks tuber chains which can lead to recruitment of new shoots. Stale seedbed techniques prior to crop sowing offer the opportunity to substantially reduce tuber infestations and decrease the amount of handweeding and other weed control inputs during the cropping season. A long term on-farm experiment was conducted to determine the impact of stale seedbeds applied between rice (*Oryza sativa* L.) and onion (*Allium cepa* L.) crops over two cropping cycles (1998 to 2000 wet and dry seasons). Stale seedbeds were achieved either by two harrowings (mechanical) or by a single harrowing followed by glyphosate application (chemical), with a 2-week interval between each operation. The treatments were imposed once before either crop or twice before both crops within a one-year rotation cycle. *C. rotundus* tubers and emerging shoots were counted and crop yields and costs of all weeding operations were recorded in each cropping season. Mechanical or chemical stale seedbed treatments once or twice in the rotation cycle resulted in tuber and shoot densities that decreased over time by 86 to 90%. Handweeding costs also decreased by 50 to 95% while crop yields and net incomes increased over time. Total net incomes from stale seedbed treatments were \$1000 more than income from farmer's practice and \$2,500 more than income from unweeded plots. Our study, which related *C. rotundus* population dynamics with reduction of its tuber and shoot densities over time, can be useful in determining mechanistic models aimed at developing alternative weed management strategies using minimum control inputs.

**Key Words:** Glyphosate, population dynamics, tillage

### INTRODUCTION

Purple nutsedge (*Cyperus rotundus* L.) is a highly dominant weed in many field and vegetable crops worldwide because of its unique biological and physiological properties. A single plant can produce 4 to 8 million tubers per hectare in a cropping season (Rao, 1968). About 2000 tubers/m<sup>2</sup> have been reported in heavily infested fields, 85% of which are in the top 15 cm of the soil (Siriwardana and Nishimoto, 1987), contributing to recruitment of new weeds upon sprouting.

In rainfed rice-onion rotations in the tropics, tubers of *C. rotundus* growing with onion (dryland) are carried over into the rice crop (flooded). Over the years, because of

the continuous rainfed rice-onion cropping pattern, *C. rotundus*, which is the most dominant weed in onion, is now also emerging as a major weed in rainfed rice. It has been shown to cause yield losses of up to 90% in onion and by 40% in upland rice (Okafor and De Datta, 1976; Baltazar et al, 2000). Handweeding, which is the only selective postplant weed control option in onion constitutes 20% of the total production costs (Baltazar et al., 2000). Replacing handweeding with cheaper alternative management practices against *C. rotundus* can reduce production costs.

Tuber dormancy, which is the principal reason why *C. rotundus* is difficult to control, is also the key to successful management of this weed. Tubers in intact chains are controlled by apical dominance and majority of these are dormant, surviving conventional control methods (Muzik and Cruzado, 1953). To break dormancy and enhance sprouting, tubers have to be separated from the chain. Soil disturbance in the form of tillage or cultivation can break tuber chains apart and sprouted tubers are killed by mechanical or chemical means. Using this concept to reduce tuber populations of the *Cyperus* species, early studies employed cultural systems that integrated repeated tillage for several months in fallow areas and later, as systems that integrated stale seedbed techniques in annual crops (Smith and Mayton, 1938; Glaze, 1987).

Previous studies have determined the effectiveness of tillage or stale seedbed treatments on reducing *C. rotundus* populations in fallow areas or in single crop systems. However, none or very limited data are available on effectiveness of stale seedbeds on reducing this weed's populations and on its dynamics in multiple cropping systems. The objectives of this study then were to: 1) quantify the population dynamics and fate of *C. rotundus* tubers throughout the rice-onion rotation; 2) identify the best combination of tillage and chemicals in stale seedbed techniques for reduction of tuber and shoot populations; 3) to assess cost-effectiveness of stale seedbed treatments compared to farmer's practice; and 4) to quantify the yield losses due to *C. rotundus* in rainfed rice-onion rotation systems. Data of this kind, which are limited in the tropics, are vital in the generation of IWM technology involving cost-effective alternative cultural control strategies. Information derived from this study will also be useful in the formulation of mechanistic models of weed population dynamics which are necessary as theoretical basis of improved weed management systems (Sagar and Mortimer, 1976).

## MATERIALS AND METHODS

The study was conducted in a farmer's field in San Jose, Nueva Ecija, Philippines, lat 15-16°N and long 121 °E, 50 to 100 m above sea level. Annual rainfall averages 1900 mm from 1980 to 1999, with a monthly average of 300 mm from May to October (wet season) and 30 mm from November to April (dry season). The monthly average day and night temperatures range between 20°C and 32°C, with a yearly mean average of 27°C. Soil is clay loam, classification Inceptisols (Eutropepts with Dystropepts). The sequence of cropping seasons, tillage practices, stale seedbed treatments, and tuber census points are in Figure 1. Rice was grown in the wet seasons of 1998 and 1999 from June to October. Onion was grown in dry seasons of 1999 and 2000 from November to March. An area with uniform infestation of *C. rotundus* was selected. Plots were marked and laid out. Fixed reference points were marked so that the same treatments and sampling sites were maintained in the same plots across crops.

After mowing the area, land was plowed twice and harrowed thrice with an animal-drawn implement. Two weeks after land preparation, stale-seedbed treatments were imposed on selected plots. After 1 week, 28-day old seedlings of rice 'IR 64' were transplanted at 20 cm between rows and 20 cm between hills, with 2 to 3 seedlings per hill. Plots treated with the farmer's practice were applied with 1.0 kg ai/ha butachlor (*N-butoxymethyl-2-chloro-N-2,6-diethylphenyl acetamide*) at 4 days after transplanting (DAT) in the 1998 wet season and with 1.0 kg ai/ha butachlor + propanil [*N-(3,4-dichlorophenyl) propanamide*] (pre-mix formulation) at 6 DAT in the 1999 wet season. At 1 day after herbicide application, plots were irrigated from a deep-well pump. At least 2 cm of standing water was maintained in the paddies throughout the season. All plots were handweeded at 20 and 40 DAT. Yields were harvested about 90 DAT. At the end of the fallow periods following the 1998 and 1999 rice crops, weeds in all plots, except for those to be applied with stale-seedbed treatments, were burned down with 1.0 kg ai/ha glyphosate [*N-(phosphonomethyl)glycine*] (farmers' practice). Land was plowed twice and harrowed thrice with an animal-drawn implement in the stale-seedbed treated plots. In these plots, stale-seedbed treatments were applied 2 weeks after land preparation. Four days after the stale-seedbed treatments, 10-cm rice straw mulch was laid out in all plots. Twenty-eight day old seedlings of shallot onion 'Tanduyong' were dibbled into the mulch at 15 cm between rows and 15 cm between hills, with 2 to 3 seedlings per hill. Fluazifop [(+2-[4-[5-(trifluoromethyl)-2-pyridinyl] oxy] phenoxy] propanoic acid) (0.9 kg ai/ha) was applied 20 DAT into the plots treated with farmer's practice for both cropping seasons. All plots were handweeded at 20 and 40 DAT. Irrigation was obtained from a deep-well pump and soil moisture was maintained at field capacity. Yields were harvested about 90 DAT.

The following treatments were maintained in the same plots across crops: 1) farmer's practice; 2) mechanical stale-seedbed before rice; 3) mechanical stale seedbed before onion; 4) mechanical stale seedbed before both crops; 5) chemical stale seedbed before rice; 6) chemical stale seedbed before onion; 7) chemical stale seedbed before both crops; 8) untreated, unweeded control, and 9) untreated control where all other weeds except *C. rotundus* were removed manually. Mechanical or chemical stale-seedbeds were applied 2 weeks after the last harrowing during land preparation to allow the *C. rotundus* tubers to sprout and grow for maximum efficacy of treatments. At treatment time, the seedlings were about 6 to 8 cm with two to four leaves. Plots treated with mechanical stale-seedbed were harrowed two to three times with a hand-pushed machine-driven rotavator at a depth of 10 to 15 cm. Plots treated with chemical stale-seedbed were applied with 1.0 kg ai/ha glyphosate. Herbicides were applied with a hydraulic knapsack sprayer in 500 L/ha using flat fan nozzles.

Tuber counts were taken prior to land preparation and after harvest in each crop. A soil core of 0.25 x 0.25 x 0.20 m was dug at two points along the diagonal (lower left and upper right corners) of each plot. The soil was sieved to separate the tubers from the soil. The tubers were handled carefully in order not to break the tuber chains because tubers separated from chains will sprout. The tubers in the chains were counted and the tuber chains were returned intact to the same points in the plots from where they were taken. Decayed and dead or rotting tubers were not counted. At 20 and 40 DAT non-destructive shoot counts were made from a 0.5 m x 0.5 m area at the same points where the tuber counts were made. The time spent in handweeding each plot at 20 and 40 DAT were recorded. Crop yields were taken from a 2 x 4 m<sup>2</sup> at the

center of each plot and from two 1 x 1 m<sup>2</sup> areas over the points where tuber counts were made, a total of 10 m<sup>2</sup>. Costs of all weed control inputs (handweeding, harrowing, herbicides) were recorded. The treatments were laid out in a Randomized Complete Block Design (RCBD) with four replications. Plot size was 4 m x 5 m. Data were subjected to Analysis of Variance (ANOVA) using General Linear Models Procedure (Statistical Analysis Systems). Treatment means were compared using LSD at 5% level of significance.

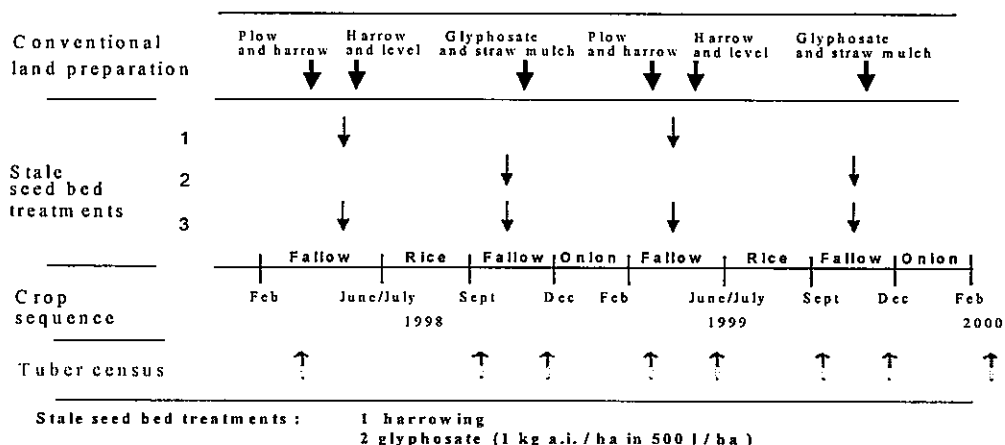


Figure 1. Sequence of cropping seasons, tillage practices, stale seedbed treatments, and tuber census points from 1998 wet season to 2000 dry season

## RESULTS AND DISCUSSION

### Tuber and shoot densities

Initial pre-treatment tuber populations, which averaged 155 tuber/0.25 m<sup>2</sup> are considered high populations since these are much greater than the economic threshold levels of 50/m<sup>2</sup> determined by Keeley (1987). Tuber densities in all plots decreased across the four crops (Fig. 2a). At the end of the 4<sup>th</sup> crop, tuber densities in plots treated with stale seedbeds and farmer's practice decreased to an average of 14 tubers/0.25 m<sup>2</sup>, a reduction of 90% of initial densities. Tuber densities in the unweeded plots decreased to about 70 tubers/0.25 m<sup>2</sup>, a reduction of only 50% of initial densities, and were 80% more than tuber densities in the treated plots (Fig. 2a).

Shoot densities, which peaked at 132 shoots/0.25 m<sup>2</sup> at midseason of the first crop, also decreased in all plots across the four crops (Fig. 2b). At the end of the fourth crop, shoot densities in plots treated with mechanical or chemical stale seedbeds once (before onion) or twice (before rice and onion) were reduced to 16 shoots/0.25 m<sup>2</sup>, a reduction of 88% of initial densities. Shoot densities in plots treated with the farmer's practice, or with stale seedbed once (before rice), and in unweeded control plots were reduced by 82% of initial densities. Thus, shoot densities in these plots were 30 to 47% more than those in plots with stale seedbed treatments before onion or before both crops.

In both tuber and shoot densities, the greatest rate of decline occurred at the beginning of the second crop after imposition of a maximum of two stale seedbed treatments (Fig. 2a & 2b). No further substantial reductions in populations occurred with additional stale seedbed treatments in the third and fourth crops.

The tuber densities in unweeded control plots showed a fast rate of increase at low populations and slow rate at high populations (Fig. 3). This suggests that population expansion rates are regulated by a density-dependent process. This could explain why tuber and shoot densities in the unweeded control plots were higher than those in *C. rotundus* control plots (Fig. 2a and 2b).

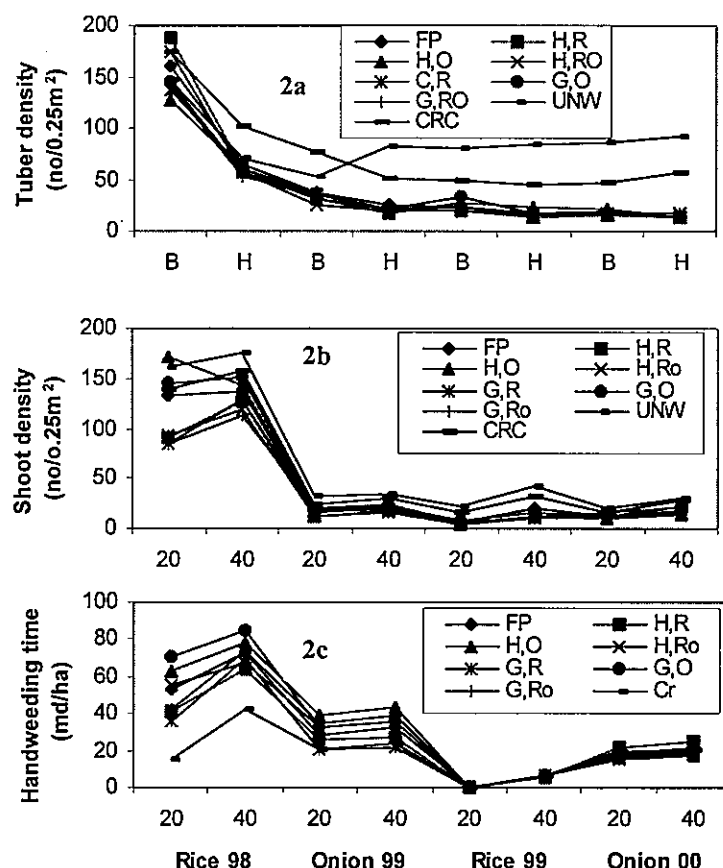


Figure 2. Dynamics of *C. rotundus* tuber (2a) and shoot (2b) populations and time spent in handweeding (2c) over four cropping seasons. Tuber counts were taken before planting (B) and after harvest (H); shoot counts and handweeding times were taken 20 and 40 days after transplanting in each cropping season. (Legend: FP=farmer's practice, H,O=harrow (onion), H,R=harrow (rice), H,RO=harrow (both), G,R=glyphosate (rice), G,O=glyphosate (onion), G,RO=glyphosate (both), UNW=unweeded control, Cr or CRC = *C. rotundus* control).

### Crop yields

Rice and onion yields in plots treated with chemical or mechanical stale seedbeds once or twice in the rotation (before either crop or before both crops) were higher than yields in untreated (including farmer's practice plots) or unweeded plots (Fig 4a and 4b). Higher yield losses in unweeded control plots and more pronounced increase in yields from stale seedbed treatments in onion than in rice confirm the lower competitive ability of onion than rice against *C. rotundus*. Yields in plots treated with stale seedbeds before the onion crop or before both rice and onion crops were higher by

2 t/ha over those of plots treated with farmer's practice and those with stale seedbed treatments before the rice crop, 5 t/ha more than those of *C. rotundus* control plots, and 7 t/ha more than yields in unweeded plots (Fig 4b). The treatments with the highest yields also had the lowest shoot counts particularly in the second season of the onion crop.

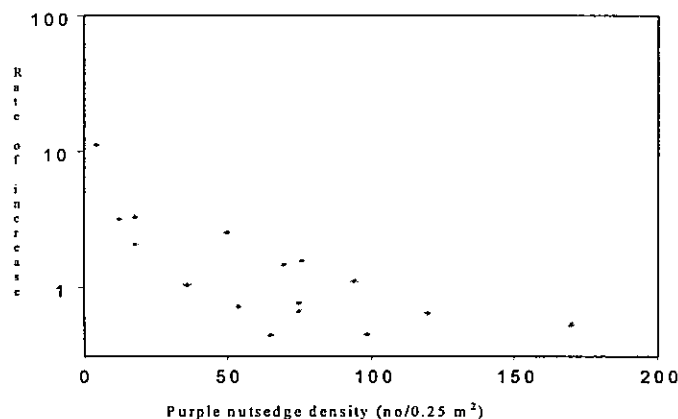


Figure 3. Population expansion rates of *C. rotundus* in relation to density in unweeded plots at the start of the 1999 onion season.

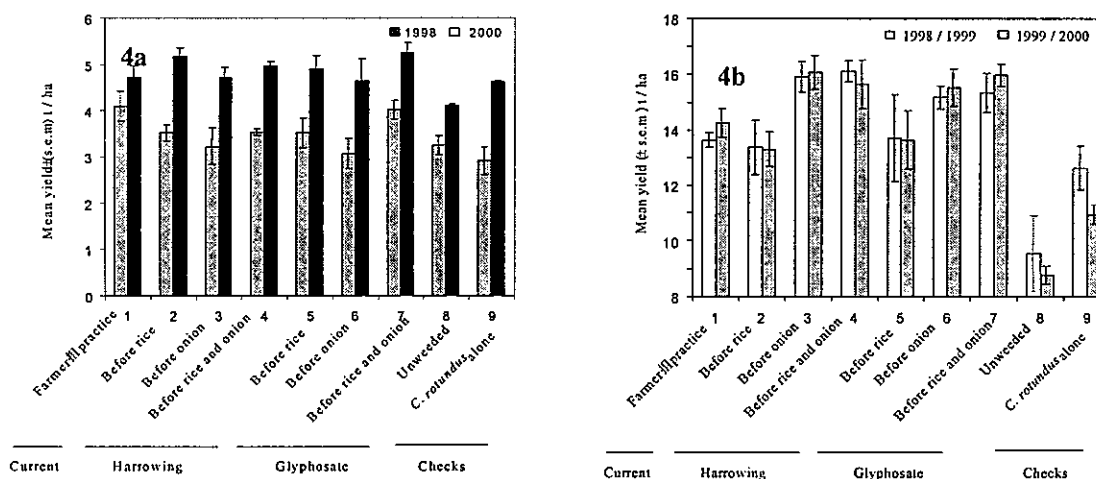


Figure 4. Yield of rice (4a) and onion (4b) in relation to cropping season and stale seedbed treatments.

### Weed control costs

Total weed control costs include herbicide costs and labor costs for handweeding, spraying herbicides and harrowing. Across the four crops, plots treated with farmer's practice had highest total weed control costs (Table 1). Weed control costs were highest during the first year of each crop due to high initial shoot populations, thus high handweeding costs, which accounted for a greater portion of the total weed control costs. Handweeding times decreased in the succeeding crops as the shoot populations decreased with imposition of stale seedbed treatments. In the second year of rice, no handweeding was done at 20 DAT because of very low shoot populations at this period (Fig 2c).



Although tuber and shoot densities due to stale seedbed treatments were reduced drastically within the first year, reductions in weed control costs did not occur until the second year of both crops. In rice, weed control costs during the second year were reduced by 80 to 95% of costs in the first year. In onion, reduction in weed control costs from the first to the second year was lower, about 10 to 48% (Table 1). This was due to the more drastic reductions in *C. rotundus* shoot densities from the first to the second rice crop. Shoot densities from the first to the second onion crop were not as drastically reduced.

Table 1. Total weed control costs in rice and onion crops treated with stale seedbeds from 1998 to 2000 wet and dry seasons, San Jose, Nueva Ecija. <sup>1</sup>Consists of: cost of two handweeding (\$2.50/manday); harrowing (10 mandays/ha at \$5.00/manday); spraying labor (2 mandays/ha at \$2.50/manday); glyphosate (1.0 kg ai/ha) at \$25/ha; and butachlor or butachlor + propanil (1.0 kg ai/ha) at \$11/ha. Figures in parenthesis indicate percent reduction over the first crop).

TREATMENT	Total weed control costs (\$/ha) <sup>1</sup>				
	Rice 98	Onion 99	Rice 99	Onion 00	Total
Farmers' practice	324	246	27 (92)	196 (20)	793
Harrow (rice)	313	129	64 (80)	116 (10)	622
Harrow (onion)	353	258	18(95)	134 (48)	762
Harrow (both)	356	221	65 (82)	134 (39)	776
Glyphosate (rice)	289	108	41 (86)	96 (11)	534
Glyphosate (onion)	389	197	18 (95)	115 (42)	719
Glyphosate (both)	315	208	41 (87)	108 (48)	671

### Net income

Net returns from weed control costs increased across the four crops, with higher net returns from the second year of both crops (Table 2). High initial tuber and shoot populations of *C. rotundus* in the first crop which required high handweeding costs resulted in lower net returns in the first years of both crops. As stale seedbed treatments were imposed through the seasons, *C. rotundus* shoot populations decreased, reducing handweeding costs and increasing net returns in the second year of both crops. Highest total net income across crops were obtained from plots treated with mechanical or chemical stale seedbeds before both crops. Total net income in these treatments were higher by about \$2500 over unweeded plots, by \$1600 over *C. rotundus* control plots, and by \$1000 over farmer's practice plots (Table 2). The farmer's practice of two herbicides and two handweeding reduced tuber populations at rates similar to those of the stale seedbed treatments. However, the rate in shoot decline was slower, resulting in higher shoot densities and handweeding costs, thus lower net income obtained from farmer's practice than from the stale seedbed treatments.

Although *C. rotundus* populations were reduced after the first year of both crops after two stale seedbed treatments, the reduction in weed control costs and increase in net income was realized only in the second year after the imposition of additional stale seedbeds. Thus, imposition of stale seedbed treatments at least once a year in a one-year crop rotation cycle is needed to maintain tuber and shoot populations of *C. rotundus* to reduced levels which will reduce handweeding costs and increase net incomes.

Mechanical or chemical stale seedbed treatments once or twice in a one-year rice-onion rotation cycle resulted in tuber and shoot populations and handweeding costs that decreased over time by 86 to 90%, with corresponding increase in crop yields and net incomes. Our data support the hypothesis of Neeser et al (1998), who, in using a mechanistic population dynamics model, determined that in fields with high tuber populations, control aimed at reduction of tuber survival would be more effective than control aimed at reducing production rate of new tubers. Our study, which was conducted in a field with high densities (more than 50 tubers and shoots/m<sup>2</sup>) of *C. rotundus* was aimed at reducing tuber survival through tillage and use of a translocated herbicide in the form of stale seedbed treatments.

Table 2. Net income in rice and onion crops treated with stale seedbeds from 1998 to 2000 wet and dry seasons, San Jose, Nueva Ecija. <sup>1</sup>Gross income minus weed control costs (partial budgeting, does not include other production costs). Farmgate price of rice : \$0.25/kg (1998); \$0.21/kg (1999). Farmgate price of onion cv. Tanduyong: \$0.25/kg (1998); \$0.20/kg (2000).

TREATMENT	Net Income (\$/ha) <sup>1</sup>				Total
	Rice 98 WS	Onion 99 DS	Rice 99 WS	Onion 00 DS	
Farmers' practice	539	936	3154	2664	7293
Harrow (rice)	501	1002	3220	2544	7268
Harrow (onion)	392	946	3718	3086	8141
Harrow (both)	458	960	3804	3026	8248
Glyphosate (rice)	524	964	3317	2664	7470
Glyphosate (onion)	331	926	3578	3045	7880
Glyphosate (both)	615	1025	3618	3091	8350
Unweeded	767	841	2375	1760	5743
<i>C. rotundus</i> alone	532	926	3039	2097	6594

While previous studies have shown that stale seedbed technique is highly effective in depleting *C. rotundus* tuber populations in single crop systems, our study determined its effectiveness in multi-crop systems. Our study is probably one of the first attempts at relating tuber depletion over time with the weed's tuber and shoot population dynamics and how it results in increased weed control efficacy and economic returns over time. Our data also support the newly emerging paradigm shift from a single season approach to a long-term population management approach. The latter, which considers the carry-over effects of weeds across crops, requires an understanding of weed population dynamics (Jones and Medd, 2000). Data from our study can be useful in developing mechanistic models which are vital in developing alternative approaches aimed at reducing weed populations using minimum inputs. Hopefully, this will enhance global competitiveness of farmers in rice-based cropping systems in the tropics.

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## WEED EMERGENCE AND CONTROL IN SOME DIFFERENT TYPES OF RICE (*ORYZA SATIVA*) CULTIVATION WITH CONTINUOUSLY REPLICATED CULTURE IN KOREA

I. B. Im, E. S. Kyoung, and S. C. Kim

National Honam Agricultural Experiment Station, Iksan, Jeonbuk, Korea  
imweed@rda.go.kr

**Abstract:** This study was conducted to investigate the ecology of weed occurrence and to establish its economical weed control system under different cultivation methods in the paddy field. Herbicides, sulfonylurea compounds, were continuously applied at the rice field for four years. The main problem weed among annual and perennial weeds was *Eleocharis kuroguwai* that increased with number of cultivated year and the amount of its tuber was high at direct seeding on dry paddy field. Most of annual weeds including *Echinochloa crus-galli* were controlled in machine transplanting, water and dry direct seeding within 2 years. The tuber formation of *Eleocharis kuroguwai* was highly inhibited at the middle and latter period, and the sulfonylurea compound herbicide treatment by two times inhibited about 75~84%, and the additional treatment of bentazone showed 84~98%. This trend of control effect was similar in machine transplanting, water and dry direct seeding. The tuber formation of *E. kuroguwai* was highly inhibited by bentazone SL application at the middle stage of rice cultivation. The control of *E. kuroguwai* could be effective by the application of sulfonylurea system herbicides at the early stage of rice and the additional application on 20 days after the first treatment or bentazone SL application on 40~50 days after the first treatment.

**Key words:** Rice, weed control, *Eleocharis kuroguwai*, Korea

### INTRODUCTION

Recently, rice cultivation in Korea is gradually changing to dry and water direct seeding culture with the development of farming machine and decrease of rural population. Weed emergence and competition injury in direct seeded rice field is much more than transplanted because growth period in direct seeded rice field is longer than transplanted rice field. For weed control, sulfonylurea compounds herbicides have been used in rice cultivation paddy field in Korea for the last 10 years. By that reason, some of particular weeds are dominant and troublesome to control in the paddy fields. Therefore, the research was tried to establish weed control system with the investigation of weed emergence ecology in associated with different rice cultivation methods in Korea.

### MATERIALS AND METHODS

The paddy field which has been used for machine transplanting until 1995 to investigate weed emergence ecology according to different types of rice cultivation. In 1996, pyrazosulfuron-ethyl • molinate GR, were applied two times to uniform weed emergence into all paddy field. Weed control for the rice cultivation was followed

standard method in Korea (Table 1).

Table 1. Standard weed control methods of each rice cultivation types in Korea

Cultivation types (Planting or seeding date)	Standard weed control system		Additional weed control
	1st	2nd	2nd or 3rd
Machine transplanting Paddy field	Pyrazosulfuron- ethyl • mefenacet(0. 07+3.5% GR)(10	-	Molinate •cinosulfuron (5+0.08GR): 30 DAT, bentazone(40% SL)
Direct seeding on Water paddy field (12th May)	Azimsulfuron • moli nate (0.05+7%GR) (12 DAS)	-	Molinate •cinosulfuron GR: 32 DAS, bentazone SL
Direct seeding on dry paddy field (26th Apr.)	Cyhalohop- butyl • bentazone(3 +16.5%ME) (20 DAS*)	Azimsulfuron • molinate GR (28 DAS)	Molinate •cinosulfuron GR: 48 DAS, bentazone SL

\* DAT : Days after transplanting, DAS : Days after Seeding

Table 2. Rice cultivation methods of these experiments

Cultivation types	Seeding date	Transplantin g date	Seeding amount	Planting distance	Fertilizer amount
Machine transplanting	3rd May	28th May	150g/tray	30x13cm	110-70-80kg/ha
Direct seeding on water paddy field	12 May	-	40kg/ha	-	110-70-80kg/ha
Direct seeding on dry paddy field	30th April	-	60kg/ha	-	160-90- 110kg/ha

## RESULTS AND DISCUSSION

### Changes of weed emergence

Several kinds of Weeds occurred such as *E. crus-galli*, *E. kuroguwai*, *S. juncoides*, *Monochoria vaginalis*, *Lindenia procumbens*, *Cyperus difformis*, and *Rotala indica*, etc. in this experiment. *E. crus-galli* has been completely controlled from the first to the third year in all rice cultivation methods (Fig.1). The emergence amount of *E. kuroguwai* tended to increase from year to year (Fig. 2).

The control of *E. kuroguwai* was not effective by the usage of sulfonylurea system herbicides year by year. On the other hand, Perennial *S. juncoides* was almost controlled in machine transplanting paddy, water and dry direct-seeded paddy(Fig. 3).

In conclusion, the main problematic weed was *E. kuroguwai* in machine transplanting, water and dry direct seeding on rice paddy field when sulfonylurea system herbicides were used in the same field every year. In addition, the weed control effects tended to decline gradually by year.

### Control characteristics of problematic weed (*Eleocharis kuroguwai*)

*E. kuroguwai* is a perennial weed propagated by underground tuber, especially, it needs much cumulative temperature for emergence among the perennial weeds

emerging on paddy field. So, *E. kuroguwai* is difficult to control because of late and continuous emergence. Accordingly, it is emphasized that *E. kuroguwai* should be controlled in the two points. One is to escape of competition in that year, the other is to inhibit the formation of tuber propagation body.

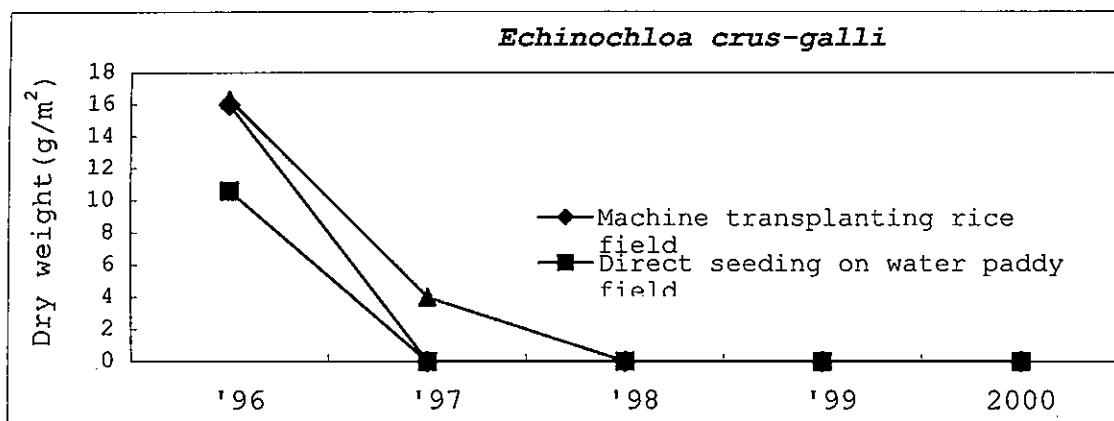


Figure 1. Yearly fluctuation for emergence of *Echinochloa crus-galli* according to standard weed control of several rice cultivating types.

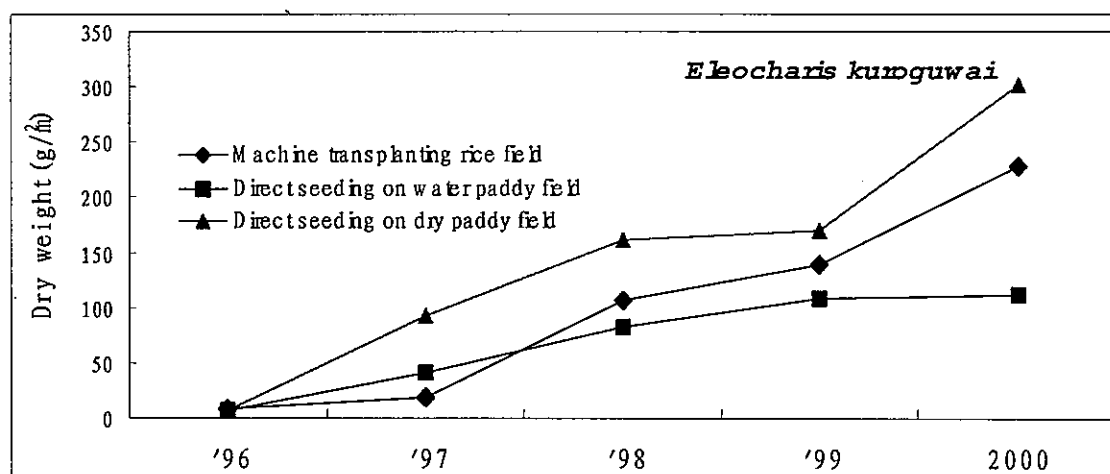


Figure 2. Yearly fluctuation for emergence of *Eleocharis kuroguwai* according to standard weed control of several rice cultivating types.

Table 3, 4 and 5 show the effects of the control and tuberous inhibition of *E. kuroguwai* according to weed control system of several rice cultivation types. In machine transplanting paddy field, the control effect of *E. kuroguwai* by pyrazosulfuron-ethyl • mefenacet GR application on 10 days after transplanting was about 70% on 23 days after application, but it was decreased to 11% at middle and late stage, on 63 and 94 days after transplanting. In the case of pyrazosulfuron-ethyl • mefenacet GR application on 10 days after transplanting followed by molinate • cinosulfuron GR on 20 days after application, weeding effect were over 90% at the middle and latter period. The addition treatment of bentazone on 50 days after transplanting was over 96% for weed control and 84 % for tuberous inhibition.

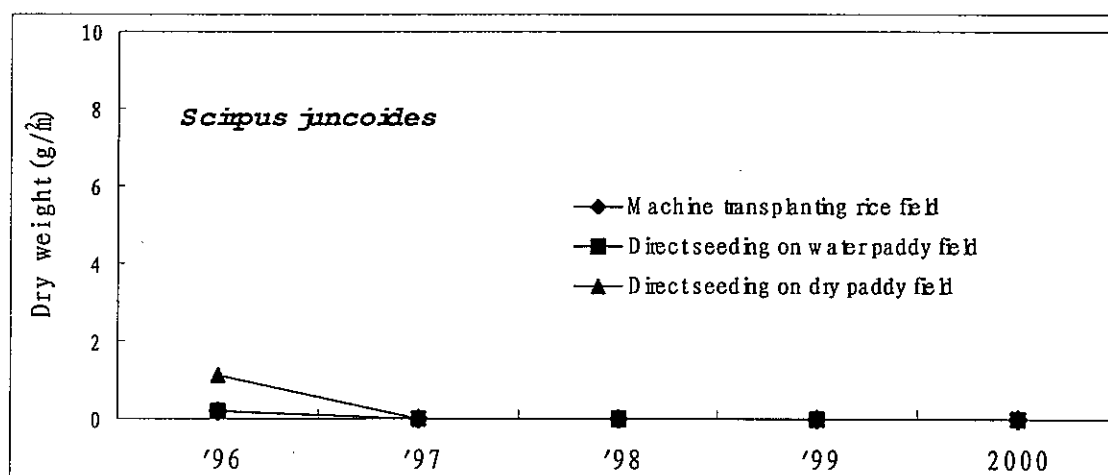


Figure 3. Yearly fluctuation for emergence of *Scirpus juncoides* according to standard weed control of several rice cultivating types.

Table 3. Weed control effects and tuber formation inhibiting rate of *Eleocharis kuroguwai* in machine transplanting paddy field by each herbicide application system

Herbicides	Control effects			Inhibiting rate of tuber formation (%)
	Jun. 30	Jul. 30	Aug. 30	
Pyrazosulfuron-ethyl • mefenacet GR	70	11	11	4
Pyrazosulfuron-ethyl • mefenacet GR fb Molinate • cinosulfuron GR	70	95	93	77
Pyrazosulfuron-ethyl • mefenacet GR fb bentazone	70	96	97	84

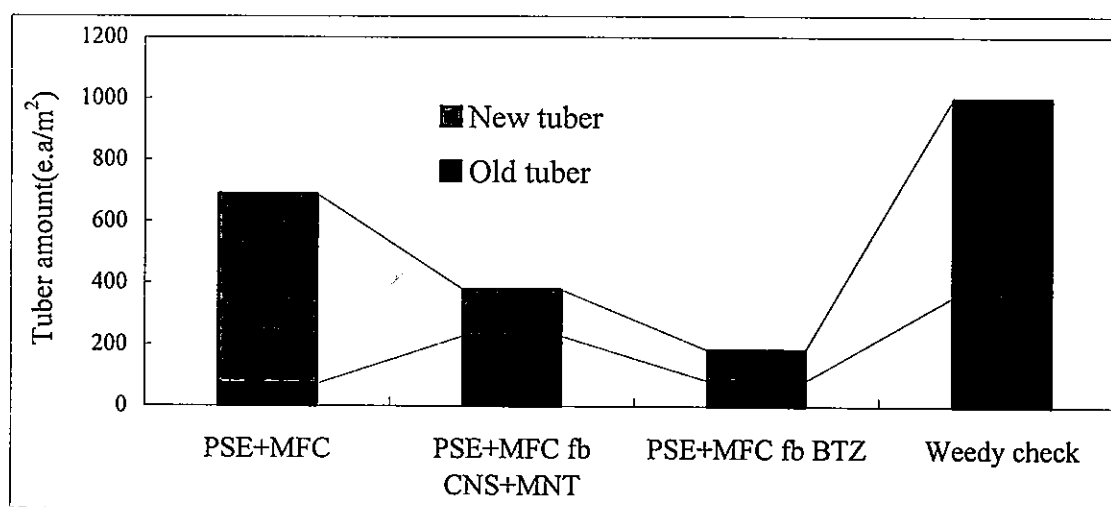


Figure 4. New forming and old dormant tuber amount of *Eleocharis kuroguwai* under soil of machine transplanting paddy field by each herbicide application system. \* PSE+MFC : pyrazosulfuron-ethyl • mefenacet GR, CNS+MNT : cinosulfuron • molinate GR, BTZ : bentazone SL

In machine transplanting paddy field, Tuber amount of *E. kuroguwai* under soil by each weed controls system after rice harvest(Fig. 7). The dormant tubers of *E. kuroguwai* by 1 time application of pyrazosulfuron+mefenacet GR were very few to 10% of all, but application of pyrazosulfuron+mefenacet GR and cinosulfuron+molinate GR were not a few to 60% of all (Fig. 4). This indicates that the dormancy of *E. kuroguwai* tubers is induced by force of herbicide(the second times).

In direct seeding on water paddy field, the control effects of *E. kuroguwai* by azimsulfuron • molinate GR application on 12 days after seeding were 62% at early stage, 39~52% at the middle and late stage of rice. The control effect by additional application of molinate • cinosulfuron GR on 20 days after early treatment were 90% at the early period, but weeding effect dropped into 80-73% at the middle and the latter period. Also, azimsulfuron • molinate GR application on 12 days after seeding followed by bentazone on 43 days after application showed 91~95% control effect at the middle and the latter period.

Table 4. Weed control effect and tuber formation inhibiting rate of *Eleocharis kuroguwai* in direct seeding on water paddy field by each herbicide application system

Herbicide	Control effects			Formation inhibition rate of tuber(%)
	Jun. 30	Jul. 30	Aug. 30	
Azimsulfuron • molinate GR	62	39	52	16
Azimsulfuron • molinate fb	90	80	73	84
Molinate • cinosulfuron GR				
Azimsulfuron • molinate GR fb	59	91	95	87
Bentazone SL				
Azimsulfuron • molinate GR fb				
Molinate • cinosulfuron GR	90	80	100	96
fb bentazone SL				

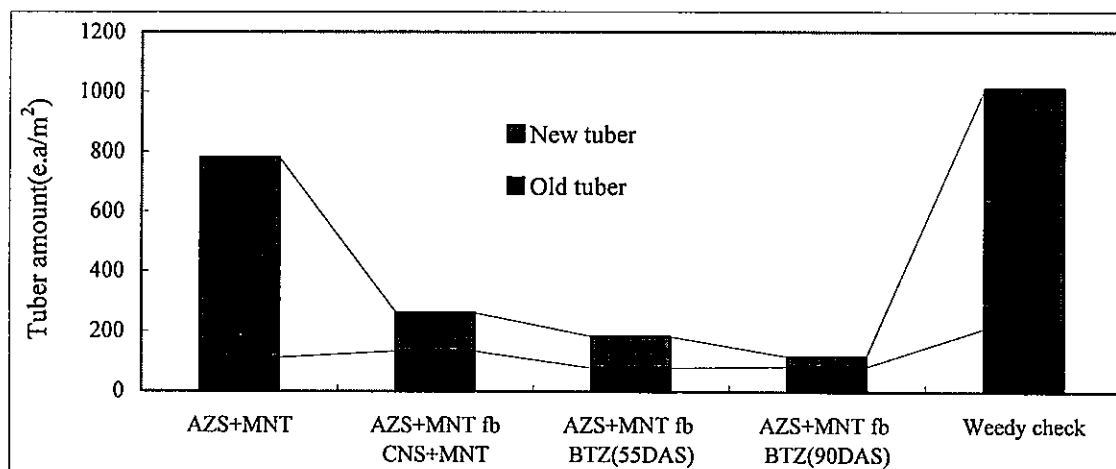


Figure 5. New forming and old dormant tuber amount of *Eleocharis kuroguwai* under soil of direct seeding on water paddy field by each herbicide application system. \* AZS+MNT: azimsulfuron • molinate GR, CNS+MNT: cinosulfuron • molinate GR, BTZ: bentazone SL



The weed control effects with cyhalohop-butyl • bentazone ME on 20 days after seeding in direct seeding on dry paddy field showed in Table 5. The control effect of *E. kuroguwai* by azimsulfuron • molinate GR application on 28 days after seeding was very low at the middle and latter period. The control effects by additional application of molinate • cinosulfuron GR on 48 days after seeding became low gradually at the latter of rice growth. But the control effect of *E. kuroguwai* by bentazone application on 60 days after seeding was over 90% at the middle and the latter period and the tuber formation was inhibited over 95%.

The control effect of *E. kuroguwai* with application of sulfonylurea compound herbicide by two times tended to increase according to the order of machine transplanting, water direct-seeding and dry direct-seeding in three rice cultivation types. These were thought to be the herbicide application time was late relatively.

Table 5. Weed control effect and tuber formation inhibiting rate of *Eleocharis kuroguwai* by each herbicide application system in direct seeding on dry paddy field

Herbicide	Control effects			Formation inhibition rate of tuber(%)
	Jun. 30	Jul. 30	Aug. 30	
Cyhalohop-butyl • bentazoneME fb Azimsulfuron • molinateGR	86	29	36	20
Cyhalohop-butyl • bentazoneME fb Azimsulfuron • molinateGR fb Molinate • cinosulfuronGR(Jun.13)	89	75	68	75
Cyhalohop-butyl • bentazoneME fb Azimsulfuron • molinateGR fb bentazoneSL(60DAS : Jun.25)	89	98	99	98
Cyhalohop-butyl • bentazoneME fb BentazoneSL(50DAS : Jun.15)	85	96	96	83
Cyhalohop-butyl • bentazoneME fb Azimsulfuron • molinateGR	86	29	100	96

The tuber formation of *E. kuroguwai* was highly inhibited at the middle and latter time, and the sulfonylurea compound herbicide treatment by two times inhibited about 75-84%, and the additional treatment of bentazone showed 84-98%. All of three rice cultivation types showed similar trend.

#### Weed control system on several rice cultivation types

**Machine transplanting paddy field** Annual weeds *E. crus-galli*, *Lindernia procumbens*, *Cyperus difformis*, *Scirpus juncoides*, *Rotala indica* and so forth in 3 years continuous cultivation paddy of machine transplanting were completely controlled by the one time application of pyrazosulfuron-ethyl • mefenacet GR on the first year. The control effect of *E. kuroguwai* was very low as 11 %, but on the second application of molinate • cinosulfuron GR on 30 days or bentazone on 50 days after transplanting, the effects were 95 and 96% respectively, therefore total weed control effect showed higher effect of 98% by two times than 59% by one.

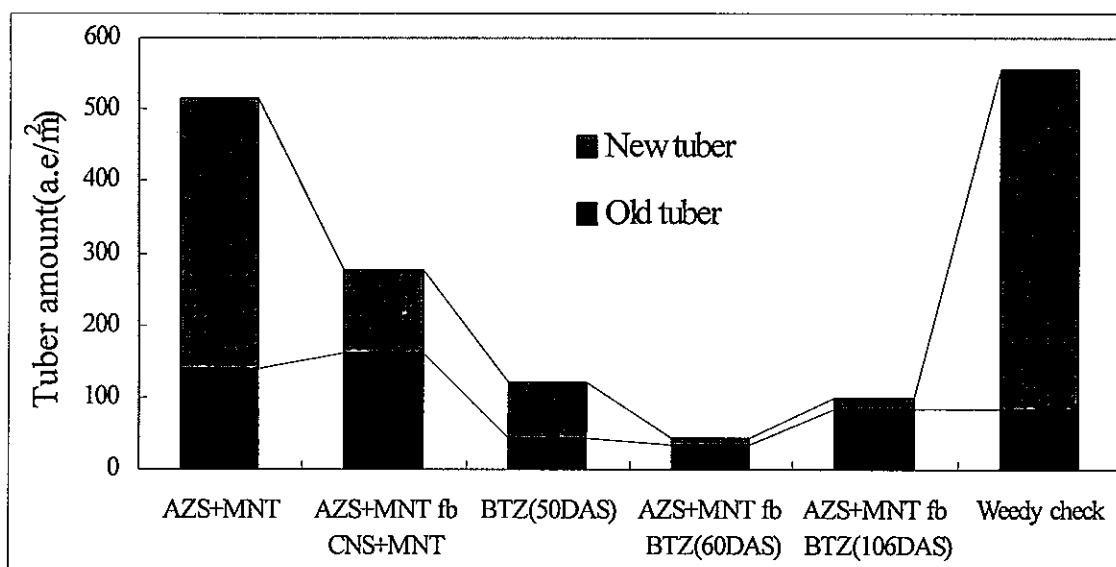


Figure 6. New forming and old dormant tuber amount of *Eleocharis kuroguwai* under soil of direct seeding on dry paddy field by each herbicide application system. \* AZS+MNT: azimsulfuron • molinate GR, CNS+MNT: cinosulfuron • molinate GR, BTZ: bentazone SL

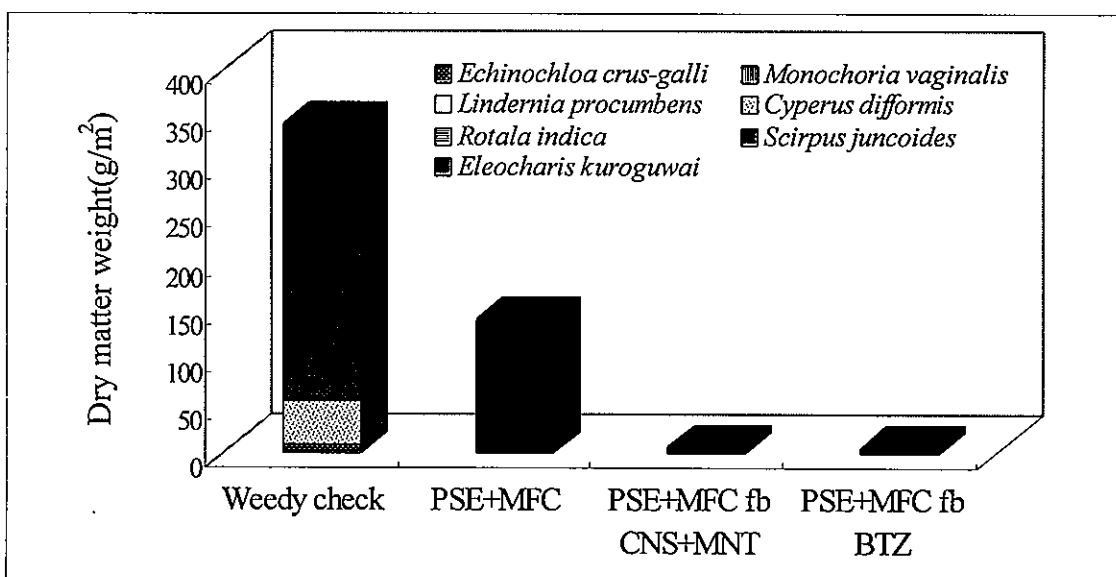


Figure 7. Weed control effects in machine transplanting paddy field by several herbicide application systems. \* PSE+MFC: pyrazosulfuron-ethyl • mefenacet GR, CNS+MNT: cinosulfuron • molinate GR, BTZ : bentazone SL

**Direct seeding on water paddy field** Annuals emerged on water direct-seeded rice field for 3 years were completely controlled by one time application of azimsulfuron • molinate GR on 12 days after seeding, but the control effect of *E. kuroguwai* became very low with 39% about 78 days after seeding. Therefore, the second addition application of molinate • cinosulfuron GR on 32 days after seeding or

bentazone SL on 55 days after seeding was able to increase to each 80 and 91% for the control effect of *E. kuroguwai*. The effect of all weed control was over 90% by two times application.

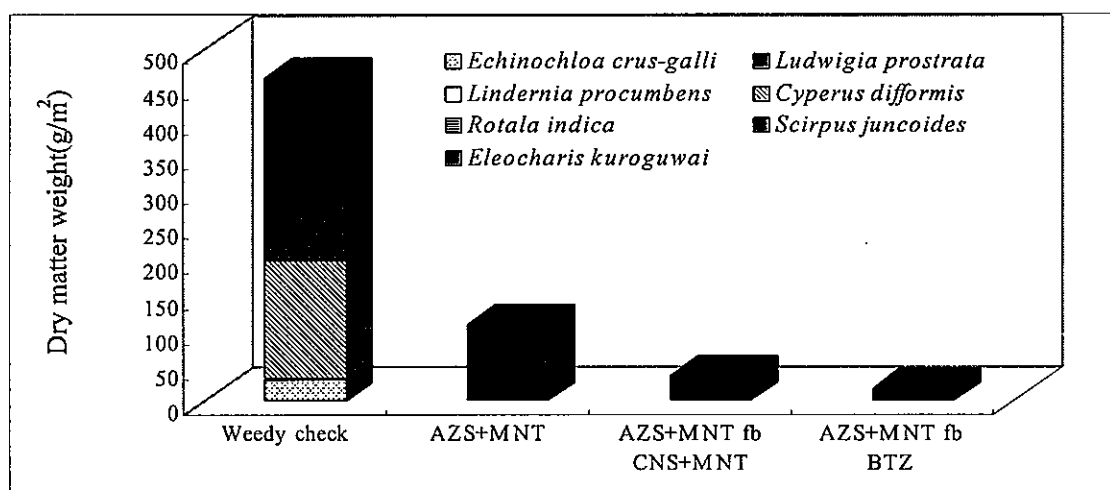


Figure 8. Weed control effects in direct seeding on water paddy field by several herbicide application system. \* AZS+MNT: azimsulfuron • molinate GR, CNS+MNT: cinosulfuron • molinate GR, BTZ : bentazone SL

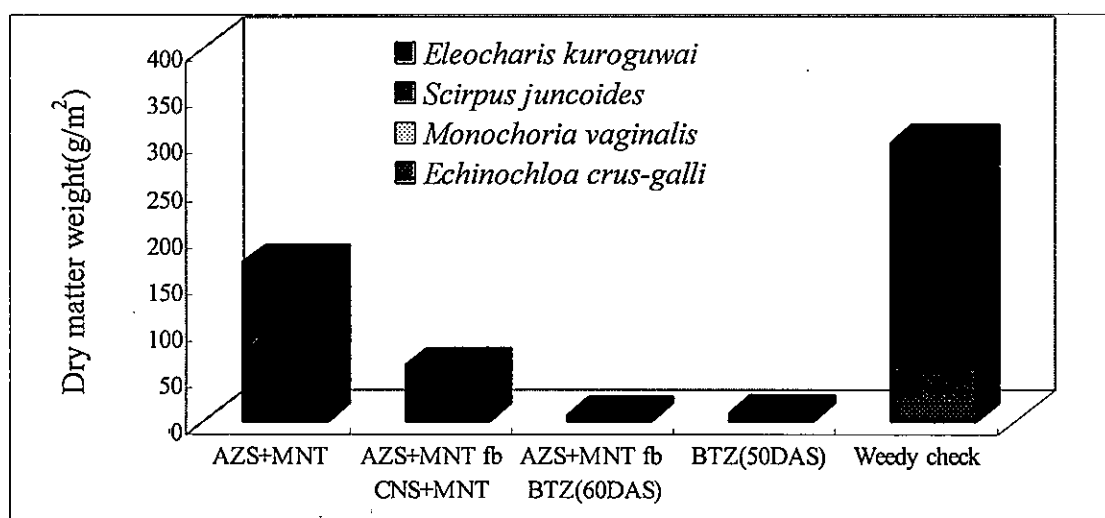


Figure 9. Weed control effects in direct seeding on dry paddy field by several herbicide application systems. \* AZS+MNT: azimsulfuron • molinate GR, CNS+MNT: cinosulfuron • molinate GR, BTZ : bentazone SL

**Direct seeding on dry paddy field** The weed control of dry paddy period on dry direct-seeded paddy was conducted by cyhalohop-butyl • bentazone ME application on 20 days after seeding, and then the paddy was irrigated on 26 days after seeding.

The weeds occurred on the same paddy field were almost controlled except *E. kuroguwai* with the second treatment of azimsulfuron • molinate GR on 2 days after irrigation. The middle and latter control effect of *E. kuroguwai* could be raised to 80-

92% with Molinate • cinosulfuron GR on 48 days or over 97% with bentazone SL on 60 days after seeding.

In conclusion, the main problematic weed with continuous cultivation for 3 years was perennial weed *E. kuroguwai* in machine transplanting, water direct seeded and dry direct seeded rice paddy field. For the effective weed control, the first year weed control system on all rice cultivation types were the additional treatment of bentazone SL on the early or middle in July with the standard weed control of each rice cultivation types. These weed control systems were able to inhibit the tuber formation of *E. kuroguwai* and to avoid the competition between rice and weed on the year.

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## EFFECT OF CONTINUOUS APPLICATION OF GLYPOSATE AND PARAQUAT ON WEED CONTROLLING EFFICACY, PHYTOTOXICITY AND YIELD OF WET SEEDED RICE

A.S.K. Abeysekera, W.M.J. Bandara, and U.B. Wickrama

Rice Research and Development Institute, Batalagoda, Ibbagamuwa. anuru@sltnet.lk

**Abstract:** A long-term experiment was carried out to evaluate perennial weed controlling ability, phytotoxicity and yield of rice by continuous application of Glyphosate and Paraquat as pre-plant herbicides in wet seeded rice cultivation. Five treatment viz. 1) Glyphosate, one ploughing, one harrowing and leveling (Gly+P+H+L); 2) paraquat, one ploughing, one harrowing and leveling (Para, P+H+L), 3) no chemical, two ploughing, one harrowing and leveling (NoC2P+H+L); 4) no chemical, one ploughing, one harrowing and leveling (NCP+H+L), 5) no chemical, no ploughing, one harrowing and leveling (Noc+NoP+H+L) were used in randomized complete block design with 3 replicates. Chemical application, one ploughing, one harrowing and leveling is the common farmers practice of controlling weeds in Sri Lanka. Experiment was conducted for three major seasons and three minor seasons starting from 1996 major season in the rice fields. Soil is sandy clay loam classified as low humic gley soil of pH 6.2 with 2.8% organic matter content. Dominant weeds were *Ischane globosa*, *Panicum repens*, *Cyperus* species, *Commelina diffusa* and *Murdania nudiflora*. Results showed that Glyphosate treatment has significantly highest weed controlling ability followed by NoC NoP+H+L and NoC 2P+H+L, Para P+H+L, NoC P+H+L treatments in decreasing in sequence, but Gly+P+H+L and NoC 2P+H+L treatments were not significantly different in weed control. Glyphosate treatment reduced the perennial weed biomass by 30% in 1996 minor season, by 40% and 45% in 1997 major and minor seasons and by 70% and 80% in 1998 major and minor seasons respectively. Paraquat treatment reduced the weed biomass but this reduction was significantly lower than the glyphosate treated plot and higher than the plots with out chemicals. No phytotoxicity damage was observed in paraquat and Glyphosate applied treatments. Highest grain yield of rice was given by 2P+H+L treatment but this yield was not significantly different from the yield given by Gly+P+H+L and Para +P+H+L treatments. Rice yield by NoC P+H+L treatments was the lowest. . Therefore this experiment revealed that Glyphosate and paraquat can be used continuously as preplant herbicides for wet seeded rice without damaging the rice plant.

**key words:** glyphosate, paraquat weed control/efficacy

### INTRODUCTION

Rice (*Oryza sativa* L) is the main crop as well as the staple food of nation in Sri Lanka. It is grown in diverse soil and climatic environments and weed control is the most important agronomic practice to obtain high yield of rice. It has been observed nearly 30-40% yield losses due to weeds in rice cultivation in Sri Lanka. It is also reported that initial weed control at land preparation is the most determinant factor that affects the subsequent growth of rice crop (Sarkar and Moody, 1981). Shortage of water, increasing labour cost and slow adaptation of low-cost mechanized farming and timeliness are the critical issues involved in controlling of weeds in rice production.

With standard practice of land preparation higher amount of water is required to control weeds. This operation needs about 30% of the total water requirement, 20% of the cost of cultivation and takes 20-25% of cropping time. An alternative package for tillage system is needed to solve these problems which can control weed and prepare a good seed bed for rice seedlings and also to conserve water. In Sri Lanka standard tillage practice is 2 ploughing, one harrowing and leveling with a minimum of one-week interval between each operation. However due to many problems farmers common practice is combination of preplant herbicide with reduce number of tillage. These herbicides are popular among Sri Lankan farmers but there is a misconception that continuous application of preplant herbicide cause adverse effect on soil and subsequent rice crop.

The experiment was therefore conducted to evaluate and compare the effect of long-term application of glyphosate and paraquat with other tillage systems on the crop safety, weed-controlling ability, and on the period taken for land preparation to reduce water losses.

## MATERIAL AND METHOD

The experiment was conducted for 6 seasons for 3 years from 1996-1998 in low humic gley soils (Plinthtaquils) which are lower member of the drainage catena in association with red yellow podzolic soils at the Rice Research and Development Institute, Batalagoda in Low Country Intermediate Zone of Sri Lanka. Soils of the experimental sites were sandy clay loams classified as imperfectly drained low humic gley soil. They were low in organic matter (1.8 - 2.1 %) near neutral in reaction (pH 6-6.2) low cation exchange capacity (10-12 meq/100 soil). These soils contained low levels of extractable P (8-9 ppm) exchangeable K (30-40 ppm) and total nitrogen (0.08-0.1%). The annual rainfall of the region is 1500-1585mm with an average temperature of 23°C - 28°C. Rice. Bg 300, (3 month age) was used as the test crop, direct seeding was done @100kg/ha in puddle soil, of low land plots of 6x3 m<sup>2</sup> surrounded by 30cm bunds. Dominant weed flora in the experimental site was *Ischne globosa*, *panicum repens*, *cyperus* species, *paspalum* species and *commelina diffusa*. Pre plant herbicides Glyphosate 360 g/ha at the rate of 4 l/ha, {N phosphor (methyl glycine)} and paraquat 200 g/ha at the rate of 3 l/ha {1:1 dimethyl - 4.4 dipyridylum di iodide} were included in the experiment. Five treatment viz 1) Glyphosate followed by one ploughing, one harrowing and leveling (Gly+P+H+L), 2) paraquat followed by one ploughing harrowing and leveling (para+P+H+L), 3) no chemical two ploughing one harrowing and leveling (Noc+2P+H+L), 4) no chemical one ploughing harrowing and leveling (Noc+P+H+L), 5) no chemical no ploughing one harrowing and leveling (No C+NOP+H+L) were used in randomized complete block design with 3 replicates. 2P+H+L is the standard practice and preplant herbicide P+H+L is the common practice by rice farmers in Sri Lanka. Fertilizer application and other cultural practices, insect and disease control were carried out according to the recommendation given by the Department of Agriculture (Technoguide 1999). During the experimental period annual weeds were removed manually at two and four weeks after sowing (WAS). Data on rice stand count at 2WAS, annual and perennial weed count and their dry weight just before implementing the experiment and 6 WAS were taken. Visual assessments were made for herbicides efficacy and phytotoxicity on rice. Grain yield and yield computer package components were recorded. Results were statistically analysed using SAS.

## RESULTS AND DISCUSSION

Results showed that Glyphosate provided excellent herbicidal performance against annual and perennial weeds with out reducing the grain yield of rice. . Table 1 indicated that the period of 10-14 days were needed to get good weed control with glyphosate while 2-4 days with paraquat. However perennial weed controlling ability of paraquat is very poor compared with glyphosate treated plots (Table 1), This may be due to the glyphosate herbicide is a non-selective, post emergence, foliar applied and translocated herbicide, while paraquat herbicide is a non-selective post emergence contact herbicide. Paraquat cannot kill the perennials but it can suppress the growth of perennials. However one objective of the use of preplant herbicide is either to accelerate land preparation or to shorten the period taken for land preparation by suppressing the weed population. With that point of view paraquat herbicide can be selected as a pre plant herbicide for land preparation within a short period with good crop safety. Moody and De Datta (1980) also reported that reduction in turn around time is possible if the number of tillage is reduced or chemicals are substituted for the standard tillage treatments of one ploughing followed by two harrowing.

Table 1. Weed killing ability of Glyphosate and Paraquat at 1-15 days after the application.

Herbicide	No.of days after Herbicide	Minor 96	Major 96	Minor 97	Major 97	Minor 98	Major 98
Glyphosate	1	0	0	0	0	0	0
	3	0	0	0	0	0	0
	5	0	0	2	0	2	0
	7	2	4	2	4	2	4
	9	4	4	4	4	4	4
	11	6	6	6	6	6	6
	13	6	6	6	6	6	6
	15	6	6	6	6	6	6
Paraquat	1	2	2	2	2	2	2
	3	6	4	6	6	4	4
	5	6	6	6	6	6	6
	7	6	6	6	6	6	6
	9	6	6	6	6	6	6
	11	8	6	8	8	8	8
	13	8	8	8	8	8	8
	15	8	8	8	8	8	8

0 – No damage on weeds and fallow crop –all plants look green; 2 – Weeds and fallow crop have turned slightly brown; 4 – Weeds and fallow crop have turn mostly brown but plant are still strong; 6 – Most of the weeds kill; 8 – Re growth from vegetative parts of weeds has initiated.

Figure 1 shows the weed-controlling efficacy of continuous application of glyphosate, paraquat and other practices of different land preparation method. Except NoC+NoP+H+L treatment all other practices reduced the perennial weeds population. Vargas (1978) also reported that weed growth did not decrease even 2-3 harrowings of the field. Reduction of weed population was higher in glyphosate treated plots.

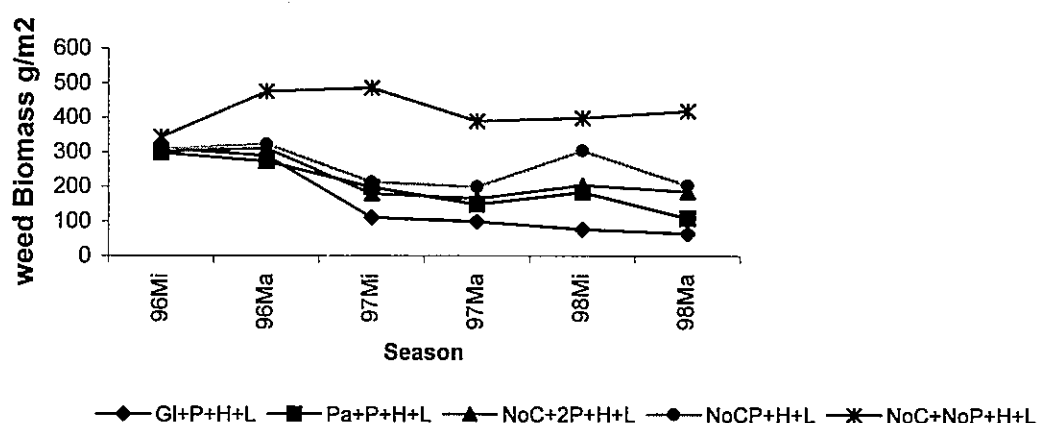


Figure 1. Continuous application of pre plant herbicides and different tillage practices on perennial weed biomass at the commencement of the experiment (1996 minor to 1998 Major season).

Table 2. Effect of different tillage practices on Rice Stand Count at two weeks after sowing (Plants/m<sup>2</sup>).

Treatments	Season					
	96	96	97	97	98	98
	Minor	Major	Minor	Major	Minor	Major
Gly+P+H+L	328.0a	265.6a	332.4a	291.5a	301.3a	257.6a
Para+P+H+L	310.4a	298.5a	301.6a	304.5a	299.5a	308.7a
Noc+2P+H+L	288.3ab	302.6a	275.6a	208.4ab	231.3b	242.0a
Noc+P+H+L	210.4b	189.8b	172.6b	185.4b	142.3c	138.9b
Noc+Nop+H+L	137.4a	142.3c	125.1b	148.5c	185.4bc	118.2b

In a column, means followed by the same letter are not significantly different at the 5% probability levels of Duncan multiple range test.

The effective control of dominant perennial weeds were *Ischne globosa*, *panicum repens* *Commelina diffusa* and *paspalum* species. Paraquat treated plots and two ploughing adapted treatments also reduced those perennials but re-growth of stolen were observed in those plots at crop establishment time. In glyphosate treated plots, weed emergence period was very long compare with other treatments. During the cropping period in each season 6 WAS lower perennial weed biomass was observed from Gly+P+H+L treatment. Higher weed biomass were observed in NoC+NoP+H+L treatment in each season (Fig.2). Table 2 showed the rice plant density (No/m<sup>2</sup>) obtained at two weeks after sowing Rice seedlings counts were significantly reduced in no chemical, no ploughing adapted treatment (NoC NoP+H+L). Increase emergence of annual and perennial weeds are the main reason for reducing rice seedling emergence in no chemical, no ploughing adapted (NoC+NoP+H+L) plot.

Glyphosate, Paraquat and two ploughing adapted treatments gave higher yields, which are not significantly different. No ploughing, no harrowing treatment gave the lowest yield (Table 3). This experiment showed that ploughing of the field is important



to increase the grain yield of rice. Noc+Nop+H+L treatment gave the lowest yield, but it was not significantly different from Noc+P+H+L treatment, it may be due to reduce number of grains per panicle and panicles per square meter (Data not shown). Chemical treatment is also important operation here because it shorten the land preparation time, reduces the water and labor requirements for land preparation. Therefore preplant herbicides as well as ploughing at initial land preparation are important in rice cultivation in Sri Lanka. De Data (1979) also reported that Glyphosate followed by ploughing is as good as conventional tillage in controlling weeds and result similar grain yields.

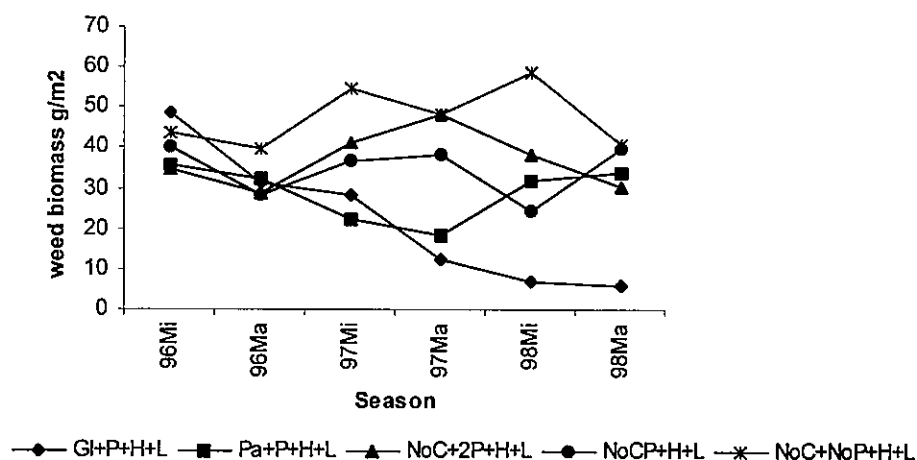


Figure 2. Continuous application of pre plant herbicides and different tillage practices on perennial weed biomass at 6 WAS (1996 minor to 1998 Major season)

Table 3. Effect of different land preparation method on grain yield of rice (t/ha).

Treatments	Season					
	96 Minor	96 Major	97 Minor	97 Major	98 Minor	98 Major
Gly+P+H+L	3.8a	3.4 a	4.6 a	4.1 a	4.5 a	4.8 a
Par+P+H+L	2.7a	3.0 a	3.7 ab	3.5 b	2.8 b	3.0 b
Noc+2P+H+L	3.4a	3.2 a	4.7 a	4.0 a	4.8 a	5.0 a
Noc+P+H+L	3.6a	2.6 b	3.0 b	2.8 bc	2.6 b	3.3 b
Noc+Nop+H+L	2.1b	1.8 c	2.3 b	2.1 c	1.6 c	2.9 b

In a column, means followed by the same letter are not significantly different at the 5% probability levels of Duncan multiple range test.

### Summary

The results suggest that paraquat + P+H+L can be used to accelerate land preparation or to shorten the period taken for land preparation by increasing the rate of weed suppression. However with heavy perennial weed infestation condition, if the main objective is to reduce weed population, then Gly+P+H+L can be recommended. Both herbicides shows the no visual phytotoxicity to rice with continuos applications for six seasons which appeared to be attributed to the continuous application. The two

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ploughing harrowing and leveling system followed by flooding seem to have increase grain yield by increasing no of grains per panicle and panicles per square meter, but with this tillage system, reduction of perennial weed population was low compared with Glyphosate treated plots. Rice seedling emergence and their growth was reduced in no ploughing adapt treatment. So the use of Glyphosate and paraquat as preplant herbicides with reduce tillage or increase number of tillage system are critical to reduce both annual and perennial weed population and subsequently higher rice yield.

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## GREEN MANURE APPLICATION AND ITS EFFECT ON WEED MANAGEMENT AND GRAIN YIELD OF RICE CULTIVATE IN SRI LANKA

A. S. K. Abeysekera, D. N. Sirisena, and U. B. Wickrama

Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka.

anuru@sltnet.lk

**Abstract:** These studies compared the effect of different organic manures with, with out application of chemical fertilizer on nitrogen availability, weed bio-mass, and grain yields in wet land rice culture. Results revealed that application of green manure enhanced the mineral nitrogen content in the soil irrespective of the plant species. Combined application of green manure and chemical fertilizer recorded more mineral nitrogen than the application of both the sources individually. Weed dry weight of 64g/m<sup>2</sup> of the plots treated with chemical fertilizer alone was significantly higher than that of the plots treated neither fertilizer nor manure. Effect of organic manure on weed bio mass was differ among organic manure sources. In the presence of chemical fertilizer, straw and *Gliricidia sepium*, recorded higher weed dry weight of 60g/m<sup>2</sup> and 63g/m<sup>2</sup> respectively. With *Tithonia diversifolia* and *Croton lacifer* weed weight of 27 g/m<sup>2</sup> and 28 g/m<sup>2</sup> was recorded. There was a yield reduction of 29% due to competition of weeds in the plots treated with chemical fertilizer alone. Application of with *Tithonia diversifolia* and *Croton lacifer* significantly reduced the weed dry weight and as such there was a yield increment of 27% and 23% over the plots treated with chemical fertilizer alone and 11% and 6% over the plots treated with both *Gliricidia sepium* and chemical fertilizer. In this experiment it is proved that application of some green manures enhances mineral N content in soil while reducing weed biomass of the paddy filed which is an important factor to encourage farmers to apply organic manure application to paddy fields.

**Key words:** Green manure, Nitrogen availability, Rice, Weed bio mass,

### INTRODUCTION

Green leaf manure for rice fields, or other wards the practice of incorporating undecomposed plant materials in to the soils with the object of increasing the supply of plant nutrients especially nitrogen is an age old manuring method known to farmers in almost all parts of the world. Before sowing plant materials were brought to the field and ploughed in. Many years before the advent of chemical fertilizer in the country Department of Agriculture showed considerable interest in promoting the increased use of plant materials in rice cultivation. From the early 20's Department of Agriculture took efforts to promote the use of plant residues in rice cultivation by providing information, instruments and planting materials to the farmers (Department of Agriculture, 1922 )

The common plant materials used by the farmers are rice straw, leaves and twigs of Keppetiya (*Croton laifer*), Wild sunflower (*Tithonia diversifolia*) and *Gliricidia* (*Gliricidia sepium*). The use of sufficient quantities of the material was observed to cause an appreciable increase in rice yields ( Molegoda, 1921 Amarasiri and Wicramasinghe, 1977, Nirmala Gunapala and Amarasiri, 1989)

Since the introduction of chemical fertilizer in the early 50's and the increased adoption of their use by farmers, the practices of addition of organic manure have gradually become less important. Today it is an insignificant practice in rice cultivation (Statistics of Rice cultivation in Sri Lanka, 1999). It is obvious that application of plant residues to paddy fields can be encourage if the other advantages of the practice would be understood. Through long term experience farmers have identified the specific type of plants in the surrounding desired when added to soil. It is interesting that supplying of plant nutrient to the rice crop is often not only the benefit intended in the practice. Controlling ability of pest and diseases is also taken in to consideration. It has been reported that some plant residues have the ability of controlling pests in cultivated filed (Grainge and Ahmed, 1988 Sivakaddacham, 1988) and some plant material have the ability to check the emmergence of weeds (Abeysekara ,1992 and Park, 1991) but little information is available how the commonly used organic manures effect on weed management in paddy fields which is one of the costlier practice in Sri Lankan rice cultivation.

Therefore this paper reports the results of the experiments carried out to study the effect of organic manures on nitrogen availability and weed management in Sri Lanka paddy fields.

## MATERIALS AND METHODS

Experimental plots were laid down at Rice Research and Development Institute of Sri Lanka. The soils were Low Humic Gley, sandy loam in texture, pH 5.7, organic matter 1.6% total N 0.14% Olsen's P 3.5 ppm and exchangeable K 0.06 meq/100g. Plant materials tested in the experiment and application rates are presented in the Table 1. Green leaf and straw were applied at the rate of 2.5 t/ha.

Table 1. Plant materials, their application rates and chemical fertilizer rates of the different treatments.

Treatment number	Type of plant material and rate of application	Rate of chemical fertilizer	Weeding*
T1	<i>Gliricidia sepium</i>	100kg/N, 30kg/ P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	NW
T2	<i>Gliricidia sepium</i>	Nil	NW
T3	<i>Tithonia diversifolia</i>	100kg/N, 30kg/P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	NW
T4	<i>Tithonia diversifolia</i>	Nil	NW
T5	<i>Croton lacifer</i>	100kg/N, 30kg/P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	NW
T6	<i>Croton lacifer</i>	Nil	NW
T7	Rice Straw	100kg/N, 30kg/P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	NW
T8	Rice Straw	Nil	NW
T9	Nil	100kg/N, 30kg/P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	NW
T10	Nil	Nil	NH
T11	Nil	100kg/N, 30kg/P <sub>2</sub> O <sub>5</sub> , 20 Kg/K <sub>2</sub> O	HW
T12	Nil	Nil	HW

\*NW : no weeding, HW: Hand weeding

All the plant materials were tested in the presence and absence of chemical fertilizer. There were 12 treatments in the experiment including treatments with no fertilizer and no organic manure. Treatment with straw application was included in the

experiment since straw application was common in paddy fields. Weeding was not done in the organic matter treated plots to study the effect of organic matter/green leaf manure on weed bio mass. Treatments were replicated 3 times in a randomized complete block design. The plot size was 6 m x 3 m. Each plot had independent inlets and independence outlets for irrigation and drainage. The experiment was initiated in yala 1999 and planted to rice variety Bg 357.

Soil samples were taken at 2 and 6 weeks after application of plant materials. Three soil samples were taken randomly to a depth of 15 cm from each plot and composite to make one sample for each plot. Soil samples were stored at deep freezer until the extraction within 3 days.

Weed biomass was collected in 1m<sup>2</sup> area from each plot at six weeks after sowing. Weed samples were oven dried at 80°C for three days and weighed.

A middle part (13 m<sup>2</sup>) of each plot was mechanically harvested at 105 days after planting. The harvested grains were weighed at time of harvest and subsampled for moisture determination.

A sub sample (80g) of saturated soil was weighed in to 200 ml conical flask a 50ml aliquot of 1M KCl was added and the flasks were stoppered and shaken mechanically for 1hr. Samples were extracted through No. 42 Whatman filter paper washed with 25M KCl. Filtrate was analysed for NH<sub>4</sub>-N using spectrophotometer.

## RESULTS AND DISCUSSION

Measurements of KCl extractable NH<sub>4</sub>-N shows in the Fig 1 are reflected the amount of N readily available for plant use at different times of sampling.

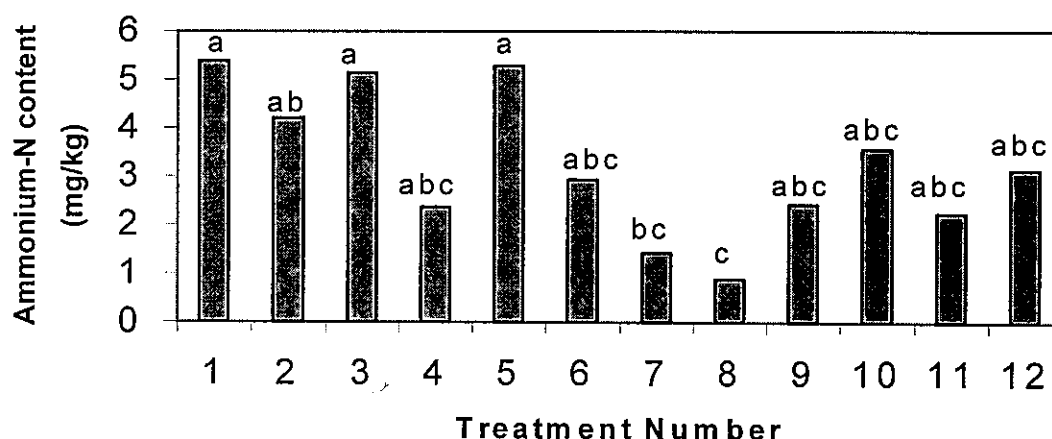


Fig 1. Ammonium-N content of the soil at 2 weeks after application of green leaf manure and straw with and without chemical fertilizer.

This parameters includes mineral N both in solution and in the exchange complex that can readily replace N removed from solution by uptake, immobilization and various mechanism of losses the system. This fraction will be referred to here as available N since no nitrate was detected at any point in the sampling and analytical procedure typical for flooded soil.

Initial concentration of ammonium and nitrate in the experimental plots were 20.8 mg/kg and 1.68 mg/kg respectively.

Table 2. C:N ratio of plant materials.

Type of plant material	C:N ratio
<i>Gliricidia sepium</i>	10.3
<i>Tithonia diversifolia</i>	10.7
<i>Croton lacifer</i>	9.8
Rice Straw	81.2

Concentrations were very high at 6 weeks after application and plots treated with green leaf materials plus chemical fertilizer showed the highest values. Lowest values were recorded in the plots where green manure or chemical fertilizer was not added. Comparatively higher ammonium content was recorded in the weeded plots even though only chemical fertilizer was applied. C: N ratio of the straw

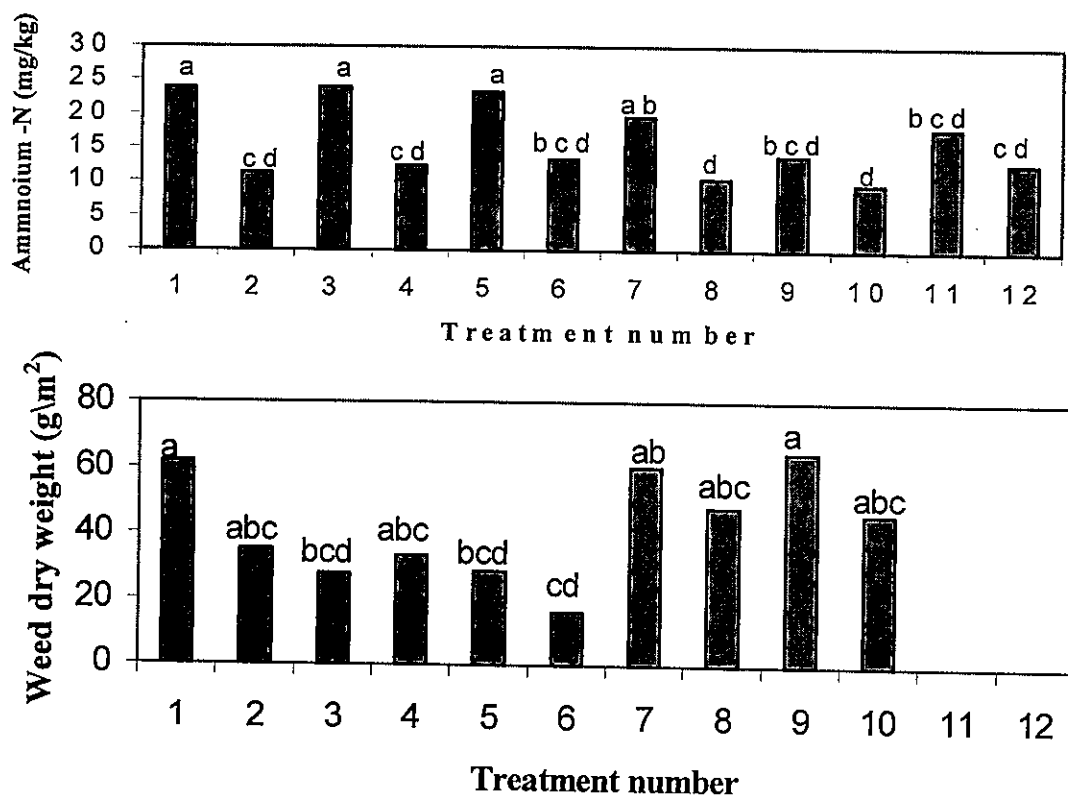


Fig 2. Ammonium-N content in soil at 6 weeks after application of green leaf manure and straw with and with out chemical fertilizer.

Therefore, mineralisation of the plant materials may have taken place within two weeks time. As such concentration of ammonium-N measured in the treatemnts showed significant difference at the first sampling date (2 weeks, Fig1).

Fig 3. Weed dry weight at 6 weeks after application of green leaf manure and straw with and without chemical fertilizer.

Concentrations were greater with the addition of *Gliricidia* and *Tithonia* along with chemical fertilizer. Plots treated with straw showed least amount of ammonium irrespective to the application of chemical fertilizer. Concentrations of ammonium were ranged from 1ppm to 5.5 ppm. Comparatively higher ammonium N content was observed in the plots at 6 weeks after application of green leaf manure (Fig. 2). was high (Table 2); as reported by Nagarajah (1987) and Amarasiri and Wickramasinghe (1977), low ammonium-N content of plots treated with straw may be due to immobilization of nitrogen by microbes in soil.

Effect of green leaf manure on weed biomass was differ among different plant species. Weed dry mass of the plots treated with Wild sunflower (*Tithonia diversifolia*) plus chemical fertilizer was comparative to that of *Tithonia diversifolia* plus chemical fertilizer (Fig 3). The highest weed dry mass at 6 weeks after sowing ( $64 \text{ g/m}^2$ ) was recorded in the plots treated with chemical fertilizer alone. The lowest weed dry biomass was observed ( $15 \text{ g/m}^2$ ) in the plots treated with *Croton lacifer* alone. The values were not significantly higher even with the application of *Croton* plus chemical fertilizer.

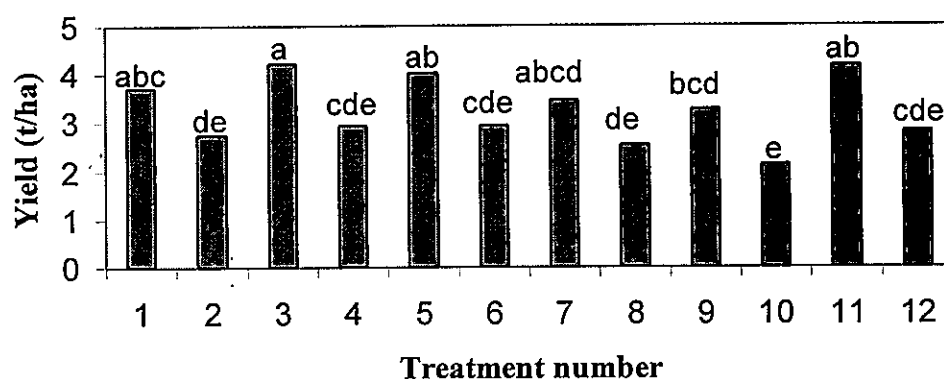


Fig 4. Grain yield of rice with the application of green leaf manure and straw with and without application of chemical fertilizer

Grain yield obtained from the plots incorporated with plant materials is shown in Table 3. Grain yield was significantly higher in the plots that received plant materials except straw. Grain yield was very much higher when green leaves were applied along with chemical fertilizer. The highest yield of 5.5t/ha was recorded from the plots which received *Tithonia* and chemical fertilizer while the lowest yield of 3.5t/ha was recorded in the plots which received neither organic manure nor chemical fertilizer and no weeding was done. Yield levels of the other plots treated with green leaf manure and chemical fertilizer were comparative to the yield level of the plot treated with *Tithonia* and chemical fertilizer.

It was observed in the experiment that application of green manure has increased the available nitrogen (ammonium -N) in the rice fields. Availability was at the highest at 6 weeks after application. Plots treated with green plant materials showed significantly higher available nitrogen than straw. All the plant materials except straw tested here had very low C: N ratio (Table 4), therefore, mineralisation of the plant materials may have taken place within two weeks time. As such comparatively higher ammonium N content has been observed. C:N ration of the straw was high and as reported by Nagarajah (1987) and Amarasiri and Wickramasinghe (1977) low

ammonium-N content of straw treated plots may be due to immobilization of nitrogen by microbes in the soil. Even with the uptake of nitrogen by rice plants ammonium-N were higher in the plots treated with green leaf manure.

Weed dry matter contents were significantly lower in the plots which treated with *Croton lacifer* and *Tithonia diversifolia*. It was reported by Amarasiri and Nirmala Gunapala (1989) that availability of nutrients other than nitrogen are also higher in soil when green plant materials are incorporated. On the other hand it was observed in this experiment that ammonium contents are comparatively higher when *Croton* and *Tithonia* were incorporated in. Phosphorus and potassium were added at the rates recommended by the Department of Agriculture. It was reported by Nagarajah (1987) that availability of nutrients other than nitrogen are also higher in plots when green plant materials are incorporated into the soil. Phosphorus and potassium were added at the rates recommended by the Department of Agriculture. On the other hand it was observed in this experiment that ammonium concentrations are comparatively higher when *Croton* and *Tithonia* were incorporated in. Pest controlling ability of the above two plant materials have been already reported by Grainge and Ahmed (1988) and Sivakaddcham, (1988). Therefore, lower weed biomass in the plots treated with *Croton lacifer* and *Tithonia diversifolia* may be due to allelopathic effect of the aforesaid plant materials.

Yield levels obtained from the experimental plots were comparative to that of the area (Annual reports of the RRDI, 1999) The highest yield of 5.5 t/ha was recorded in the plots treated with *Tithonia* and chemical fertilizer. Yield levels of the plots treated with green leaf manure were comparative to the above yield. Yield of the plots, which received chemical fertilizer alone but mechanical weeding was done, was also non significant to the yield of the plots treated with green leaf manure and chemical fertilizer showing the importance of weeding in paddy cultivation of Sri Lanka.

The results of the experiment revealed that application of green leaf manure enhances the nitrogen supply for paddy cultivation giving better yield of grain. Application of *Tithonia* and *Croton* as green leaf manure should therefore be encouraged because they have the advantage of weed controlling ability.

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## WEED INTEGRATED MANAGEMENT IN TRANSPLANTED RICE FIELD IN NORTH CHINA

D. Z. Lu, X. J. Li, G. Q. Wang, and B. H. Li

Institute of Cereal and Oil Crops, Hebei Academy of Agricultural Sciences,  
Shijiazhuang 050031, China. hbifoc@public.sj.he.cn

**Abstract:** The author provided general information on weed species, their dynamics and the relationship between weed density and rice yield in transplanted rice field. Based on the above study, integrated weed management mainly chemical control was proposed.

**Key words:** weed, integrated management, rice.

### Rice Planting and Weeds in North China

**Rice planting in north China** Rice is one of the important crops in China with the planting area of 34 million hectares in the whole country. Due to the relatively low temperature, rice only cultivated once a year in north China. The temperature during the rice growing season usually from early April to late October is at least 10°C and with the sufficient sunshine rice can get a high yield in this area. Rice seeds are planted in the nursery in late April and early May, transplanted in early June and harvested in late September or early October. The fields are fallow during winter after rice harvested. Most of the rice field are located along the shore of Bohai where there are relatively less farmers and much land, therefore chemical weed control is necessary to develop.

**Weeds in transplanted rice field in north China** There are at least 40 weed species in transplanted rice field in north China according to the weed survey results by the National Farmland Weed Survey Group, of which *Echinochloa crusgalli* is the most widespread and harmful weed which infests about 50% of the rice field in China and the other weeds such as *Cyperus difformis*, *Monochoria vaginalis*, and *Potamogeton distinctus* rank the second with 5-10% infested area. With regard to the density of weeds, Gramineae takes up 8.3%, Cyperaceae 7%, Potamogetonaceae 5% and 32% in the other families. Weeds in the rice fields can be divided into four groups, annual 39.6%, biennial 2%, perennial 21% and the other types 6% according to the life span. Among them, *Scirpus planiculmis*, *Scirpus triquetus*, *Phragmites communis* and *Typha angustifolia* grow only in the slightly alkaline soil with the pH between 7 to 8. The following are two examples of weed community in rice field in north China (Table 1 to 2).

Table 1. The structure of *Oryza sativa* - *Echinochloa crusgalli* community

Scientific name	Height (cm)	Abundance (Plants m <sup>-1</sup> )	Coverage (%)	Biomass (g m <sup>-1</sup> )	Phenophase
<i>Oryza sativa</i>	100	90	75	512	Fruiting
<i>Echinochloa crus-</i> <i>galli</i>	105	363	60	3200	Heading
<i>Scirpus planiculmis</i>	85	125	10	500	Flowering
<i>Scirpus triquetus</i>	65	95	10	125	Flowering
<i>Eleocharis</i> <i>pauciflora</i>	5	2358	40	125	Flowering
<i>Vallisneria spiralis</i>	5	25	5	32.2	Flowering

<i>Diplachne fusca</i>	85	19	<5	75	Grain filling
<i>Alisma orientale</i>	15	1	+	5.0	Fruiting
<i>Potamogeton distinctus</i>	15	1	+	5.0	Seedling
<i>Ecftia prostrata</i>	20	1	+	6.8	Seedling

Table 2 The structure of *Oryza sativa* - *Scirpus planiculmis* community

Scientific name	Height (cm)	Abundance (Plants/m <sup>2</sup> )	Coverage (%)	Biomass (g/m <sup>2</sup> )	Phenophase
<i>Oryza sativa</i>	100	90	75	512	Fruiting
<i>Scirpus planiculmis</i>	95	361	70	1510	Flowing
<i>Eleocharis pauciflora</i>	5	2358	40	125	Flowing
<i>Scirpus triqueter</i>	80	16	<5	25	Flowing
<i>Ecftia prostrata</i>	20	2	+	12	Seedling
<i>Diplachne fusca</i>	85	1	+	4	Heading

The seasonal aspects of weed communities are distinct in rice field in north China, which is closely related to weed control. Around the Bohai Bay, Hebei province, introduced below, it may reflect that of weed succession in single-cropping system rice field in north China (Figure 1). The different kinds of phycophyta such as *Nostoc spp.* and *Oscillatoria spp.* occur before transplanting of the rice, *Echinochloa crusgalli* and *Scirpus planiculmis* come out then from rice transplanting to heading, afterward, flatsedge such as *Scirpus juncoides*, *S. triqueter*, *Juncellus serotinus* and *Eleocharis palustris* emerge and finally before rice harvesting *Potamogeton distinctus*, *Vallisneria spiralis* and *Najas minor* emerge. The fertile single-crop fields often have *Potamogeton distinctus*, indent land, *Vallisneria spiralis* and the slightly alkaline fields with bad soil structure, *Scirpus planiculmis*.

*-- <i>S. juncoides</i> and <i>E. pauciflora</i>	Height field
*-- <i>Potamogeton distinctus</i>	Low field
*-- <i>Vallisneria spiralis</i> and <i>N. minor</i>	Indent field
*-- <i>Echinochloa crusgalli</i>	Bad drainage
*-- <i>S. yagara</i>	Heavy saline
*-- <i>Najas spp.</i>	Alkali soil

Growing stage		Seedling				Tillering		Flowering to maturing	
Cultivation		Nursery		Transplanting		Herbicides and fertilizer applying			
Date		March	April	May	June	July	August	September	

Figure 1. Seasonal distribution of weed communities in coastal rice belt

### Rice yield reduction due to weeds

The negative correlation is found between weed density and rice yield. We take *Echinochloa crus-galli* as an example. Rice grain yield will be reduced by 42.9kg mu<sup>-1</sup> when the weed density increases by 10000 stems mu<sup>-1</sup>. Furthermore, *Echinochloa crus-galli* in rice hill creates more yield reduction by decreasing the spike numbers, grain numbers and grain weight with the models as follows.

$$Y_1=11.0399-0.1733X, Y_2=643.6360-12.0636X \text{ and } Y_3=18.1182-0.3479X$$

where X is the density of *Echinochloa crus-galli* growing in the rice hill,  $Y_1$  is the rice spike number,  $Y_2$  and  $Y_3$  are the rice grain number and grain weight in each hill.

Within the scope of 1-55 stems of *E. crus-galli* in each rice hill, the rice spike number, grain number and grain weight will decrease by 0.17, 12.06 and 0.35g, respectively.

## **Integrated weed management in transplanted rice field in north China**

### **Concept of integrated weed management**

Integrated weed management means the systematic management of weed communities in a biological ecosystem, that is, limiting weed population to the threshold values by integrated measures such as physical, cultural, chemical and biological methods, so that crops and other beneficial living creatures can grow well and the activities of man can be conducted efficiently.

### **Measures of integrated weed management**

Each method of weed control has its own role to play in weed management system. They must be connected with each other and conducted according to different weed problems, geographical areas, climates and cropping conditions.

It is very important to clean the rice seeds before sowing by cleaning sieves, wind and salt water. At present, specific weight method is popular in North China. By dipping seeds into common salt solution with specific weight 1:1.5, the heavy rice seeds settle to the bottom of the bucket while the light weed seeds float to the surface. After the weed seeds are removed, the rice seeds thus separated must be washed with plenty of fresh water at once.

Weed seeds in fresh manure do not lose their viability which can keep for a long time. They should be piled up for months so that a high temperature will reach and weed propagative organs and seeds will be killed.

Pre-transplanting irrigation that induces weeds to germinate ahead of rice is an important husbandry method of weed control. After weeds germinate, tillage implements and non-selective herbicides which can be degraded by sunlight and microorganisms could be provided to eradicate weeds.

Adequate fertilizers and irrigation can develop vigorous growth of rice which becomes more competitive in rice growing duration, that is especially important in early stage, from reviving to tillering, of rice.

Weed can also be suppressed by rational density of rice. It is proved that when rice is transplanted in density of 11.5, 12.7, 14.7, 15.1, 17.3, 18.0 thousand plants  $ha^{-1}$ , the number of *E. crus-galli* will be 30.9, 34.5, 19.5, 10.5, 1.6 plants  $m^{-2}$ , respectively.

In rice growing duration, controlling weeds in row and at rice hills by hand pulling and machinery tillage are usually provided as an accessory measure in China.

Irrigation and drainage ditches, farm boundaries and other uncropped areas should be cleaned before weed seeds can scatter.

In autumn, soil deep tillage after rice harvest can bring the underground hibernating storage organ of some perennial weeds such as the radical buds of *Potamogeton distinctus* and the rhizomes of *Scirpus juncoides* to the soil surface, thus they can be killed for cold and dry weather in the winter, at the same time, the weed seeds on the soil surface can be buried to deeper soil layer so that they can not germinate.

Thoroughly eradicating weeds in the nurseries in which weeds are easy to be controlled for their smaller areas and using fixed nurseries can avoid weeds getting transplanted from the nurseries to the main fields along with rice seedlings. That can get double results with half the effect.

Herbicide control is the major practice in weed integrated control system in China and this will be discussed in following paragraph.

All harmful organisms which attack rice including weeds, pests, Diseases and microbes can effect and benefit each other when they grow and reproduce. That must be considered in weed control and paid more attention to. Effective measures which are suitable to weed control should suppress other harmful organisms simultaneously.

#### **Principle of weed eco-economic control**

The concept that weeds are always harmful to humans and should be eradicated as soon as they appear has been considering to the principle of weed control in past years in China. But it is proved that is not always right in the view of ecology and economy. For a weed-infested crop is a complex dynamic system with interference taking place between crop and weeds, between weeds and between crops. In the presence of only very few weeds, between weeds and between crops. In the presence of only very few weeds or on the seedling stages of them, there will be no detectable effect on the rice yield. On the contrary, the existence of these weeds which are beneficial in regulating fertility, preserving soil structure, stabilizing the soil surface in areas subject to erosion, protecting native predators, connecting the chain of living organisms, etc. would be the important supplementary angle to farm biological ecosystem. Therefore, the eradicating of these weeds will cause subsequent problems such as increasing control cost, destroying soil structure, polluting environment, inducing herbicide-resistant weeds, etc. So weed eco-economic control should be considered as an important part in weed management system in the modern world. The key of eco-economic control is suppress the weed numbers or weight in rice field below the threshold value, so called eco-economic control threshold, which is weed population, usually density or weight, when the cost of weed control is equal to the net loss of rice yield caused by weeds.

Eco-economic control threshold in the rice field which can get by field experiments differs according to different rice growing area, soil fertility, rice cropping condition, weed species, etc.

By the formula below, permitting loss of rice yield (Y) can be calculated.

$$Y = C / (P \times V \times E) + F / (P \times V)$$

Y, C, P, V, E and F represent permitting loss percentage of rice, the cost of weed control, rice yield, rice market price, effect of control method and the benefit of weeds when the weed population is equal to the eco-economic control threshold, respectively.

With precise field experiment, the sigmoid line which show the relationship between weed density or weight (X) and rice yield loss (Y') can be reached and the equation between X and Y' will be calculated. Replacing Y' by Y, we can get X, that is, the eco-economic threshold in this rice field.

#### **Chemical control in transplanted rice field in north China**

**Nurseries** *E. crus-galli* appears in both dry nursery, which is also called plastic paper mulched nursery, and water-layered nursery. In dry nursery, propanil can be applied in 1.5-2 leaf stages of *E. crus-galli* at the rate of 200g mu<sup>-1</sup> (active ingredient, as the same in the following) before the plastic paper mulching. If *E. crus-galli* is above 3 leaf

stages, quinclorac should be applied at the rate of 20-25g mu<sup>-1</sup> after rice reached 3 leaf stages and the plastic paper should be pulled off after application in case high temperature injures the seedlings. And another herbicide, the mixture of butachlor and prometryne, is also popularly used in the nurseries in north China. This mixture has a broad spectrum, high control efficacy and low price but the nurseries should be well prepared with good seeding quality and the seeds should be covered above with 3 cm soil layer so that no rice seeds are injured by the herbicide. To control the *Cyperus* spp., we can post apply propanil and chloridazon at the recommended rate above plus bentazon at the rate of 50 to 60g.

In water-layered nursery, propanil and chloridazon are popularly used herbicides but butachlor and prometryne are seldom applied.

**Transplanted rice field** If the dominant weed species is *E. crus-galli*, butachlor at 60-80g applied before transplanting or 15days after transplanting or oxidiazon at 10g applied 4-5 days before transplanting will give good control. To control the *Cyperus* spp. bensulfuron at 3-4g and pyrazosulfuron-ethyl at 1.5g can be applied before transplanting or bentazon and MCPA are applied after transplanting. As to the aquatic weeds, bensulfuron and pyrazosulfuron-ethyl are sprayed before transplanting and to control the weeds in the sideway Rounup is the best selection.

## OCCURRENCE OF WEEDS IN WET-SEEDED RICE FIELDS AND APPLICATION OF NOMINEE (BISPYRIBAC-SODIUM) IN ZHEJIANG, CHINA

Q. Wang<sup>1</sup>, X. P. Zhao<sup>1</sup>, C. X. Wu<sup>1</sup>, F. Dai<sup>1</sup>, L. Q. Wu<sup>1</sup>, H. Xu<sup>1</sup>, M. S. Li<sup>2</sup> and J. H. He<sup>3</sup>

<sup>1</sup> Institute of Plant Protection, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China. qwang@mail.hz.zj.cn

<sup>2</sup> Wenzhou Academy of Agricultural Sciences, Zhejiang 325006, China

<sup>3</sup> Jinhua Institute of Agricultural Sciences, Zhejiang 321017, China

**Abstract:** A comprehensive survey was made on weeds and application techniques of Nominee (bispyribac-sodium) were studied for weed control in fields of wet-seeded rice (FWSR) in Zhejiang, China in 1996-2000. Based on weed frequency, density and damage to rice, there appear 15 species of dominant weeds, and *Echinochloa crusgalli* is most important. Weed species and their proportions composing weed communities differ on areas and culture systems. Weeds occur and damage to rice much more seriously in FWSR than in transplanted rice fields. Weeds germinate 3-4 days after rice seeded (DARS), reach an emergence peak 15-21 DARS for early rice or 7 DARS for single season rice, and there is a small second emergence peak 42-70 DARS. Gramineae weeds germinate first, then most broad-leaf weeds emerge, and lastly sedge and some broad-leaf weeds germinate. Field trials show Nominee sprayed at 15-45 g/ha during 4-5 leaf-stage of barnyardgrass is highly effective on weeds including *E. crusgalli* after 2 leaf-stage and perennial weeds, but not on *Leptochloa chinensis*. For control of *L. chinensis*, field trials show Sofit (pretilachlor+fencloirim), Saturn (thiobencarb), MY100 (oxaziclomefone), YRC2388 (fentrazmide), Clincher (cyhalofop), Whip (fenoxaprop-p-ethyl) are effective. The weed can also be effectively controlled by water management. Sprayed with Nominee, Japonica rice shows plant dwarf and leaf yellow 4-10 days after spraying, recovers 10-20 days after without loss of rice yields, while Indica rice and hybrid rice are tolerant to the chemical. Phytotoxicity of Nominee to Japonica rice is affected by rice variety, leaf stage, herbicide dosage, soil, water management, temperature and safener. By *in vivo* biochemical test, acetolactate synthase (ALS) is prevented by Nominee and then recovers quickly in rice, while in barnyardgrass inhibition of ALS by Nominee is never recovered. It seems in residue assay that Nominee degrades a little faster in rice than in weed.

**Key words:** application technique, Nominee (bispyribac-sodium), phytotoxicity, water management, weed, wet-seeded rice

### INTRODUCTION

Direct seeding, one of main types of less-labor cultivation for rice, has become more and more popular in southern China since 90's, as big farms grow and lot labor shift from countryside to cities. There are 219 120 ha of directly seeded rice in Zhejiang in 1999, while only 38 600 ha in 1995. In fields of wet-seeded rice (FWSR), weeds occur widely, damage seriously to rice and are difficult to be controlled, compared with fields of seedling-transplanted rice. Since weeds in southern China are different from those in northern China and other countries due to soil, climate, water management and last season crops, a comprehensive survey on occurrence of weeds in FWSR and a study

on the control of weeds by application of Nominee were made in Zhejiang, China in 1996-2000.

## MATERIALS AND METHODS

### Survey on weeds

According to ecology, climate and cultivation, 4 main plantation areas of wet-seeded rice were selected in Zhejiang for weed survey, each area was further divided into 3 to 6 regions by geography, soil and culture habit. Investigation was made in several typical villages for each region and more than 50 field plots for each village in 1996-2000. Weeds were surveyed by random eye assessment of 5 grade with 5 samples (1 m<sup>2</sup> each) in each plot 1 week before rice harvested, occurrence frequency, damage rate and damage index for weeds were recorded and calculated.

Weed germination was also observed in FWSR in 1996-1998. Early rice and single season rice were chosen with 2 plots (no herbicide use) for each type of rice and 3 fixed samples (0.11 m<sup>2</sup> each) for each plot. Weed number of each species just germinated was recorded 3-7 days after rice seeded (DARS) and then once a week, and weed seedlings were removed after each survey.

### Application of Nominee and weed control

Field trials were made in 1996-1998 on efficiency and application techniques of Nominee for controlling weeds. Plot tests were conducted by random arrangement of plots (20 m<sup>2</sup> each) and 4 repetitions for each treatment, and demonstration tests with no repeat and 0.5 ha for each treatment. Herbicides tested were applied to fields by a "Worker 16" sock-back sprayer or a "Red East 18" electricity-powered sprayer, and water in fields was managed by draining before application and irrigating 2 days after. Effect of herbicides on weeds and safety to rice were assessed by eye with 10 grade method 7-15 days after spraying, and control efficiency (including weed plant and weight control) and phytotoxicity (quality and quantity of rice seedling) were surveyed by taking 3-4 samples (0.11-0.25 m<sup>2</sup> each) for each plot or treatment 25-50 days after spraying. Lastly, plant height, number of effective ear, grain number of each ear, weight of thousand grains and yields of rice were also measured when rice harvested.

Phytotoxicity of Nominee, activity of acetolactate synthase (ALS) and residue of Nominee in rice and weed plants were further studied. Nominee was sprayed on rice seedling in pot by an "ASP-1098" auto-direction sprayer with 35 cm/s of speed and 0.4 MPa of pressure, Nominee dosages 1.25-160 times higher than normal, 8 rice varieties, rice seedling with 2-7 leaves, 3 types of soil, 2 situations of water layer, 4 temperatures and a safener were designed for assessment of factors on phytotoxicity of Nominee. Symptom of damaged rice seedling was observed and photographed; plant height and fresh weight of upper or underground part of rice were recorded. The activity of ALS was determined by an acetoin-creatine-naphthol complex on spectrophotometer, and inhibition of Nominee to ALS in rice and weeds assessed. Residue of Nominee in rice and weed plants after spraying was also assessed by HPLC with Waters 244.

For the control of *L. chinensis*, water management and herbicide mixtures were studied in fixed rice plantation groove and fields. Different time to start irrigation after rice and weed seeded and different period to keep water were designed, and weed occurrence and rice growth were observed. Fields trials were made as above on efficiency of herbicides and mixtures for the control.



## RESULTS AND DISCUSSION

### Weed occurrence in wet-seeded rice fields

Weed species and damage Zhejiang, locating in southeastern China and with sub-tropic climate and multiple natural ecological system, is rich in light, heat and water resources and suitable to weed growth. Based on a throughout survey during 1996-2000, there are 131 species and 2 varieties belonging to 77 genus, 41 families of weeds appearing in FWSR in Zhejiang. Most species are occupied by *Cyperaceae* (31 species, 9 genus), *Gramineae* (11 species and 2 varieties, 9 genus), *Scrophulariaceae* (11 species, 5 genus), then are *Hydrocharitaceae* (5 species, 4 genus), *Lemnaceae* (5 species, 3 genus), *Lythraceae* (5 species, 2 genus), *Polygonaceae* (5 species, 1 genus), *Juncaceae* (5 species, 1 genus), and less species in other families. According to weed frequency, density and damage to rice, there mainly appear 41 species and 1 variety belonging to 35 genus, 21 families in the fields, and most are species of *Gramineae*, *Cyperaceae*, *Scrophulariaceae*, *Lythraceae*, *Amaranthaceae*. Among them, the dominant species are *Echinochloa crusgalli*, *Leptochloa chinensis*, *Cyperus difformis*, *Monochoria vaginalis*, *Sagittaria pygmaea*, *Rotala indica*, *Lindernia procumbens*, *Ludwigia prostrata*, *Eclipta prostrata*, *Eleocharis yokoscensis*, *Scirpus planiculmis*, *Paspalum distichum*, *Alternanthera philoxeroides*, *Leersia japonica*, *Murdannia triquetra*.

The above main weeds have a distribution in whole of Zhejiang, but weed species and their proportions composing weed communities differ on areas and culture systems. Barnyardgrass (*E. crus-galli*) is the most important weed throughout the whole province. In early rice fields, weed communities are simple and consist of *gramineae* (mainly barnyardgrass) and few other weeds, while for single season rice fields *gramineae*, sedge and broadleaf weeds compose the communities. In fields cultivated with seeded rice for years, *L. chinensis* becomes more and more series, whereas in fields just shifted from seedling-transplanted rice to directly seeded rice *M. vaginalis*, *S. pygmaea* are dominant. In some big farms farming by machines and less labor, *P. distichum* occurs frequently and is very difficult to be controlled. *S. planiculmis* appears often in fields with saline-alkali soil in coast, and *A. philoxeroides*, *L. japonica* and *M. triquetra* increase recently in some areas. In single season rice fields in northern Zhejiang, numbers of weed species are 25.0%, 18.8%, 56.2% and weed density 11.2%, 11.6%, 77.2%, whereas in early rice fields in southern Zhejiang weed densities are 61.2%, 9.4%, 29.4% for *gramineae*, sedge, broadleaf weeds, respectively.

Weeds have heavy damages to directly seeded rice, and single season rice is injured more seriously than early rice. An investigation in central Zhejiang shows weeds have a density as high as 501-538 plant/m<sup>2</sup> and weight of 850-2679 g/m<sup>2</sup>, and barnyardgrass is 59-216 plant/m<sup>2</sup> and 402-2301 g/m<sup>2</sup>. Weeds occur and damage to rice much more seriously in seeded rice fields than in transplanted rice fields, although weed species appeared are same. A survey in southern Zhejiang shows weeds have damage index of 78.8% and frequency rate higher than the 3<sup>rd</sup> grade of 95.4% in FWSR, compared with those (46.1% and 37.4%, respectively) in transplanted rice fields. *E. crusgalli*, *L. chinensis*, *P. distichum*, *A. philoxeroides*, *E. yokoscensis*, *L. japonica*, *M. triquetra* are much more common and *M. vaginalis*, *S. pygmaea* less in seeded rice fields than in transplanted rice fields, while *C. difformis*, *R. indica*, *L. procumbens*, *E. prostrata* are same in two types of rice fields.

**Weed emergence and fluctuation** Observation in early rice fields in 1996-1998 shows weeds start to germinate 3 to 4 DARS, then emerge much more 7-14 DARS, and reached an emergence peak 15 DARS in southern and central Zhejiang or 21 DARS in northern Zhejiang. After that, number of weed germinated decreases dramatically, keeps a very few level, and there might be a small second emergence peak 42-70 DARS. In single season rice fields in northern Zhejiang there are a main first peak for weed emergence at 7 DARS and a second peak at 49-56 DARS, and the second peak is more obvious than in early rice fields.

For different species of weeds, *E. crusgalli* and *L. chinensis* germinate first, and reach emergence peaks before or at the same time as the peak of total weeds. Then, *R. indica*, *L. procumbens*, *L. prostrata*, *E. prostrata* and other most broad-leaf weeds emerge with the same peak time as total weeds. Lastly, *C. difformis*, *M. vaginalis* and *S. pygmaea* germinate with emergence peaks behind. In single season rice fields, some broadleaf weeds such as *R. Indica* and *L. procumbens* have a obvious second peaks of germination to contribute to the second emergence peak of total weeds. Barnyardgrass grows simultaneously as rice or a little quicker than rice, and up to tilling stage of rice it may have 1 to 1.5 more leaves than rice.

### Effect and application techniques of Nominee

**Effect and target weeds** Field plot trials in 1996-1997 showed Nominee (bispiribac-sodium) is highly effective on most species of weeds including gramineae, sedge and broad-leaf weeds in FWSR, with rice yield increase of 226.7-356.7%. Especially, it controls the dominant weed *E. crusgalli* with more than 2 leaves and perennial weeds such as *P. distichum*, *A. philoxeroides*, *M. triquetra* and *S. planiculmis*. Its effect on sedge weeds (*C. difformis*) and most broadleaf weeds (*R. indica*, *L. procumbens*, *L. prostrata*, *E. prostrata*, *M. vaginalis*, *S. pygmaea*) is excellent with more than 95.1% of control efficiency, and it is also good for *Ammannia arenaria* with 55.6-90.4% of plant control and 85.3-98.9% of fresh weight control. However, Nominee is not effective on *L. chinensis* with control efficiency less than 76.0%. The effect of Nominee on weeds was then proved again by field demonstrations in 1998-1999. Compared with other herbicides, Nominee is obviously much better than Sofit (pretilachlor + fenclorim) or the mixture of "quinclorac + methy bensulfuron or bentazone" in controlling barnyardgrass and perennial weeds.

**Dosage and time** Field plot trials shows the efficiency of Nominee on weeds increases as the dosage increases, optimum dosage for use of Nominee is 15-22.5 g/ha for most broad-leaf weeds, 22.5-30 g/ha for gramineae, sedge and some broad-leaf weeds (such as *M. vaginalis* and *S. pygmaea*), and 30-45 g/ha for some perennial gramineae weeds (*P. distichum*).

Based on trials for different spraying time, Nominee has good effect on barnyardgrass at 2 to 12 leaf-stage and the optimum time for spraying is 4 to 5 leaf-stage. In the field plot trial in Hangzhou in 1997, plant control effect on barnyardgrass of Nominee at 22.5 g/ha sprayed 2.5 and 4.5 leaf stage of the weed is 90.4% and 100%, and weight control 96.1% and 100% respectively. Even for barnyardgrass at 12 leaf stage, Nominee at 45 g/ha also gets 98.3% control on it, and 82.7% to 100% for *S. planiculmis*, *P. distichum* and *M. vaginalis* at same time.

**Surfactant and water management** High efficacy of Nominee on weeds can be gained by spraying with 450 liter/ha of water. Moreover, a special surfactant ("A-100" or "WK", polyoxyethylen dodecylether) is needed to add in herbicide solution when

spraying, another surfactant "885" is also helpful to the herbicide, but the effect of Nominee without any supplementary surfactant is low. Field tests in Hangzhou in 1997 shows Nominee at 22.5 g/ha added "A-100" 0.1% and 0.05% controls barnyardgrass by 90.4-96.1% and 70.8-87.5% respectively, and 82.1-92.1% of control is gotten by adding "885" at 0.1%. In the plot trial in Wenzhou in 1998, the effect of Nominee at 22.5g/ha added "A-100" 22.5 g/ha is 94.8%, 97.0%, 92.7% for *E. crusgalli*, *C. diffomis*, *R. indica* respectively, while only 83.4%, 77.3%, 77.7% for Nominee without any supplementary surfactant.

For water management, it is necessary to drain off water before spraying, keep wet on the surface of soil when spraying, and irrigate 2 days after spraying. The pot test in Wenzhou shows sprayed with Nominee 22.5 g/ha during 4 leaf stage of barnyardgrass, 99.5% of effect is got if no water on soil during spraying and only 58.1% if soil surface with water 3-5 cm deep.

**Safety to rice** It is observed that Nominee is toxic to Japonica rice 4-10 days after spraying in some regions. Sprayed with Nominee, Japonica rice showed plant dwarf, leaf yellow, brown pots on sheath, stem base widened, and root lessened. However, rice recovers from poisoned 10-20 days after spraying and rice yield is not affected when harvesting.

#### Weed control in wet-seeded rice fields

Due to non-effect of Nominee on *L. chinensis* and phytotoxicity to Japonica rice, control of the weed and factors on phytotoxicity of Nominee were also studied before the new herbicide is popularized.

**Control of *L. chinensis*** Based on survey in fields, Chinese sprangletop, *L. chinensis* become more and more serious in continently wet-seeded fields for years. An investigation in Cailu village, Dongyang shows occurrence frequency of the weed increases from 14.3% in 1996 to 56.4% in 1997 and density from 2.3 plant/m<sup>2</sup> in 1996 to 9.0 plant/m<sup>2</sup> in 1997. It germinates to have an emergence peak at 14 DARS in single season rice fields or 21 DARS in early rice fields. The weed grows faster than rice and barnyardgrass by 1 to 2 leaves, but the weed plant is much shorter before tilling stage. Field trials prove that some new herbicides are very effective on *L. chinensis*. They are Sofit (pretilachlor+fencloirim) 450 g/ha used at 0-4 DARS, Saturn (thiobencarb) 750 g/ha, MY-100 (oxaziclomefone) 30 g/ha and YRC-2388 (fentrazmide) 125 g/ha at 1.5-2.5 leaf-stage of the weed, Clincher (cyhalofop) 45-60 g/ha at 4-6 leaf-stage, Whip (fenoxaprop-p-ethyl) 10-30 g/ha at 5-9 leaf-stage. Spraying with the mixture of Nominee 22.5 g/ha and Whip 10 g/ha at 4-5 leaf-stage of the weed is a good choice for the control of barnmyardgrass, Chinese sprangletop and other weeds. From simulated tests on rice plantation groove; the weed can also be controlled by water management. Treatments of keeping water 3-5 cm deep on soil all the time after 1.5, 2.5, 3.5 leaf-stage of rice kill the weed by 100%, 100%, 33.7% respectively; 3 day-shift, 5 day-shift, 7 day-shift of water-dry rotation on soil after 2.5 leaf-stage of rice get weed control of 67.6%, 72.1%, 100%.

**Phytotoxicity of Nominee to rice** Rice varieties have different susceptibility to Nominee, Japonica rice is vulnerable to the chemical and Indica rice and hybrid rice are tolerant. At high dosage (90 g/ha) of Nominee, Japonica rice varieties "Xiushui 11" and "ES98-3-2" show plant dwarf by 35.1-55.9% of height inhibition and die at last, while for Indica rice and hybrid rice varieties only 15.2-25.5% of plant height repressed and no dead plants are found. When Nominee is sprayed at 2, 3, 4, 5, 6 leaf-stage of rice,

inhibition rate for plant height of Japonica rice was 46.27%, 33.20%, 17.12%, 8.36%, 8.97%, respectively, so Nominee has less effect on more than 4 leaf-stage rice than on 2-3 leaf-stage rice. The higher dosage of bispyribac-sodium is applied, the more seriously rice becomes poisoned. Mortality of rice sprayed with Nominee at 37.5, 75, 150, 600, 2400 g/ha is 0%, 10%, 10%, 43.3%, 100% respectively. Rice planted in different soil shows different degrees of phytotoxicity by Nominee, and the herbicide causes less toxic to rice if there is a thin layer of water on soil during spraying. Pot test shows rice plant height of the treatment of spraying Nominee with a layer of water on soil during spraying, the treatment of spraying Nominee with no water and the untreated is 21.7 cm, 20.5 cm and 30.8 cm, and fresh weight of underground part 0.309 g, 0.098 g and 0.338 g respectively. Rice shows poisoned by the chemical quicker and heavier at 30-40°C than at 20-30°C, and mortality of rice treated with Nominee 90 g/ha 13 days after treatment is 90% for 35-40°C, 60% for 30-35°C, 50% for 25-30°C and 20-25°C. A safener seems to reduce phytotoxicity of Nominee to Japonica rice. Plant height of rice treated by Nominee and untreated is 28.2 and 17.9 cm, while 20.1, 18.8, 18.6 cm for rice treated by mixtures of Nominee and the safener with 1:1, 2:1, 4:1 of ratio respectively. Observation also shows that fertilizer of nitrogen seems to be helpful to the recovery of rice from injuring by Nominee.

Nominee kills weeds and causes phytotoxicity to rice by inhibiting acetolactate synthase (ALS) and preventing biosynthesis of branched-chain amino acids in plants. In order to understand mechanism of phytotoxicity of Nominee to rice, activity of ALS was determined by an acetoin-creatine-naphthol complex on spectrophotometer and inhibition of Nominee to ALS in rice and weeds assessed. The results show absorb peak of the complex for rice and barnyardgrass is 520 nm. Hybrid rice variety "Xianyou 63" and Japonica rice variety "Xiouhui 11" have the highest ALS activity (0.098 and 0.0975 A 520nm), Indica rice variety "Jiayu 293" has the lowest one (0.0725 A 520nm), and glutinous rice variety "Xianhu 84" with the medium (0.0815 A 520nm). For different weeds, the order of ALS activity is *E. crusgalli* (0.054 A 520nm), *E. prostrata* (0.053 A 520nm), *C. difformis* (0.030 A 520nm), *L. chinensis* (0.0243 A 520nm). By in vivo test, ALS is prevented by Nominee and then recovers quickly in rice, while in barnyardgrass the inhibition of ALS by Nominee is never recovered, and the weed dies at last. For rice varieties, ALS is repressed most quickly in "Jiayu 293", most strongly in "Xiouhui 11", whereas in "Xianhu 84" and "Xianyou 63" the herbicide has slow and week inhibition to ALS. By in vitro test, the inhibition of Nominee to ALS is stronger than another similar herbicide pyanchor.

Residue assay by HPLC shows 1.048 and 2.597 mg/kg of Nominee are detected in rice ("Xiouhui 11") and barnyardgrass at 4 days after spraying, and 0.173 and 0.742 mg/kg at 7 days after spraying. Therefore, it seems that Nominee degrades a little quicker in Japonica rice than in barnyardgrass.

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## EFFECTS OF STRIP LIMING DOSES UNDER CONSERVATION TILLAGE ON WEED DEVELOPMENT AND MAIZE (*ZEA MAIZE*) YIELD IN RUBBER (*HEVEA BRASILIENSIS*) BASED INTERCROPPING SYSTEM.

G. Wibawa<sup>1</sup>, H. Suryaningtyas<sup>1</sup>, E. S. Saragih<sup>2</sup>, and Y. K. Leksana<sup>2</sup>

<sup>1</sup>Indonesian Rubber Research Institute, Sembawa Research Station, P.O. Box 1127  
Palembang, Indonesia. irri-sbw@mdp.co.id

<sup>2</sup>PT. Monagro Kimia, Jl. MH. Thamrin 57, Jakarta- 10350, Indonesia

**Abstract:** This research was carried out at Sembawa Research Station, Palembang to determine the effects of lime doses under conservation tillage on weed development, yield of maize in rubber based intercropping system. Data presented were recorded from two years maize harvest. Soil was prepared using two tillage systems called Conventional tillage and Reduced tillage only at the first year. Four lime doses was applied, each year, as width as 20 cm along maize planting rows. The optimal lime dose applied to maize rows at Conventional tillage plots, at the first and the second year were between 1100 and 1650 kg of  $\text{CaCO}_3/\text{ha}$  or equivalent to application of 1.5 and 2.25 x  $\text{Al}_{\text{ech}}$ , respectively, while at Reduced tillage plots, those optimal lime doses were lower i.e. between 550 and 1100 kg of  $\text{CaCO}_3/\text{ha}$  or equivalent to application of 0.75 and 1.5 x  $\text{Al}_{\text{ech}}$ , respectively. Significant relation between maize yield and Al saturation was observed, with regression equation as follows  $y = -52.7x + 6315.9$ ,  $R^2 = 0.96$ , where y and x were maize grain yield (grain moisture of 14%) and Al saturation (%), respectively. Effects of treatments on changes in soil properties, weed development and rubber growth were also discussed. Financial benefit of planting maize with conservation tillage in rubber based intercropping was also presented.

**Key words:** Conservation tillage, rubber-based intercropping, lime doses, weed development, maize yield.

## INTRODUCTION

Research on conservation tillage systems (defined as tillage system that applied to prepare the land to enable the optimal crop grow and yield, with always concern to soil and water conservation) such as minimum tillage (MT) and no tillage (NT) has been carried out for food crop production in upland as reviewed by Utomo (1998). Upland area in Sumatra and Kalimantan is mainly Ultisol type or locally known as *Podsolik Merah Kuning (PMK)*, with the main characteristics: susceptible to erosion, low mineral contents especially on N, P, K, Mg, low pH or high Al saturation and low cation exchange capacity (Wibawa dan Thomas 1997). The roles of organic matter are very significant, especially on liberation of P from Al or Fe, and on improvement of soil structure. Conservation tillage increase soil organic matter through incorporation of mulches to soil and increase the availability of soil nutrients, improve soil structure and increase the activity of soil micro-fauna (Utomo 1998).

In Indonesia, rubber area is about 85% (about 2.5 millions ha) managed by smallholders (Ditjenbun 1996). Rubber based intercropping systems are considered to be more adaptive to the farmers' conditions, due to its capacity to provide short term income to farmers, while rubber in immature phase for about 5-6 years. Rubber growth, in the environment where intercrops is managed intensively by applying chicken manure of 5

tonnes/ha, for chilly, was 25% better than that where rubber was managed extensively (Wibawa 1998).

The constraints on the development of annual food crops, especially for maize and soja bean, were: high Al saturation of the Ultisol (> 75%), however the ideal condition for the crops' growth is when the Al saturation is below 50% (Sujadi and Effendi 1991). Liming is the practical way to decrease Al saturation. However, the availability at farmers' level and the expensive price of lime limite its use in crop production. Stip liming applied a long crops' rows each crop season is the way to decrease the dose and cost of production.

The goal of this research is to study, in long term, the effects of two soil tillage systems and four lime doses on weed development and maize yield planted in between rubber (IRR 39 clone) rows. This paper presents changes of soil properties, weed development, maize yields and rubber growth during the first and second year, completed with financial benefit of this practice to farmers.

### MATERIALS AND METHODS

This research was conducted at Sembawa Research Station, Palembang - South Sumatra, Indonesia, during two different rainy seasons in 1998 and 1999. Research plots was previously of rubber GT1 clone replanted in 1998. Old rubber trees were felled and cut at 60 cm height above ground in September 1998. Initial weed condition was homogenous which was dominated by grass *Ottochloa nodosa* with around 90% coverage. Other weeds were found sporadically i.e. *Melastoma* sp. and *Borreria* sp.

In the first year, December 1998, the research was set as *Split Plot Design* with three replications. Main plots were two tillage systems:

A. Conventional full tillage system: rubber trunks were removed by bulldozer, and soil was plowed and harrowed 2 times, mechanically, respectively;

B.Reduced tillage system: rubber trunks were not removed, and done only one plowing and one harrowing respectively; leaving some chopped roots of ex-rubber and weed residue on the ground;

For treatments of A and B in the second planting year, December 1999, weeds was also controlled with glyphosate 2 weeks prior to planting. Full tillage and reduced tillage systems were done once in a year. Main plot size was 1000 m<sup>2</sup>. Sub-plots were four levels of lime doses applied at the beginning of each maize planting season.

Lime application, was banded as width as 20 cm along planting rows; with 80 cm distance in between rows this banded application was equal to 25% of dosage of broadcasting application. Lime dose treatments in sub-plots were applied for 14m x 7m. The treatments in sub-plots were : a. No lime, b. 550 kg CaCO<sub>3</sub>/ha, c. 1100 kg CaCO<sub>3</sub>/ha and d. 1650 kg CaCO<sub>3</sub>/ha. Maize, of hybrid C7 was used in those two planting years.

Latex timber rubber clone, IRR 39, was planted in along the previous row with spacing 3.5m x 7m in May 1999. Budded stump was planted in between un-removed trunks in treatment B (reduced tillage). Maize seed planting was applied with 4-row *No-till Planter* (NTP), a mechanised seeder or planter which was introduced and tested under Conservation Tillage condition in Indonesia by PT. Monagro Kimia (Monsanto Company subsidiary in Indonesia). The machine was pulled by a four-wheel tractor. Between row distance could be set 75 - 90 cm, while in-row distance could set 15 - 33 cm. In this study, spacing was selected 80cm x 20cm to provide around 60,000 plant population. The 4-row

NTP was also equipped with 4 hoppers to distribute granular pesticide, for instance carbofuran to control soil-borne pest.

In all treatments, basal fertilizers at time of planting was applied manually by using: Urea 100kg/ha, SP-36 125 kg/ha and KCl 100 kg/ha, and additional Urea was applied 75 kg/ha at 4 and 6 weeks after planting respectively. Soil properties and other environmental factors (rainfall distribution, etc), weed development, growth and yield of maize and rubber growth were measured during the study.

## RESULTS AND DISCUSSION

### Rainfall, evaporation and changes of soil properties during study

Rainfall between December 1998 and March 2000, indicated that water was not limiting factor for the growth of maize (Figure 1). Water consumption for maize was predicted about 80 - 100mm/month (Kung 1971). The critical periods for maize growth to water stress were at early growth (2-3 leaves) and during flowering, in December 1998 and March 1999 respectively for the first year and December 1999 and March 2000 respectively for the second year.

Soil condition during the two planting seasons is presented in Table 1. Soil nutrient content and soil pH several weeks after soil tillage, in all plots, were low. Conventional tillage increased Al saturation at 20cm depth, while with Reduced tillage, Al saturation was almost unchanged from the initial condition, before tillage. Soil organic matter decreased with rubber trunk removal in conventional tillage (Table 1).

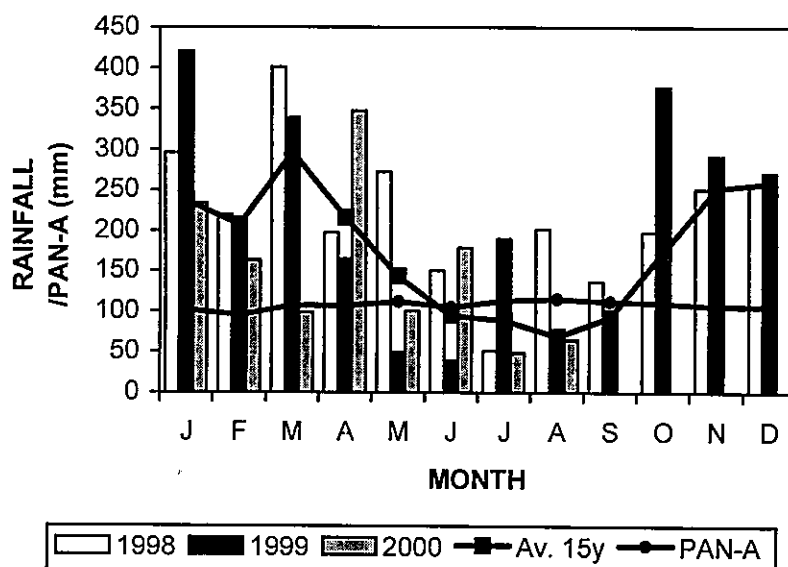


Figure 1. Rainfall during research period, 15 years of rainfall average and Pan-A evaporation in research site.

At the end of maize harvest in the second year, May 2000, at 0-20cm soil depth, soil pH decreased at the two soil tillage treatments, however Al saturation decreased compared to that of the first year, until at the level below 70%, that not harm to maize growth (Sujadi and Effendi, 1991). The significant different in Al saturation between soil in Conventional



tillage and Reduced tillage observed at the beginning of the research, reduced in the second year. Soil pH and soil nutrient decreased with soil depth, addversely the more the soil Al content, the more the Al saturation increase. This result was coherent to that found in Ultisol in West Sumatra by other researchers (Arya, *et al.* 1992; Zainol, *et al.* 1993).

Soil analysis at different lime dose treatments, soil tillage systems and at two different soil depths (0-20cm and 20-40cm), after the second year harvest (May 2000) showed that increment of lime doses influenced only on Ca and Al contents. Al saturation increased significantly only on the first soil surface (0-20cm). At 20-40cm soil depth, no effect of lime dose was detected. Al saturation was still at high level (more than 70%). Application of lime at  $0.75 \times Al_{ech}$  at Reduced tillage system and at  $1.0 \times Al_{ech}$  at Conventional tillage system reduced Al saturation to below 70% (Figure 2).

Table 1. Soil nutrient contents and pH at various tillage treatments at 0-20cm soil depth, at two dates of observation (Feb. 99 and May 2000) and at 20-40 cm depth (May 2000).

Tillage Systems	pH	Organic matter (%)		Bray II ppm	Cation, Anion or CEC (me/100g)					Al saturation (%)
		C	N		K	Ca	Mg	CEC	Al <sub>ech</sub>	
Feb, '99										
0-20 cm										
Conv.Till.	5.70	1.35	0.12	0	0.30	0.06	0.04	3.7	2.60	87
Red. Till.	5.16	3.35	0.23	4	0.59	0.17	0.14	9.4	2.38	73
Pre Till.*	5.34	2.08	0.22	0	0.64	0.05	0.06	10.8	2.38	76
May, '00										
0-20 cm										
Conv.Till.	4.76	2.09	0.10	9	0.11	0.14	0.10	7.3	1.64	54
Red. Till.	4.60	2.49	0.10	7	0.10	0.06	0.06	7.0	1.43	47
20-40 cm										
Conv.Till.	4.60	1.41	0.06	6	0.08	0.19	0.04	6.6	2.27	81
Red. Till.	4.49	1.30	0.06	6	0.06	0.05	0.04	6.3	2.69	91

\*Conv.: Conventional, Red. : Reduced, Till.: Tillage, Pre Tillage: prior to soil tillage

Conversely, soil bulk density data at two treatments, in second year, showed that soil structure increased, mainly on the first soil surface (Table 3). At the following depths, no significant bulk density change was observed. Soil profil (Figure not shown) indicated that iron concretion was detected at more than 50cm soil depth, where the soil bulk density was very high, more than  $1.7 \text{ g/cm}^3$ .

Increment of soil bulk density, that occurred only on the first soil surface was due to the use of tractor during each maize planting time. This point was very important to avoid soil compaction. Incorporation of mulch, harvest waste to soil was very important to conserve soil structure in the future.

#### Weed development due to lime doses and soil tillage systems

Weed development during the two maize seasons showed no significant difference, especially among plots applied with lime. Measurement of weight and weed coverage at two sampling times in the third maize season (Figure 2 and 3) showed that those variables appeared to decrease in the highest lime dose. This might be related to the stronger competitiveness of maize growing at the highest lime dose. After three seasons of maize

planting, it also showed no change in the weed flora composition. Weed species dominating the treatment plots included: *Digitaria adscendens*, *Paspalum conjugatum*, *Eleusine indica*, and *Axinopus compressus*.

Table 2. Soil analysis at two tillage and lime dose treatments, at two soil depths (0-10cm, 20-40cm) after the second maize harvest (May 2000).

Soil depth, Tillage Systems and Lime doses	pH	Organic matter (%)		Bray II Ppm	Cation, Anion or CEC (me/100g)					Al Saturation (%)
(kg CaCO <sub>3</sub> /ha)		C	N	P	K	Ca	Mg	CEC	Al <sub>ech</sub>	
0-20cm										
Conv. Till.										
0	4.51	2.09	0.09	8	0.08	0.14	0.05	7.2	2.44	87
550	4.76	2.31	0.10	10	0.10	0.83	0.11	6.8	2.16	63
1100	4.82	1.84	0.09	7	0.14	1.45	0.11	7.6	1.14	37
1650	4.96	2.13	0.10	10	0.10	1.79	0.12	7.7	0.83	29
Red. Till.										
0	4.44	2.45	0.12	10	0.12	0.19	0.03	6.7	2.56	86
550	4.54	he	0.11	7	0.08	0.46	0.06	6.6	1.60	48
1100	4.91	3.29	0.10	6	0.09	1.30	0.07	8.6	0.70	28
1650	4.53	1.54	0.08	6	0.11	1.06	0.06	6.8	0.87	25
20-40cm										
Conv. Till.										
0	4.60	1.05	0.05	7	0.07	0.80	0.03	5.9	2.44	90
550	4.57	1.35	0.06	5	0.07	0.20	0.04	7.4	2.40	81
1100	4.62	1.60	0.05	7	0.07	0.27	0.05	6.4	2.35	81
1650	4.61	1.63	0.06	4	0.09	0.43	0.05	6.6	1.87	73
Red. Till.										
0	4.46	1.10	0.06	9	0.06	0.11	0.05	8.0	2.51	89
550	4.40	1.02	0.06	5	0.06	0.12	0.05	3.9	2.63	90
1100	4.51	1.49	0.05	4	0.08	0.16	0.05	6.2	2.90	91
1650	4.59	1.60	0.06	5	0.05	0.11	0.03	7.0	2.72	94

Conv. Till.=Conventional tillage, Red. Till.=Reduced tillage.

Table 3. Soil bulk density at two sampling dates and several soil depths.

Items	Soil depth (cm)					
	0-10	10-20	20-30	30-40	40-50	60-80
Beginning of trial						
Row	1.16	1.21	-	-	1.41	-
1.5m from row	1.19	1.23	-	-	1.46	-
Middle row	1.11	1.24	-	-	1.40	-
May 2000						
Conv. Till.	1.25	1.28	1.53	1.64	-	1.78
Red. Till.	1.26	1.27	1.49	1.55	-	1.78

(-): no measurement

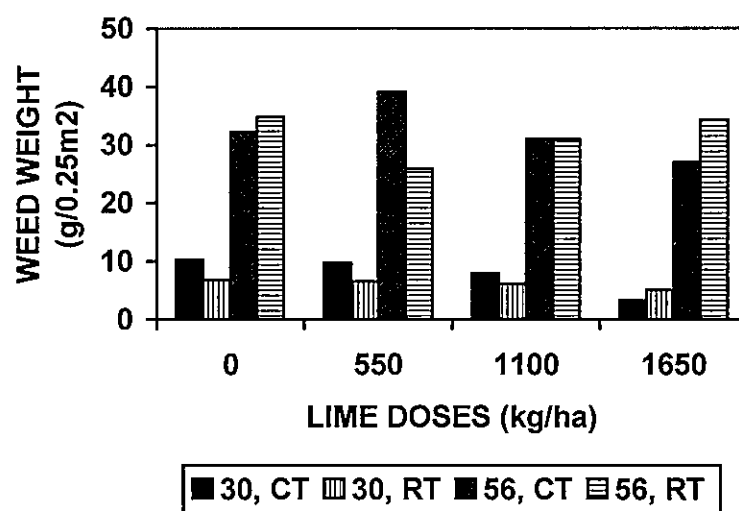


Figure 2. Weed weight at two sampling times, 30 and 56 days after maize shawing (DAS) and at two tillage systems, Conventional tillage (CT) and Reduce tillage (RT) due to different lime doses applied to maize rows.

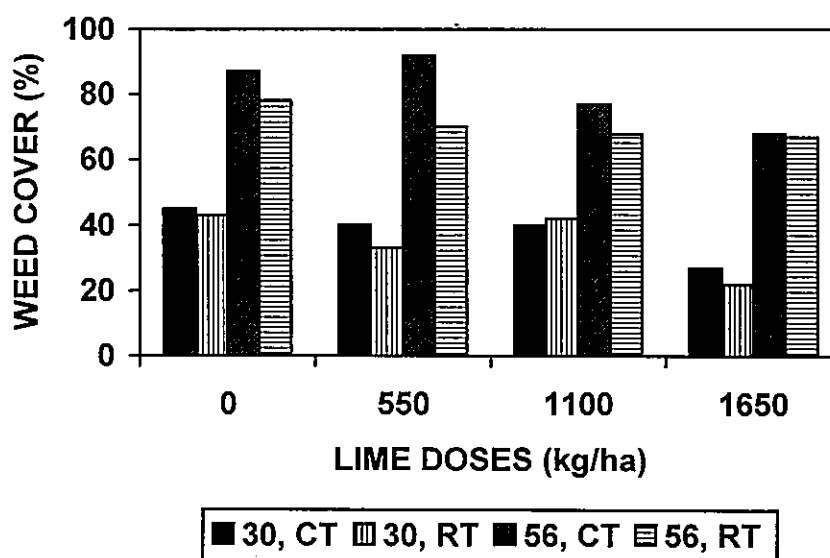


Figure 3. Weed cover at two sampling times, 30 and 56 days after maize shawing (DAS) and at two tillage systems, Conventional tillage (Conv. Till.) and Reduce tillage (Red. Till.) due to different lime doses applied to maize rows.

#### Response of maize yield to tillage systems and lime doses.

Lime influenced number of plants per ha, only at treatment with and without lime. (data not shown, Wibawa *et al.* 1999). This effect was observed in both treatments of soil tillage. This was reasonable because Al saturation at plot without lime application was still high e.i above 70% (Table 1 and 2). As indicator, plant leaf was violet in colour and was stunted in growth.

Until two years of maize harvest (Note: the third maize harvest is not done yet), maize yields at plots with Reduced tillage were still better than those at plots with Conventional tillage (Figure 4). No significant interaction between tillage systems and lime doses was observed, either at the first or the second year.

Biomass without grain and root, maize yields in both maize seasons were very responsive to lime application. Dependent on the tillage treatments, those variables increased significantly with lime dose increment i.e. between 1100 and 1650 kg of  $\text{CaCO}_3/\text{ha}$ . The optimal lime dose at Conventional tillage plots, at the first and the second year were between 1100 and 1650 kg of  $\text{CaCO}_3/\text{ha}$  or equivalent to application of 1.5 and 2.25 x  $\text{Al}_{\text{ech}}$ , respectively, while at Reduced tillage plots, those optimal lime doses were lower i.e. between 550 and 1100 kg of  $\text{CaCO}_3/\text{ha}$  or equivalent to application of 0.75 and 1.5 x  $\text{Al}_{\text{ech}}$ , respectively.

Relation between maize yield and Al saturation was very significant (Figure 5). Reduce of Al saturation increased maize grain yield significantly. The regression equation was  $y = -52.7x + 6315.9$ ,  $R^2 = 0.96$ , where y and x were maize grain yield (grain moisture of 14%) and Al saturation (%), respectively. Soil Al saturation of around 25% was needed to obtain maize yield of around 5000 kg/ha.

Table 4. Biomass without grains and root of maize of C7 variety, at different lime doses and soil tillage systems.

Tillage system	Lime doses (kg/ha)	Biomass without grain and root (kg/ha)	
		Year I	Year II
Conv. Till.	0	265	1064
	550	1141	2280
	1100	2687	2755
	1650	2809	3595
Red. Till.	0	793	1039
	550	2819	2381
	1100	3321	2854
	1650	3363	3149
Conv. Till.		1726	2424
Red. Till.		2574	2356

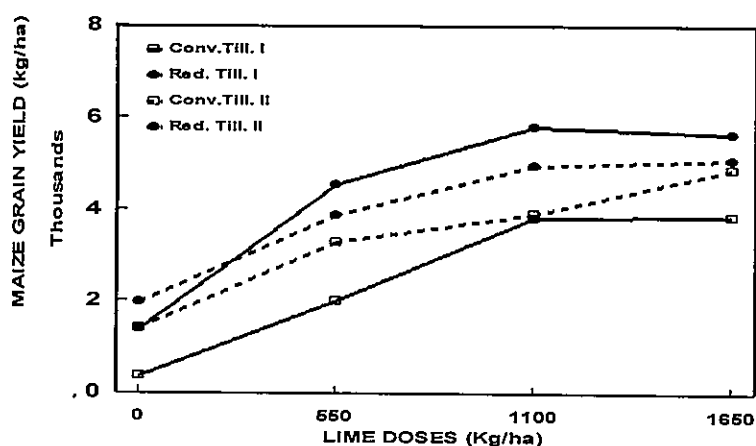


Figure 4. Relation between lime doses and maize grain yield in Conventional tillage and Reduced tillage systems, at the first (I) and the second (II) year.

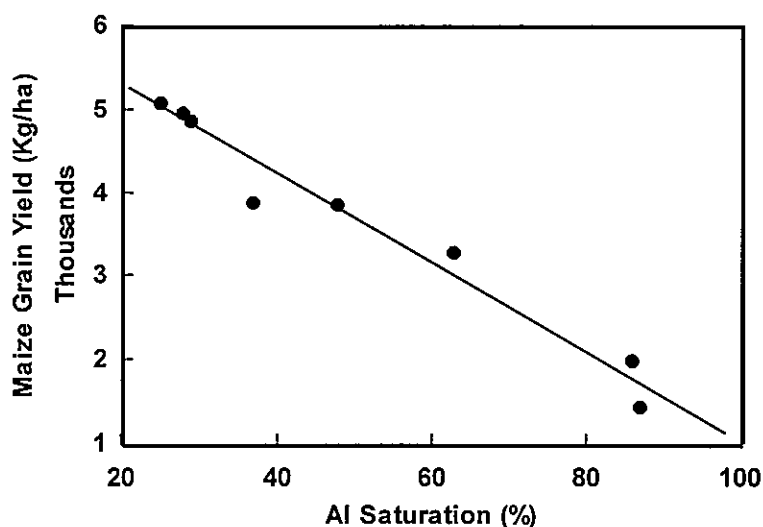


Figure 5. Relation between Al saturation (%) and maize grain yield (kg/ha) at grain humidity 14% in Sembawa. Regression equation  $y = -52.7x + 6315.9$ ,  $R^2 = 0.96$

#### Rubber growth at two different soil tillage systems

Rubber growths (height, number of whorls, and stem diameter) at Conventional tillage and at Reduced tillage plots were comparable (Table 5). Furthermore, the big rubber stems diameter (more than 2.5cm) a year after planting indicated that rubber growths in this experiment were relatively fast compared to the normal condition. (Wibawa 2000). Based on these results, until the beginning of the second year of rubber, intercropping rubber with maize planted mechanically using tractor and *no-till planter* not influenced negatively rubber growth. Rubber canopy growth was still limited and possible to plant maize for the third planting season.

Table 5. Rubber growth at two soil tillage systems.

Treatment	Variable	Age (months)			
		3	7	10	13
Conv. Till.	Height (cm)	29	79	-	-
	Number of whorls	1.04	3.25	5.6	-
	Stem Diameter (cm)*	-	1.21	2.05	3.06
Red. Till.	Height (cm)	28	107	-	-
	Number of whorls	1.16	3.83	6.5	-
	Stem Diameter (cm)*	-	1.26	3.01	3.48

\* : measured at 10cm from union (-): not measured.

#### Financial analysis of producing maize using conservation tillage systems under rubber plantation

Financially, intercropping rubber with maize during the first two to three years can give an additional income to farmers. The value of R/C more than 2 indicated that this intercropping was highly beneficial. (Table 6). With the current level of yield, if the price of a kg of maize is Rp 1200 and the cost of production is of around 2 million of rupiah, then the break even point (BEP) of production will be reached at 1700kg of

grain/ha of rubber plantation, or with the same analogy then the BEP of price will be reached at Rp 430 to Rp 500/kg of grain.

This farming practice will be better if it is done within farmer groups, to increase the efficiency of mechanized equipment used in this intercropping system. Joint cooperation between farmers and feedmills to shorten the market chain will be very helpful to farmers in Indonesia, who faced mainly to marketing problems. To close the relation between estate plantation and farmers living around the plantation, this alternative technology may be very interesting to propose. Estate plantation may provide mechanized equipment, land between plantation crops and inputs of production, while farmers provide labor. The net income can be shared between farmers and estate. This practice will be more interesting compared to use leguminous cover crops (LCC) as soil cover.

Table 6. Financial analysis of producing hybrid maize variety C7, planted using no-till planter in conservation tillage systems under rubber at the first and the second year.

Items	Grain yield / input needed (unit/ha)		Cost unit (Rp/unit)	Value (Rp)	
	Year I	Year II		Year I	Year II
Income from maize (kg)*	4.600	4.000	1.200	5.520.000	4.800.000
Total income				5.520.000	4.800.000
Cost					
Land preparation**					
1. Plowing	2 times	-	96.000	192.000	-
2. Harrowing	2 times	-	32.500	32.500	-
3. Weed spraying (WD)	-	5	7500	-	37.500
Planting ***					
1. Tractor+no-till planter (TWH+PWH)	0,8	-	110.000	88.000	88.000
Maintenance and harvest (WD)					
1. Weeding	25	25	7.500	187.500	187.500
2. Fertilizing	10	10	7.500	75.000	75.000
3. Pests and diseases control	10	10	7.500	75.000	75.000
4. Harvest	15	15	7.500	112.500	112.500
5. Hulling (mechanized)	3	3	7.500	22.500	22.500
Materials					
Herbicide (glyphosate) (ltr)	-	5	27.000	-	135.000
Maize seeds (kg)	16	16	17.500	280.000	280.000
Urea (kg)	200	200	940	188.000	188.000
SP-36 (kg)	100	100	1.540	154.000	154.000
KCl (kg)	80	80	1.500	120.000	120.000
Lime (kg)	1000	1000	300	300.000	300.000
Insecticides (ltr)	1 packet	1 packet	250.000	250.000	250.000
Total cost				2.077.000	2.025.000
Total net income (total income-total cost)				3.443.000	2.775.000
R/C				2,65	2,37

\*Maize density was 80% of the maize monoculture density. Yield of maize in monoculture plot (variety C7) was 5750 kg/ha at the first year and 5000 kg/ha at the second year; \*\*Tractor was calculated as material and operational cost; \*\*\*If plating was done manually, then 20 Working Days (WD) was needed, (1 WD= 7 hours/day); Price was based on Palembang price, January 2000. 1 US Dollar = Rp 8500.

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## ROUNDUP TANK MIXTURES WITH ATRAZINE PLUS ACETOCHLAR FOR WEED CONTROL IN ZERO-TILLAGE CORN

X. J. Li<sup>1</sup>, D. Z. Lu<sup>1</sup>, Y. H. Li<sup>2</sup>, and R. E. Blackshaw<sup>3</sup>

<sup>1</sup> Institute of Cereal and Oil Crops, Hebei Academy of Agric.Sciences, Shijiazhuang 050031, China. hbifoc@public.sj.he.cn

<sup>2</sup> Department of Agronomy, Nanjing Agricultural University

<sup>3</sup> Research Center, Agr. and Agri-Food Canada, Lethbridge, AB, Canada

**Abstract:** Field experiments were conducted to evaluate preapplied roundup tank mixtures with atrazine plus acetochlar for weed control in zero-tillage corn field. Both preemergence-applying atrazine plus acetochlar and roundup alone provided relatively worse weed control. Roundup tank mixtures with atrazine plus acetochlar showed excellent weed control with the control efficiency of 90.3% for weed density and 96.6% for weed biomass. No crop damage was observed for each treatment.

**Key words:** Roundup, atrazine plus acetochlar, zero-tillage corn, weed control

### INTRODUCTION

Corn is one of the important crops in north China. The no-tillage summer corn covers more than 70% of corn planting area (Li and Li 1996). In this corn production system, corn is planted as soon as winter wheat harvested. Without till the soil, weeds such as crabgrass, barnyardgrass and redroot pigweed emerge in late growing season of the winter wheat remain alive in the following corn field and reach their threshold before corn emerge (Li and Li 1996).

A common production of chemical control in no-tillage corn in wheat corn double cropping system is to preapplying herbicide called Yia mixture, a registered trade product made of atrazine plus acetochlar. However atrazine and acetochlar are not so effective on such emerged weeds especially when they are above 3 leaf stages. Therefore, no-tillage corn yield is usually reduced a lot even after atrazine and acetochlar are sprayed preemergence in no-tillage corn (Lu and Lu 1992).

Roundup 41%SL with the active factor of glyphosate is a widely used, nonselective, broad-spectrum postemergence herbicide produced by Monsanto Co. It is commonly used in China to control existing vegetation as burndown in roadside, orchard and the place without crops but seldom used in no-tillage corn field. Because Roundup provides no residual control, it is also used plus a soil-residual herbicide as preemergence application for grass and broadleaf weed control in crop field (Wilson and Worsham 1988). But tank mixtures of Glyphosate with residue herbicides often result in antagonism. For example, the addition of atrazine to glyphosate reduced efficacy on quackgrass Appleby and Somabhi 1978). Until now, not so much information has found on glyphosate tank mixture with atrazine and acetochlar on the existing weed species such as crabgrass, barnyard grass and redroot pigweed etc. in the no-till corn in winter wheat-summer corn double cropping system.

The objective of this study was to evaluate control efficacy of weed species in no-till corn field with roundup applying in combination with atrazine and acetochlar mixtures when they were in different rate.



## MATERIALS AND METHODS

The experiment was conducted in winter wheat summer corn double cropping system in the research farm in the Institute of Cereal and Oil Crops, Hebei Province, China. Corn was planted as soon as wheat harvested on June 10, 1998 and June 14, 1999. Fertilizers were broadcast and incorporated to bring the total N and P 150 and 50 kg/h, respectively, before wheat planting and fertilizers were broadcast with the total N of 50 kg/h in the seedling stage of corn. Wheat was harvested in early June and corn was harvested in later September. The soil was sandy clay loam with the pH 7.5 to 7.3 and the organic matter 2.1 to 2.3%.

Weed species in the experiment field are crabgrass (*Digitaria ciliaris*), goosegrass (*Eleusine indica*), hairy cupgrass (*Eriochloa villosa*), redroot pigweed (*Amaranthus retroflexus*), purslane (*Portulaca oleracea*), abutilon (*Abutilon theophrasti*) and copperleaf (*Acalypha australis*). Weed density was 50.0 plants/m<sup>2</sup> and most of weeds were on 1-6 leaf stages in 1997 and 27.2 plants/m<sup>2</sup>, 1-5 leaf stages, respectively, in 1998 before corn planted.

Plots were 4 by 8 m and were arranged in randomized complete block design. Treatments were replicated four times and consisted of Roundup 41 SL provided by Monsanto Co. at 0, 1500 ml/ha, 2250 ml/ha applied alone, Yia 40 FL provided by Xuanhua Pesticide Co, Hebei Province, at 0, 1500 ml/ha, 2250 ml/ha applied alone and Roundup in above rate mixture with Yia 40 FL at 1500 ml/ha, 2250 ml/ha.

All herbicides were applied with a CO<sub>2</sub>-pressureized small plot sprayer delivering 450 L/ha at 205 Kpa. herbicide were applied on June 13, 1997 and on June 17, 1998 before corn emerged.

The density of weeds was recorded at the time of herbicide application by measuring four spots with 0.25 m<sup>2</sup> of each in every plots. Plots were rated for weed control by species at 15, 30, 45 day after the herbicide applications and weed biomass was measured 45 day after application. All control ratings were based on a scale of 0 (no control) to 100% (complete control). Corn injury was visually determined at the same time. Corn yield was measured from each plot by hand harvesting.

All data were subjected to analysis of variance and means of the data averaged over years (no year interaction occurred for any parameters) were separated by a Fisher's Protected LSD test at the 5% level of probability and all possible interaction of years and treatment factors were tested.

## RESULTS AND DISCUSSION

### The control efficacy when Roundup and Yia applied alone

15 days after application of the herbicide, the control efficacy of Roundup at 2250 ml/ha on crabgrass, goosegrass and redroot pigweed reached 91.2%, 95.9% and 99.8% and the control efficacy of abutilon and copperleaf were relatively lower, no more than 80%, at the same rate (Table 1). In both years, grass and broadleaf weeds emerged after the preapplying of Roundup for no residual effect in the soil of the herbicide. Therefore weed control efficacy reduced 25 days after application. Control efficacy of weed biomass of the whole weed community was 23.6% and 42.4%, respectively, 45 days after application of the herbicide at the two rates (Table 3).

Yia failed to control the grass weeds when the weeds were above three-leaf stages, but it gave good control on broadleaf weeds. Yia applying alone at the rate of 2250 ml/

ha controlled crabgrass and hairy cupgrass by 39.2 % and 55.8 % 15 days after application (Table 1) and it controlled purslane, abutilon, redroot pigweed and goosegrass nearly 100%. 45 days after application of Yia at 1500ml/ ha and 2250ml/ ha, control efficacy of weed biomass of the whole weed community was 41.3% and 64.4%, respectively.

Table 1. Weed control efficacy 15 days after application (%)

Herbicide	Rate ml/ha	Crabgrass	Goose grass	Cup grass	Purs lane	Redroot pigweed	Abutilon	Copper leaf
Roundup	1500	85.5	93.0	89.8	60.3	90.5	46.5	70.8
Rounup	2250	91.2	95.9	93.7	63.3	99.8	45.7	75.5
Yia	1500	30.0	90.5	43.5	99.5	98.3	95.0	88.0
Yia	2250	39.2	98.0	55.8	100	100	98.9	87.5

Weed control efficacy of Roundup and Yia was related to the environment factors before and after application. Due to the 3.2mm precipitation in the same day of the application in 1997, weed control was relatively lower than that in 1998 at the same rate of Roundup. In the other way, more wheat stubble reduced weed control of Yia in 1998.

#### The control efficacy when Roundup and Yia mixed

When Roundup was tank mixed with Yia, weed control efficacy ranged from 83.5 to 99.5% 25 days after application, depending on the rate of Roundup and Yia and weed species (Table 2). Because of the burndown effect of Roundup and the residual activity of Yia, weed control efficacy was much better than Yia applied alone, especially the control efficacy of crabgrass and cupgrass was improved. 45 days after application of Roundup at 2250 ml/ha mixture with Yia at 2250ml/ ha, the control efficacy of weed community in corn field was 90.3% in density and 96.6% in biomass and the control efficacy of weed biomass in this treatment was significantly higher at the level of  $P=0.05$  than the other treatments, except the treatment of Roundup at 2250 ml/ ha plus Yia at 1500ml/ ha (Table 3). Therefore, excellent weed control would be observed preapplying Yia at 2250 ml/ ha alone for broadleaf weed. But for grass weed control at least 2250ml/ ha Roundup should be added.

Table 2. Weed control efficacy 25 days after application (%)

Herbicide	Rate (ml/ha)	Crabgrass	Goose grass	Cup-Grass	Purs-lane	Redroot pigweed	Abutilon	Copper leaf
Roundup	1500	18.9	23.5	11.8	50.0	55.5	23.5	63.3
Roundup	2250	28.5	58.8	35.5	51.5	58.0	33.0	61.4
Yia	1500	32.0	94.3	23.3	98.5	99.8	95.3	88.0
Yia	2250	35.3	96.5	48.5	99.5	99.5	96.0	87.8
Roundup+Yia	1500+1500	85.0	90.2	83.5	95.5	95.8	94.5	89.3
Roundup+Yia	2250+1500	90.5	93.3	91.0	94.8	96.0	95.3	90.0
Roundup+Yia	1500+2250	92.0	91.0	83.5	95.5	95.0	95.0	85.3
Roundup+Yia	2250+2250	93.5	96.8	92.3	96.8	99.8	96.5	93.3
None		0	0	0	0	0	0	0

#### Corn yield when Roundup mixed with Yia

Both Roundup and Yia did not injured corn growth and yield. Corn yield of the untreated check was 4.9kg/plot in 1997 and 10.4kg/plot in 1998. Roundup and Yia

applied preemergence alone improved corn yield a lot compared with the untreated check. Tank mixing Roundup 1500 to 2250ml/ ha with Yia 1500 to 2250ml/ ha increased corn yield significantly compared with the untreated check and herbicide applied alone. Among the mixed treatment, Roundup 2250 ml/ ha added Yia 2250 ml/ ha showed the highest yield. In the plot of Roundup and Yia applied alone, corn density and height were decreased due to the weed competition. Corn density, height and yield severely decreased for dense weed in 1997(Table 4).

Table 3. Weed control efficacy 45 days after application (%)

Herbicide	Rate ml/ha	Grass weed		Broadleaf weed		Weed community	
		Density	Biomass	Density	Biomass	Density	Biomass
Roundup	1500	13.2	25.0	38.3	19.7	23.5	23.6 e
Roundup	2250	38.4	48.2	43.6	26.2	40.5	42.4 d
Yia	1500	42.5	23.1	93.2	91.8	63.2	41.3 d
Yia	2250	64.4	52.5	96.2	97.5	77.3	64.4 c
Roundup+Yia	1500+1500	70.9	84.5	90.0	92.5	78.7	86.6 b
Roundup+Yia	2250+1500	80.0	86.3	92.8	97.0	85.2	89.1 b
Roundup+Yia	1500+2250	78.7	92.6	96.0	95.3	85.7	93.3 ab
Roundup+Yia	2250+2250	86.5	96.0	96.0	98.2	90.3	96.6 a
None		0	0	0	0	0	0

Table 4. Corn yield when treated with Roundup mixed Yia

Treatment	Rate (ml/ha)	Density (No./plot)		Corn height (cm)		Yield (kg/plot)	
		1997	1998	1997	1998	1997	1998
Roundup	2250	90	148	105.0	165.0	11.3 bc	16.0 b
Yia	2250	110	151	132.3	171.1	14.0 b	15.0 b
Roundup+Yia	2250+2250	115	150	149.5	168.9	18.2 a	19.2 a
None		50	148	69.0	175.6	4.9 d	10.4 c

Previous study has shown that tank mixtures of Glyphosate with other herbicides for example atrazine and alachlor, would cause antagonism on some weed species (Appleby and Somabhi 1978). In this study, Roundup and Yia was not antagonistic at any of the rates evaluated. That was probably because of the different weed species in experiments. Tank mixing Roundup with Yia alleviated the need for Yia rate and would be beneficial to the farmers, since the corn growers usually applied Yia in a great rate to control the emerged grass weeds which would cause more damage to the growth and yield of the following winter wheat.

### ACKNOWLEDGMENTS

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## THE DOMINANT WEED SPECIES AND THEIR CONTROL IN CORN FIELD IN HEBEI PROVINCE

Y. J. Yang<sup>1</sup>, F. R. Yuan<sup>2</sup>, and X. J. Li<sup>3</sup>

<sup>1</sup>General Plant Protection Station of Hebei Province, Shijiazhuang 050031, China.  
hbifoc@public.sj.he.cn

<sup>2</sup>Plant Protection Station of Chengde, Hebei 067000, China

<sup>3</sup>Institute of Cereal and Oil Crops, Hebei Academy of Agric. Sciences, Shijiazhuang 050031, China,

**Abstract:** Weed survey was conducted in the corn field in Hebei province from 1997-2000. According to the survey, there were more than 30 weed species including the dominant weeds of crabgrass (*Digitaria ciliaris*), barnyardgrass (*Echinochloa crusgalli*), field bindweed (*Convolvulus arvensis*), goosegrass (*Eleusine indica*), green foxtail (*Setaria viridis*), purselane (*Portaulaa olerancea*), redroot pigweed (*Amarinthes retroflexes*). It was difficult to control the weeds in the corn field for they growing flourish in the high temperature and rainy season. Chemical control is the effective method in this case and also wheat straw mulching could help to reduce the herbicide rate.

**Key words:** Dominant weeds, weed control, corn field

### INTRODUCTION

Corn is one of the important crops in Hebei province north China. The corn planting area covers about 2 million hectares every year. Weed is the main problem that effects the corn yield. During the corn growing season weed growing flourishing due to the high temperature and the raining season. Hand weeding of weeds seems very difficult because it is not so easy to work in the field in this kind of climate. Corn yield is reduced by 10% to 30% according to the situation. Therefore, it is necessary to make clear the dominant weed species, their dynamics and yield reduction ratio in the corn field so that the appropriate control technology should be provided. The objective of this paper is to give a general introduction of the dominant species of weeds and their control method in the corn field based on the author's study in the past few years.

### MATERIALS AND MATHODS

#### Weed survey

Weed survey was conducted in the whole province from 1997-2000, which covered more than 30 counties with ten-one square meters samples of each according to the corn distribution. Weed species, height, frequency, coverage and the biomass were measured in every spot.

#### Weed dynamics and corn yield reduction study

This study is conducted in Shijiazhuang. Which located in south Hebei province. 10 one square meter spots were set up with the distance of 50 meters between every

two spots in corn field in mid June. Corn was planted as soon as winter wheat harvested. Weed density and biomass were measured during corn growing season.

In yield reduction study, weeds were allowed to grow together with corn for 10,15,20,30,40,50,60 days after corn planting. Following this competitive period, corn was maintained weed-free for the rest time of the growing season to determine the duration for weed competition. Corn yield was measured in the harvesting time.

### **Chemical weed control study**

The soil was disked as soon as winter wheat harvested and then soil surface was made smooth and no weeds by human labor before corn planted. Different herbicides and their mixtures were applied before corn emerged. A human air-pressured sprayer was used to deliver 750L/h.

Plots were rated 90 days after the herbicide applications and weed biomass were measured 45 day after application. All control ratings were based on a scale of 0(no control) to 100%(complete control). Corn injury was visually determined at the same time. Corn yield was measured from each plot by hand harvesting.

### **Wheat mulching study**

The crop growing system was winter wheat-summer corn double cropping system. Corn was planted as soon as wheat harvested in early June. Wheat was harvested in early June and corn was harvested in later September. The plots were mulched with dry wheat straw of 0, 2250, 3375 and 4500kg/h just after corn planted. The plots were irrigated as soon as they were mulched. The density and biomass of weeds of three 0.25m<sup>2</sup> areas in each plots were measured 30 days after corn emerged.

Economic effect of three control methods including Atrazine 1500ml/h plus lasso 1500ml/h, hand weeding and weed straw mulching were compared by weed control efficacy, corn yield, human labor consuming and net income of the farmers .

All data were subjected to analysis of variance and means of the data averaged over years(no year interaction occurred for any parameters) were separated by a Fisher's Protected LSD test at the 5% level of probability and all possible interaction of years and treatment factors were tested.

## **RESULTS AND DISCUSSION**

### **Weed survey**

30 weed species were found in the corn field with the dominant weed species of crabgrass (*Digitaria ciliaris*), barnyardgrass (*Echinochloa crusgalli*), green foxtail (*Setaria viridis*), india lovegrass (*Eragrostis plosa*), common purslane (*Portulaca oleracea*), field bindweed (*Convolvulus arvensis*), redroot pigweed (*Amarinthes retroflexes*) and so on (Table1). Crabgrass is the most dominant weed species with the frequency of 92.8% and the corn yield reduction of 73%.

### **Weed dynamics**

Weed dynamics in corn field was showed in Figure 1. Weeds were emerged at the same time of corn emergence. Most of the weeds emerged when corn was at 5-6 leafstages. Then weed density decreased due to weed competition. Weed biomass continued increasing. Yield reduction study indicated that from the time of corn planted to 20 days after corn planted was the duration for weed competition. During

this period weeds grown faster than corn seedlings in the humid climate. Corn yield was reduced a lot if weeds were not very well controlled.

Table 1. The dominant weeds in Hebei province in the corn field

Weed species	Frequency %	Yield reduction ratio %
crabgrass	92.8	73.0
purslane	78.0	14.0
copperleaf	76.0	0
goosegrass	74.4	15.0
redroot pigweed	52.8	7.0
goosefoot	41.0	10.0
nutgrass flatsedge	25.0	2.0
barnyardgrass	19.0	0
green foxtail	10.0	0
siberian cocklebur	9.0	0
india lovegrass	<5	0
field bindweed	<5	0

### Chemical weed control study

Different weed control efficacy was obtained from the different herbicides.

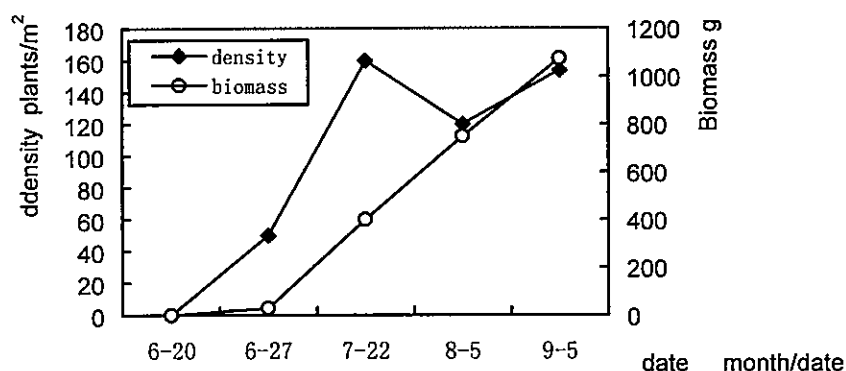


Fig.1 Weed dynamics in the corn field

Herbicide mixtures usually shown better weed control comparing with applying only one herbicide. Atrazine tank mixture with alachlor or metolachlor brought both the best weed control and the highest yield among the applying herbicides. Applying atrazine only controlled broadleaf weed better but shown relative worse control on grassweeds. Besides, atrazine applying at a large rate would injure the following crops for example winter wheat. Applying alachlor or metolachlor only would increase the herbicide cost of the farmers even they gave good weed control for the high price of the two herbicides(table 2).

### Wheat straw mulching experiment

Table 3 illustrated the weed control efficacy in corn field after winter wheat mulching. It is obvious that weed control efficacy increased as the mulching rate increasing especially when the straw was cut shorter and spread uniform. The appropriate winter

wheat straw mulching rate was 4500kg/h considering both weed control efficacy and corn yield. Higher mulching rate would cause the worse corn emergence and weak seedlings that decreased the corn yield..

Table 2. Weed control efficacy in different herbicides

Treatments *	Weed Density (m <sup>-1</sup> )	Weed Biomass (g)	Weed control efficacy(%)	Corn yield (kg ha <sup>-1</sup> .)	Yield increasing (kg ha <sup>-1</sup> .)
A	10.1	54.58	88.7	6600	555
B	16.8	62.09	85.6	6975	930
C	15.5	57.59	86.1	6990	945
D	12.8	43.8	92.6	7140	960
E	14.3	43.4	91.8	7050	1005
F	8.3	37.5	95.7	7056	1020
G	25.3	72.5	85.3	6975	930
H	174.0	500.8	-----	6045	-----

\*A,B,C,D,E,F,G and H indicate atrazine 4500ml ha<sup>-1</sup>, alachlor 3000ml ha<sup>-1</sup>, metolachlor 3000ml ha<sup>-1</sup>, atrazine 1500ml+alachlor 1500ml ha<sup>-1</sup>, atrazine 1500ml+ tavron1500ml ha<sup>-1</sup>, atrazine 1500ml+metolachlor 750ml ha<sup>-1</sup>, atrazine 1500ml+ acetochlor1125ml ha<sup>-1</sup> and weedy check, respectively.

Table 3. The weed control efficacy and corn yield in wheat straw mulching

Mulching rate (kg ha <sup>-1</sup> )	Weed control efficacy (%)	Corn yield (kg ha <sup>-1</sup> .)	Yield increasing (kg ha <sup>-1</sup> .)
4500	93.4	7114.5	811.5
3750	89.3	6601.5	610.5
2250	85.6	6247.5	346.5
6000	62.3	5882.7	200.5

In the 4500kg h<sup>-1</sup> plot, the organic matter, the rapidly available phosphorus, potassium and nitrogen increased by 0.14%, 17.2mg kg<sup>-1</sup>, 23mg kg<sup>-1</sup> and 30.4mg kg<sup>-1</sup>, respectively. Therefore, whe

at straw mulching also had the effect of improving the soil structure and increasing fertility content. But wheat mulching also had some worse effects such as it benefited some pests which caused the damage of the corn and the following crops.

Table 4. Economic effect of different method of control weeds.

Treatments*	Weed control efficacy (%)	Corn yield (kg/ha.)	Yield increasing (kg/ha.)	Human labor per hectare	Net income increasing (RMB yuan/ha.)
A	92.6	7005	960	7.5	445.5
B	71.3	6750	705	67.5	204.0
C	87.65	7110	796.5	4.5	361.5

\*A,B,C indicate atrazine1500ml+alachlor1500ml/ha, hand weeding and wheat mulching, respectively.

According to the economic effect evaluation of different weed control methods, chemical control which used atrazine and alachlor mixtures provided the highest



economic effect for the best weed control efficacy and the highest corn yield (Table 4).

The dynamics and yield reduction of the weed species was studied after then. Flixweed as the most dominant biannual weed with the frequency of 98.5%, the density of 19.1 plants m<sup>-1</sup> and the coverage of 80% based on the study caused significant yield reduction of winter wheat if their density reached 10 plants m<sup>-1</sup>. Different herbicides were tested to control the weeds while Express and Harmony were well worked herbicides.

## WEEDS AND THEIR CONTROL IN WHEAT IN JIANGSU

X. Y. Ma

Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences,  
Nanjing 210014, China. wlppi@jaas.ac.cn

**Abstract:** The strata cover and multiplied dominance ratio methods have been employed in extensive survey of weediness and crop-weed community composition and have promoted monitor and survey of weed shifts in wheat. Investigations showed that in post-rice wheat of Jiangsu, dominant and troublesome weed species are mostly hygrophilous grasses, eg. *Alopecurus japonicus*, *Beckmannia syzigachne*, *Sclerochloa kengiana*. Change from traditional cultivation to minimum and zero-tillage in post-rice wheat planting has favored and significantly increased infestation of hygrophilous grass species which prefer higher soil moisture. Minimum and zero-tillage combined with serial application of chlortoluron from middle to the end of 1980's resulted in shift of pre-dominant weed species from *Alopecurus aequalis* to *A. japonicus*. On basis of soil residual studies and weed shifts in 1990's, mixture formulations of low-dosage chlorsulfuron with isoproturon, chlortoluron or fenoxaprop-p-ethyl have been applied extensively in neutral or acidic soils of wheat-rice double cropping districts for effective control of *A. japonicus*, *B. syzigachne*, *S. kengiana*, *Galium aparine* and other broad-leaved weeds.

**Keywords:** *Alopecurus japonicus*, chlorsulfuron, hygrophilous grass, multiplied dominance ratio, post-rice wheat.

### INTRODUCTION

Since the 1970's, as a result of reduction of green manure crop, barley and enlargement of wheat planting acreage, the proportion of rice-wheat continuous cropping has reached 70%~80% in many districts. Extensive application of minimum and zero-tillage in wheat planting from the 1980's facilitate preservation of soil moisture, early sowing of wheat, encourage early germination of weed seeds, consequently increase rapidly infestation of hygrophilous grass weeds. Wheat yield loss caused by weeds surpassed that by diseases and insect pests from the middle of the eighth decade till the beginning of the ninth decade in ordinary year.

By joint effort of agricultural research institutes, university (college), extension organizations and herbicide industries, significant progress has been achieved.

### THE IMPORTANT WEED SPECIES IN WHEAT

Jiangsu province is a transitional district from the sub-tropical zone to the warm temperate zone.

There are 161 weed species in wheat, belonging to 32 families and 96 genera. Most of the weed species are over-winter annuals, some are spring annuals and perennials.

There are three main types of double-cropping systems: wheat-rice, wheat-upland crops, and wheat-rice (1st year)—wheat-upland crops (2nd year). Over 60% wheat areas are post-rice wheat. Weed flora of post-rice wheat fields is distinctly different from that of upland wheat (Table 1).

Table 1. Important weeds in wheat of Jiangsu

Family	Species	Post -rice wheat	Upland wheat
Gramineae	<i>Alopecurus aequalis</i>	+	
	<i>A. japonicus</i>	++++	
	<i>Sclerochloa kengiana</i>	+++	
	<i>Avena fatua</i>	++	++
	<i>Beckmannia syzigachne</i>	+++	
	<i>Polypogon fugax</i>	++	
	<i>Poa annua</i>	+	+
Rubiaceae	<i>Galium aparine</i>	+++	++
Scrophulariaceae	<i>Veronica persica</i>		++
Leguminosae	<i>Vicia sativa</i>	++	++
Boraginaceae	<i>Lithospermum arvense</i>		++
Caryophyllaceae	<i>Malachium aquaticum</i>	++	
	<i>Silene conoidea</i>		+
Cruciferae	<i>Descurania sophia</i>		++
	<i>Capsella bursa-pastoris</i>	++	+++
Compositae	<i>Hemistepta lyrata</i>	++	+
	<i>Lapsana apogonoides</i>	++	
Euphorbiaceae	<i>Euphorbia helioscopia</i>	++	++
Convolvulus	<i>Calystegia hederacea</i>	+	+
Geraniaceae	<i>Geranium carolinianum</i>	++	++

Among the 20 important species listed seven species infest only post-rice wheat, four species harm only upland wheat, whereas nine species infest both post-rice wheat and upland wheat. In post-rice wheat, dominant and troublesome weed species are mostly hygrophilous grasses (eg *A. japonicus*, *S. kengiana*, *B. syzigachne*) with noxious broad-leaved weeds as sub-dominant species (eg *G. aparine*, *V. sativa*). In upland wheat dominant species are mostly broad-leaved weeds, with the exception of *A. fatua* in some districts. Infestations of weeds are usually heavier in post-rice than upland wheat, due to higher soil moisture and fertility.

Weeds in wheat may be classified into three categories according to their adaptations to soil moisture.

1. Hygrophilous weeds: seeds over-summer and survive well in paddy fields, viability decreases significantly when over-summer in upland fields; seeds small, seed germination requires higher soil moisture; infest only post-rice wheat, eg *A. aequalis*, *A. japonicus*, *B. syzigachne*, *S. kengiana*, *M. aquaticum*.

2. Xerophytic weeds: seeds lose viability when over-summer in paddy fields, infest only upland wheat eg *V. persica*, *D. sophia*.

3. Weeds adapted to different soil moisture conditions, their seeds over-summer and survive well both in paddy and upland fields, eg *G. aparine*, *V. sativa*, *C. bursa-pastoris* (Lou et al. 1998; Ma 1993, 1994).

Since the 1980's large area surveys of weed infestations in wheat have been carried out constantly to make clear the distribution and weediness of important weeds, shifts of dominant weed populations in the six wheat-ecological districts. Surveys of wheat-weed communities have been conducted in different districts since the end of the eighth decade.

The "3 strata, 3 grade method for survey of weediness" according to strata coverage has been widely used in Jiangsu (Ma 1983). In 1988 the author proposed the multiplied dominance ratio (MDR) method in the study of crop-weed communities, when investigating wheat communities in Southern Jiangsu. The MDR value is obtained by multiplying coverage (C), relative height (H') and frequency (F).

$$\text{MDR}_2 (\%) = (C \times H') \times 100 \quad \text{MDR}_3 (\%) = (C \times H' \times F) \times 100$$

$$\text{MDR}' (\text{relative MDR}) \text{ of a given species} = (\text{MDR of the species}) / (\text{the sum of MDR of major species}) \times 100$$

MDR<sub>2</sub> and MDR<sub>3</sub>' represent respectively the importance value of the weed species in a field and a district. This method has been applied successfully in quantitative analysis of floristic composition, classification of weed communities and assessment of weediness in wheat and many other crops in several districts of Jiangsu and also in other provinces (Ma 1993). It has been applied by the Wuxian County Agricultural Research Institute to monitor yearly and predict the trends of population shifts and succession of weed communities in wheat since 1992 (Qian et al. 1998).

Investigations showed that there have been important shifts of dominant weed populations in post-rice wheat during the last twenty years. From the middle to the end of 1980's, *A. japonicus* has replaced *A. aequalis* as the predominant grass species; among broad-leaved weeds, *G. aparine* has surpassed *V. sativa* and *M. aquaticum* to become the first important weed species. Since the 1990's, *Sclerochloa kengiana* and *B. syzigachne* have been spreading rapidly, occupying second-position among grass weeds in Northern and Southern Jiangsu respectively; *A. fatua*, *P. fugax*, *P. annua* and dicotyledonous weeds *L. apogonoides*, *E. helioscopia* and *G. carolianum* are also increasing. Factors contributing to shifts of weed populations are as follows:

1. Shift of soil cultivation system: non and minimum tillage of post-rice wheat fields preserve well soil moisture and favor the development of hygrophilous grasses which prefer higher soil moisture, eg *A. japonicus* and *B. syzigachne*. *B. syzigachne* infestations are usually heavier in lowland and fields under poor drainage.

2. Continuous application of chlortoluron and MCPA in 1980's promoted the spread of less chlortoluron-susceptible *A. japonicus* and MCPA tolerant *G. aparine*. Long term application of chlorsulfuron has been encouraging the spread of less susceptible *B. syzigachne* and tolerant species *A. fatua*, *S. kengiana* and *L. apogonoides* since the 1990's.

3. Adaptation or adaptive evolution of weed species Several weed species have been spreading rapidly from upland wheat, field ridges or roadside to post-rice wheat, eg *A. fatua* (post-rice wheat ecotype), *E. helioscopia* and *G. carolianum*.

4. Rapid and ignored transmission of weed seeds by contaminated wheat seeds, eg *A. fatua*, *G. aparine*, *V. sativa*, *E. helioscopia*. Species with small and light seeds can be transmitted by irrigation water, eg *B. syzigachne* and *S. kengiana* (Lou et al. 1998; Qian et al. 1998).

## CHEMICAL CONTROL OF WEEDS

In the 1980's, chemical weed control was focused on the widely distributed *A. aequalis*, *A. japonicus* and *G. aparine*. Frequent drought in autumn often brought trouble to the control of *A. aequalis* by chlortoluron. Integrated with minimum and non-tillage of wheat planting, timing of chlortoluron application was changed from post/

rototiller) in minimum tillage to make full use of the soil moisture after rice harvest and increase control efficacy. *G. aparine* and other broad-leaved weeds were controlled by dicamba+MCPA or bentazon+MCPA.

The shift of dominant weed population from *A. aequalis* to *A. japonicus* + *G. aparine* mixed populations in most parts of the wheat-rice double cropping area since the middle of 1980's has made control by chlortoluron difficult. Rapid, extensive and heavy infestation of weeds urgently requires highly effective, broad spectrum and economic herbicides. Chlorsulfuron was the only fitted new herbicide. Experiments showed that carryover of this herbicide caused injury and yield losses of succeeding upland crops eg. maize, soybean, cotton etc. In districts with neutral or light acidic soil pH, under wheat-rice double cropping, autumn application of chlorsulfuron 15g a.i./ha in post-rice wheat, residual activity disappeared quickly after transplanting, rice seedlings recovered rapidly without deleterious effects to *O. japonica* variety (safety margin  $\geq 2$ ), high dosages caused yield losses of rice (Ma et al 1992).

Residual study of chlorsulfuron in wheat-rice double cropping district of Southern Jiangsu (soil pH 5.95) revealed that autumn application of 15g a.i./ha chlorsulfuron in wheat, chlorsulfuron residue in soil was 0.22 $\mu$ g/kg at rice transplanting the next year. 96.8% of applied chlorsulfuron was degraded and the degradation half-life of chlorsulfuron in soil was 38.6d. *O. japonica* was injured at residue concentration of 0.17 $\mu$ g/kg. Although *O. japonica* is highly sensitive to chlorsulfuron, as chlorsulfuron is easily degraded in flooded soil, its actual damage to succeeding rice is light, and the improving of soil fertility may affiliate to some extent the effects of chlorsulfuron to plant (Cai et al. 1995; Shan et al 1995). Since degradation rate of chlorsulfuron in soil is affected by many factors eg climate, soil pH, and soil moisture, it was suggested that rate of chlorsulfuron in herbicide mixture be limited at 7.5g a.i./ha. Mixtures of chlorsulfuron with isoproturon, chlortoluron and fenoxaprop-p-ethyl as one shot herbicides, give good control of *B. syzigachne*, *S. kengiana*, *G. aparine*, other important grass and broad-leaved weeds.

From 1990-1994 chemical weed control area in wheat of Jiangsu attained 1334-1667 thousand ha each year. From 1992 to 1995, application area of mixtures including chlorsulfuron in wheat-rice double cropping districts of Southern and Middle Jiangsu reached over 667 thousand ha each year and contributed considerably to control of weeds and increase of yield. Due to deviations in recommendation and application, there were also carry over injury of chlorsulfuron to succeeding upland crops and sometimes to rice. The less persistent sulfonylurea herbicides eg tribenuron and their mixtures are going to extend for the control of grass and broad-leaved weeds in post-rice wheat of light alkali soil districts and in upland wheat.

Bioassay and test of ALS activity showed that, after 6 successive years of chlorsulfuron application in experiment field in Kun Shan, significant resistance was expressed by *B. syzigachne* and *M. aquaticum* (Li 1996). Resistance of weeds in large area is potential and not pronounced.

## INTEGRATED CONTROL OF WEEDS

Herbicide application integrated with local cultural measures have been practiced in many regions and received good results.

1. Rotation of wheat with rape facilitates the control of problematic grass weeds by application of highly effective graminicides in rape combined with strong crop

competition. Rotation of upland crops with rice significantly decreases the longevity and viability of hygrophilous grass weeds eg *A. japonicus*, *S. kengiana* and *P. fugax*. Proceeding adjustments of agricultural production and cropping structure, reduction of wheat sowing area, will be beneficial to weed control in wheat.

2. Cultivation Hygrophilous grasses eg *A. japonicus* and *B. syzigachne* germinate early and massively before rice harvest. Shallow cultivation by rototiller while or just after wheat sowing kills efficiently weed seedlings and germinating seeds, and facilitates chemical control of heavy weed infestations. Good and early drainage is implemented for control of *B. syzigachne* and *A. japonicus*, especially in non-tillage fields.

3. High yield culture of wheat to maximize crop competition and cleaning of wheat seeds to eliminate seeds of *A. Fatua*, *G. aparine*, *V. sativa*, *L. arvense* etc.

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## **EFFECT OF THICKNESS OF POLYETHYLENE AND DURATION OF SOIL SOLARIZATION ON HEAT CONDUCTANCE, WEED GROWTH AND YIELD OF GROUNDNUT (*ARACHIS HYPOGAEA*)**

H. V. Nanjappa, B. K. Ramachandrappa, and H. C. Appajigowda  
Department of Agronomy, College of Agriculture, University of Agricultural Sciences,  
GKVK, Bangalore - 560 065, India. priya\_n@satyam.net.in

**Abstract:** Field experiment was carried out for two years during 1998-99 at the Main Research Station of the University of Agricultural Sciences, Bangalore, India to study the effect of thickness of polyethylene sheet and duration of soil solarization on heat conductance, weed control and yield of groundnut. The study comprised of 10 treatments consisting of combination of three thickness of transparent polyethylene sheets viz., 0.05, 0.10 and 0.15 mm and three durations of soil solarization viz., 15, 30 and 45 days during April and May months and a control. The results revealed that 0.05 mm transparent polyethylene (TPE) was efficient in enhancing the soil temperature and reducing the weed growth as compared to 0.01 and 0.15 mm thickness. Increasing the duration of soil solarization from 15 to 45 days resulted in higher heat conductance and consequent reduction in weed number and dry weight. Soil solarization with 0.05 mm TPE for 45 days registered significantly higher pod yield over control.

**Key words:** Duration, heat conductance, solarization, thickness, weed control.

### **INTRODUCTION**

Eco-friendly methods of weed management attained greater significance in the recent past in view of increased global awareness about the healthy environment and originally raised foods. Soil solarization is a method of heating the surface soil by using polyethylene sheets placed on moist soil to trap solar radiation to raise the soil temperature and killing of weeds in the soil. The soil temperature in solarized plots at 5 cm soil depth reached 50°C (Katan, 1980). The effective control of weeds due to solarization was reported by Yaduraju and Ahuja (1990), and Mudalagiriappa et al. (1999) in different crops. Hence, a comprehensive field study was conducted to study the effect of thickness of polyethylene and duration of soil solarization on heat conductance of soil solarization on weed growth and yield of groundnut under alfisols.

### **MATERIALS AND METHODS**

Field experiment was conducted during 1998 and 1999 on sandy loam soil at the Agronomy field unit, Main Research Station, Hebbal, University of Agricultural Sciences, Bangalore. The pH was 6.5 and the organic carbon was 0.37 per cent. The study comprised of 10 treatment consisting of combination of three thickness of transparent polyethylene sheets such as 0.05, 0.10 and 0.15 mm and three durations viz., 15, 30 and 45 days and a control tried in Randomized Block Design with three replications. The land

was brought to fine replications. The land was brought to fine tilth and laid out according to plant. Each plot was levelled and irrigated to bring the soil moisture to field capacity. The transparent polyethylene sheets of required thickness and size were spread on the respective plots depending on the treatments during April and May months and were sealed on all the sides to make it air tight using moist soil. Soil temperature was recorded using piercing type of mercury thermometer at 5 and 10 cm soil depths in both covered and uncovered plots between 2 PM and 3 PM. The hole made in TPE film while recording soil temperature was pasted with transparent gum tape.

The polyethylene sheets were removed after the respective period of soil solarization. Groundnut cv. TMV-2 was sown immediately after solarization in line at a spacing of 30 cm x 10 cm. The crop received 25 : 75 : 38 kg N : P<sub>2</sub>O<sub>5</sub> : K<sub>2</sub>O ha<sup>-1</sup> at sowing. The observations on weed count, dry weight and yield were recorded.

## RESULTS AND DISCUSSION

### Weed flora

In groundnut the important monocotyledonous weeds observed were *Cynodon dactylon*, *Digitaria marginata*, *Dactyloctenium aegyptium* and *Dicanthium annulatum*. The dominant dicotyledonous weeds were *Acanthospermum hispidum*, *Commelina benhalensis*, *Amaranthus viridis*, *Lagascea mollis*, *Euphorbia hirta*, *Portulaca oleracea*, *Parthenium hysterophorus*, *Phyllanthus niruri*, *Bidense pilosa*, *Borreria hispida* and *Polygonum pletezum* while, the *Cyperus rotundus* was the prominent sedge in the crop.

### Effect on soil temperature

Soil solarization had marked influence on soil temperature during 1998 soil solarization with 0.05 mm TPE raised the soil temperature from 36.67°C in control to 51.33°C (Table 1). However, during 1999 the raise in soil temperature was least due to interference of rains and cloudy weather. The degree of raise in soil temperature degreased as the thickness of TPE increased from 0.05 mm to 0.15 mm owing to higher radiation transmittance. Thus agreeing with the findings of Lodha (1989). The increase in duration of soil solarization from 15 to 45 days further increased the soil temperature during both the years. This was due to longer period required for CO<sub>2</sub> buildup and leaf generation under soil solarization.

### Effect on weeds

Total weed count and dry weight varied significantly due to thickness of polyethylene and duration of soil solarization except weed dry weight of 30 DAS (Table 2). Soil solarization with 0.05 mm TPE for 45 days significantly reduced the weed count dry and weight compared to control as a consequence of rise in soil temperature (Elmore *et al.*, 1993). Greater rise in soil temperature by 0.05 mm TPE caused the death or damage of the weed seeds present in the soil to a greater extent resulting in reduced weed count and number. In general, the efficacy of soil solarization decreased with respect to weed control as the thickness of polyethylene increased and the duration of soil solarization decreased.



### Effect on crop yield

Pod yield of groundnut different significantly due to thickness of polyethylene and duration of soil solarization during 1999 (Table 3). Solarization with 0.05 mm TPE for 45 days caused significantly higher pod yield (25 q ha<sup>-1</sup>) compared to all other treatments. This increase in yield was a consequence of higher pod weight (26 g plant<sup>-1</sup>) number of pods (22.60) and 100 kernel weight (51 g). Similar trend was observed during 1998. But, there was no significant difference in pod yield and 100 kernel weight. The increase in pod yield and yield components could be attributed to the better control of weeds as evident from reduced weed count and dry weight due to soil solarization.

Thus, it can be inferred that soil solarization with 0.05 mm TPE for 45 days during summer months of April and May, when the land is vacant would be effective in rising the soil temperature which has lethal effect on dominant weeds in groundnut and is the cause for enhancing productivity of groundnut.

Table 1. Soil temperature (°C) as influenced by thickness of polyethylene and duration of soil solarization.

Treatments	1998					
	15 DAPT		30 DAPT		45 DAPT	
	0-5	5-10	0-5	5-10	0-5	5-10
TPE 0.05 mm for 15 days	52.00	51.33	-	-	-	-
TPE 0.05 mm for 30 days	52.33	51.00	46.33	45.00	-	-
TPE 0.05 mm for 45 days	52.33	51.00	46.67	45.33	54.33	52.67
TPE 0.10 mm for 15 days	51.33	51.00	-	-	-	-
TPE 0.10 mm for 30 days	51.00	49.67	45.00	44.00	-	-
TPE 0.10 mm for 45 days	51.33	49.67	45.33	44.00	53.67	52.00
TPE 0.15 mm for 15 days	50.00	48.67	-	-	-	-
TPE 0.15 mm for 30 days	50.33	48.67	44.67	43.67	-	-
TPE 0.15 mm for 45 days	50.33	49.00	44.33	43.67	53.00	51.67
Control	36.67	35.67	40.00	37.67	44.00	40.67

Table 1. Continued.

Treatments	1999					
	15 DAPT		30 DAPT		45 DAPT	
	0-5	5-10	0-5	5-10	0-5	5-10
TPE 0.05 mm for 15 days	48.60	47.60	-	-	-	-
TPE 0.05 mm for 30 days	48.60	40.60	46.30	45.30	-	-
TPE 0.05 mm for 45 days	47.00	45.60	47.30	45.30	53.60	53.00
TPE 0.10 mm for 15 days	48.30	48.30	-	-	-	-
TPE 0.10 mm for 30 days	47.30	46.00	45.00	43.60	-	-
TPE 0.10 mm for 45 days	48.30	45.30	46.30	46.30	52.30	51.00
TPE 0.15 mm for 15 days	48.00	47.00	-	-	-	-
TPE 0.15 mm for 30 days	48.30	47.00	45.00	44.60	-	-
TPE 0.15 mm for 45 days	47.60	45.00	45.00	44.00	51.00	51.30
Control	47.60	46.30	41.00	41.00	40.60	39.60

DAPT - Days after polyethylene tarping.

Table 2. Weed count (No. m<sup>-2</sup>) and dry weight (g 0.25 m<sup>2</sup>) in groundnut as influenced by thickness of polyethylene and duration of soil solarization.

Treatments	1998			
	Weed count		Weed dry weight	
	30 DAS	60 DAS	30 DAS	60 DAS
TPE 0.05 mm for 15 days	6.82(46.10)	7.77(60.49)	2.52(5.87)	3.45(11.45)
TPE 0.05 mm for 30 days	4.61(20.77)	6.32(39.50)	2.25(4.60)	3.04(8.75)
TPE 0.05 mm for 45 days	2.73(6.99)	3.58(12.33)	1.81(2.79)	2.24(4.53)
TPE 0.10 mm for 15 days	7.15(50.75)	8.46(71.20)	2.89(7.89)	3.79(13.91)
TPE 0.10 mm for 30 days	5.17(26.32)	7.10(49.99)	2.29(4.75)	3.12(9.28)
TPE 0.10 mm for 45 days	3.84(14.81)	5.36(28.32)	2.11(3.96)	2.70(6.82)
TPE 0.15 mm for 15 days	7.84(61.08)	9.11(82.55)	3.08(8.99)	4.24(17.55)
TPE 0.15 mm for 30 days	5.79(33.10)	7.47(55.44)	2.38(5.18)	3.38(10.99)
TPE 0.15 mm for 45 days	4.20(17.21)	5.98(35.33)	2.10(3.93)	2.83(7.54)
Control	6.99(48.48)	11.84(139.87)	3.99(15.46)	5.39(28.64)
SEm±	1.11	0.96	1.21	0.89
CD at 5%	3.27	2.62	NS	-

Table 2. Continued

Treatments	1999			
	Weed count		Weed dry weight	
	30 DAS	60 DAS	30 DAS	60 DAS
TPE 0.05 mm for 15 days	9.51(93.33)	9.64(96.00)	7.54(57.33)	7.40(54.66)
TPE 0.05 mm for 30 days	10.17(105.33)	11.66(136.00)	4.94(25.33)	5.69(34.66)
TPE 0.05 mm for 45 days	9.72(96.00)	9.63(81.33)	6.99(49.33)	7.50(57.33)
TPE 0.10 mm for 15 days	5.98(49.33)	9.24(85.33)	8.27(68.00)	8.82(77.33)
TPE 0.10 mm for 30 days	9.11(84.00)	8.74(77.33)	5.69(32.00)	6.65(44.00)
TPE 0.10 mm for 45 days	8.78(77.33)	9.22(85.33)	6.95(48.00)	7.94(62.66)
TPE 0.15 mm for 15 days	9.82(97.33)	10.29(106.66)	8.02(64.00)	8.65(74.66)
TPE 0.15 mm for 30 days	9.45(94.33)	10.01(105.33)	7.14(50.66)	7.85(61.33)
TPE 0.15 mm for 45 days	7.95(65.30)	8.81(80.00)	7.76(60.00)	8.51(72.00)
Control	12.69(165.33)	13.56(189.33)	11.99(145.33)	12.29(153.33)
SEm±	1.06	0.88	0.68	0.63
CD at 5%	3.09	2.58	1.99	1.85

DAS - Days after sowing; Numbers in the parenthesis indicate original values.

Table 3. Yield and yield components of groundnut as influenced by thickness of polyethylene and duration of soil solarization.

Treatments	No. of pods plant <sup>-1</sup>		Pod weight(g plant <sup>-1</sup> )		100 kernel weight(g)	
	1998	1999	1998	1999	1998	1999
TPE 0.05 mm for 15 days	16.3	15.6	9.1	19.3	23.9	48.1
TPE 0.05 mm for 30 days	18.7	19.0	11.2	20.5	25.1	50.0
TPE 0.05 mm for 45 days	22.1	22.6	13.0	26.0	26.51	51.0
TPE 0.10 mm for 15 days	15.0	17.0	8.11	16.6	23.3	42.66
TPE 0.10 mm for 30 days	17.8	17.0	10.0	17.6	24.9	45.3
TPE 0.10 mm for 45 days	20.6	19.0	11.5	19.1	26.2	46.33
TPE 0.15 mm for 15 days	14.6	14.3	8.0	12.0	22.35	40.6
TPE 0.15 mm for 45 days	19.8	20.0	11.2	10.6	26.1	48.1
Control	10.3	11.3	5.26	7.40	21.6	36.3
SEm±	3.63	0.58	2.29	0.39	2.07	0.50
CD at 5%	10.79	1.69	6.83	1.15	NS	1.48

Table 3. Continued

Treatments	Pod yield (q ha <sup>-1</sup> )		Haulm yield (q ha <sup>-1</sup> )	
	1998	1999	1998	1999
TPE 0.05 mm for 15 days	20.2	14.0	18.0	16.1
TPE 0.05 mm for 30 days	24.9	16.1	19.0	17.4
TPE 0.05 mm for 45 days	28.8	25.0	20.7	19.0
TPE 0.10 mm for 15 days	18.0	14.6	18.0	15.3
TPE 0.10 mm for 30 days	22.2	15.4	18.5	17.1
TPE 0.10 mm for 45 days	25.6	17.6	19.5	18.6
TPE 0.15 mm for 15 days	17.7	15.0	17.7	14.1
TPE 0.15 mm for 30 days	21.7	14.6	18.0	16.5
TPE 0.15 mm for 45 days	24.9	17.1	19.5	16.3
Control	11.6	8.4	15.3	12.8
SEm±	5.11	0.50		0.35
CD at 5%	NS	1.48		1.03

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## CONTROLLING OF COGONGRASS (*IMPERATA CYLINDRICA* (L.) RAEUSCHEL) BY SESAME (*SESAMUM INDICUM* L.) CULTIVATION

C. Premasthira<sup>1</sup>, S. Zungsontiporn<sup>1</sup>, P. Meesilpa<sup>2</sup>, and P. Chaosettakul<sup>2</sup>

1. Weed Science Sub-division, Botany and Weed Science Division, Department of  
Agriculture, Chatuchak Bangkok 10900 Thailand. cpremas@doa.go.th

2. Srisaket Sericultural Research Center, Sericultural Research Institute, Department of  
Agriculture, Srisaket Province 33000, Thailand

**Abstract:** The interference of sesame (*Sesamum indicum* L.) and cogongrass (*Imperata cylindrica* (L.) Raeuschel) was investigated in glasshouse at Weed Science Sub-division. The 2, 4 and 8 of sesame plants were grown in associated with 2, 3 and 4 cogongrass plants in each pot. The same number of these plants were solely grown in each pot as the control. The results indicated that dry weight of cogongrass grown competing with sesame plants in all treatments were less than cogongrass grown alone. But the cogongrass number did not affect on dry weight of sesame in all treatments. These results was repeated under field condition. The three sesame seed rates at 6.25, 12.5 and 18.75 kg/ha were sown in the field with high density of cogongrass. Cogongrass was cut at the soil level before sown sesame seeds. The growth of cogongrass was measured at the harvesting time of sesame. The results revealed that the growth of cogongrass in sesame cultivation plot was significantly reduced. The stem numbers and dry weight of cogongrass growth was reduced 43, 54 and 66% and 38, 60 and 68 % of the control at 6.25, 12.5 and 18.75 Kg/ha of sesame seeding rates, respectively.

**Key words:** Interference, allelopathy, competition, sesame (*Sesamum indicum* L.), cogongrass (*Imperata cylindrica* (L.) Raeuschel), growth inhibition, plant population,

### INTRODUCTION

Weed interference is a cause of growth and yield reduction. The interactions between crops and weeds were related to their species and density which were grown in association. Crop production was a reverse proportion to weed populations. Weed density increased as crop production decreased. (Buchanan *et al.* 1980, Patterson, 1990 and Stauber *et al.* 1991). Each weed species had different effect on growth and crop production (Eaton *et al.* 1976, Crowley, 1978). Depending on their competitive ability to use physiological factors or on toxic substances released by weeds to crops grown in association. These substances were called allelopathic substances (Elmore, *et al.*, 1983). In some cases, crops had more competitive ability than weeds and thus reduced weed growth. Evetts and Burnside (1975) found that dry weight of milkweed (*Asclepias syriaca* L.) grown in associated with sorghum (*Sorghum bicolor* L.) was decreased but not of sorghum. Harrison and Peterson (1991) reported that growth of yellow nutsedge (*Cyperus esculentus* L.) were decreased more than 50 % by 8-12 weeks old of sweet potatoes (*Ipomoea batatas*). These toxic substances were found in skin root of sweet potatoes. Crop plants can inhibit some crop growth. Iron and Burnside (1982) found that sunflower (*Helianthus annuus* L.) had root exudates effecting hight, fresh and dry weight of sorghum and soybean. At the present, it is believed that long term using herbicides in agriculture causes environmental pollution. Thus, the research of natural product from plants was conducted to develop for weed control.

Sesame is a common crop which has long been grown in Thailand, its products are locally used in farmer families. Sesame contains plant growth inhibiting substances that inhibit crop and weed growth (Premasthira and Zungsontiporn, 1987; Premasthira and Zungsontiporn, 1996). In the past, sesame was grown before or after major crops such as rice, corn, sorghum etc. But, now sesame was grown as a minor crop and common use for cropping system.

Cogon grass is one of 10 world worst weeds which is a perennial grass. It is a troublesome weed in orchard and rubber plantations in Thailand. Particularly, when the main crop were still young because there were wide spacing areas between these young trees which is suitable for cogon grass growth. The preliminary data indicated that the inhibitory effect of sesame on growth of cogon grass depended on sesame population. The suppression effect of sesame on the growth of cogon grass became evident when 2 sesame plants were grown in association with such weed species. (Premasthira and Zungsontiporn, 1992). Since sesame and cogon grass are allelopathic plants, thus, the interaction between these plants were investigated in glasshouse and the field.

## **MATERIALS AND METHODS**

### **Glasshouse experiment**

2, 4 and 8 plants of sesame were grown in association with 2, 3 and 4 cogon grass in each pot. These plants at the same number were solely grown in each pot as the control. These treatments were conducted in greenhouse at Weed Science Sub-division, Botany and Weed Science Division. The growth of sesame and cogon grass was measured at the harvesting time of sesame. The treatments were four replications.

### **Field experiment**

This experiment was conducted in the field at Srisaket Sericultural Research Center, Sericultural Research Institute, Srisaket Province. High density of cogon grass area was selected for this experiment. Cogon grass were cut at the soil level. Three rates of sesame seeds, 6.25, 12.5 and 18.75 Kg/ha were sown on this area. Cogon grass and sesame growth were measured at the harvesting time of sesame. These treatments were carried four times.

## **RESULTS AND DISCUSSION**

### **Glasshouse experiment**

The interaction between cogon grass and sesame was evaluated by growth measurement at the harvesting time of sesame. The results shown in table 1 indicated that dry weight/plant, height and number of rhizome of cogon grass grown in association with sesame were less than those grown alone.

Dry weight/plant of two cogon grass grown alone in pot was 29.27 g the dry weight of the same number of cogon grass grown associated with 2, 4 and 8 sesame plants were 12.38, 8.90 and 7.66 g respectively. They were significant difference from the control. Plant dry weights of two cogon grass grown associated with two, four and eight plants of sesame were decreased 57.50, 69.59 and 73.82 % of the control respectively.

Dry weight/plant of three cogon grass grown in the pot was 24.01 g while those grown in association with 2, 4 and 8 sesame were 9.80, 11.06 and 9.50 g respectively.

These plant dry weight were decreased 59.18, 53.98 and 60.43 % of the control respectively.

Dry weight/plant of the four cogongrass grown in the pot was 21.21 g while plant dry weight of these plants grown in associated with 2, 4 and 8 sesame were 7.59, 9.59 and 7.29 g respectively. These plant dry weights were decreased 64.21, 54.78 and 65.62 % comparing with control respectively.

Dry weight/plant of 2 cogongrass grown in the same pot was greater than 3 and 4 cogongrass/pot. Dry weight/plant of 4 cogongrass/pot was significant less than 2 and plants/pot.

The cogongrass grown in associated with sesame produced less rhizomes than cogongrass grown without sesame competition. The numbers of rhizome per plant of two, three and four cogongrass grown alone in the pot were 21.25, 12.4 and 13.75 when grown association with 2, 4 and 8 plants of sesame respectively. While the numbers of cogongrass rhizome were less than those without sesame.

Table1. Effect of sesame (*Sesamum indicum* L.) population on growth of cogongrass (*Imperata cylindrica* (L.) Raeuschel).

Treatment	Dry weight/plant (gm)	Shoot no./ plant	Height (cm)
2 cogon-grass ( 2 C )	29.27 a	21.25 a	100.02 a
2 C + 2Sesame ( 2 S )	12.38 c	16.75 ab	80.07 b
2 C + 4Sesame ( 4 S )	8.90 c	9.75 bcd	81.40 ab
2 C + 8Sesame ( 8 S )	7.66 c	9.60 cd	78.02 b
3 cogongrass ( 3 C )	24.01 ab	12.4 ab	94.75 ab
3 C + 2S	9.80 c	8.8 bcd	78.75 b
3 C + 4S	11.06 c	4.28 bcd	90.32 ab
3 C + 8S	9.50 c	6.81cd	94.95 ab
4 cogongrass ( 4C )	21.21 b	13.75 bc	85.10 ab
4 C + 2S	7.59 c	7.63 d	73.35 b
4 C + 4S	9.59 c	7.00 d	90.95 ab
4 C + 8S	7.29 c	8.05 cd	78.25 b

Values within column followed by same letters are not significantly different at 0.05 level according to Duncan s multiple range test

These results showed that plant dry weights and rhizome numbers of 2 cogongrass grown in associated with 2, 4 and 8 sesame plants were decreased 57.70, 69.60 and 73.83 and 21.18, 54.12 and 54.82 % respectively.

While plant dry weights of cogongrass when grown 3 plants in associated with 2, 4 and 8 sesame were decreased 59.18, 53.98, 60.43 and the numbers of rhizomes were decreased about 29.03, 65.48 and 45.08% respectively.

Similarly plant dry weights of 4 cogongrass plants grown in associated with 2, 4 and 8 sesame plants were decreased about 64.21, 54.78, 65.62 and decreasing of rhizome numbers in these treatments were 44.50, 49.09 and 41.45 % respectively.

The increase in populations of cogongrass from 2 to 3 and 4 plants/plot resulted in decrease in their dry weights and shoot numbers. The rates of reduction from 2 to 3 was greater than that from 3 to 4 plants/plot (Table 1)

It means that cogongrass has intraspecific competition which affect to plant dry weight and rhizome initiation. The intraspecific competition of plant was related to the plant populations.

The effects of cogongrass on sesame growth when they were grown in association shown in Table 2. The results revealed that plant dry weight of 2, 4 and 8 sesame grown in association with 2,3 and 4 cogongrass were significantly reduced. Plant dry weight of eight sesame was less than that of the 4 and 2 when they were grown association with 2,3 and 4 cogongrass. But, different populations of sesame grown in association with 2, 3 and 4 cogongrass were not significantly different in plant dry weight. This suggested that decreasing of sesame plant dry weight in each population was not affected by neighboring cogongrass. Sesame has intraspecific competition effect on plant dry weight. Even though the plant height of sesame was decreased when cogongrass population increased. So, decreasing of sesame plant dry weight in the same population was not caused by the increasing of cogongrass population. Its intraspecific competition may be the cause.

Table 2. Effect of cogongrass (*Imperata cylindrica* (L.) Raeuschel) population on growth of sesame (*Sesamum indicum* L.)

Treatment	Dry weight/plant(g)	Height (cm)
2 Sesame + 2 cogongrass	8.20 b	94.7 abc
2 Sesame + 3 cogongrass	10.93 a	83.57 cdef
2 Sesame + 4 cogongrass	8.59 b	76.42 ef
4 Sesame + 2 cogongrass	3.35 c	102.37 a
4 Sesame + 3 cogongrass	4.14 c	89.55 bcd
4 Sesame + 4 cogongrass	3.84 c	79.30 def
8 Sesame + 2 cogongrass	1.37 d	96.5 ab
8 Sesame + 3 cogongrass	1.49 d	82.25 bcde
8 Sesame + 4 cogongrass	2.46 d	74.42 f

Values within column followed by same letters are not significantly different at 0.05 level according to Duncan's multiple range test

These results indicated that two sesame grown associated with 2, 3 and 4 cogongrass in 490cm<sup>2</sup> reduced cogongrass dry weight more than 50 %. The rhizome numbers were also reduced 21-44 %. The growth of cogongrass was decreased as the sesame population increased. The similar experiment was undertaken in the field condition

### Field experiment

Three seed rates of sesame were sown on the uniform density on cogongrass area. The growth of cogongrass in the experimental plots was measured at the harvest time of sesame. The results shown in Table 3. Three seed rates of sesame, 6.25, 12.5 and 18.75Kg/ha reduced the growth of cogongrass. The numbers of plant, fresh and dry weight of cogongrass were less significantly than control. The cogongrass plants including of the above ground stem and under ground stem (rhizome) in the untreated plot (control) was 46.31 plants, while those of the treated plots with 6.25, 12.5 and 18.75Kg/ha of sesame seed rates were 26.88, 21.01 and 15.88 plants respectively. Dry weight/plant of cogongrass in the untreated plot was 92.04 g, while those of the treated plots were 57.01, 36.34 and 29.11g with 6.25, 12.5 and 18.75Kg/ha of sesame seed rates



respectively. The growth of cogongrass was significantly reduced by the different sesame population. The cogongrass plant number was reduced by 42, 54 and 66% and its dry weight was reduced by 38, 60 and 68% with the same sesame seed rates respectively. The growth of cogongrass decreased when sesame seed rates increased. Fresh weight of sesame were 268, 276 and 333g while dry weight were 94, 73 and 74 g in the plots of 6.25, 12.5 and 18.75 Kg/ha sesame seed rates respectively.

Table 3. The effects of sesame (*Sesamum indicum* L.) population on growth of cogongrass (*Imperata cylindrica* (L.) Reaueschel).

Treatment (Sesame seed rates)	No. of stem	No. of shoot	No. of Rhizome	fresh weight (g)	dry weight(g)
Control	46.13a	32.88a	13.25a	249.31a	92.04a
Sesame 6.25Kg/ha	26.88b	18.88b	8.0a	101.49b	57.01ab
Sesame 12.5Kg/ha	21.01b	12.63b	8.38a	100.0b	36.34b
Sesame 18.75Kg/ha	15.88b	9.50b	6.38a	82.7b	29.11b

Values within column followed by the same letter are not significantly different at 0.05 level according to Duncan multiple range test

These results indicated that sesame can inhibit growth of cogongrass. The cause of interference between sesame and cogongrass were competition and allelopathy. The competition was competitive ability of plant species to use the limited factor for plant growth. Decreasing of cogongrass growth, particularly the decreasing number of rhizome it may be a cause of sesame root exudates. Sesame was proved that it is an allelopathic plant and its substances inhibited growth on many weed seedlings. It can release some toxic substances via root exudates. It might be a cause of rhizome initiation reduced. Sesame cultivation is alternative method for cogongrass control.

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## USE OF MULCHES FOR WEED CONTROL IN ORCHARDS.

A. Rahman<sup>1</sup>, M.J. Hartley<sup>2</sup>, K.C. Harrington<sup>3</sup> and T.K. James<sup>1</sup>

<sup>1</sup>AgResearch, Ruakura Research Centre, Hamilton, New Zealand

anis.rahman@agresearch.co.nz

<sup>2</sup>367 Lawn Road, R D 2, Hastings, New Zealand

<sup>3</sup>Institute of Natural Resources, Massey University, Palmerston North, New Zealand

**Abstract:** Comparisons have been made over several years of various mulches for weed control in apple orchards in Hawkes Bay, New Zealand. The mulches tested included the organic mulches straw, sawdust, bark, compost, wooldust and low growing ground cover species creeping red fescue (*Festuca rubra*), white clover (*Trifolium repens*), dichondra (*Dichondra micrantha*) and hydrocotyle (*Hydrocotyle heteromeria*). They were compared with conventional residual and non-residual herbicide treatments for weed suppression, effect on tree growth, yield and soil bioactivities. Creeping fescue and white clover were established from seed while dichondra and hydrocotyle were planted as established plants in soil plugs, all with and without herbicide assistance. Seedlings were difficult to establish in the mature orchard. Periodic mowing was necessary with all species to prevent weeds over-growing the young ground covers. Creeping red fescue and white clover persisted without herbicide assistance while dichondra persisted only with winter herbicide treatment. Hydrocotyle failed to persist under dry summer conditions. Of the organic mulches, straw gave the best weed control and compost generally encouraged weed growth. All the other mulches allowed some weed ingress and the few weeds that established became very large and required spot treatment. Established dichondra provided good weed suppression, but only with herbicide treatment. Creeping red fescue also gave good weed suppression, with or without herbicide assistance, but needed mowing to restrict its growth. Soil temperatures in the mulch plots were slightly lower and fluctuated less than in the herbicide treated plots. Compost treatment had the highest soil respiration, soil microbial activity and earthworm numbers. There was a strong relationship between trunk growth and the total fruit yield. In the newly planted orchard the ground cover species reduced tree growth and fruit yield in the first season compared to herbicide and bark mulch.

**Key words:** Apples, green mulches, ground covers, herbicides, organic mulches, orchards, soil bioactivity

## INTRODUCTION

There is an increasing demand worldwide for a reduction in pesticide use in food production. In most New Zealand orchards weeds are controlled by herbicides applied along the tree line and mowing between rows (Dastgheib and Frampton 2000; Harrington et al. 1992). As bare soil provides a favourable environment for establishment of weeds, either residual herbicides or several applications of post-emergence knockdown herbicides are necessary throughout the growing season. Herbicide applications made during the active growing period of the tree increase the risk of chemical residues being deposited on or within the developing fruit.

One area in which pesticide use in orchards could be reduced is in weed control by the use of mulches and cover crops. An environmentally desirable strategy might be to establish a low growing ground cover species which requires little mowing and which prevents competitive weed species from establishing during the growing season. If the ground cover tolerated herbicides, any weeds which did establish could be killed during tree dormancy, so no herbicides would be necessary during the growing season when fruit could be contaminated by chemical application.

This paper provides a brief review of results from trials conducted over the past 8 years in apple orchards in Hawkes Bay, New Zealand. The objective of these trials was to compare organic mulches viz., straw, sawdust, bark, compost and wooldust and low growing ground cover species viz., creeping red fescue (*Festuca rubra*), white clover (*Trifolium repens*), dichondra (*Dichondra micantha*) and hydrocotyle (*Hydrocotyle heteromera*). They were compared with conventional residual and non-residual herbicide treatments for weed suppression and effect on tree growth and soil bioactivity. These species were selected following extensive screening work testing herbicide tolerance of ground cover species (Harrington 1995; Harrington and Grant 1993; Harrington and Rahman 1998).

## MATERIALS AND METHODS

Two large experiments have been conducted in young apple orchards near Havelock North, Hawkes Bay, New Zealand. The first experiment initiated in 1992 concentrated on organic mulches while the second experiment had the emphasis on the establishment and long term influence of low growing ground cover species on weed suppression, tree growth, fruit yield and soil bioactivity.

The first experiment had six replicate blocks, three containing 2-year old 'Braeburn' variety and three containing 3-year old 'Fiesta' variety trees. Each plot contained three apple trees. Details of the methods are given in Hartley and Rahman (1994) and Hartley et al. (1996). Briefly, soil treatments consisted of a control (no mulch, no residual herbicide), a residual herbicide (terbuthylazine originally applied in August 1992 at 4 kg ai/ha and repeated annually), or one of four organic mulches. The mulches, laid in September 1992, were untreated pine sawdust (10 cm), barley straw (10 cm), commercial compost topped with sawdust (5 cm), and wooldust (short-fibre combings containing sheep dung, 10 cm). Mulches were also laid at half thickness with a half rate of the residual herbicide underneath. Straw mulch was replenished on replicates 1-3 in September 1993, and on all replicates the following year. Sawdust was re-applied in the third year, but other mulches were not re-applied. Non-residual herbicides (glyphosate or paraquat) were applied over all plots, including the no-mulch control, as needed to prevent excessive weed growth. In a later modification of the trial, two mulches, compost and wooldust, were replaced by low growing creeping red fescue or white clover seeded directly over the old mulches (Hartley and Rahman 1998).

For this experiment visual estimates were made of percent weed cover and major species present before mulches were laid in early spring and at intervals throughout the following 18 months. Later, when ground cover species had established, total vegetation biomass was recorded by cutting samples. Soil bioactivity was assessed in two ways: (1) by measuring respiration through CO<sub>2</sub> emission (Springett et al. 1994) and (2) by cellulose degradation through weight loss of cotton strips (Harrison et al. 1998). Every six months (summer and winter) the bacterial and fungal bioactivity in soil was

measured from soil cores taken at two depths, 0-50 mm and 50-100 mm. Soil temperatures were recorded at 50 mm soil depth every 2-3 weeks during early morning and late afternoon. Trunk diameter of all trees was measured before commencement of the trial and subsequently at the end of each growing season. Fruit yield was also recorded each year.

The second experiment was started in September 1998 in a newly planted block of apple trees. Each plot contained three trees and treatments were replicated three times. The height and trunk diameter of each tree was measured soon after planting. Details of the methods have been provided by Hartley et al. (2000). Briefly, the low growing ground cover species were established (creeping red fescue and white clover from seed, dichondra and hydrocotyle by plugs) into cultivated ground in a newly planted orchard. The ground covers were established with and without herbicide assistance and all plots were mown periodically to control initial weed growth. A bark mulch treatment was applied to one third of the plots for initial weed control with the intention of establishing the ground covers at a later date. The bark plots were spot treated with non-residual herbicide for additional weed control as necessary. Weed numbers and ground cover of weeds and sown species were assessed at regular intervals. At the end of the growing season trunk diameter of each tree and fruit yield were measured.

## RESULTS AND DISCUSSION

### Weed control

Best weed control was given by the straw mulch with equally good weed control from thick straw as from half thickness of straw plus a half rate of terbuthylazine. Deep layers of sawdust were also as effective as reduced depths of sawdust plus herbicide but wooddust was improved by the addition of herbicide while compost encouraged weed growth even with herbicide (Hartley and Rahman 1994).

One and two years after their introduction, the fescue and clover treatments were assessed for their ability to suppress weeds. The fescue suppressed broadleaf weed effectively but allowed ingress of other grasses while the clover had little effect on the ingress of weeds. However, the vegetation biomass on the fescue and clover plots was two to three times greater than on the untreated plots though the vegetation height was reduced by the fescue.

In the second experiment dichondra, with a residual herbicide treatment at establishment, reduced weed cover to about 40% in the first summer and 20% in the second summer but only with a further herbicide treatment in the second winter. Without herbicide assistance dichondra did not give any reduction in weed cover even though it maintained a good cover itself. The hydrocotyle did not persist under hot summer conditions sufficiently to be of any real value for weed suppression. Creeping fescue gave very useful weed suppression with and without herbicide assistance. Total vegetation produced in all treatments during the first season was highest where no ground cover was planted or where dichondra or hydrocotyle were planted without herbicide. It was reduced to about 45% of untreated by creeping fescue with or without herbicide and hydrocotyle with herbicide, and to about 10% by dichondra with herbicide (Hartley et al. 2000).

### **Tree growth and fruit yield**

The organic mulches had very little effect on established tree growth in the first trial, except for a slight increase in trunk diameter on compost plots but this was associated with a slightly lower fruit yield (Hartley and Rahman 1994). Three years after planting, the fescue reduced total fruit yield compared to all other treatments but also had the largest proportion of reject fruit. The highest fruit yield was on the herbicide plots (both non-residual and residual), with straw and sawdust being intermediate (Hartley and Rahman 1998). With the young trees in the second experiment, the first year growth was significantly reduced by all ground cover treatments, with and without herbicide assistance, compared to herbicide only and bark mulch treatments. This trend was also reflected in a five-fold increase in fruit yield for the latter treatments in the second year (Hartley et al. 2000).

### **Soil temperature**

The organic mulches reduced fluctuation in soil temperature (5 cm depth) compared to bare ground and the overall mean soil temperature was also reduced (Hartley and Rahman 1994).

### **Soil respiration**

Soil respiration, measured under the organic mulches over a 19 month period, was highest overall under compost and consistently lower under straw and wooldust. There was no difference between residual and non-residual herbicide treatments (Hartley et al. 1996).

### **Cellulose degradation**

Cellulose degradation activity, a measure of soil microbial ability to degrade dead plant material, was initially stimulated by all mulch treatments but declined rapidly and remained low under wooldust. Activity under sawdust declined after 6 months but remained higher under straw and compost than in the untreated plots. Activity was also slightly higher following residual herbicide than non-residual herbicide (Hartley et al. 1996).

### **Soil microbial biomass**

At each measurement bacterial biomass in the top 50 mm of soil was significantly higher under grass than under the organic mulches or the herbicide treatment. By the second winter bacterial biomass was significantly lower under wooldust than all other treatments. The difference was less at the deeper sampling depth. The fungal biomass was also significantly greater, at both depths, under grass and less under wooldust than under herbicide and sawdust at all times (Hartley et al. 1996).

### **Earthworm population**

Earthworm populations surveyed 9 months after organic mulches were applied were highest under compost, though one species was most numerous in the non-residual herbicide plots. Straw also had high numbers of earthworms and newly hatched earthworms were most numerous under straw and sawdust indicating these mulches were likely to maintain higher populations (Hartley and Rahman 1994).

### **Soil chemical analysis**

Soil samples taken from the top 100 mm after 4 years showed wooldust (applied at the commencement of the trial) had a major effect on the chemical composition of the soil. It had decreased the calcium, potassium, magnesium and sodium levels significantly and increased the sulphur level four fold. This reduced the pH from 6.18 to 5.03 while compost and straw raised the pH of the soil. Compost raised the calcium and phosphate levels while straw raised the potassium and phosphate levels. Sawdust reduced the calcium and phosphate levels (Hartley and Rahman 1997).

### **Leaf analysis**

In spite of major effects on the chemical analysis of the soil the organic mulches had little effect on nutrient status of the apple leaves over the five year period in the first experiment (Hartley and Rahman 1997). In the second experiment the ground cover species without herbicide treatment reduced leaf nitrogen and potassium levels and increased phosphate and calcium levels compared to those in the herbicide treated plots. The bark mulch had no effect on the leaf nutrient levels.

Effective weed control by means of organic mulches or ground cover species may be more difficult to obtain than by herbicides. Organic mulches would appear to require annual replacement or mechanical or chemical assistance to maintain reasonable weed control. Of those we tested, straw appears the best but it had the disadvantage of grain within the straw growing as a weed. Pea straw might avoid this disadvantage. Sawdust and bark were also useful but wooldust should be avoided. Though organic mulch treatments had some measurable beneficial effects on soil bioactivity, they had no effect on tree growth or yield up to the end of the third growing season. However, treatments may take longer to affect the trees. Baxter (1970) found straw mulch round apple trees doubled the fruit yield after 5-6 years when compared to a cultivation treatment for weed control.

Ground cover species are unlikely to be of value unless assisted by at least a herbicide application in winter when trees are dormant. Even with such treatment mowing may also be necessary. *Dichondra* has shown good promise as a low growing ground cover species capable of reducing weed growth, when assisted by herbicide during establishment and mown afterwards. Its long term sustainability without the aid of a herbicide treatment is yet to be determined. *Hydrocotyle* was unable to establish under the dry conditions of the Hawke's Bay region, even with herbicide assistance and irrigation. *Creeping red fescue* established well from autumn sowing and provided moderate weed suppression.

Tree growth was reduced by all vegetation treatments including *dichondra* with herbicide, which had the least amount of weeds present. The competition affected all aspects of tree growth in the first year and fruit yield in the second year but this could have been influenced by tree size in the second year. It would appear from the results of this trial that even if low growing ground covers could be established to give satisfactory control of larger weeds in organic orchards it is probable that some reduction in tree growth and loss of yield will occur. *Dichondra* ground covers have been shown to cause no decrease in fruit yields when grown under well established apple trees (Harrington et al. 1999). Ground covers should probably be kept away from the base of trees for the first few years, and would do better in systems where occasional use of herbicides in winter is permitted to remove weeds that do establish.

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## INVESTIGATIONS INTO THE WEED INFESTATION, WEED SEEDBANK AND TEA GROWTH IN RELATION TO THE DIFFERENT WEED MANAGEMENT METHODS IN YOUNG TEA (*CAMELLIA SINENSIS* [L.] KUNTZE) PLANTATION

K. G. Prematilake<sup>1</sup>, R. J. Froud-Williams<sup>2</sup>, and P. B. Ekanayake<sup>3</sup>

<sup>1</sup>Low Country Station, Tea Research Institute, Ratnapura, Sri Lanka. aglctri@mail.ac.lk

<sup>2</sup>Department of Agricultural Botany, University of Reading, 2, Earley Gate, Berks, Reading RG 6 6 AU, United Kingdom.

<sup>3</sup>Mid Country Station, Tea Research Institute, Kandy, Sri Lanka.

**Abstract:** A field experiment was conducted in the Low-Country of Sri Lanka, during the period 1994 to 1995 to investigate weed seedbank density in soil and severity of weed infestation and growth of tea in relation to weed management methods during early tea establishment. Manual weeding at various intervals was compared with the use of various herbicides, with and without mulching. Weed seedbanks were determined using "seed germination method" and "Malone's seed extraction method" before and 12 months after imposition of treatments. Seedbanks were reduced marginally (6%) ( $p>0.05$ ) with the use of herbicides, but increased (40%) when manual weeding was less frequent than six week intervals. Glufosinate ammonium at 0.2 kg a.i. ha<sup>-1</sup> and 2, 4-D at 0.94 kg a.i. ha<sup>-1</sup> followed by at 0.17 paraquat kg a.i. ha<sup>-1</sup> were less effective in weed control and had little influence on reducing seedbank density. Weed control with herbicides was superior to that of manual weeding ( $P<0.05$ ). Manual weeding at six-week intervals and herbicide treatments affected tea growth similarly, but the former was not cost effective. Neither agronomic nor economic advantage was achieved with manual weeding at two-week intervals. Plots allowed to remain weedy for 12 weeks or more adversely affected tea growth. Application of oxyfluorfen at 0.29 kg a.i. ha<sup>-1</sup> followed by paraquat at 0.17 kg a.i. ha<sup>-1</sup> or glyphosate at 0.99 kg a.i. ha<sup>-1</sup> was more cost effective and superior to manual weeding ( $p<0.05$ ) at six-week intervals due to minimum weed infestation. Mulching of tea inter-rows with grass loppings (at 38 t. ha<sup>-1</sup>) was more beneficial for tea than not mulching, whilst a live mulch of weeds suppressed tea growth. A combination of mulching and chemicals followed by manual weeding at 10-week intervals would appear to be the most appropriate integrated weed management system during establishment of young tea.

**Key words:** Weed management, tea plantation, infestation

### INTRODUCTION

Weed management in tea plantations is a critically important operation, particularly during the young stage and post-pruning phases when fields are more exposed due to inadequate ground cover. It was reported that 5-15% tea yield is lost due to delayed weeding exceeding four months (Wettasinghe 1971a, b). Moreover, weeds have become a burden as they disturb the major field operations mainly plucking.

Although, many plantations adopt manual, chemical and some cultural measures to manage the weeds at present, weeds perpetuate in tea fields. Thus, the expenditure on weed management in tea, accounts for about 10-14 per cent of the cost of all field

operations and is attributed to the high cost of chemicals and labour. An understanding of the causes for weediness, in particular estimation of weed seed density in soil, an in-depth knowledge on weed infestation and an understanding of how and when weeds affect growth of young tea through competition will provide insights as to when and how weed control measures should be imposed. These information will be more useful in formulating integrated approaches for economically viable weed management systems in tea. The objectives of the present study was therefore, to investigate into the behaviour of weed flora and changes in weed seed population and growth performance of tea under different weed management practices in newly established tea under lower elevation of Sri Lanka and; to further develop the present weed management strategies based on these investigations.

## MATERIALS AND METHODS

A field experiment was conducted at the Low-Country Station, Tea Research Institute, Ratnapura, Sri Lanka where the Elevation is about 60 m amsl and the latitude & longitude are 6° 41' N and 80° 24' E, respectively. The soils belong to the Red Yellow Podsollic group (Ultisol) with sandy loam. The annual rainfall is about 2500-3000mm with a bimodal distribution. January to February are relatively drier months. The mean temperature is 28° C.

Tea (Clone-Kenilworth 16/3) was planted in a field rehabilitated with the grass (*Cymbopogon confertiflorus*) for 18 months previously. Eight month old nursery tea plants were planted on holes dug to a depth of 45 cm at the spacing of 1.2 m \* 0.6 m on contours in June, 1994 and; 40 plots were marked. Thus, each plot, 21.6 m<sup>2</sup> in size consisted of 30 plants (five rows x six plants/row). Guard rows of tea and shade were properly maintained.

### Treatment combinations

Five manual weeding treatments imposed to thatched plots included, hand pulling alone at intervals of 2, 6, 12 and 18 weeks and another to unthatched plots at 6 week-interval. Chemical weeding treatments imposed on thatched plots included glyphosate [0.5%] at 0.99 kg a.e.ha<sup>-1</sup> with kaolin at 3.42 kg ha<sup>-1</sup>], 2, 4-D at 0.73 kg a.i.ha<sup>-1</sup> + paraquat at 0.15-0.22 kg a.i.ha<sup>-1</sup>, glufosinate ammonium at 0.2 kg a.i.ha<sup>-1</sup> and oxyfluorfen at 0.29 kg a.i.ha<sup>-1</sup> + paraquat at 0.15-0.22 kg a.i.ha<sup>-1</sup>. The same rates of oxyfluorfen + paraquat were also treated to an unthatched plots. Experiment was assigned to a Randomized Complete Block design with 4 replications.

All plots were clean weeded prior to allocation of treatments. Plots to be thatched according to the treatments were mulched with *Cymbopogon* grass loppings at 37 tonnes ha<sup>-1</sup> soon after planting tea. Rethatching was done in Oct. '94, Feb. and July, '95. In unthatched manual weeding treatment, weeds were allowed to grow as a live mulch on tea inter-rows and slashed every 6 weeks. Goal-2E was applied on tea inter-rows soon after planting tea, prior to thatching, using a knapsack sprayer to which a Flood-Jet nozzle [Yellow] (Orifice size, 042) was fixed. Other herbicides were carefully sprayed when weeds grew to a height of 10-15 cm on thatched plots. Those weeds, which were unattended and resistant to herbicides, were manually removed. Plants were manured with a mixture containing N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and MgO in 8.2%, 5.5%, 12% and 4.8% respectively at a rate of 1500 kg ha<sup>-1</sup> yr<sup>-1</sup> in 6-split applications.

## Assessments

### Weed sampling for identification of species and density assessments.

a) 12 quadrat (0.4 m<sup>2</sup>) samples, representing the entire land block, were taken prior to plot demarcation. At 18 months after imposition of treatments (MAIT) two quadrat samples were taken from each plot for identification and counting.

b) Total fresh weight of weeds (above ground) per plot at each manual weeding. In chemically treated plots, only the weight of unaffected and resistant weeds was recorded. 500g fresh weed sample was oven dried for 48 h at 65<sup>o</sup>c. to estimate the total dry weight.

### Weed seedbank assessments.

Soil sampling for the determination of weed seedbank followed the 'Germination method' (Brenchley & Warington, 1930) and additional seed extraction (Malone, 1967). A total of 15 soil samples were randomly taken to two depth horizons of 0-5 and 5-15 from an area of 202 cm<sup>2</sup> within each plot, using a metal pipe 4.3 cm diameter, prior to imposition of treatments and 12 MAIT. Samples from the same depth in each plot were bulked and put in several plastic pots. Five pots filled with sterilised sand were used as controls. The pots of soil were placed on benches in the screen house and were well protected from contamination by air-borne weed seeds. Soil was kept moist throughout to facilitate weed seed germination.

The identification, counting and removal of each emerged weed was continued until no further emergence occurred. Soil was disturbed at intervals to facilitate germination of buried seeds. Subsequent to germination of all non-dormant viable seeds, the balance of viable seeds remaining were isolated using Malone's seed extraction method.

### Growth assessment of tea

a) Per plant leaf area, fresh and dry weight were measured from two uprooted plants from each plot at 4.5 month intervals. Roots, stem and leaves were separated. The leaf area of 10 g was measured using leaf area meter (Delta T devices).

b) Total pruning weight and leaf area were also measured 12 MAP [June, 95] from first Pruning (at 30cm height) and; 18 MAP [Nov. '95] from second cut. The shoot dry weight and leaf area were also measured.

c) Number of casualties were recorded at 4.5, 9.0, 10, 12 and 18 MAP in each plot.

## RESULTS AND DISCUSSION

### Weed flora composition prior to imposition of treatments

About 23 weed species were present within the experimental area during June, 1994 prior to the imposition of treatments. The largest and the smallest weed species density /0.04 m<sup>2</sup> i.e. 23 and 7 were found in plots which were to be weeded at 12 and 18 week-intervals, respectively. Most of the weeds were broad-leaved species, the predominant weed species were *Oxalis barrelieri* (45 ± 20), *Caladium bicolor* (28 ± 13). Species such as *Borreria latifolia* (13 ± 3) and *Hyptis suaveolence* (18 ± 5) were also prominent. Among sedges, only *Cyperus rotundus* (13 ± 5) was found. Weeds such as *Mollugo pentaphylla*, *Desmodium heterophyllum*, *Lindernia cordifolia* and *Stomedia verticillata* could be considered as "favourable" or "soft weeds" as they cover the soil surface and generally have no adverse effects on growth of tea. Among grasses *Pennisetum purpureum* (Fox tail) and *Paspalum* species were found in abundance.

### Effectiveness of various weed management methods on weed infestation

**Manual weeding** Of the manual weeding treatments, the lowest weed dry weight was always recorded for clean weeding treatment (Fig. 1) and was significantly less ( $p < 0.05$ ) than other manual weeding treatments throughout. This may be attributed to lesser weed population due to low *in-situ* seed rain as a result of arresting weed growth at seedling stage. Moreover, the greatest species diversity was observed in clean weeded plots. This would be as a consequences of less intense intraspecific competition.

Overall weed dry weight for 18 month-period which showed 1.4-2.0 times increase in manual weeded plots every 18 weeks compared with plot weeded every 12 and 6 weeks, respectively. Undisturbed weed infestation for a long duration would have caused proper establishment and proliferation of such weeds. Thus, the weed production increase can be attributed to the frequency of manual weeding. Species diversity decreased with less frequent manual weeding, in particular the "soft weed" were excluded by the dominants. A few uncommon weed species also occurred in these plots.

**Chemical weeding** The mean weed dry weight of chemically weeded plots were significantly lower ( $P < 0.05$ ) than that of manual weeding every 6, 12 weeks (Fig.1). Thus, it is apparent that herbicide followed by manual control of weeds is agronomically feasible against manual method alone. Manual weeding alone resulted in fragmentation of vegetative parts, aggravating the weed problem. In contrast, herbicides could kill some weeds, resulting in a lower dry weight of the survived. Generally, fewer species were recorded with herbicide treated than for manually weeded plots (Table 1).

Oxyfluorfen + paraquat and glyphosate (0.5%) + kaolin had caused the lowest weed dry weight among herbicide treatments as a result of controlling many of the weeds and in turn a low weed seed return (Fig.1). The plots were maintained with 90-95% weed-free situation for 3-4 month period following spraying with these combinations. The proper weed control with oxyfluorfen/paraquat which was mainly attributed to the pre-emergent action of the former, was also witnessed by (Sandanam *et al.*, 1985).

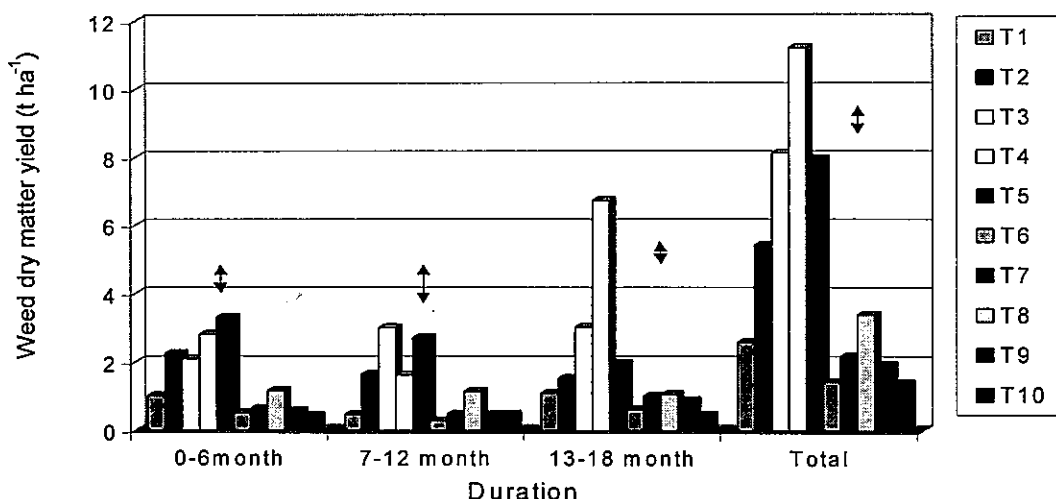


Figure 1. Mean weed dry matter yield as affected by manual and chemical weeding methods. **Manual weeding (thatched):** T1-every 2 weeks; T2-every six weeks; T3-every 12 weeks; T4-every 18 weeks; and **(unthatched):** T5-every 6 weeks. **Chemical weeding (thatched):** T6-glyphosate (0.5%); T7-2, 4-D/paraquat; T8- glufosinate ammonium; T9-oxyfluorfen /paraquat & **(unthatched):** T10- oxyfluorfen /paraquat.

Many authors have proved the efficient control of weeds by glyphosate (Ekanayake 1994; Kabir *et al.* 1991). With 2, 4-D/ paraquat, a certain number of species was survived comparing to oxyfluorfen and glyphosate. The greatest weed dry matter yield among the chemical treatments was recorded with glufosinate. Onsando *et al* (1989 a & b) claimed that glufosinate has minor performance when compared with glyphosate. High density of seeds in the soil seedbank may also have caused for an increased weed population.

**Impact of thatching on weed growth** Thatched plots, manually weeded at frequent intervals below 6 weeks produced a lower ( $p<0.05$ ) weed biomass than unthatched plots weeded every 6 weeks and the difference was significant (Fig.1). Weeds present in unthatched plots are mostly characterised by a fast recovery following slashing of weeds.

In contrast, unthatched, oxyfluorfen/paraquat treated plots had less weed dry weight ( $p<0.05$ ) than that of thatched treatment entire 18 month-period (Fig.1). The weed flora composition present under these two treatments resulted in such dry weight differences. The great occurrence of weeds could be ascribed to the favourable soil environment accrued from thatching (Table 1). Moreover, tying up of herbicide spray due to incomplete breakdown of the previous mulch at the time of subsequent applications of oxyfluorfen might have impaired the performances of oxyfluorfen. Crutchfield & Wicks (1983) also reported that wheat mulch reduced weed control by tying up herbicides.

Table 1. Mean effect of various weed control treatments on weed species number 6 and 18 months after planting.

Treatment	Mean species number	
	6 MAP	18MAP
1) Hand weeding <sup>\$</sup> every 2 weeks	22	26
2) " " " 6 "	16	19
3) " " " 12 "	15	22
4) " " " 18 "	10	18
5) " " " 6 "	19	16
6) glyphosate (0.5%)	18	11
7) 2,4-D/paraquat	12	17
8) glufosinate ammonium	12	13
9) oxyfluorfen/paraquat	16	14
10) " +	9	13

\$ = Thatched plots; + = Unthatched plots

### Assessments on weed seedbank in soil

#### Prior to imposition of treatments

There were no significant differences in seed density between treatments at 0-15 cm depth. Collectively, the plots with and without thatching which were to be weeded at 12 and 6 week intervals, respectively had a greater number of seeds buried at 0-15 cm depth. The plots to be treated with glufosinate and unthatched plots, which were to be sprayed with oxyfluorfen/paraquat had the greatest and the least seed number, respectively (Fig.2).

The majority of weed seeds were of broad-leaved "soft weed" species as listed out previously. Their occurrence may be attributed to seed transmission from nearby blocks

and *in situ* production following tea establishment. Portsmouth (1951) also reported that some "soft weeds" produced seeds within a month from germination in tea fields.

#### Weed seed density in soil at 12 months after imposition of treatments

There was no great difference in seed density between year ( $p < 0.05$ ) 1994 and 1995, although relatively more seeds were present in plots manually weeded with the exception of plots weeded every 2 weeks, 12 MAIT (Fig.2). However, there was a significant difference in seed number between treatments, 12 MAIT in 1995. The increase in seed density after one year period may be attributed to the subsequent replenishment of the seedbank with *in-situ* seed production which was depended upon the frequency of weeding and the type of weeds present.

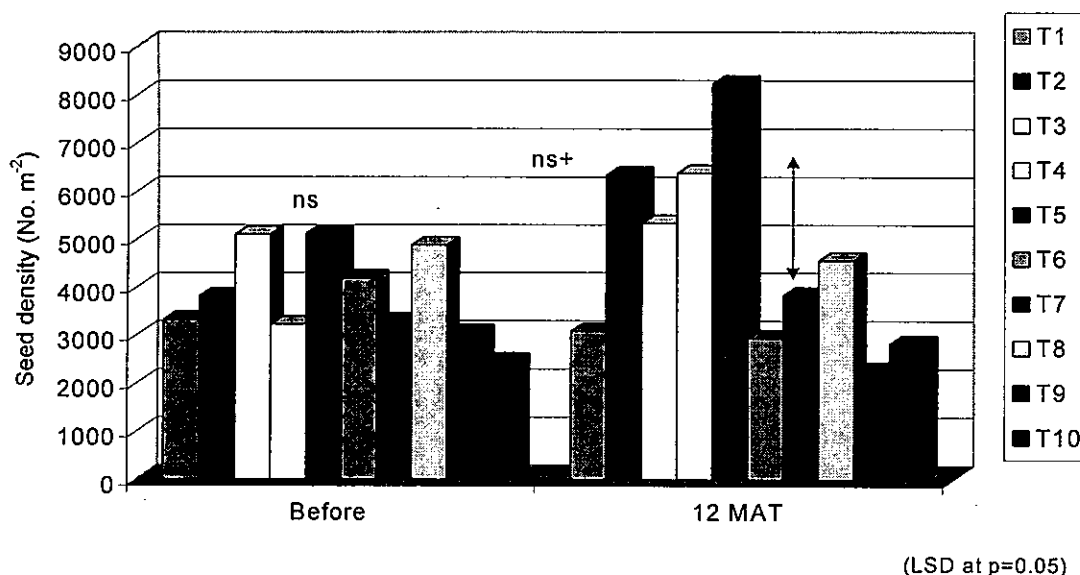


Fig.2: Weed seed density in soil seedbank at 0-15 cm depth, prior to & 12 months after imposition of various weed control methods.

**Manual weeding (thatched):** T1-every 2 weeks; T2-every six weeks; T3-every 12 weeks; T4-every 18 weeks; and **(unthatched):** T5-every 6 weeks; **Chemical weeding (thatched):** T6-glyphosate (0.5%); T7-2,4-D/paraquat; T8- glufosinate ammonium; T9-oxyfluorfen/paraquat & **(unthatched):** T10- oxyfluorfen /paraquat.

Comparable seed densities occurred in manually weeded plots at 6, 12 and 18 week intervals and; with glufosinate and paraquat/2, 4 D. Seeds present in surface 5 cm contributed almost 60% of the total. The reduced seed burden in clean weeding plots may be attributed to lower seed influx from *in-situ* production. Weed removal during the onset of vegetative growth prevented reproductive output. The greatest seed density in unthatched plots every 6 weeks has resulted from an initial high seed density as well as *in-situ* production of seeds from weeds present as a live mulch.

Similarity in seed number present in all chemically treated plots initially and 12 MAIT indicated maintenance of the *status quo* from weed control. Successful control of many weeds with both glyphosate and oxyfluorfen/paraquat treatments resulted in such fewer weeds which prevented further replenishment. Authors have earlier concluded that effective weed control with herbicides has led to a decreased seedbank (Fogelfors, 1991). The inadequate weed control in glufosinate ammonium and 2, 4-D/paraquat treatments

has reflected in the seedbank too indicating a slight increase in seed number of tolerable weeds.

### Effectiveness of the method of weed management on early growth of tea

**Manual weeding:** The differences between various weed control methods in total leaf area of shoots removed at first pruning were significant ( $p < 0.05$ ) 12 MAP (Table 2). The lowest leaf area 12 MAP was in plots weeded manually every 18 weeks and was significantly smaller when compared with that of other treatments except for unthatched plots weeded 6 week intervals. However, the differences between treatments in total shoot dry weight and leaf area removed at second pruning 18 MAP were significant at ( $p < 0.10$ ) level. Although non-significant, shoot growth and leaf area were slightly reduced in plots weeded manually at 12 weeks intervals compared with manual weeding every 6 weeks. The greatest mortality was recorded in March 95 for plots manually weeded at 12 and 18 week-intervals. Unthatched plots weeded every 6 weeks also had relatively more casualties.

Thus, manual weeding not exceeding 6 week-intervals in thatched plots seemed to be most favourable for young tea growth. Arresting the weed growth at shorter intervals caused minimum competition of weeds on tea growth. Weeding every 2 weeks seemed to be slightly less beneficial to tea than manual weeding every 6 weeks. Fernando (1967) stressed that the mere presence of weeds did not adversely affect plant growth and is indicative that small weeds may modify the micro-climate in favour of the tea plant. Pronounced growth retardation compared to herbicide treatments provides an indication that weeding earlier than 3 months is beneficial. Furthermore, adverse effects on young tea growth with delayed weeding for more than three months is well documented and is ascribed to competition (Prematilake *et al*, 1999). Competition set by weeds may perhaps be for moisture, nutrients and light (Eden, 1947, 1949; Wettasinghe, 1972 & Akobundu, 1987).

Table 2. Mean leaf area and dry weight of pruned shoots of tea as affected by various weeding methods 12 and 18 MAP\*.

	1 <sup>st</sup> Pruning (12 MAP)		2 <sup>nd</sup> Pruning (18 MAP)	
	Leaf area (cm <sup>2</sup> )	Shoot dry wt (g/plot)	Leaf area (cm <sup>2</sup> )	Shoot dry wt (g/plot)
1) Hand weeding every 2 weeks**	18115a	395 a	57538 a	788 a
2) " " " 6 "	33300a	553 a	58008 a	811 a
3) " " " 12 "	23540a	341 a	46420 a	471 a
4) " " " 18 "	6135b	183 a	19938 b	268 a
5) " " " 6 "	14290ab	226 a	38647 ab	477 a
6) glyphosate (0.5%)	23443a	416 a	49655 a	730 a
7) 2, 4-D/paraquat	24915a	373 a	48405 a	584 a
8) glufosinate ammonium	30863a	432 a	53430 a	826 a
9) oxyfluorfen/paraquat	31095a	505 a	75520 a	927 a
10) " " ***	17938a	321 a	40783 ab	528 a

\* significant at  $p < 0.10$ ; \*\*Thatched plots; \*\*\*Unthatched plots.

**Chemical weeding** There was a significant difference ( $P < 0.05$ ) in leaf area of shoots removed 12 MAP, between all chemical and manually weeded every 18 weeks

treatments (Table 2). All herbicide and manual weeding treatments (at 6 and 12 week intervals) 12 and 18 MAP had comparable leaf area and shoot dry weights. Higher growth attributes in oxyfluorfen/paraquat treatment was due to low competition of weeds. 2, 4-D/paraquat treated plots had relatively a poor growth and greater casualties due to the weed interference with tea. Limited growth impediment occasionally observed with glyphosate treatment could be inferred as a result of some phytotoxicity of glyphosate in tea.

**Effect of mulching** There was a consistent dry weight increase in pruned shoots in thatched plots compared with unthatched plots weeded every 6 weeks. Shoot dry weight, leaf area were also relatively greater in thatched plots sprayed with oxyfluorfen/paraquat than in unthatched plots (Table 2). The conducive soil conditions created by the mulch comparing with its unthatched plots were also attributable for a better growth of tea. Growth suppression and mortality in unthatched manually weeded plots was due to the interference by weeds as a live mulch. This indicates that a weed live mulch was detrimental to tea particularly under drought. Wijeratne & Ekanayake (1990) also emphasized that live mulches of leguminous cover crops compete with young tea for soil moisture during drought.

### **Economics of weed management in young tea**

The implications are that the longer the manual weeding interval, the lesser the weeding cost. Thus, the longer intervals of manual weeding alone, beyond 6 weeks were unsuccessful not only because of weed competition with tea, but also its lesser cost effectiveness relating to chemical weeding supplemented by hand pulling. The cost of chemical weeding was 44% lower compared with manual weeding every 6 weeks in thatched plots.

The higher cost of manual weeding in unthatched plots (Rs.2517/-) compared with thatched plots (Rs.2298) was attributed to the type of weeds which characterise a prostrate growth, poor soil moisture condition which had lead to have more time for hand pulling. The least expensive treatments were oxyfluorfen/paraquat (Rs. 1140/-) followed by glyphosate (Rs. 1243/-). Such a lower cost was attributable to the greater efficacy of these herbicides in weed control. The high cost of weeding with oxyfluorfen/paraquat was recorded in thatched plots compared to unthatched treatments due to the occurrence of many weeds.

## **CONCLUSION**

Weed control by herbicide followed by manual weeding treatments was superior to that of manual weeding alone at 6week intervals. Neither agronomic nor economic advantage was achieved with manual weeding at 2 week intervals. Manual weeding every 6 weeks and herbicide treatments affected tea growth similarly but the former resulted in a slight increase in the soil seedbank and not cost effective. Plots allowed to remain weedy for 12 weeks or more adversely affected tea growth. Oxyfluorfen/paraquat and glyphosate were relatively cost effective. Further, the latter had little influence on reducing the soil weed seedbank. However, glyphosate seemed to be little phytotoxic to tea. Thatching tea inter-rows with grasses was more beneficial for tea than not thatching whilst, the live mulch of weed caused a suppressed tea growth. Finally a combination of chemical method with oxyfluorfen/paraquat followed by



thatching with grasses and manual weeding practised at shorter (8-10 weeks) intervals would be the most appropriate integrated weed management system in new clearing tea fields.

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## CONTROL OF *ACACIA MANGIUM* IN YOUNG RUBBER PLANTATION

M. A. Ahmad Faiz

Crop Improvement and Protection Unit, Malaysian Rubber Board, 47000 Sungai Buloh,,  
Selangor, Malaysia. afaiz@lgm.gov.my

**Abstract :** *Acacia mangium* Willd is a fast growing tree legume which has encroached and become a serious weed problem in rubber growing areas located in vicinity of *A. mangium* plantations. Slashing did not provide effective control and herbicides were evaluated in pots and in a young rubber plantation to obtain effective control of the plant. In pot trials, triclopyr ( $\geq 0.06$  kg ae/ha), fluroxypyr ( $\geq 0.04$  kg ae/ha), metsulfuron methyl ( $\geq 0.01$  kg ai/ha) and glyphosate isopropylamine ( $\geq 0.48$  kg ai/ha) affected growth when sprayed on to the seedlings. Mixture of triclopyr, fluroxypyr, metsulfuron methyl, glyphosate or imazapyr with diesel in a ratio of 1:8 by volume of commercial formulation provided complete kill of young plants of about 2m tall when sprayed around base of the stems. In field trials, seedlings of the plant were effectively controlled by spraying glyphosate isopropylamine or glyphosate trimesium each at 1.44 kg ai/ha direct on to the plants. Fluroxypyr from 0.1 to 0.4 kg ae/ha and metsulfuron methyl at 0.04 to 0.05 kg ai/ha provided moderate control. After slashing, stumps of the plant were effectively controlled from regeneration by spraying mixtures of triclopyr with diesel in ratios of 1:5, 1:10 and 1:15 by volume of commercial formulation or mixtures of premixed picloram triisopropanolamine + 2,4-D triisopropanolamine with diesel in ratios of 1:10, 1:15 and 1:20 by volume of commercial formulation. Young trees of about 3m in height were killed after spraying mixtures of triclopyr with diesel in ratios of 1:0, 1:4, 1:8, 1:12 and 1:16 by volume of commercial formulation around base of the stems. Since growth of the plant is rapid, its control should not be neglected as it could give rise to more serious problem later.

**Key words:** *Acacia mangium*, control, herbicides, rubber,

## INTRODUCTION

*Acacia mangium* Willd., a leguminosae, was introduced to Malaysia for timber and pulp. It is a fast growing tree legume and produces abundant seeds which enable it to spread easily. Rubber plantations which are located in vicinity of these *Acacia* plantations have been invaded by this legume and has become a problem weed. Its rapid growth could surpassed height of the rubber trees within a short period and this could interfere rubber growth and development. Undesirable growth of the legume is generally controlled by slashing. In areas where its infestation is extensive and labour availability is limited, control of the legume has become a problem.

Hardly any study has been done to control extensive infestation of this legume in rubber plantations. Probably use of herbicides may be a solution as less labour is required and some herbicides have been known to be effective against woody weeds. Triclopyr has been reported effective against woody weeds (Teoh et al, 1982; Yeoh and Faiz, 1985; Sabudin and Abdul Jalil, 1990). Metsulfuron methyl and fluroxypyr had been reported effective against *Clidemia hirta* under rubber (Faiz, 1993). Picloram had also been used for killing unwanted rubber seedlings and *Chromolaena odorata* (Riepma, 1968). *C. hirta* was also reported susceptible to triisopropanolamine salts of picloram +

2,4-D although less compared with triclopyr (Faiz, 1993). This paper reports results of trials conducted in pots and in the field to determine cost-effective herbicides for controlling *A. mangium* in rubber plantations.

## MATERIALS AND METHODS

### Pot trial

Trials were conducted in plastic pots measuring 21cm height and 28cm diameter and containing clay loam soil. Seedlings of *A. mangium* were collected from a field in Batu Arang, Selangor during 1999. Watering of pots were done twice daily and fertilizer applied at monthly interval. Herbicides used were Roundup (glyphosate isopropylamine - 41% w/w), Assault 100A (imazapyr - 9.4% w/w), Ally 20DF (metsulfuron methyl - 20% w/w), Starane 200 (fluroxypyr, 1-methyl heptyl ester - 29.6% w/w) and Garlon 250 (triclopyr, butoxy ethyl ester - 32.1% w/w).

### Seedling

The pots were arranged in complete randomised design (CRD) in five replicates. Each pot contained one seedling of about 0.5m in height. Herbicides used for the treatments were commercial formulations of glyphosate isopropylamine (0.48, 0.96, 1.44, 1.92 and 2.40 kg ai/ha), imazapyr (0.2, 0.3, 0.4, 0.5 and 0.6 kg ae/ha), metsulfuron methyl (0.01, 0.02, 0.03, 0.04, 0.05 kg ai/ha), fluroxypyr (0.04, 0.08, 0.12, 0.16, 0.20 kg ae/ha) and triclopyr (0.06, 0.12, 0.19, 0.25, 0.31 kg ae/ha). The herbicides were sprayed direct on to the plants using a knapsack sprayer. Phytotoxic effects of these herbicides on the legume were assessed based on survival, height, phyllode number and fresh weight.

### Young plant

A trial was conducted on young plants of *A. mangium* of about 2m tall planted in the pots. The pots were arranged in CRD in three replicates. Commercial formulations of metsulfuron methyl, fluroxypyr, triclopyr, glyphosate and imazapyr were mixed with diesel as carrier in a ratio of 1:8. The mixture was sprayed around base of the stem about 30 cm from the ground level using a knapsack sprayer. Phytotoxicity of these herbicides was assessed visually based on percentage kill.

### Field trials

The trials were conducted in a rubber plantation of about three years old in Batu Arang, Selangor on sandy clay loam soil in 1999 and 2000. Herbicides used were Roundup (glyphosate isopropylamine - 41% w/w), Touchdown (glyphosate trimesium - 39.8% w/w), Ally 20DF (metsulfuron methyl - 20% w/w), Starane 200 (fluroxypyr, 1-methyl heptyl ester - 29.64% w/w), Rumputox (2,4-D butyl ester - 45% w/w), Garlon 250 (triclopyr, butoxy ethyl ester - 32.1% w/w), Banvel 400 (dicamba dimethylamine - 49.0% w/w), Tordon 101 (picloram triisopropanolamine - 10.2% w/w + 2,4-D triisopropanolamine - 39.6% w/w), 2,4-D Amine 720 (2,4-D dimethylamine - 60.0% w/w) and Assault 100A (imazapyr isopropylamine - 9.4% w/w). The plots were arranged in CRD in three replicates. Phytotoxicity of these herbicides were assessed visually based on percentage kill.

**Seedling** The trial was conducted in plots of 20m<sup>2</sup> each on *A. mangium* seedlings of less than a meter in height. The plants were sprayed with herbicides using a knapsack sprayer. Herbicides used were commercial formulations of glyphosate isopropylamine (1.44, 1.92, 2.40, 2.88 and 3.36 kg ai/ha), glyphosate trimesium (1.44, 1.92, 2.40, 2.88

and 3.36 kg ai/ha), metsulfuron methyl (0.01, 0.02, 0.03, 0.04 and 0.05 kg ai/ha), fluroxypyr (0.05, 0.1, 0.2, 0.3 and 0.4 kg ae/ha) and 2,4-D butyl ester (0.45, 0.90, 1.35, 1.80 and 2.25 kg ae/ha).

**Stump** The trial was conducted in 5m<sup>2</sup> plots on stumps left after slashing *A. mangium* plants. The plots were arranged in CRD in three replicates. Commercial formulations of herbicides were mixed with diesel in ratios of 1:0, 1:5, 1:10, 1:15 and 1:20 and sprayed around the legume stumps using a knapsack sprayer. Herbicides applied were triclopyr, fluroxypyr, metsulfuron methyl, dicamba, premixed picloram triisopropanolamine + 2,4-D triisopropanolamine and 2,4-D dimethylamine.

**Young tree** The trial was conducted on young trees or saplings of about 3m in height with 20 trees per plot. Commercial formulations of herbicides were mixed with diesel in ratios of 1:0, 1:4, 1:8, 1:12 and 1:16 and sprayed around base of the stem at about 30cm from the ground level using a knapsack sprayer. Herbicides used were triclopyr, fluroxypyr, metsulfuron methyl, premixed picloram triisopropanolamine + 2,4-D triisopropanolamine, dicamba, 2,4-D dimethylamine, 2,4-D butyl ester, glyphosate isopropylamine, glyphosate trimesium and imazapyr.

## RESULTS AND DISCUSSION

### Pot trial

**Seedling** Among the herbicides tested, glyphosate isopropylamine and triclopyr were most effective against *A. mangium* seedlings assessed at more than three months after treatment. Glyphosate isopropylamine provided complete kill of the seedlings at 0.96 kg ai/ha or higher while triclopyr at 0.19 kg ae/ha or higher. The seedlings were less susceptible to fluroxypyr and much less to metsulfuron methyl.

**Young plant** All herbicide treatments by basal stem application with triclopyr, fluroxypyr, metsulfuron methyl, glyphosate isopropylamine and imazapyr killed the legume completely at six weeks after treatment. Reaction of the legume to these herbicides was quite rapid with almost complete kill at four weeks and complete kill at six weeks after treatment. Probably a lower herbicide concentration could be effective as all the plants were killed even at the lowest concentration.

### Field trials

**Seedling** Glyphosate isopropylamine and glyphosate trimesium were most effective among the treatments (Figure 1). Effectiveness of glyphosate isopropylamine

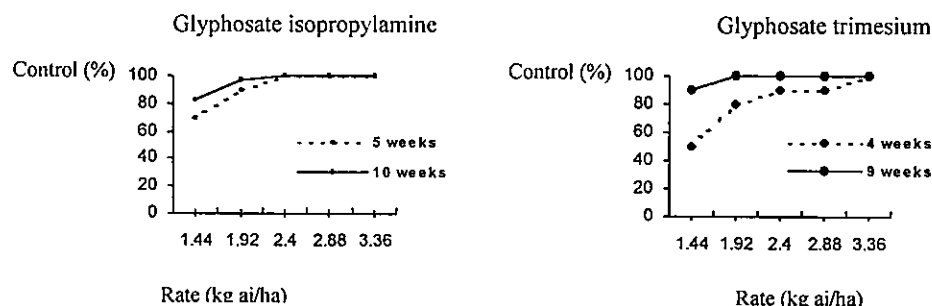


Figure 1. Effectiveness of herbicides in controlling *A. mangium* seedlings by foliar spraying

against the seedlings agree as in the pot trial. Glyphosate although commonly used against noxious grasses had also been reported effective on woody weeds (Armstrong,

1993; Toth et al, 1993). *A. mangium* seedlings in the field had higher tolerance to metsulfuron methyl and fluroxypyr as effectiveness was reduced. Low susceptibility to metsulfuron methyl could be due to poor absorption as this herbicide has low water solubility. Probably addition of surfactant could improve weed kill. Triclopyr was not included as a treatment for fear of phytotoxic effects on the young rubber plants. Since glyphosate has low phytotoxicity to young rubber, this herbicide can be recommended for controlling *A. mangium* seedlings under young rubber.

**Stump** Triclopyr was most effective among the herbicides tested and agree with result of pot trial using basal stem application on the young plant (Table 1). Effectiveness of picloram + 2,4-D or dicamba was less than triclopyr while fluroxypyr, metsulfuron methyl and 2,4-D dimethylamine were ineffective. Generally effectiveness of these herbicides declined with increase volume of diesel probably due to dilution of the herbicide. Herbicide formulated as esters or salts could probably reacted variably to diesel and hence influenced effectiveness on weed control.

Table 1. Effectiveness of herbicides plus diesel in controlling *A. mangium* stumps at about four months after treatment

Treatment	Percent control at different ratios of herbicide : diesel				
	1:0	1:5	1:10	1:15	1:20
Triclopyr	82	95	95	85	65
Fluroxypyr	0	0	0	0	0
Metsulfuron methyl	0	0	0	0	0
Dicamba	10	63	60	47	30
Picloram + 2,4-D	23	70	50	33	30
2,4-D dimethylamine	0	0	0	0	0

Table 2. Effectiveness of herbicides in controlling young *A. Mangium* trees by basal stem spraying at about two months after treatment

Treatment	Percent control at different ratios of herbicide : diesel				
	1 : 0	1 : 4	1 : 8	1 : 12	1 : 16
Triclopyr	100	100	100	100	100
Fluroxypyr	30	80	80	90	90
Metsulfuron methyl	10	10	15	25	25
Picloram triisopropanolamine + 2,4-D triisopropanolamine	97	87	77	65	73
Dicamba dimethylamine	51	37	57	42	63
2,4-D dimethylamine	12	15	15	30	35
2,-D butyl ester	3	4	20	23	36
Glyphosate isopropylamine	37	47	75	80	80
Glyphosate trimesium	57	67	37	30	33
Imazapyr	2	5	13	28	38

**Young tree** Most effective treatment as basal stem application was triclopyr (Table 2). Effectiveness of triclopyr plus diesel as arboricide had also been reported (Sabudin and Abdul Jalil, 1990; Lim and Abdul Aziz, 1981). Fluroxypyr, picloram triisopropanolamine + 2,4-D triisopropanolamine and glyphosate isopropylamine each mixed with diesel although effective did not provide complete kill. Effectiveness of triclopyr or fluroxypyr in mixture with diesel as basal spray on honey locust had been reported (Armstrong, 1993). Efficacy of picloram + 2,4-D had also been reported against *Eucalyptus populnea* (Young et al., 1979) and *C. hirta* (Faiz, 1993).

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## **PRESENT STATUS AND FUTURE PROSPECTS OF CHEMICAL WEED CONTROL ON FARMLAND IN CHINA**

Y. F. Piao and H. Huang

National Agro-Technology Extension and Service Center, Ministry of Agriculture,  
Beijing 100026, China

**Abstract:** It had gained great achievements in chemical weed control in China since 1950's, while it still has some problems. Strategy for weed management in following years is: develop an integrated weed management program gradually; set up weed inspect system to predict weeds occurrence and resistance; better farmer training to use pesticides; reinforce research on chemical weed control technique; reinforce technique training, propaganda and popularization to promote weed control in sustainable agriculture.

**Key words:** Weed, chemical weed control, integrated weed management

### **WEED OCCURRENCE IN CHINA**

Agriculture is very important in China. Crop planting area is 154 million hectares in 1998, mainly rice, wheat, maize, soybeans, oil seed rape, cotton, vegetables and fruit crops. According to field survey, there are 580 species weeds of 77 families in China, including 33% Compositae (77 species), 11% Gramineae (66 species), 6% Cyperaceae (35 species). There are 15 species weed which are most damaging and hard control, including barnyardgrass in paddy and dry fields; barnyardgrass, diffomed galingale, sheathed monochoria, district pondweed, flatstalk bulrush in paddy fields; wild oat, common crabgrass, goosegrass wiregrass, green bristlegrass, nutgrass galingale, bunge knotweed, redroot amaranth, equal alopecurus, lalang grass in dry fields. There are 31 species of most damaging and widely spread weeds, including 9 in paddy fields, 20 in dry fields and 2 in paddy and dry fields. There are 23 species of most damaging weeds in certain region. In conclusion, there are over 60 million hectares of lands were infested with weeds in 1998, including 20.45 million hectares in rice, 18.80 million hectares in wheat, 13.16 million hectares in maize, 4.37 million hectares in soybeans, 5.24 million hectares in oil seed rape.

### **PRESENT STATUS OF CHEMICAL WEED CONTROL ON FARMLAND IN CHINA**

Chemical weed control started from 1956 in China, but developed slowly in the first 20 years. With development of economy and technique, change of labor force from farm to city, it stepped into a new era since 1980's. Also with application of high active and efficiency herbicides such as Sulfometuron-methyl, chlorsulfuron and bensulfuron, with farmer's consciousness of weed control, chemical weed control developed rapidly and achieved remarkably.

Technology of control weeds had developed in all respects like target weeds, crops and cultivation. 19 types of crops such as rice, wheat, maize, vegetables, fruits, soybeans, oil seed rape, cotton and peanut had been treated with herbicides for weed control. 102 herbicides and 1214 formulations from both domestic and overseas

agrochemical companies have been registered in China. Herbicides used in paddy rice are butachlor, benthocarb, acetochlor, molinate, oxadiazon, quinclorac, bensulfuron, pyrazosulfuron-ethyl, bentazone, MCPA, some mixtures made by bensulfuron, pyrazosulfuron-ethyl and sulfometuron-methyl; herbicides used in wheat are 2,4-D butylate, MCPA, difenzoquat, triallate, isoproturon, fluroxypyr, express, fenoxaprop-p-ethyl, amidosulfuron, thifensulfuron-methyl and some mixtures; herbicides used in maize are atrazine, acetochlor, butachlor, MCPA, 2,4-D butylate, alachlor, metolachlor, glyphosate, paraquat, nicosulfuron and some mixtures; herbicides used in soybeans are pursuit, chlorimuron-ethyl, fomesafen, nabusethoxydim, fluazifop-p-butyl, quizalofop-p-ethyl, bentazone, haloxyfop-R-ethyl, acetochlor, flumetsulam, fenoxaprop-p-ethyl and some mixtures; herbicides used in cotton are butralin, trifluralin, alachlor, acetochlor, butachlor, metolachlor, methoxydiuron, fluometuron, oxadiazon, fluazifop-p-butyl, haloxyfop-R-ethyl, nabusethoxydim, glyphosate, paraquat; herbicides used in rape are acetochlor, trifluralin, butachlor, metolachlor, quizalofop-p-ethyl, haloxyfop-R-ethyl, nabusethoxydim, fluazifop-p-butyl, benazolin-ethyl.

Chemical weed control area in China has been expanding these years: 0.32 million hectares in 1967, 1.7 million hectares in 1975, 8.6 million in 1985, 1.8 million in 1990, 4.13 million in 1995, 4.87 million in 1997, 5.8 million in 1998, 6.7 million in 2000.

Chemical weed control has achieved great economical and social benefits to save yearly average yield losses of grain crops 11.43 million tons (including 5.67 in rice, 3.11 in wheat, 2.05 in corn), 0.6 million tons of soybean and 0.48 million tons of oil crops. It had promoted agricultural economy enormously with planting techniques such as zero tillage and mulching film cover.

Table 1. Chemical weed control in China in 1998 (million hectares, million tons)

Items	Sum	Rice	Wheat	Corn	Soybean	Rape	Vegetable
Planting area	31.76	31.76	30.06	23.77	8.35	10.10	11.29
Weed area	73.85	20.45	18.80	13.16	4.37	5.24	3.20
Weed control area	58	19.98	13.70	9.07	3.38	3.65	2.06
Loss saved	15	5.67	3.11	2.05	0.60	0.48	1.80
Loss quantity	3.80	0.69	1.01	0.64	0.13	0.18	0.62

Herbicide industry thrived gradually with the development of pesticide industry in China. Both outputs and varieties had been increasing far more rapidly than other pesticides. Product of herbicides was eight thousand tons in 1986 (counted as 100% active ingredient) and 117 thousand tons in 2000, technical was 13 kinds in 1986 and 76 kinds in 2000, which were classified into 20 types such as sulfonyleureas (17), amides (12), triazines (10), carbamates (8).

## PROBLEMS IN CHEMICAL WEED CONTROL IN CHINA

Because the dominant races or biotypes of main weeds changed more rapidly, it became more difficult in weed control. For example, after chlortoluron was used in wheat in Changjiang River region for many years, *Alopecurus aequalis*, *Malachium aquaticum* decreased, while *Beckmannia syzigachne*, *Alopecurus japonicus*, *Sclerochloa kengiana*, *Galium aparine* increased greatly; after 2,4-D butyl used in north China for many years in wheat to control broad-leaved weeds like *Chenopodium album*, perennial weeds such as *Descurainia sophia*, *Chorispora tenella*, *Capsella bursa-*



*postoris*, *Lithospermum arvense*, *Silene conoidea*, *Convolvulus arvensis*, *Sonchus brachyotus*, *Cephalanoplos segetum* have increased greatly; after butachlor and Molinate to control barnyardgrass in rice in Yangzi River region for many years, *Leptochloa chinensis*, *Juncellus serotnus*, *Sagittaria pygmaea*, *Scirpus planiculmis*, *Paspalum distichum* and *Alternanthera philoxeroides* have increased greatly; after trifluralin, butralin to control gramineae such as *Digitaria sanguinalis* in cotton, dicotyledon weeds such as *Amaranthus retroflexus*, *Veronica didyma* have increased greatly; after 2,4-D butyl was used in maize to control broad-leaved weeds such as *Chenopodium albm*, gramineae weeds such as *Digitaria sanguinalis*, *Eleusine indica* increased greatly and *Eriochloa villosa*, *Sorghum halepense* which were rarely seen formerly had occurred.

Herbicides having long residue period have hidden troubles in agriculture. Although sulfometuron-methyl, chlorsulfuron, pursuit, clomazone, chlorimuron-ethyl, ethametsulfuron and atrazine which were used in rice, wheat, maize, soybeans, rapeseed since 1990's have contributed to weed control greatly due to its high efficiency, low price, wide control spectrum, but some of those had some disadvantageous for their long residue period in soil, such as accumulation in soil, damage to next crop and yield reduction in crop rotation fields. This happened many times and was more serious in Yangzi River region and northeast region.

Weed resistance increased. Atrazine has been used in maize in northeast region for a long time, which lead to resistance of *Digitaria sanguinalis*. chlortoluron had been used in wheat in southeast region and led to resistance of *Alopecurus japonicus*, *Beckmannia syzigachne*, *Malachium aquaticum*. butachlor and benthocarb used for many years are easy to enhance resistance of *Echinochloa crusgalli*.

## FUTURE AND PROSPECTS

As China would become a membership of WTO and agricultural structure in China would regulate, strategy for chemical weed control henceforth is: exert all our powers to do chemical weed control well, make chemical weed control active in promoting agricultural product, structural regulation, cultivation system reform and farmer's earnings. Our aim is to realize "both weed control and prevention, both technique guidance and use management, both technique popularization and research"

Develop an integrated weed management program gradually. Chemical weed control had developed forty years and had some bad effects on agricultural product and ecological environment. So, we should stress "integrated control" in weed control. In practice, chemical control is still the main methods aided by mechanical methods and hand-pulling and based on ecological regulation and agricultural measure.

Set up weed inspect system gradually to predict weeds occurrence and resistance. As weed community changed more rapidly, chemical weed control became more difficult. It is necessary to establish inspection station in each region and form a net of field weed system inspection.

Better farmer training to use pesticides: evaluate effect of herbicide; propagandize new herbicide of safe, high efficiency, economy; guide farmer to use herbicide of not safe and long residual period; restrict or forbid to use if necessary. Because degradation of sulfonylureas herbicide affected largely by soil pH, temperature and humidity, wheat field herbicide containing chlorsulfuron or Sulfometuron-methyl are restricted to use in paddy and dry rotation wheat field and acid soil in South; rice field herbicide containing

chlorsulfuron are restricted to use in rice transplanting field in South. It should be forbidden to use in time if herbicide residue accumulates beyond the limit.

Reinforce research on chemical weed control technique. As China would become a membership of WTO and agriculture restructuring in China would regulate, area of crops with high earning would enlarge and agriculture export would increase annually, herbicide residue in agricultural products would become a key point.

Reinforce technique training, propaganda and popularization to promote weed control in sustainable agriculture, improve farmer's technique and make great contribution to chemical weed control on farmland and guarantee agriculture production.

## INTEGRATED WEED MANAGEMENT IN GOLF TURF OF SOUTHERN CHINA

G. Xue<sup>1</sup> and J. X. Ma<sup>2</sup>

<sup>1</sup>East China Weed Technology, Nanjing, Jiangsu 210014, China.  
xueguang@jlonline.com

<sup>2</sup>Institute of Botany of Jiangsu Province and Chinese Academy, Nanjing, Jiangsu  
210014, China

**Abstract:** This paper is based on the investigation during 1998-2000 in 46 Golf courses in southern China to determine that there are more than 159 species weeds includes 39 species grasses, 15 species sedges and 105 species broad-leaved weeds. Among them, the most trouble weeds are *Digitaria sanguinalis*, *Paspalum distichum*, *Panicum repens*, *Paspalum conjugatum*, *Axonopus comperssus*, *Cyperus rotundus*, *Kyllinga brevifolia*, *Hydrocotyle sibthorpioides*, *Oxalis cornidila*, *Kummerwia stviata*, *Alternanthera philoxeroides*, *Desmodium triflorum*, *Mimosa pudica*, *Centella asiatica*, *Phyllanthus urinaris*, *Voila japonica*, *Hedyotis corymbosa*, *Euphorbia hirta*, *Lobelia asiatica*, *Alysicarpus vaginalis*. The author present 10 items of proposal for improving weeds integrated management in golf turf. The mains are strict quarantine; control weeds before sowing; Selecting the best turfgrass cultivar; mixing seeding; keep successful establishment; thicken sands; set up isolation areas; change and replace fertilizer; use pre- & early post-emergent herbicide; give good maintenance.

**Key words:** Integrated weed management, turf, golf course

### INTRODUCITON

Golf course is rapidly developing in China recently. More than 100 Golf courses had set up by the end of 2000. About 14 golf courses are in building. Above 85% of the Golf courses are in Southern part of China. Weed control is a key component in any successful turfgrass management program. Most people in turf maintenance department in Golf turf try to control weeds with chemical. But they fail. They have not followed the right ideas and right ways to use herbicides. Several factors, including herbicide, rate, time and number of applications, temperature, irrigation, light, mow and turfgrass cultivar may influence the effect of weed control. To increase the efficacy of weed control, we made investigation during 1998-2000 in 46 Golf courses in southern China to determine the weed species. 10 items of proposal for improving weeds integrated management in golf turf in China presented based on the investigation.

### MAIN WEEDS IN GOLF CLUB IN SOUTHERN CHINA

According to the investigation during 1998-2000 in 46 Golf courses in southern China, there are more than 159 species weeds includes 39 species grasses, 15 species sedges and 105 species broad-leaved weeds (Table 2).

#### Problem of weed control

Weeds can be disseminated by seed and/or vegetative means, with most weed species being prolific seed producers. Many weeds have a strong dormancy factor that

enable then to survive during period of extended drought, very cold winter, flooding etc. At most of Golf courses, strategy of weed control is not enough to fit weed situation. Any single measure in weed control can not achieve thoroughly successful. There two kind of weed control: one is "initiative weed control", another is "passive weed control". Most managers, who are in charge of turf maintenance in Golf club, had been driven to the place at "passive weed control" for some reasons. Some of them rely on hand weeding after weed interfering. Some of them rely on herbicide to kill the "adult weed" as foliage application. Some of them rely on mowing frequently to prevent "new weed seed". Some of them rely on sorting fertilizer to try hunger weed. They are as if "to take just palliative treatment". Why is it difficult to achieve thoroughly successful, when use a single measure in weed control? Perhaps, there are 6 causes.

Table 2. Some of the main weeds in Golf turf in southern China 1998-2000.

Grasses	Broadleaved
<i>Alopecurus aequalis</i>	<i>Acalypha australis</i>
* <i>Axonopus comperssus</i>	* <i>Alternanthera philoxeroides</i>
<i>Chrysopogon aciculatus</i>	* <i>Alysicarpus vaginalis</i>
<i>Cynodon dactylon</i>	* <i>Centella asiatica</i>
<i>Dactyloctenium aegyptium</i>	* <i>Desmodium triflorum</i>
* <i>Digitaria sanguinalis</i>	<i>Eclipta alba</i>
<i>Echinochloa crus-galli</i>	<i>Emilia sonchifolia</i>
<i>Eleusine indica</i>	<i>Erigeron annuus</i>
<i>Eragrostis aspera</i>	* <i>Euphorbia hirta</i>
<i>Eragrostis ferruginea</i>	<i>Euphorbia humifusa</i>
<i>Eragrostis unidoides</i>	* <i>Hedyotis corymbosa</i>
<i>Imperata cylindrica</i>	<i>Hydeotis diffusa</i>
<i>Imperata flavida</i>	* <i>Hydrocotyle sibthorpioides</i>
<i>Ischaemum indicum</i>	* <i>Hydrocotyle sibthorpioides</i> Var. <i>tatrachium</i>
<i>Leersia hexandra</i>	* <i>Kummerwia striata</i>
* <i>Panicum repens</i>	<i>Lantana camana</i>
* <i>Paspalum conjugatum</i>	* <i>Lobelia asiatica</i>
* <i>Paspalum distichum</i>	<i>Mazus japonicus</i>
<i>Paspalum orbiculare</i>	* <i>Mimosa pudica</i>
<i>Polypogon frugax</i>	* <i>Oxalis cornidilta</i>
<i>Roegneria kamoji</i>	* <i>Phyllanthus urinaria</i>
<i>Setaria viridis</i>	<i>Portulaca oleracea</i>
<i>Sporobolus indicus</i>	<i>Rostellularia procumbens</i>
	<i>Sagina japonica</i>
	<i>Sphenoclea zeylanica</i>
	<i>Striga asiatica</i>
	* <i>Trifolium repens</i>
	<i>Vernonia patula</i>
	* <i>Voila japonica</i>
Sedges	
<i>Cyperus compressus</i>	
<i>Cyperus iria</i>	
* <i>Cyperus rotundus</i>	
<i>Fimbristylis milliacea</i>	
* <i>Kyllinga brevifolia</i>	

\* Most trouble weed

### **Large seed bank and weed flora shift**

Most Golf turf was build up on rice field or other plantation or seashore. There is a large seed bank in the soil. There are also new weed seeds spreading every season. The number of weed seeds in soil is tremendous! If we pick up weed roots by hand every day, we have to turn up the soil! The weed seeds in soil surface turn up from button soil by hand, get good condition to emergent including oxygen, light and water. In this case, weed is getting serious. Some weed seeds were shifted from the border of rough areas by wind or violent storm. The weed control is over years job.

### **Old weeds are difficult to control**

Good weed control can be obtained in right application. In general, it is easy to control when weed is below 3-leave stage. At this stage, weeds are so short that can not be found if one do not pay attention carefully. People do not want pay any cost on herbicide if weeds have not threaten the turf. But it will fail to control weeds when weeds have imperiled the turf!

### **Some weeds and turf have similar looks**

Three kinds of weed are difficult to control by hand or chemical. First one is grass with creeping or straggling at the base like *Digitaria sanguinalis*, *Paspalum conjugatum*; Second one is sedges with tuber-forming or rhizomes underground like *Cyperus rotundus*, *Kyllinga brevifolia*; Third one is broad-leaved weeds with crawling or creeping rhizomes at the base like *Hydrocotyle sibthorpioides*, *Desmodium triflorum*, *Centella asiatica*, *Voila japonica*, *Euphorbia hirta*, *Alysicarpus vaginalis*. Such weeds have strongly spreading ability. If we pick up such weeds by hand, they will turn up pretty soon!

### **Competition**

There is a competition between weeds and turf. If someone rely on sorting fertilizer to try hunger weed, it will let turf hungry. Weed will invade a turf that is subject to poor cultural practices.

### **Irrigation or rain**

Proper water is good for turf growing. Frequency irrigation or rain will reduce of the efficacy of weed control. Soon after foliage application of chemical, watering wash out the herbicide from the surface of weed leave to soil or sand. The "actual rate" is far less than "design rate". In Hainan and south of Guangdong, weeds invade turf in rain season more heavily than that in dry season.

### **Mow**

We can find that most golf turf weeds are shorter and smaller than it in crop. Mowing frequency made it! After each time of mowing, some single low weeds escape from the mowing machine. They breed short stains. Their progeny become shorter and sorter. It always troubles the "turf keeper".

## **INTEGRATED WEED MANAGEMENT IN GOLF TURF**

Based on the investigation and practice during 1998-2000, we present 10 items of proposal for weed integrated management in Golf turf.

### **Strict quarantine**

Main way to reproduce progeny for weed is from seed or rhizomes. Strict quarantine can keep weed away from turf. Some weed like *Zoysia* in Bermudagrass, was infested into turf from seed. *Cyperus rotundus*, *Kyllinga brevifolia*; *Hydrocotyle sibthorpioides* in Bermudagrass, were also infested from seed or rhizomes. As soon as these weeds settle down in turfgrass, it will be difficult to wipe out and eradicate such weeds. Strict quarantine is cheap way to prevent weeds.

### **Control weeds before planting**

Before turfgrass planting, weeds may or may not have emerged, treatment can be by fumigation, soil sterilization, or non-selected or selective herbicide. Application of systemic herbicide to kill both existing weeds and rhizomes is needed.

### **Selecting the best turfgrass cultivar**

Tifdwarf, one of the Hybrid couches (*Cynodon dactylan* × *C.transvaalensis*) was recommended to golf green and fairways. The grass develops a deep, Strong root system. It is more tolerant of heat and resistant to drought than bent grass. It has a high tolerance to wear and tear. Tifdwarf forms a fine dense turf is more tolerant of herbicide and competitive to weeds, which is ideal for golf tee, green and fairways, particularly in southern China.

### **Mixing seeding**

In Shanghai, Jiangsu and Zhejiang region, bermudagrass will go to dormancy in winter when the temperature is below 0°C. For preventing the winter weeds, the best choice mixing seeding kentucky bluegrass. It can increase the competition ability of turf.

### **Keep successful establishment**

Most weeds can be found in varying part of a course. The kind of weed in a course will vary depending on the locality of the area, the weed found in the area, and the management imposed on that section of the course; eg. green, tees and fairways. Weeds will invade a turf that has become weak and this due to environmental stress. A dense, vigorous turf is best able to compete against weeds and this is only possible if the turf is well drained, receives adequate nutrition. Keep successful turf establishment is first important point to prevent weeds.

### **Thicken sands**

As a "soil" bedding on the base of turf, the ideal of sand thickness is more than 40cm. The sand should be treated by fumigation or "soil sterilization" to keep good turf establishment.

### **Set up isolation areas**

Bad environment, including wasteland, uncultivated hill or weedy plantation, brings a distant and continuously source of weed seed bank. Weed dispersal occurs by wind, water movement, animals, birds, in soil and sod, and on contaminated maintenance equipment. Set up weed free isolation areas with the width of 10-15 m can keep weed seeds or rhizomes away from course. Reducing the interfering of weeds greatly.

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### **Change and replace fertilizer**

Some weed like *Kyllinga brevifolia* used to high level fertilizer. If we change and replace fertilizer every season, the sedge will go weak. In this case, chemical control is very easy.

### **Use pre- & early post-emergent herbicide**

Pre- & early post-emergent herbicides are applied to establish turf prior to the emergence or early post-emergence of a specific weed and generally cannot control established weeds. They usually have a longer residual life than most post-emergent herbicide in order to control germinating weed seeds. Pre-emergents should be applied at least several weeks before the anticipated germination period of the weed seed to ensure control. Early post-emergents should be applied before weeds at 2 leave stage. Some of such chemical can keep weed control for more than 120days. Herbicide should not be regarded as the only way to control weeds but an important component of the total management program.

### **Give good maintenance**

Weed invasion into established turf is usually a result of the turf becoming weak and thin providing an opportunity for weeds to establishment. This may be due to damage by turf pests, improper cultural practices or intense wear. Severe weed problem may indicate unfavorable turf conditions (eg. Acid pH, waterlogged soils, shaded areas, low fertility) and all facets of turf management should be examined. Every measure should be applied coordinately. If we apply pre- & post-emergent herbicide 2~3 times every year with a good maintenance, we will achieve the weeds control.

## **EFFECT OF SHALLOW TILLAGE ON WEED SEED, SOIL MOISTURE, AND CROP YIELD**

C. Y. Xin, Q. Y. Guo, and X. L. Qiu

Plant Protection Institute, Qing hai Academy of Agriculture and Forestry, Xi ning  
810016, China

**Abstract:** Field experiments were conducted to determine the effect of tillage system on distribution of weed seed, soil moisture, and crop yield. Results indicated that timely shallow tillage after harvest could put 85-90% weed seed into 0-10 cm depth of soil. Shallow tillage system could provide suitable conditions for weed seed germination and emergence, subsequently increased the possibility of weed control by herbicide. Shallow tillage in combination with herbicide application could decrease the impact of weed to crop in 2-3 years. Further more, shallow tillage system reduced 50% of irrigation water and increased crop yield compared to that of deep tillage system.

**Key Words:** shallow tillage, seed distribution, soil moisture

### **INTRODUCTION**

Weed control by shallow tillage is mainly used to develop dry and semi-arid agriculture. In northern of China, water shortage in the soil is the main limited factor for agriculture. To receive more rainfall by farmland and to control weed, deep tillage in autumn as traditional tillage system was proceeding. This technology has two disadvantages, the first one is it spends lots of labors and powers. The second one is more transpiration of soil water in deep tillage field than that of in shallow tillage field and therefore soil drought is accelerated. Meanwhile weed seed fallen on the land is ploughed into 0-30 cm depth of soil by deep tillage, it is difficult to control by herbicide, because of the difficulty of weed seed germination in such a depth.

This study focuses on effect of shallow tillage on soil moisture, weed seed distribution in soil, and the efficacy of herbicides, so as to provide some advisement for dry land farming.

### **MATERIALS AND METHODS**

#### **Experimental design**

The Experiment includes the shallow tillage by disk harrow in year 1, 2 and 3, depth of 10 cm and deep plough by 5 moldboard in year 1,2, and 3, depth of 30 cm. In the year 1 wheat was planted, year 2 oil-seed rape was planted, and year 3 highland barley was planted. In the seedling stage dicotyledon weed was controlled by 2.4-D at 540g/ ha. Soil was treated to control wild oat and annual dicotyledon weed with Trefanocide at 1260g /ha before oil-seed rape planting. In the untreated control field no weed control was applied.

#### **Investigation**

Soil moisture in 0-10 cm, 10-20 cm and 20-30 cm depth of soil was measured before wheat planting and after harvesting. The distribution of weed seed in different soil layer was investigated after soil ploughed, and the total number of weed seed in the



tilling layer affected by herbicide was also measured. Costs of plough machine, irrigation water and power were measured in shallow tillage and deep tillage field, respectively, according to the price at that time. Crop yield was calculated through sampling when harvesting.

### **Experiment sites**

Experiment field located at the Tang Gemu Farm, Gonghe Basin in Hai Nan Tibetan with 2960 m above sea level. The experiment field belongs to dry farming with the average 260 mm rainfall a year. Irrigation was carried out in growing season. The soil was desert pedocal and the organic content was around 1.8%. Main crop is highland barley, wheat, and seed-oil rape.

## **RESULTS AND DISCUSSION**

### **Effect of shallow tillage on the moisture content in soil layers**

After 3 years of continuous shallow tillage, on April 4 of the third year, the moisture content in the soil of depth of 0-10cm, 10-20cm, 20-30cm were measured. The results showed that moisture content in shallow tillage field were 17.6%, 21.2%, 18.0%, respectively, and 18.8%, 22.7%, and 19.1% in deep tillage field, respectively.

So soil water was almost the same in different tillage layers of soil and it could meet the need of crop germination and emergence. The second measure was conducted on June 13, and the moisture content in the depth of 0-5 cm, 5-10cm, 10-15 cm, 15-20 cm, 20-25 cm, 25-30 cm were 1.5%, 10.8%, 16.8%, 16.1%, 3.4%, and 16.6% respectively, in the field of shallow tillage, and 1.2%, 9.8%, 9.3%, 19.6%, 8.5%, and 18.9% respectively, in the field of deep tillage. In the field of shallow tillage, the highest moisture content was in the 5-20cm, and decreased in the depth of 20-25cm. This is explained by the forming of plough sole under 25 cm without ploughed. In this case crops could obtain enough water and grow well. In the deep tillage field the lowest moisture content was in the layer of 0-15cm, and the moisture was higher in the depth of 15-20 cm, it further increased below the layer of 25 cm due to the plow sole was lacerated by the deep plowing. In this case, seedlings were few and grow weakly. In the dry non-irrigation area, the moisture content evaporates quickly, due to deep plough and the soil was greatly short of water. This was one of the main causes of soil drying.

### **Effect of shallow tillage on distribution of weed seeds in soil**

Field experiment showed that about 85-90% weed seed fallen on the ground were ploughed into 0-10 cm depth of soil by shallow tillage and kept these seeds from falling into deeper soil. Subsequently weed seeds in this layer germinate easily with getting enough water, air and heat, so weeds might be controlled after shallow tillage with applying herbicide within 2-3 years.

The vertical distribution of weed seeds in soil varied with the time of shallow tillage. There were 98.2% of weed seed in the depth of 0-10 cm when shallow tilled 2 days after harvest in 1987; 95.9% of weed seed when shallow tilled 11 days after harvest in 1988; and 100% of weed seed 2 days after harvest in 1989. Weed seeds below 10 cm were only 1.8%, 4.1%, 0% respectively, in different years. While in deep tillage, weed seeds were ploughed into 0-30 cm depth of soil. The percentages of weed seeds in the 0-10 cm depth of soil were 17.1% in 1987, 18.3% in 1988, and 24% in 1989. There were 75.6%, 73.2% and 65.2% of weed seeds in the layer of 10-20cm

respectively, in the three years experiments. In the layer of 20-30 cm weed seeds were 7.3%, 8.5%, and 10.8% respectively. We concluded that there were much more weed seeds below 10 cm depth of soil in the deep tillage than that of in the shallow tillage, but few of them could germinate and emerge because of the limitation of environmental factors, so it is impossible to control weeds effectively. This might be the reason of difficulty to control weeds in deep tillage field.

#### Effect of shallow tillage in combination with herbicide on weed seeds in the soil

Table 1 shows the results from the experiments of shallow tillage with herbicide, and deep plough with herbicide for 3 years. Results showed that the best way to control weed was shallow tillage in combination with herbicide for three years, and the amount of weed decreased by 75.4% every year. Shallow tillage for two years and then deep tillage for one year was also a better way to control weed, in this system the amount of weed decreased by 53.2% every year. With shallow tillage for 1 year and continuous deep plough for 2 years, the amount of weed decreased by 27.6% every year. With continuous deep tillage for 3 years, the amount of weed decreased by 27.3% every year. The amount of weed increased up by 43% in the field without applying herbicide and weed cutting.

Table 1. Effect of weed control by shallow tillage in combination with herbicide\*

Weed Seed	Investigation time 4,10,1988		Investigation 4,6,1989		Investigation 10,4,1989		Means ( $\pm$ %)
	Treat	Seed ( $\pm$ %)	Treat	Seed ( $\pm$ %)	Treat	Seed ( $\pm$ %)	
2502	DT+ h	2124-15.1	DT + h	450-78.8	DT + h	504+12	-27.3 c
2502	ST + h	666-73.4	DT	120-82.0	DT + h	207+72.5	-27.6 c
2502			ST + h	303-54.5	DT + h	207-31.7	-53.2 b
2502					ST + h	5-98.3	-75.4 a
1348	DT	1505+10.4	DT	6354+76.3	DT	11034+42.4	+43.0 d

\*Means in each column followed by the same letter are not significantly different at 5% level according to Duncan's Multiple Range Test. DT: deep tillage; St: shallow tillage; h: herbicide.

#### Effect of shallow tillage on yield, cost and irrigating water

Comparing with deep tillage, shallow tillage system used in this study could save 50% of irrigation water and 60% of power cost by tractors (Table 2). Deep tillage severely wore out agricultural machine, increased spending of power and water,

Table 2. Effect of shallow tillage on barley yield, cost and irrigation water\*.

Tillage	Cost of Plough (yuan/ha)	Cost Save (%)	Irrigation Times	Irrigation Water (m <sup>3</sup> /ha)	Irrigation Save (%)	Yield (kg/ha)
ST	75	66.7	1 Seedling Water	1650	50	4350 a
			1 Winter Water	1050	36.4	
DT	225		1 Seedling Water	3300		4371 a
			1 Winter Water	1650		
ST	75	66.7	1 Winter Water	1650	50	2085 b
DT	225		1 Winter Water	3300		1552.5 c

\*Means in each column followed by the same letter are not significantly different at 5% level according to Duncan's Multiple Range Test. DT: deep tillage; St: shallow tillage; h: herbicide.

therefore increased the cost of production. There was little difference of yield between shallow tillage and deep plough with one irrigation in winter and in spring, if only one

irrigation in winter, the yield of shallow tillage was 2085kg/ha, and in deep tillage it was 1552.5kg/ha.

## SPECIES, OCCURRENCE AND REGIONAL DISTRIBUTION OF THE MAJOR WEEDS IN UPLAND CROPS OF SHANDONG PROVINCE

R. T. Jiang, W. J. Tan, Z. D. Liu, M. Ji, S. Z. Liu, X. Zheng, and M. Li

Development and Extension Center for New Herbicides and Technology of Shandong Province, Agricultural Science Research Institute of Taian City, Taian 271000, Shandong, China

**Abstract:** Extensive field investigation on species, occurrence and regional distribution of the weeds in upland crops was made in Shandong province. Totally 254 weed species were found in the upland crops of Shandong province, which belong to 171 genera from 53 families. Of the weed species, about 22 weeds belonging to 19 genera from 12 families occur in winter wheat, while 25 species belonging to 17 genera from 10 families occur in the spring and summer crops. Among the weed species in winter wheat, those belonging to the family of Cruciferae, Caryophyllaceae, and Borage occur universally. Their emergence peaks normally twice a year. They first occur starting mid October till mid November before winter, then peak starting end March till early April after winter and mature all during mid-end May. Of the weeds in spring and summer crops, most of them are annuals, with annual grasses, *Chenopodium album* and *Amaranthus tricolor* as dominant species. Soil, temperature, moisture and farming practice are the key factors that affect the infestation and abundance of the weeds in upland crops. Based upon the difference in climate, soil and crop cultivation practice, Shandong province is divided into the following six agricultural regions in term of weed infestation and distribution: Jiaodong region, Bohai region, Lubei region, Luxi region, Hudong region, and Lunan region. However, weeds in each of the region have the same biological-ecological characteristics. In addition, there is a trend that weeds that favor moisture reduce gradually and at the same time those that could resist cold and drought increase markedly along with the movement of latitude from the south to the north. Meanwhile, with the movement from Bohai to Hudong region, the number of the salt-resistant weeds reduces gradually, while that preferring fertilizer and moisture increase accordingly.

**Key words:** weeds, upland crops, occurrence, emergence, Shandong

### INTRODUCTION

Being a large agricultural province, Shandong locates in a wheat-corn rotational agro-ecological zone, with a total cropping area of 10 million ha. Winter wheat is the leading crop which share about 4 million ha each year. The second biggest crop is maize, with a seeding area of 2.4 million ha each year. In addition, there are 0.6 million ha of sweet potato and 0.1 million ha of corn. The rest are peanut (0.6 million ha), soybean (0.45 million ha), tobacco (0.1 million ha) and vegetables (0.7 million ha). Yielding two crops per year is the main characteristic of the farming system. Along with the agricultural development of high efficiency, quality and yielding as well as the adjustment of cropping structure and system, the three-dimension farming technology has been extended rapidly in recent years. For example, in order to achieve better economic benefit per acreage, a lot of farming technology such as wheat inter-planting with maize, peanut, soybean or vegetables has been introduced and applied, which

might affect the weed occurrence and distribution.

## MATERIALS AND METHODS

Extensive investigations on weed species, occurrence and distribution in various upland crops by means of visual and counting assessment were conducted in 79 representative counties and cities within Shandong province.

## RESULTS AND DISCUSSION

Totally 254 weed species were found in the upland crops within the province, which belong to 171 genera and 53 families.

### Species and occurrence of common weeds in winter wheat

There are 22 weeds belonging to 19 genera and 12 families found in winter wheat, among which Cruciferae, Caryophyllaceae, Boraginaceae, Chenopodiaceae are the more widespread species. They are as follows:

Cruciferae: Tansy Mustard (*Descurainia Sophia* (L.) Schur), Shepherdspurse (*Capsella bursa-pastoris* (L.) Medic), Hairy Bittercress (*Cardamine hirsuta* L.) and Orange Erysimun (*Erysimum bungei* (Kitag));

Caryophyllaceae: Cone Silene (*Siolene conoidea* L.), Common Chickweed (*Stellaria media* (L.) Cyr.) and Thymeleaf Sandwort (*Arearia sepyllifolia* L.);

Boraginaceae weeds: Orange Erysimun (*Erysimum bungei* (Kitag)), Corn Giromwell (*Lithospermum arvense* L.) and Labiatae Henbit Deadettle (*Lamium amplexicaule* L.);

Rubiaceae: Slender Catchweed Bedstran (*Galium aparine* L. Var. *tenerum* (Gren. et Godr));

Chenopodiaceae: Lambsquarters Goosefoot (*Chenopodium album* L.) and Serotinous Goosefoot (*Chenopodium serotinum*);

Moraceae: Japanese Bromegrass (*Bromus japonicus* Thunb);

Polygonaceae: Doorgrass (*Poygonm aviculare* L.) and Pale-flowered Knotweed (*Poygonm lapathifolium* L.);

Leguminosae: Bush Vetch (*Vicia sepium* L.);

Convolvulaceae: Common headgeparsley (*Torils scabra* (Thunb.) DC.) and European Binduweed (*Convthium. Arvensis* L.);

The composite family: Chinese ixeris (*Ixeris chinensis* (Thunb.) Nakai);

The graminaceous family: Equal Alopecurus (*Alopecurus aequalis* Sobol.) and Japanese Bromegrass (*Bromus japonicus* Thunb).

As regional problem weed, Sun euphorbia (*Euphorbia helioscopia* L.) of Euphorbiaceae occurs mainly in the regions of Zaozhuang, Tengzhou and Linyi.

The occurrence of the weeds in winter wheat has two peaks. The first one is from mid October to early November before winter. The major species emerging in this period are Shepherdspurse (*Capsella bursa-pastoris* (L.) Medic), Orange Erysimun (*Erysimum bungei* (Kitag)), Tansy Mustard, Corn Gromwell, and Thymeleaf sandwort. Except some natural mortality, most of them can survive through winter safely. They blossom in March or April the next year and grow up gradually during mid-end May. The second peak is end March. Besides the weeds mentioned above, Lambsquarters Goosefoot, Bush Vetch (*Wicia sepium* L.), Japanese Hop, Doorgrass, Common

headgeparsley (*Torilis scabra* (Thunb.) DC.) occur in this time, which blossom and grow up before the wheat harvest in June.

### **Dominant species and emergence pattern of weeds in spring and summer crops**

There are 25 familiar weeds found in spring and summer crops, which belong to 17 genera of 10 families. The dominant species are Lambsquarters Goosefoot, Serotinous Goosefoot, Chinese City Goosefoot (*Chenopodium urbicum* L. subsp. *sinicum* Kung et G.L.Chu) of Chenopodiaceae; Redroot Amaranthus, Green Amaranthus Roxburgh Amaranth (*Wicia sepium* L.) of Amaranthaceae; Copperleaf (*Acalypha australis* L.) of Euphorbiaceae; Chingma Abutilon Piemaker (*Abutilon theophrasti* Medic) of Malvaceae; Siberian Cocklebur (*Xanthium sibiricum* patrin, White Eclipta (*Eclipta prostrata* L.), Common Cephalanopios (*Cephalanoplos segetum* (Bge.) Kitam) of Solanaceae; Goosegrass, Common Crabgrass, Barnyardgrass, Green Bristlegrass (*Setaria vividis* (L.) Beauv) of the hebenke; Rice Galingale, Diffomed Galingale, Nutgrass galingale of Cyperaceae; Common dayflower (*Commelina communis* L.) of Commelinaceae.

Maize and cotton in Shandong province are usually seeded or planted in early-mid May. Those covered with plastic sheeting are seeded even in mid-end April. If the humidity of soil is fit, the first spring or spring-summer weed species may occur starting mid-end May. As a rule, serotinous Goosefoot and Common Purslane emerge first, followed by Lambsquarters Goosefoot, Redroot Amaranthus, Green Amaranth, Indian Hemp, Common Crabgrass, and Goosegrass. The summer crops, such as Maize, Peanut etc, are seeded in early or mid June. As inter-planted rotational crops between winter wheat rows, they are usually seeded starting the end May till the early June. At this moment, the temperature is high and if the humidity of soil is also suitable, most weeds can occur in 6-7 days after the rotational crop seeding. Normally only a little emergence peak occurs right after irrigation or rainfall. After early or mid of July, most weeds enter the period of blossom and fruitage and their seeds are ripe gradually.

### **Characteristics and regional distribution of the weeds in upland crops in Shandong province**

Based upon the difference in the climate and soil factors in shandong province, shandong has been divided into six agricultural regions in term of weed infestation and distribution, namely Jiaodong, Bohai, Lubei, Luxi, Hudong and Luzhong agricultural region. Because the distribution of weeds in each region is influenced largely by the farming systems, crops, and measures of weeding, the species, the dominant population composition and the density of weeds are different certainly from each other. Especially in recent years, due to the extensive use of herbicides and the North-South introduction of crops, great changes and difference in the dominant weed species and population have been found in each of the agricultural regions.

#### **Agricultural Region of Jiaodong**

Wheat and maize are the major crops in this region, followed by peanut. Soil in this plain is loam mainly, while that in mountain area is sand loam or sandy soil mainly. Due to the regulation of the sea, the humidity in this region is high in spring so that the spring drought is relatively light. In autumn, it rains much more due to the influence of the typhoon. The dominant species in winter wheat in this region are Tansy Mustard, Orange Erysimum (*Erysimum bungei* (Kitag)), Thymeleaf sandwort, Common Chickweed and Equal Alopecurus etc, among which the infestation by Tansy Mustard is

100%, whereas Orange Erysimum, Thymeleaf sandwort, and Common Chickweed are around 30%. The population complex of dominant weeds in winter wheat are Tansy Mustard - Thymeleaf sandwort - Thymeleaf sandwort - Common Chickweed - Orange Erysimum.

The major weed species in spring and summer crops include Goosegrass, Common Crabgrass, Common Purslane and Flower Gentle *etc.*, among which the infestation of Goosegrass and Common Crabgrass is as high as 88%. The dominant population is Goosegrass - Common Crabgrass - Common Purslane and Goosegrass - Common Crabgrass - Flower Gentle - Lambsquarters Goosefoot.

#### **Agricultural region of Bohai**

Wheat, cotton, soybean and peanut are the main crops in this region. Soil here is influenced by drought, water-logging and alkali seriously, so the weeds that can bear salt and drought often occur, such as Oakleaf Goosefoot (*Chenopodium glaucum* L.), Common Reed, Common seepweed (*Suaeda glauca* Bge.), Tansy Mustard and Convolvulaceae *etc.* Meanwhile, rainfall in July and August in this region is excessive, so that Goosegrass, Barnyardgrass and Common Crabgrass occur here and there. The infestation of Tansy Mustard is more than 70%, and that of Oakleaf Goosefoot, Common Reed and Pale-flowered Knotweed 30%, respectively. The dominant weed population in winter wheat is Tansy Mustard - Oakleaf Goosefoot - Common headgeparsley - Common seepweed.

Major weeds occurring in spring and summer in this region include Common headgeparsley, Common Cephalanopios, Lambsquarters Goosefoot, Common Purslane and the grasses such as Goosegrass, Wiregrass, Barnyardgrass and Common Crabgrass. The infestation of the species of Convolvulaceae is ca. 80%, and that of annual grasses 100%. The dominant population is Goosegrass - Barnyardgrass - Common Crabgrass - Flower Gentle - Lambsquarters Goosefoot and Goosegrass - Common Crabgrass - Common headgeparsley - Common Cephalanopios.

#### **Agricultural region of Lubei**

This region lies in the half arid zone. Drought in spring and water logging in summer are very serious, with a little drought in autumn. Cotton is the major crop, followed by soybean, wheat and maize *etc.* Weed infestation and species diversity here is less than that in other regions. The main weeds in spring and autumn crop are mainly annual grasses, such as Common Crabgrass, Goosegrass, and Green Bristlegrass. As broad-leaved weeds, there are Common Purslane, Lambsquarters Goosefoot, and Flower Gentle *etc.* In addition, Goosegrass - Common Crabgrass - Common Purslane or Goosegrass - Common Crabgrass - Lambsquarters Goosefoot - Flower Gentle is one of the dominant weed communities in this region, with a highest infestation up to 90% by Goosegrass and Common Crabgrass.

Tansy Mustard is the worst weed in winter wheat, the second is Orange Erysimum, followed by Thymeleaf sandwort and so on. Lambsquarters Goosefoot, Common headgeparsley and other weeds only emerge in the spring next year. The dominant weed population is Thymeleaf sandwort - Lambsquarters Goosefoot - Common headgeparsley - Cone Silene. As to the infestation, Thymeleaf sandwort is about 90%, Common headgeparsley 30%, Lambsquarters Goosefoot 25% and Cone Silene 12%.

#### **Agricultural region of Luxi**

The climate in this region belongs to that of grassland. Soil here is barren due to alkalization and the content of organic matter is also lower. Wheat, cotton and soybean are the major crops. Major weeds, which are barren-tolerant and favor humidity or half

humidity, include Branched Horsetail (*Equisetum ramosissimum* Desf), Puncturevine Caltrap (*Tribulus terrestris* L.), Bush Vetch (*Vicia sepium* L.), Black Nightshade (*Solanum nigrum* L.), and White Eclipta etc. The density of weeds in this region is lower than that in other regions. Weeds which survive through the winter are mainly Tansy Mustard. Broad-leaved weeds, such as Common hedgeparsley and Common Cephalanopios (*Cephalanoplos segetum* (Bge.) Kitam), occur mainly in the next spring while Chenopodiaceae, Amaranthaceae, Cyperaceae weeds, Barnyardgrass and Common Crabgrass are the major weeds occurring in the spring and summer crops.

#### **Agricultural region of Hudong**

Water resource here is plentiful and soil belongs to light loam with good quality which makes this region possible to achieve a highest level of productivity in Shandong province. Wheat, maize and sweet potato are the major crops planted here in upland crops. Many species of weeds occur in this region with high population density and serious injury to the crops. The dominant weed species in winter wheat include Tansy Mustard, Shepherdspurse, Orange Erysimum, Thymeleaf sandwort, Corn Gromwell, Cone Silene, Common Chickweed and Slender Catchweed Bedstran, while those in low and wet fields are Hairy Bittercress, Longstamen Onion (*Allium macrostemon* Bunge), White Eclipta, Sun euphorbia, and Common dayflower (*Commelina communis* L.) etc.

In recent years, Equal Alopecurus, Wild Oat (*Avena fatua* L.) and Sun euphorbia are becoming more and more severe in winter wheat in this region. The dominant weed population complex in winter wheat are Tansy Mustard - Shepherdspurse - Orange Erysimum - Corn Gromwell, or Tansy Mustard - Cone Silene - Slender Catchweed Bedstran. The infestation by Tansy Mustard is more than 90%, while that of Thymeleaf sandwort, Cone Silene and Orange Erysimum are ca. 50% respectively and Shepherdspurse 65%. The dominant weed population complex in spring and summer crops is Goosegrass - Common Crabgrass - Lambsquarters Goosefoot - Flower Gentle - Common Purslane, among which the infestation of Common Crabgrass and Goosegrass is as high as 100%, followed by Lambsquarters Goosefoot and Flower Gentle with more than 90% infestation.

#### **Agricultural region of Lunan**

This region has the highest terrain and the most complicated natural geomorphology. Tansy Mustard, Orange Erysimum, Lambsquarters Goosefoot, Shepherdspurse, Japanese Hop, Corn Gromwell, Nutgrass galingale are the major weeds in winter wheat, among them the infestation of Tansy Mustard is more than 90%, Orange Erysimum 20%, Shepherdspurse 15%, Cone Silene 10%, and Lambsquarters Goosefoot 30%. The dominant population complex is Tansy Mustard-Orange Erysimum - Corn Gromwell - Cone Silene or Tansy Mustard - Shepherdspurse - Lambsquarters Goosefoot. The major weeds in spring and summer crops in this region include annual weeds such as Common Crabgrass, Goosegrass, Green bristlegrass, Amaranthaceae, Chenopodiaceae, Copperleaf and some perennials such as Common Cephalanopios (*Cephalanoplos segetum* (Bge.) Kitam), Lalang Grass (*Imperata cylindrica* (L.) Beauv. Var. *major* (Nes) C.E.Hubb), Nutgrass galingale, Bermudagrass (*Cynodon dactylon* (L.) Pers), and Common reed (*Phragmites communis* Trin.) etc.

In one word, despite of certain difference in distribution among the mentioned agricultural regions, most weeds in Shandong province share the similar biological-ecological characteristics, such as exuberant vitality, high reproduction, and high adaptability and resistance to drought, water-logging, cold, salt and barren conditions. In addition, there is a trend that weeds that favor moisture reduce gradually and at the



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same time those that could resist cold and drought increase markedly along with the movement of latitude from the south to the north. Meanwhile, with the movement from Bohai to Hudong region, the number of the salt-resistant weeds reduces gradually, while that which prefer fertilizer and moisture increase accordingly.

## **ON THE DEVELOPMENT OF CHINA WEED INFORMATION SYSTEM BASED ON THE WEB**

S. H. Wei and S. Qiang

Weed Research Laboratory, Nanjing Agricultural University, Nanjing 210095, China,  
wrl@njau.edu.cn)

**Abstract:** China Weed Information System (CWIS), a web based information system about weed sciences in China is developed using modern computer and network technologies. It could be used to obtain large amount of information on weed species and its management. In SQL Server, several databases were created, such as databases of weed species and specimens, herbicides, quarantine weeds and reference materials. By ASP pages and ODBC, these databases were connected to the query interface of web pages and the weed information could be shared online with weed researchers all over the world. In CWIS, information of 2270 weed species belonging to 144 families, 823 genus in China were stored, which included their name, prevalence, damage, distribution, morphological description, photos, control methods, specimens etc. You could query the information of those weed species by Chinese name, English name, Latin binomial or their biological characteristics, or you could use multiple parameters to narrow the search results. These data could be obtained rapidly and conveniently. In addition, you could make statistical analysis of weeds in China based on these databases. The decision-making features of the CWIS allow you to select herbicides or other measures to implement your weed control strategy. In CWIS you also could get the reference materials, identify the weed species and manage the information of weed specimens. The system is available on the WWW at the site <http://weed.njau.edu.cn/>.

**Key words:** Weed, information system, weed management, weed specimen, quarantine weeds, Web based

### **INTRODUCTION**

Weed infestation is the major factor causing the yield loss of farm crops. As weed control measures continue to increase, the way choose to control weeds becomes more complex and there are more restraints for weed managers to implement their control measures (Renner et al. 1999). To control weeds safely and effectively in sustainable agriculture, rapid access to information on weed management is a prerequisite for weed scientist to make their decisions. Computerized information system could provide accurate and rapid access to information. SOYHERB and CORNHERB are weed management systems (Renner and Black 1991), but they are not web based and only used in determining herbicide usage.

Internet stretches rapidly during these years, many websites about weed sciences are mushrooming. INVADERS Database system (<http://invader.dbs.umd.edu/>), USDA-APHIS Noxious Weeds (<http://www.aphis.usda.gov/ppq/weeds/>), International Survey of Herbicide Resistant Weeds (<http://www.weedscience.com/>) are all web based sites about weed science, but they are not comprehensive websites, they provides information only about exotic or noxious weeds in the local area of USA, or the information they provided is so specialized that it could not cater to most weed managements all over the world.

IWSS (<http://www.css.orst.edu/weeds/iwss/>), the European Weed Research Society (EWIS, <http://www.res.bbsrc.ac.uk/ewrs/>), and the Weed Science Society of America (WSSA, <http://piked2.agn.uiuc.edu/wssa/>) are the organizational web sites that provide a variety of information items and links to related sites about weed science. They could not provide detailed information on weed species and its management.

Currently there are no professional website constructed to provide comprehensive information on weed species and its management in China. Prior to 1990, Qiang Sheng and Li Yanghan developed a database information system about 1300 weed species in China using Foxbase<sup>+</sup>, it could be easily used to query the Chinese name, common name, Latin binominal of weed species and their prevalence, distribution, damage, habitat and biological characteristics. China Weed Information System (CWIS) is based on the previous database information system, but further research is conducted to rich the query functions of the old system, and now, it is available for more weed researchers by connecting it to the web applications.

## **MATERIALS AND METHODS**

### **Hardware and Software**

Different to the traditional management information system, CWIS is an information system based on the browser/server structure, application runs mainly on the web server. By best trade-off between function and quality of the Server, Sunway 2000 (PIII550/30G/128M/17"/50X) with Microsoft Windows 2000 Server installed is determined as the system development platform. Ms SQL Server is a relational database administrator and a professional environment for organizing information (Zhao 1998). Its standardized structural query language enables the rapid and convenient query for weed information in the database. ASP technology of IIS is used to create a connection to the database server. Macromedia Dreamweaver and Ms Frontpage are used as the page design and site management tools. Image editors such as Adobe Photoshop, Macromedia Fireworks and Flash are used to optimize the images obtained from the Scanner or Digital Camera.

### **Data Collection**

Chinese name, common name, Latin binominal and English name of weed species and their prevalence, distribution, damage, habitat and biological characteristics are gathered mainly from Flora Sinica and Weed Flora Sinica. Weed specimens information such as the date, site, number of collection and its collector, identifier, date of identification is obtained from China Weed Herbarium (located at Nanjing Agricultural University). Data of weed photos, morphological description, control methods, herbicides, reference materials and quarantine weeds are obtained from books and periodicals about weed sciences. Most photos of weed species are scanned and saved as JPEG format, the other weed photos are captured by digital camera and directly input into computer.

### **Database Creation and Connection**

A good structure of the database ensures the rapid, accurate and effective data access. Major key fields is inevitable for creating a database table, records can be indexed quickly on it. In CWIS, major key fields is set according to the code of weed species in Classification and Codes of Chinese Plants. To provide the greatest

information about weeds, data are stored using the smallest logical units. For example, scientific name of weed species are stored using 3 fields—genus, additives and authors. In ODBC, a DSN is created for the database, ASP application use a special OLE DB provider that could communicate with any ODBC driver, and the ODBC driver is then responsible for communicating with the database (Zhang 1998). ASP pages incorporate SQL sentences, VBScripts and ActiveX control with common HTML codes that could provide more interactive query options to access the database information (Du 1998).

### **Page Design and Site Management**

Dreamweaver and Frontpage are the primary page editors while Photoshop, Fireworks and Flash are the main image editors in designing the website of CWIS. When begin creating a Web site, a series of planning steps is followed to make sure your site succeeds. Deciding the function of the site is the very first step you should take when you create a Web site. Then choosing a target audience, creating sites for browser compatibility, organizing the site structure, designing the navigation scheme etc. The more sophisticated the site is—in terms of layout, animation, multimedia content, and interaction—the less likely it is to be cross-browser compatible. Note that not all browsers can run frames, DHTML and JavaScript, try to balance between maximum effect and maximum browser compatibility.

## **RESULTS AND DISCUSSION**

### **Target Visitors**

The target visitors of CWIS is set predominantly to Windows users with a resolution of 800 x 600 pixels, using browsers such as Internet Explorer or Netscape Navigator 4.0 or above. The system is available on the WWW at the site <http://weed.njau.edu.cn/>. For best view effects, you should adjust your computer to meet the requirement. The system have English version and Chinese version, this ensures the greatest access by viewers around the world.

### **Database Structure**

In SQL Server, databases of weed species, herbicides, quarantine weed species and reference materials were created and organized systematically. Weed species database contains several tables, Species table including the information of Chinese name, common name, Latin binominal, English name and their prevalence, distribution, damage, habitat, biological characteristics. Specimens table include the information of the date, site, number of collection, collector, identifier, date of identification and cabinet. Photos table contains the images of adult, seedling, seed, stem, leaf, flower or florescence, fruit and plants in colony for each weed species. The code of Chinese Plants (sppld) served as the major key fields to connect tables of Species, Specimens, Morphology and Photos in weed species and specimens database (Figure 1). Quarantine weeds database is much alike the weed species database, but additional fields such as date, port of interception, original country etc. are added to it. Herbicides database contains the common name, product name, manufacturer, molecular formula, physical state, spectrum, application methods, rates, use precautions, mode of action, toxicology and so on. Reference materials database contains the title, author, unit, abstract, key words, name and number of books and periodicals etc.

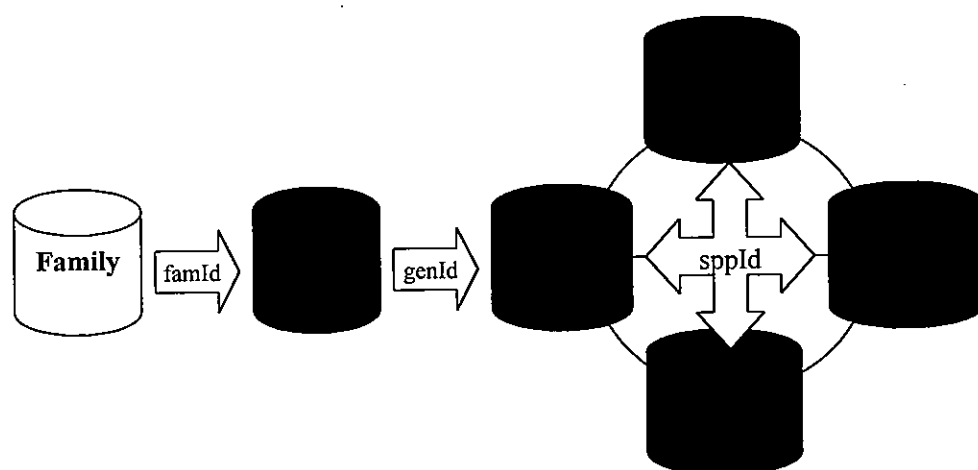


Figure 1. Structure of Weed Species and Specimens Database in CWIS

### Information Services

Web based CWIS is the subsequent of modern information society. In databases of CWIS, information about 2270 weed species belonging to 144 families, 823 genera in China were stored, which included their name, prevalence, damage, distribution, morphological description, photos, specimens etc. In addition, databases of herbicides, quarantine weeds and reference materials stored additional information on weed management. Via ASP pages and ODBC, these databases in CWIS were connected to the query interface of web applications and the information in databases is shared on WWW with the weed researchers around the world.

The query interface of CWIS greatly facilitates you to query the information. For each query, type the words or letters into the text form on any search pages in CWIS and select the corresponding item in the list menu, and then click the SEARCH button. CWIS will then look for any "matches" with your query in the database. If the search result exceeds 10 records, use the NEXT or PREVIOUS button to view more records. The search is based on a Master/Detail style, you could conduct the further search by clicking the VIEW button for each records. Phrase matching and wildcard matching is automatically used for the query. To narrow the search results, you should provide more detailed search parameters. For advance search, you could use multiple parameters to limit the search results.

In CWIS, you could query the information of weed species by Chinese name, common name, Latin binominal, English name and their prevalence, distribution, damage, habitat, biological characteristics etc. You could query the specimen information in China Weed Herbarium by the date, site, number of collection, collector, identifier, date of identification and cabinet number. The images of adult, seedling, seed, stem, leaf, flower or florescence, fruit and plants in colony for each weed species could also be obtained from the CWIS. By entering the words of morphological description in the search textbox, you could make some identification for certain weed species you are unsure of. When searching reference materials for your scientific research, you could simply use the title, author, unit, abstract, key words, name and number of books and periodicals to quickly and accurately get what you want. For weed chemical control in agricultural production, CWIS could provide herbicides information such as spectrum, application methods, rates, use precautions, mode of action, common name, product

name, manufacturer, molecular formula, physical state, toxicology etc. It allows you to choose herbicides or other measures freely to implement your weed management strategy. In addition, you could make statistical analysis of weeds in China based on the information in CWIS. Information on quarantine weeds is also available from the system as well. These data could be obtained rapidly and conveniently in your browser.

The information in CWIS is highly editable and refreshable to site administrator and a certain amount of users. On the management interfaces of CWIS, you could easily inserting, deleting and updating the records in the database. This feature is also enabled to enlarge the information in the reference material database, quarantine weeds database herbicides databases etc. to dynamically keep up with the development of weed science. The system has multiple levels of password authentication that guaranteed the good performance of security. To edit the contents in CWIS, you must have the right permission.

News and relative links about weed science are also provided in CWIS. The site is updated regularly with the latest news, conferences, advanced topics plus advice from weed experts. Check the CWIS for information about weed science to facilitate your research.

## **ACKNOWLEDGMENTS**

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# **Biological Control**

## HOST SPECIFICITY IN WEED BIOCONTROL

P. Harris

Agriculture & Agri-Food Canada, Lethbridge Research Centre, PO Box 3000,  
Lethbridge, AB, Canada, T1J 4B1.    [harris@em.agr.ca](mailto:harris@em.agr.ca)

**Abstract:** Host range determination is essential to exclude release of weed biocontrol agents harmful to desirable plants. Presently this is largely based on host range surveys in the native region and lack of larval development in no-choice tests. About 10% of the agents established in Canada have no-choice host ranges narrower than the field range, 20% are the same and 70% are broader. Over 90% of those in the last category developed in no-choice test on North American plants closely related to the host; but only 4% have attacked them in the field. The native flora is a sensitive issue so there is an urgent need to identify which are actually at risk. A combination of the native field host range and no-choice test limits risk to a plant taxon. Not releasing agents that develop on critical plants in the taxon eliminates most biocontrol agents. Four strategies less destructive to weed biocontrol are suggested for achieving the same end. 1) Extend the host range surveys to determine the candidates physical and biological needs. 2) Restrict release to candidate agents not accepting the critical plants in native-region field tests. 3) Determine risk from the relative suitability of critical plants to the host. 4) Reject candidates if host finding or acceptance of critical plants is inherited. These strategies are not mutually exclusive.

**Key words:** Host range, no-choice tests, supplementary tests.

## INTRODUCTION

Host range prediction of candidate weed biocontrol agents is necessary to exclude release of species that would harm desirable plants. The first introductions were made entirely on the agents behaviour in its native region. In the 1920's it became politically unacceptable in Australia to release agents without tests to show that crop plants were not at risk. They developed the no-choice tests in which acceptable agents had to starve on economic plants from many families, although it is clear that Dodd (1940) still found native region field studies more informative. The emphasis of the no-choice test was changed in the 1950's to determine host range limits as by default all other plants were Asafe@ (Harris, 1998). Today release decisions are largely based on a combination of native host range surveys and no-choice tests. This limits the plants at risk to a taxon. North American weeds targeted for biocontrol usually have congeneric natives on which most agents develop no-choice tests, even when they do not attack them in the field. Native plants are a sensitive issue, so even one species attacked is too many unless attack was predicted at the time of release and accepted as a cost for controlling the weed. Thus there is an urgent need for new procedures to determine which of the plants supporting development in no-choice tests are at risk in the field.

## COMPARISON OF NO-CHOICE TESTS WITH NATIVE AND NORTH AMERICAN HOST RANGES

The no-choice, native and North American host ranges used for this study are



those of 40 biocontrol agents established in Canada. Details of the host ranges and biologies of the agents are given on the web site <<http://res2.agr.ca/lethweedbio.htm>>. Roughly 10% had a no-choice host ranges narrower than their native field host range, 20% had the same host range, and 70% had broader no-choice range. Stated in these terms the no-choice test was wrong 80% of the time; but this is misleading. The no-choice test showed that all the agents are restricted to a single plant taxon, often a genus, and no plants outside the taxon have been attacked in the field. Powell (2000) reported similar results for the USA. Roughly 70% of the agents established in Canada developed on North American natives in the field, but only 4% have done so in the field. The number in the USA is 12% as they have a larger flora and more climatic zones. The attack of natives ranges from occasional to high. Thus the density of few native plants has been affected, but the point is the present tests do not indicate which are at risk.

### Narrow no-choice host range

The narrow host range arises when local populations have optimized their performance by specializing on a plant species, although other plants are used elsewhere. It is a particular concern as populations may be able change their specialization to other field hosts. This has not happened with an Ontario population of the seed capsule weevil *Gymnetron antirrhini* (Payk.) from common toadflax, *Linaria vulgaris* Mill., as it has not attacked *L. dalmatica* (L.) Mill. following its establishment in Western Canada. Another host race has now been established to attack this weed (see web site).

A contrary example is the seed-head weevil, *Rhinocyllus conicus* (Fröh), from *Carduus nutans* L. at Mulhouse, N France. The genus *Cirsium* supported development in no-choice tests, but in the weevil had three ties to *C. nutans*: oviposition poorly synchronized with the late flowering of most *Cirsium* spp., adult preference for *C. nutans* and larvae smothered by callus in some thistles (Zwölfer and Harris, 1984). In North America the weevil has become common on native *Cirsium* spp. and it has been argued that this probability was evident from the screening tests and field surveys (Gassmann and Louda 2001). However, interpretation is confounded by allozyme and morphometric analysis that showed there are two subpopulations: one in temperate Europe and the other Mediterranean, probably that described as *R. oblongatus* (Klein and Seitz, 1994). There are also smaller allozyme differences in the weevils collected from *Carduus nutans*, *C. pycnocephalus* L. and *Silybum marianum* (L.) Gaert. All have been released in the USA without further tests. Thus, in 1969 Montana obtained stock from both Mulhouse, France (*R. conicus*) and Italy (*R. oblongatus*?). By 1975 these attacked up to 43% of the heads of *Cirsium arvense*, (L.) Scop., 60% of *C. vulgare* (Savi) Ten., plants, 41% of the native thistle *C. undulatum* (Nutt.) Spreng. and well as *C. nutans* (Rees 1977). In Virginia weevils appear to be segregating into two phenotypes: an early one synchronized with the flowering of *C. nutans* and a late one on *C. acanthoides* L. Peak oviposition of the latter was delayed by two weeks and female life span increased by 17.8 days (Rowe and Kok 1984).

It is likely that releasing populations from various places and thistles has added genetic diversity to the Mulhouse population. A possible indication of this is that weevils invading Alberta from Montana seem to be more prevalent on *Cirsium* spp. than those from Mulhouse established in Saskatchewan. It is clearly bad practice to release untested populations and this is now prohibited both Canada and the USA.

However, if the attack of North American was unacceptable, the stability of the Mulhouse population should have been tested before release. If there is a need to introduce other populations because they had different host or phenology, both their host range and that of the hybrid should be determined. This is going to make the testing of a species like *R. conicus* so onerous that it would be easier to select an alternative candidate.

### **Identical host ranges**

Identical no-choice and native field host ranges arises when adult oviposition requirements match those of the larvae for development. For example, larvae of the stem boring weevil *Mecinus janthinus* Germ. require large toadflax stems for development and the adult avoids ovipositing into small stems, although it will feed on the foliage of species with small stems. Some of the other insects are gall formers where the specificity may be determined by the requirements for gall formation. This group should require no further screening tests unless the taxon in the release-region contains critical plants.

### **Broad no-choice host range**

A no-choice host range that is broader than the field host range indicates that field specificity is determined by adult habitat and host finding requirements. Thus the failure to consider the adult in host tests is bad science. In most insects the adults is responsible for host selection since its larvae lack the necessary mobility. The female is under strong selection pressure to oviposit on plants that the optimize survival of its progeny. The larva, on the other hand, has to stay on the plant, which may involve distinguishing it from adjacent species, and eat. Adult host finding involves a sequence of behavioural steps (Courtney and Kibota, 1990) that may start with the selection of a habitat, orientation to a host and then with tactile or gustatory acceptance. Any step may excluded plants that support development in a no-choice tests. Thus if a habitat, such as a swamp, only contains one plant species, this is the field host. In contrast, if a larva finds itself on a wrong plant, its best survival option is usually to try and eat it. Consequently, the adult host preference tend to be narrower and more firmly held than that of the larva (Courtney and Kibota, 1990). Over 90% of the agents in this group developed on North American plants in no-choice tests. None have attacked them in Canada, although two have attacked an ornamental and three attacked natives in the USA.

## **IDENTIFICATION OF AGENTS LIKELY TO DAMAGE NATIVE CONGENERS OF A TARGET WEED**

The no-choice test is a rapid and reliable method restricting the plants at risk to a taxon. If this taxon contains critical species in the release region, they can be safeguarding by not releasing the agent. This does not quite throw out the baby with the bath water, but it would have eliminated about 70% of those established in Canada. It would leave those restricted to the target weed, those on weeds without native congeners and the few not accepting North America congeners, which are often in a different subgenus. For the bureaucrat, who has to approve release, it is nice solution with little judgement involved; but I doubt if it is acceptable to any weed biocontrol researcher. Four suggestions are made for achieving the same end with less damage to

weed biocontrol.

### **Extend host range surveys to determine the candidates physical and biological needs**

It is little extra work to extend native-region host surveys to collect the data on the candidates physical and biological needs. These will exclude some critical species in the release region and will assist establishment. It is necessary to record the plant species, the attack rate in constant sized samples (i.e. 100 seed heads), the presence of competitors, location and factors likely to limit the insects host range in the release region. These could be shade intolerance, need for a minimum number of day-degrees, an intolerance to low winter temperatures, need for winter foliage for the beetle *Chrysolina quadrigemina* (Suffr.) or thistle seed-heads below a certain size for the weevil *Larinus planus* (Fab.). Several flea-beetles in the genus *Aphthona* introduced for the control of leafy spurge (*Euphorbia esula* L.) attack other *Euphorbia* sp. growing in leafy spurge stands, but not when alone. In Montana *A. nigriscutis* Foud. has attacked the American spurge *E. robusta* (Engelm.) Small in leafy spurge stands. The attack decreased with decline of leafy spurge, which has allowed *E. robusta* to increase (see *A. nigriscutis* on web-site). Indeed, attack of a wild native plant is of little ecological consequence unless it is shown that its population is declining.

Often habitat limiting factors are testable. Thus, the toadflax root-moth, *Eteobalea intermediella* Riedl was found to attack *Antirrhinum majus* L. when planted in an undisturbed *L. vulgaris* stand, but not in cultivated soil (Saner *et al.* 1990). Tests of this type are often lost in descriptions of biology. They need to be highlighted in a separate test section following the non-choice tests.

Two recent native-region surveys by the US (Balacuinan *et al.* 1996) and South Africa (Kluge and Gordon, 2000) weeds were based on proximity to the preferred host rather than taxonomic relationship. This approach makes me uneasy as usually specialized insects with a choice will select the preferred over the marginal host, so the survey method may generate false negatives. Also, the survey ignores the tie of most specialized insects to a plant taxon, so insects may rest on adjacent plants; but attack would not be expected.

### **Restrict release to candidates agents not accepting critical plants in native-region field tests**

The suggestions for these tests are based on proposals by Marohasy (1998). They can be done in large field cages with the danger of generating false positives, or in the open with the danger of generating false negatives as low preferences host will be ignored.

i) Expose the critical plants to the candidate agent in combinations, but without the target weed. Unaccepted plants are safe provided that the target weed is attacked in a control.

ii) Establish the preference rank of the plants failing the first test. Starting with the preferred host, record the amount of feeding and/or number of eggs laid. Accepted plants are replaced at predetermined intervals, or if unaccepted, with the previous plant. Accommodation feeding or egg dumping may occur if the test is done in a field cage. An accommodation threshold can be determined for native-region plants that are not attacked in the field and then applied to the test species. The test is similar to determining relative suitability, which is discussed later.

iii) Quantify the length of the discrimination time (the time for the candidate to feed or oviposit) in a cage. There is usually a difference of many hours between hosts and non-hosts in this regard. A threshold time for the acceptance of a non-host can be established with native-region plants. Test plants below the threshold are safe.

Some of testing of this type is being done. For example, after the defoliating beetle *Galerucella californiensis* (L.) completed development on several North American natives, they were placed near a beetle population in the native region. The test plants were attacked by newly emerged adults, but they left to breed on the preferred host. Results have been similar in Canada, although following the defoliation of *L. salicaria*, the beetles fed on the natives, laid a few eggs but no mature larvae were found. This attack is expected to decline as the beetles and host both achieve a balance at a low density (Corrigan *et al.*, 1998).

### Determine risk from the relative suitability of critical plants to the host

The need for an ovipositing female to optimize the survival of her progeny implies that she should avoid oviposition on poor larval hosts. Thus although the oviposition site is an adult responsibility, the process is driven by larval performance. This idea was tested on the *C. arvensis* flea-beetle, *Altica carduorum* Guer. In Eurasia the beetle is only known from *C. arvensis* except for one population on *C. vulgare*, but in no-choice tests the larvae developed on all tested species of *Cirsium* of which there are 93 native to North America (Wan *et al.* 1996). Six performance criteria were determined in the laboratory on the preferred host and various test thistles. 1) The proportion of adults feeding within 24 hours. In the field they feed, lay or leave within minutes of landing. 2) The amount eaten. 3) Egg production. 4) Larval survival from egg to development. 5) Developmental time. 6) Cold tolerance of adults reared on various thistles. The data for each thistle was expressed as a proportion of the value on *C. arvensis* and then multiplied to obtain a single number. Thus, if egg production and larval survival on a test thistle were 10% of that on *C. arvensis*, its relative suitability is 1% (Wan and Harris 1997).

The suitability of most North American thistles was  $5.4 \times 10^{-2}$  of *C. arvensis* in no-choice tests and  $2.3 \times 10^{-5}$  in multiple choice tests. Principle component analysis grouped the North American thistles with the non-host Eurasian species and separated them widely from *C. arvensis*. Factor analysis showed that the adult was a better discriminator of suitability than the larva. This conforms to theory, so the present tests, which are largely based on the larvae, are bad science. We also found that the beetle aggregated on *C. arvensis* and on the feces of larvae fed on *C. arvensis*, but not on other thistles. Thus they appear to respond to a chemical specific to *C. arvensis*. A caveat is that the absence of the host finding cue may protect a potentially excellent host from attack (Fox and Lalonde, 1993); but did not apply to this insect.

### Reject candidates if attack of critical plants is inherited

A common question is whether natural selection will improve survival on a non-target plant. Plant pathologists routinely test whether crop resistance to a disease can be improved by selective breeding (Wagoire *et al.* 1999). To a lesser extent the technique is used for parasitic insects (Bourchier *et al.* 1994), but I am not aware that it has been used to in weed biocontrol. The studies cited measure the improvement of development and survival. In weed biocontrol the critical factor is whether the female ability to recognize and accept the non-host is heritable. Ideally, the tests would be done in open

field releases with individual pairs. For example, the heritability of the *C. nutans* population of the weevil *R. conicus* to attack a *Cirsium* species would be reflected by a significantly increased attack rate over several generations. Individuals reared from *Cirsium* following their release would be paired and re-released as individual lines on separate *Cirsium* stands. If a dominant gene is involved, a significant improvement should be apparent in one or two generations. If the gene is recessive improvement might take several generations. It should be possible to do the test in a field cage by measuring the time for the first egg to be laid. A significant reduction would indicate an inherited acceptance of *Cirsium*.

### SUMMARY

Host specificity tests on candidate weed biocontrol agents are essential and must accurately predict the host range in the release region. I suggest that a step approach. 1) The no-choice larval developmental test is a rapid method of confining risk to a single taxon. Critical plants in this taxon in the release region need supplementary tests. 2) Some can be excluded by showing that they occur in habitats or climatic zones not used by the agent. 3) Adult field or field-cage tests on critical plants in the native region will exclude those not recognized as a host. 4) Large differences in the relative suitability of hosts and non-hosts can be compared statistically. 5) Occasional attacks on non-hosts is only a problem if the ability is inheritable.

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## ECOLOGICALLY-BASED WEED MANAGEMENT FOR THE 21<sup>ST</sup> CENTURY: BIOLOGICAL CONTROL OF FOREST WEEDS BY A MYCOHERBICIDE AGENT, *CHONDROSTEREUM PURPUREUM*

R. Prasad<sup>1</sup> and S. Kushwaha<sup>2</sup>

<sup>1</sup>Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, B.C. V8Z 1M5. RPrasad@pfc.forestry.ca

<sup>2</sup>50 Meadow St., Amherst, Massachusetts, 01002, U.S.A.

**Abstract:** Use of chemical pesticides in the forest has led to public concerns in Canada and an alternative tool, use of a bioherbicide based on (*Chondrostereum purpureum*) was found to be effective against some hardwood weeds. Field experiments carried out for a decade with this product shows good promise in suppression of resprouting in aspen (*Populus tremuloides*) Sitka alder (*Alnus viridis*), red alder (*Alnus rubra*) and in enhanced growth of the lodgepole pine (*Pinus contorta*) in British Columbia. Preliminary experiments conducted under greenhouse conditions demonstrated that this bioherbicide is equally effective in suppressing resprouting in Scotch broom (*Cytisus scoparius*).

**Key words:** aspen, bioherbicide, forest weeds, lodgepole pine, red alder, Scotch broom.

### INTRODUCTION

Even though it is a well established fact that weeds interfere with crop productivity and reduce yields in agriculture, comparable data on forestry weeds and crops are scanty. Three reasons are cited:

a) forest weeds are woody perennials (aspen, alders, maple shrubs) and difficult to manage b) they have extensive root/shoot systems or thick glabrous leaves as in salal (*Gaultheria shallon*) etc and c) special techniques such as aerial applications are needed for their efficient management.

Generally chemical herbicides (2,4-D, glyphosate, hexazinone, trichlopyr) are employed for control of the woody species but environmental and public concerns have curtailed their usage in forestry. Therefore, alternatives are being sought and recently use of bioherbicides has been gaining momentum (Prasad 1996). According to Watson (1989) bioherbicides are living entities (natural enemies) used deliberately to suppress the growth or reduce the population of a weed species. This may include an insect, a microbe (bacteria, fungi, virus) or a parasitic nematode.

Mycoherbicides are formulations containing plant pathogenic fungi that are directly applied to target weeds. Only recently, starting in 1980s, have mycoherbicides appeared commercially with the release of Devine® (*Phytophthora palmivora*) for control of strangle vine (*Morrentra odorata*) in citrus orchards and Collego (*Colletotrichum gleosporoides* var. *aeschynomene*) for control of northern joint vetch (*Aeschynomene virginia*) in rice (Templeton 1982). A Canadian product, Biomal (*Colletotrichum gleosporoides* var. *malvae*) was recently reported by Makowski and Mortensen (1992) for effective control of annual weed mallow (*Malva pusilla*). Unfortunately many of these are not commercially available. No mycoherbicide for forest weeds has been commercially developed because of a narrow market and lack of research. Therefore, we developed the native fungus, *Chondrostereum purpureum*, (Cp) found locally in our

forests in Canada. Originally its bioherbicidal properties were discovered and reported by Dutch workers (de Jong, Scheepens and Zadoks 1990) from Europe but a new formulation of the local native form was extensively researched and patented by Wall, Prasad and Sela (1996). Since this is a wound parasite and operates through cut ends, it can be integrated with manual/mechanical cleaning of hardwood weeds and thus reducing the burden of chemical herbicides. Therefore, experiments were conducted with Cp in the greenhouse for evaluation of its bioherbicidal potential on resprouting of Scotch broom (*Cytisus scoparius*) and in the field, for monitoring efficacy on red alder (*Alnus rubra*).

## MATERIALS AND METHODS

### Culture of fungi

Pure cultures of *C. purpureum* were obtained from infected red alder in Cowichan Lake forest, British Columbia, and cultivated on malt dextrose agar (MDA) in petri dishes according to a standard procedure (Wall 1994). Dishes were incubated in a sterile chamber at 22°C where vigorous mycelial growth took place and then stored at 5°C. Inoculum of the fungus was prepared by growing on sterile, moist ground corn meal in flasks and incubated for 6 weeks at room temperature (22°C). A formulation containing a thick slurry of infective mycelia was prepared by adding sterile water, an adjuvant (Bond, 0.1% v/v) and a vegetable oil (5% v/v) to the inoculum for greenhouse and field testing. This formulation was stored in a sterile squeeze bottle and ample amount (1.5 to 5 ml) of the formulation was applied at the cut surface of stems of the target plants. The mycelial concentration in such a formulation was about  $1 \times 10^6$ /ml. After applications at the target surfaces, aliquot samples of the formulation were taken from the treated site, cultured on MDA plate in the laboratory. Invariably, a profuse growth of mycelia was recovered ascertaining viability and virulence of the applied dosages on the target surfaces.

### Evaluation of the bioherbicide agent (Cp) on Scotch broom under greenhouse conditions

For this experiment, broom seedlings were grown in the greenhouse under constant condition of light (2500 fc  $18 \pm 6$  hr) temperature ( $25 \pm 1^\circ\text{C}$ ) and relative humidity ( $70 \pm 15\%$ ). When 25 cm high they were transplanted into pots containing a mixture of local soil + peat moss (3:1) and removed to a shade house to grow for 2 years. When these plants were 1 m high and 2.5 cm in diameter, sixty plants were selected; and their stems cut at 25 cm above the soil level and immediately treated with a blank (15 plants) or formulated product of Cp (15 plants). To effect comparison, 15 cut stems were treated with a commercial triclopyr herbicide (Release, Dow Chemical Company) at full strength (480 gms/L). Fifteen cut stems were also treated with sterile water to serve as control. Only cut ends were treated and care was taken to apply an ample amount (1.5 ml) of each treatment with a hand brush. Thus, each cut end of the treated stem was fully covered with the Cp formulation or the triclopyr herbicide. Cut stems with blank formulation were treated similarly. Applications were made in late August and all response (resprouting) was measured after one year. Data were analysed statistically.

### Evaluation of the bioherbicide agent (Cp) on red alders under field conditions

Field experiments were carried out for 3 years on red alders infesting a Douglas-fir



plantation in Cowichan Lake, British Columbia. Over 500 red alders (diameter 6.5 cm; height 200 cm) were cut with a handsaw and treated immediately by the formulation of Cp with and without mycelia. Two commercial herbicides (glyphosate 384 gm/L and triclopyr (Release) 480 gm/L) were also included. Thus for each treatment, 100 samples of cut stems of red alders were tested. Plots were randomised with ten replicates each consisting of ten stumps. Check (control) treatments received no formulations or formulation without the mycelia. Experiments were conducted in July-August and the degree of fructification of basidiocarps on stumps was monitored in the winter months according to a standard procedure (Wall 1994).

## RESULTS AND DISCUSSION

### Effect of the bioherbicide agent (Cp) on Scotch broom growth in the greenhouse

Measurements taken one year after treatment with bioherbicide and herbicide formulations clearly demonstrate (Table 1) that Cp applications were very efficacious and even matched the effectiveness of the chemical herbicide triclopyr.

Table 1. Influence of various treatments on resprouting growth of Scotch broom under greenhouse conditions after one year

Treatment	Sprout number	Percentage	Sprout height (cm)	Percentage (%)
Untreated (check)	5.1 <sup>a</sup> *	100	29.8 <sup>a</sup>	100
Untreated (check + blank formulation without Cp)	5.4 <sup>a</sup>	104	30.1 <sup>a</sup>	101
Treated with Cp (mycelial formulation)	0.0 <sup>b</sup>	0.0	0.0 <sup>b</sup>	0.0
Treated with triclopyr	0.0 <sup>b</sup>	0.0	0.0 <sup>b</sup>	0.0

\* Means in the same column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan Multiple Range Test.

As is evident from the above table, Cp inhibited the formation and emergence of new resprouts in a similar fashion as the chemical herbicide and it is possible both agents acted via the same pathway. However, resprouting in untreated control was not uniform and some cut stems failed to resprout or died over the course of the experimentation but absolutely no resprouting from the treated stems took place suggesting, a complete inhibition by treatments with Cp and triclopyr.

However, before any firm conclusion can be reached it is important to carry out further field trials on the efficacy of these treatments, under natural conditions. Interestingly, Scotch broom has infested some native Douglas-fir plantation in British Columbia and is affecting its growth (Prasad 2000)

### Efficacy of the bioherbicide and herbicide application on red alders under the field conditions

Table 2 shows the effects of treatments on the resprout growth of red alders after three years. As can be seen, the bioherbicide treatment with Cp was highly effective in inhibiting the resprouting by ca .80% and reducing the height growth by 65% while the herbicides (glyphosate and triclopyr) killed all stumps and regrowth. Basidiocarps

(fructification) appeared on many stumps treated with the Cp formulation suggesting that the fungus was well established and responsible for the mortality of the new growth.

Table 2. Influence of treatments with bioherbicide (Cp) and chemical herbicides (glyphosate and triclopyr) on resprouting behavior of red alders

Treatment	Sprout/stump		Height of sprouts		Fructification
	(number)	(%)	(cm)	(%)	
Untreated - check	2.9 a*	100.0	45.2 <sup>a</sup>	100.0	2.1 <sup>a</sup>
Treated with Cp	0.62 <sup>b</sup>	21.3	15.8 <sup>b</sup>	35.0	27.8 <sup>b</sup>
Treated with glyphosate	0.0 <sup>c</sup>	0.0	0.0 <sup>c</sup>	0.0	0.0 <sup>c</sup>
Treated with triclopyr	0.0 <sup>c</sup>	0.0	0.0 <sup>c</sup>	0.0	0.0 <sup>c</sup>

\* Means in the same column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan Multiple Range Test.

In conclusion, greenhouse and field experiments carried out with a new formulation of the bioherbicide (*Chondrostereum purpureum*) on two weed species (Scotch broom and red alder) in British Columbia demonstrate that it is highly effective in preventing resprout growth. It is suggested that further experiments should be carried out under many other conditions to duplicate these results. Inoculations with the mycelia of the bioherbicide were made on several conifer species (Douglas-fir, red cedar, spruce and pines) but no pathogenicity was found. In fact, a field trial carried out with lodgepole pine in British Columbia showed enhanced growth after aspens were effectively controlled by *Chondrostereum purpureum* (Prasad 2000).

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## A PLANT SUCCESSION MODEL RELEVANT TO WEED BIOCONTROL

P. Harris

Agriculture and Agri-Food Canada, Lethbridge Research Centre, PO Box 3000,  
Lethbridge, AB, Canada, T1J 4B1. harris@em.agr.ca

**Abstract:** Most targets for classical weed biocontrol do not conform to the conventional linear view of plant succession that communities are driven between a pioneer and climax state by removing or imposing disturbance and stress. This leads to disagreements whether a weed is a suitable target for biocontrol. The alternative Astates and transitions@ model provides a rationale for weed biocontrol. Herbaceous annuals, biennials and perennials that form stable states are all amenable to weed biocontrol, but for transitional species the likelihood of success decreases with the brevity of the transitional period. Thus the theory provides a means of assessing a target weed in terms of ease of control success and the difficulty with short-term transitional weeds suggests some of the agent attributes needed.

**Key words:** Weed biocontrol, states and transitions model.

### INTRODUCTION

Classical weed biocontrol is coming under increasing public challenge. The challenge that the biocontrol of a weed is needless seems to arise because most of the species targeted for biocontrol do not conform to the conventional view of plant succession: that there is a climax community determined by abiotic factors such as climate, soil and land form. In this model fire, intense grazing or other disturbances push the community towards the pioneer state; however, the climax is restored, given good management and time, once the disturbance is removed. Thus a plant community can be driven between pioneer and climax states. A metaphor used to illustrate this idea is that of a ball in a cup which returns to the equilibrium at the bottom when disturbance ceases. This implies that weed control in pasture is a matter of management, so there is no place for classical biocontrol or at best it is a cover-up for bad management. Most North American rangelands are managed on the pioneer-climax model (Clements 1916) and A good pasture@ is maintained by regulating grazing intensity to the level that keeps the abundance of key plant species known as A increasers@ and A decreasers@ at a desirable equilibrium. The model works if the overgrazing is not too great. Some weed species can be managed in this manner and these rarely, if ever, justify biological control. The model suggests no explanation why the biocontrol of annuals and biennials works sometimes or in some situations and not in others.

Often seriously disturbed land is invaded by weeds some of which, often introduced species, are not displaced by the former climax species. In Canada such species are leafy spurge (*Euphorbia esula* L.), knapweed (*Centaurea diffusa* Lam. and *C. maculosa* Lam.), St. Johns wort (*Hypericum perforatum* L.) and nodding thistle (*Carduus nutans* L.). They may have colonized following over-grazing; but continue to dominate even if all grazing is discontinued, so they do not conform to the linear succession model. They can be controlled with herbicides applied at intervals, but this is rarely economically justified on low quality grazing land. Treatment of St. Johns wort in

British Columbia with a soil sterilant killed all plants, but the weed was the first to return. In contrast when the biocontrol agent, the beetle *Chrysolina quadrigemina* Suffr., was introduced to California, it reduced the weed to about 1% of its former density and two millions acres returned to a native bunch grass community (Huffaker 1951). Cultural control to return the land to the former plant association requires massive intervention and is normally not considered.

This paper is stimulated by a comment that the biological control of nodding thistle in North America was unnecessary since it is a biennial successional that with good management would be replaced by climax grasses. The comment would have had more justification if it had been made about bull thistle, (*Cirsium vulgare*), another introduced biennial thistle. The paper is restricted to herbaceous plants. Clearly woody perennials can clearly be invasive weeds, but I have had no experience with their biocontrol, and so do not know whether the generalities I make about herbaceous species apply to them. Further details on the weeds and biocontrol agents are on the web site <http://res2.agr.ca/leth/weedbiol.htm>; but it should be possible to follow the principle without this information.

### STATES AND TRANSITIONS

The Astates and transitions@ model is an alternative to the Apioneer-climax@ model. It proposes that there can be multiple stable equilibrium communities within the limits imposed by soil and climate. Thus, following a major disturbance such as drought, fire, flood, overgrazing or mechanical disruption (Westoby et al. 1989) there is a period with transitional plant species that culminates in a new state, or stable community, which may not be the original one (Laycock, 1991). Extending the ball and cup metaphor to illustrate this model leads to an image of several state Acups@ separated by transition Ahills@, like an egg tray.

The existence of two stable adjacent states is evident on across native Canadian prairie. Roadsides tend to be dominated by brome grass (*Bromus inermis* Leyss.) or crested wheat grass (*Agropyron pectiniforme* R. and S.) with an assortment of other Eurasian plants. With the partial exception of crested wheat grass in the dark brown soils, this community rarely invades undisturbed native prairie, and few native species establish themselves on the roadside. Thus, both plant communities represent stable states with that on the roadside resulting from disturbance and seeding following road construction.

Following a disruption, a new community is composed of aggressive colonizers in the neighbourhood. These are often introduced weeds, that in part, owes their success to the lack of natural enemies. Some may be displaced by more competitive plants; but in parts of Europe and Asia, the North American ragweed and goldenrod (*Ambrosia artemisiifolia* L. and *Solidago gigantea* Ait.) form dense stable stands that do not revert to native communities. In North America, the European nodding thistle, a biennial, behaved similarly until the establishment of the seed-head weevil *Rhinocyllus conicus* Fröh.) The weevil made the thistle a transitional species that is now replaced by other plants. Leafy spurge and spotted knapweed are perennials that form a stable community that dominate disturbed sites until their natural enemies are established. These change a stable weed population to a transitional or to scattered plants in a new stable state. Biocontrol is about changing succession so that an undesirable plant dominating a

community is changed for a desirable one. It does this by reducing the competitive advantage of the weed to the advantage of associated plants.

## BIOCONTROL RESULTS WITH TRANSITIONAL AND STABLE PLANTS

Bull thistle (*Cirsium vulgare* Savi (Ten.)) is an introduced biennial in Canada that provides a contrast to nodding thistle. It was targeted for biocontrol because it forms stands of up to 30-40 plants/m<sup>2</sup> following logging in the interior of British Columbia and NW United States. This both retards forest regeneration and displaces the temporary grazing normally done while the trees are small. However, bull thistle seedling do not germinate in trash, so the stands collapse in 3-4 years. Two biocontrol agents, a rosette weevil and seed head gall fly, have been established, but do not increase fast enough to control post logging infestations. Rust disease may have the necessary rapid increase and spread, but the one specialized on bull thistle is too benign to achieve control. Bull thistle can form stable populations in pastures that are based on the number of sward breaks, but its density rarely affects grazing. Also, the number of sward breaks can be controlled by reducing grazing intensity and not grazing when the soil is wet. Biocontrol may have reduced the bull thistle seed bank for a long term benefit; but the immediate results on this short term transitional weed have not warranted the costs, as in hindsight, might have been expected.

The biocontrol of common ragweed, an annual, by a defoliating beetle (*Zyogramma suturalis* Fab.) in Russia resulted in two to three fold yield increases by destroying the weed in 700 ha in alfalfa and partially controlling it in 890 ha sainfoin (Kovalev and Vechernin, 1986), which has a shorter stand longevity. Biocontrol was unsuccessful in annually rotated crops with years with little or no weed. Beetle dispersal is sufficiently low that few, even when starving, find adjacent fields and those that do, require three years to increase to a defoliating population (Reznik et al. 1991). Thus management has made the weed into a moving target that the beetles are unable to follow. The Colorado potato beetle (*Leptinotarsus decimlineata* (Say)), a closely related genus, has the dispersal and host finding ability to find annually rotated potato fields up to 5 km away. Many of the agents that have been effective against stable weed populations are species with relatively poor dispersal abilities, so the number suitable for transitional weeds is reduced to the few with good dispersal, rapid increase and a high impact.

Obviously strong flight combined with good long range host finding is an asset in an agent that has to track a moving target; but this can be achieved by other means. A biocontrol agent with the attributes for control of a transitional weed is the rust disease (*Puccinia chondrillinae* Bub. & Syd.) that has controlled skeleton weed (*Chondrilla juncea* L.) in Australia (Cullen, 1985). In Canada the rust appeared on a small patch of the weed approximately 300 km from its nearest source in the USA. Similarly the skeletonweed gall mite (*Eriophyes chondrillae* G. Can.) appeared on another patch. Thus it appears that both rust teliospores and the overwintering stage of the mite are tracking their host by travelling on the seed.

Long term transitional species are not a problem as far as stability is concerned. For example, Scotch broom, *Cystisus scoparius* (L.), a leguminous shrub that is invading the small Garry Oak habitat in southern Vancouver Island. The park-like habitat has a unique shade intolerant herbaceous community that broom displaces by blocking up to 65% of the light (Peterson and Presad 1998). Scotch broom stands degenerate in 20-25

years, but instead of the original community, they are replaced by an introduced and a native shrub (Ussery 1997). This illustrates the states and transitions model, with broom being the disruption that causes the change. Woody plants are often harder to control biologically than herbaceous ones, but broom stands have sufficient longevity as ideally the target should be a common and stable component of a plant community for at least seven years. The remaining technical questions is whether are there organisms that can control the weed before the native community is shaded-out. If not, inundative biocontrol with a pathogen would be a better prospect, possibly with classical biocontrol reducing invasive potential of the residual broom population.

### THE NEW STATE FOLLOWING BIOCONTROL

According to the states and transitions model damage above a certain threshold results in a rapid transition to a new state, which may or may not be desirable (Laycock 1991). This is scary. However, biocontrol usually produces a new state that consists of the weed understory, or the immediately adjacent community, together with a small amount of the weed. This departure from the theory seems to arise because, in contrast to other disturbances, biocontrol selectively depresses the weed. The result is a rapid release and occupation of the site by the understory. The rapidity is important to curtail the entry of other weeds. The weed understory is usually a suppressed complex at the species present of the time of weed invasion. A site dominated by native green needle grass (*Stipa viridula* Trin.) before becoming invasion by diffuse knapweed invasion was returned by biocontrol to green needle grass with about a 10% knapweed cover. It took the agents over 10 years to adapt and increase to achieve the necessary level damage and then three years of transition to the new state.

The new plant community following the biocontrol of leafy spurge with the beetle *Aphthona cyparissiae* (Koch) was affected by previous pasture management. In a moderately cattle-grazed pasture, biocontrol returned the spurge dominated community to a native mixed grass prairie community with a little spurge. In the adjacent pasture, which had been heavily grazed by sheep in a effort to control the spurge, the shift was to a Kentucky bluegrass (*Poa pratensis* L.) dominated community. Kentucky blue grass is the result of intense grazing of sub irrigated mixed grass prairie; but to change it back is difficult and is usually not attempted. Native plants might be established in place by eliminating all the vegetation with herbicides, ground scarification and seeding.

Theoretically if the weed has no understory and the infestation is large enough that there is no nearby unaffected community, effective biocontrol, like abandoning a cultivated field, would culminate in a unpredictable new state. This situation requires some intervention to achieve a desirable plant community. A key principle in states and transitions management is to be opportunistic and thorough in achieving goals (Friedel, 1991). Unless both are possible intervention may be worthless. If there is no understory or following biocontrol intense grazing continues, the site will probably become dominated by another weed unacceptable to the cattle.

### SUMMARY

The states and transitions model provides an understanding of the changes in plant associations that are not explained by the conventional linear model. If a pasture weed

decreases with reduced grazing or other management techniques, as many do, biocontrol is probably a waste of time and money. However, herbaceous weeds that form states (stable communities) or are common transitional species persisting for at least seven years, are amenable to biocontrol. The post biocontrol state will normally be the weed understory species or nearby adjacent community with either the weed becoming a short term transitional or a minor component of the new state. Paradoxically biocontrol has made nodding thistle a transitional species and hence applicable to the pioneer-climax model of plant succession. Thus, it is now amenable to pasture management. If weed stands have no understory or nearby adjacent vegetation, the composition of the new state is unpredictable and provision should be made for establishing a desirable community as the weed declines.

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## POTENTIAL OF USING MICROBES AND MICROBIAL NATURAL PRODUCTS FOR CONTROL OF WEEDS

W. M. Zhang, M. Sulz, and T. Mykietiuk

Alberta Research Council, P.O. Bag 4000, Vegreville, Alberta, Canada T9C 1T4.

zhangw@arc.ab.ca

**Abstract:** Several projects have been initiated to evaluate the possibility of utilizing microbes and microbial natural products for control of weeds. A fungal pathogen (CL98-103) able to kill cleavers (*Galium spurium* and *G. aparine*) with a supplement of 12-16 h dew has been identified. Host specificity tests of CL98-103 on 35 plant species have demonstrated that CL98-103 is sufficiently safe as a biocontrol agent against cleavers. A fungal pathogen CW98-235 and a bacterium CW00B006C cause severe disease of common chickweed (*Stellaria media*). Both the fungus and the bacterium are non-pathogenic to nine major crops including canola, wheat, barley, oats, flax, safflower, field pea, lentil, and alfalfa. Further tests have shown that the bacterium did not attack any of five turf grass species tested. Another pathogenic bacterium (16 C) causes severe diseases on Canada thistle (*Cirsium arvense*), annual sow thistle (*Sonchus asper* and *S. oleraceus*), and dandelion (*Taraxacum officinale*). Further host range test is required to determine its potential as a bioherbicide for control of multiple weeds. Fractions of secondary metabolites from selected pathogenic fungi collected from diseased cleavers have also been isolated and evaluated for herbicidal and anti-fungal activities. One fraction possesses the ability to kill cleavers seedlings with selectivity between cleavers and canola. Some fractions also showed the potential for the control of diseases such as blackleg. Further study is needed to determine the commercial potential of the biocontrol agents and their metabolites.

**Key words:** biological control, *Galium spurium*, *G. aparine*, *Stellaria media*, *Cirsium arvense*, *Taraxacum officinale*.

## INTRODUCTION

There is an increasing demand for alternatives to chemical weed control for many weeds in cultivated and non-cultivated lands. Some of the factors contributing to this need include the increasing cost of chemical herbicide development, lack of effective chemicals for some weeds, development of herbicide resistance, and the risk of soil and water contamination by residual chemicals. It has been suggested that microbes be used as an alternative or complementary weed control strategy. The use of microbes for weed control may involve two aspects: the use of living microorganisms (Templeton, 1982; Charudattan, 1991; Watson, 1991; TeBeest, 1996; Mortensen, 1998) and the use of microbial natural products (Hoagland, 1990; Strobel et al., 1992; Duke, 2000). The purpose of this paper is to discuss these two aspects of microbial weed control and to outline our research advancements in these areas.

### The Use Of Living Microorganisms For Biological Weed Control

The use of living microorganisms, mainly plant pathogens, for control of weeds has received increasing interest in the last three decades. It usually involves two main approaches: 1) classical or inoculative and 2) inundative or bioherbicide (Watson, 1993).

The major differences between these two approaches are summarized in Table 1. Numerous review articles describe the fundamentals, the methodology, and the progress of biological weed control using living microorganisms (Templeton, 1982; Charudattan, 1991; Watson, 1991; TeBeest, 1996; Mortensen, 1998).

The use of living microorganisms in the classical approach have mainly targeted introduced weeds, on the basis that introduced weeds have escaped their natural enemies (Hasan, 1980). Generally, rusts and other fungi capable of self-dissemination by airborne spores and causing epidemics after initial release have received the most attention. Watson (1991) and Mortensen (1998) reviewed the major projects with introduced plant pathogens for weed control. A dramatic example of a successful introduction of an exotic plant pathogen is the rust *Puccinia chondrillina* Bub. And Syd. introduced into Australia for control of skeleton weed, *Chondrilla juncea* L., in wheat.

The bioherbicide approach is the augmentation of indigenous plant pathogens, commonly fungal or bacterial pathogens, to control or suppress the population of a problem weed. Typically, the pathogen is mass-cultured, formulated, standardized, and applied pre- or post-emergence when weeds are at a susceptible growth stage. Two distinct pathogen groups have been used in this approach, foliar plant pathogens and soil microorganisms. Charudattan (1991), TeBeest (1996), and Mortensen (1998) reviewed the status of foliar plant pathogens as bioherbicides in the last two decades, while Boyetchko (1996) and Kremer & Kennedy (1996) described the recent progress in biological weed control using the soil microorganisms, especially rhizobacteria. To date, the most commercially successful foliar fungal bioherbicide, is COLLEGO<sup>®</sup>, a dry powder formulation of *Colletotrichum gloeosporioides* f.sp. *aeschynomene*, for the control of northern jointvetch (*Aeschynomene virginica*) in rice and soybean in Arkansas, Louisiana, and Mississippi, U.S.A. (David Johnson, Encore Technology Inc., personal communication). The most advanced foliar bacterial bioherbicide is CAMPERICO<sup>®</sup>, *Xanthomonas campestris* pv. *poannua* for control of annual bluegrass (*Poa annua*) in golf courses (Nishino et al., 1995). The use of rhizobacteria *Pseudomonas fluorescens* for control of downy brome (*Bromus tectorum* L.) in winter wheat has been tested in large-scale field trials (Kremer and Kennedy, 1996).

### The Use Of Microbial Natural Products

Recently, the interest of weed scientists and agricultural chemists in the development of microbial natural products as biologically based herbicides or as leads for new herbicides has greatly increased (Duke, 1986; Hoagland, 1990; Strobel et al., 1992; Duke et al., 1996; Abbas & Duke, 1997; Yamaguchi, 1998; Duke, 2000). This new attention has been the result of several factors. First, rapidly evolving resistance to current herbicides has created an urgent need to find novel modes of action. Many microbial natural products have chemistries unlike those of synthetic pesticides. Study of these microbial natural products may lead to the discovery of new chemical classes that affect target sites hitherto untouched by currently used chemical pesticides. Secondly, novel compounds from nature may offer leads for new synthetic strategies. Thirdly, microbial natural products are much more likely to have some type of biological activity at lower concentrations than compounds derived from chemical synthesis programmes. Fourthly, most microbial natural products are easily and rapidly degraded or detoxified in the environment and are considered by many to be more environmentally benign than most synthetic chemical pesticides. Thus, registration of such chemicals and related chemistries is less time-consuming and less expensive than

registration of compounds that do not occur in the environment, such as most halogenated hydrocarbons. Finally, recent developments in plant cell culture, fermentation technology, molecular genetics, and genetic engineering have made cost-effective production of agriculturally useful amounts of microbial natural products a reality.

Table 1. Differences between two approaches of biological weed control with plant pathogens

Classical (inoculative) approach	Inundative (bioherbicide) approach
<u>Principles</u>	
Release of an exotic natural enemy from the site (country) of origin of the weed.	Manipulation (mass production) of the numbers of a native natural enemy.
Mostly rust fungi	Mostly facultative plant pathogenic fungi and bacteria
A small "inoculation" is released and it is hoped they become established, and spread throughout the range of the weed.	An inundative dose of the bioherbicide is released in the area (field) in which control is desired.
The aim is not to kill the weed, but to reduce the size of populations below a certain threshold, which then remains stable, in a dynamic equilibrium with natural enemy.	The aim is not necessarily to kill weeds, but to reduce their impact upon the crop
Most suitable for widespread, aggressive weeds in marginal land (eg rangelands).	Most suitable for weeds in turf grass, home garden, and agricultural land.
<u>Procedures</u>	
Identify suitable target.	Identify suitable target.
Perform domestic survey of natural enemies.	Perform domestic survey of natural enemies.
Perform foreign survey of natural enemies.	Demonstrate Koch's postulates.
Attempt to predict the efficacy of foreign (exotic) organisms and choose one (or more) to work with.	Perform efficacy trials on plants in the lab. Attempt to understand mode of action. Perform experiments to understand both the optimum and limiting conditions for the development of an epidemic on the target weed.
Perform extensive host specificity testing either in the country of origin, or in quarantine in the proposed country of release.	Perform host specificity (and other safety trials - persistence, dispersal, etc.) in the lab.
Perform host specificity trials in cages in the field.	Perform extensive host specificity trials in the field.
Release.	Investigate proper fermentation, formulation, and application technologies
Monitor.	Conduct extensive field efficacy trials in realistic cropping situations in the field.
	Patent.
	Register.
	Market and sell.

Fungal and bacterial plant pathogens often induce disease symptoms in their respective host(s) through production of phytotoxins (Strobel et al., 1992). Numerous studies on the production of phytotoxins have been done with crop disease causing fungi and bacteria, however, the disease-inducing fungi and bacteria of the vast majority of other plants (weeds, herbs, ornamentals, tropical species, forest types, important land cover forms, or aquatic species), especially those that kill weeds, have received relatively little attention in natural product-centred herbicide discovery efforts (Strobel et al., 1992; Duke, 2000). Many believe that there is a reservoir of novel natural products with herbicidal properties awaiting discovery in these organisms (Duke, 2000). The pesticide industry's natural product discovery efforts have concentrated on microbially derived phytotoxins, primarily from non-pathogenic soil microbes, instead of plant pathogens (Strobel et al., 1992, Duke, 2000).

To date, the phytotoxic microbial natural products, phosphinothricin and bialaphos, have yielded commercial herbicides (Duke, 2000). Glufosinate is the synthetic version of phosphinothrin, a breakdown product of bialaphos produced by *Streptomyces viridochromogenes* and *S. hygroscopicus*. Bialaphos, which has been produced by fermentation and sold as a herbicide in Japan, is a proherbicide that is broken down to phosphinothricin by target weed plants.

Phytotoxins vary dramatically in molecule size, chemical class (e.g. peptides, terpenoids, diketopiperazines, macrolides, and phenolics), and host specificity (ranging from the identical host specificity of the pathogen to no specificity whatsoever). With the recent registration of Messenger<sup>®</sup>, a proteinaceous natural products (harpin) produced by the bacterium, *Erwinia amylovora*, to Eden Bioscience Corporation, Bothell, Washington, U.S.A. as a fungicide (Wei et al., 1992), research interest has increased to target the protein-based herbicides as well as relevant modes of action. Bailey et al. (2000) has demonstrated that the fungal protein Nep1, produced by *Fusarium oxysporum*, can kill broadleaved weeds while remain safe to monocot plants.

### Program progress in our lab

A research program has recently been initiated in the Alberta Research Council, Alberta, Canada to evaluate the possibility of utilizing microbes (fungi and bacteria) and microbial natural products for control of weeds in Alberta and other prairie provinces of Canada. The main target weeds are cleavers (*Galium spurium* and *G. aparine*), chickweed (*Stellaria media*), Canada thistle (*Cirsium arvense*), annual sow thistle (*Sonchus asper* and *S. oleraceus*), and dandelion (*Taraxacum officinale*). These weeds are major and increasing weeds of canola and other crops in western Canada. For example, cleavers and chickweed have experienced dramatic population increase (Thomas et al., 1998) and developed herbicide resistance (Devine et al., 1991; Hall et al., 1998; Lutman & Heath, 1990). Canada thistle is a serious perennial weed in almost all crops - cereal, oilseed, and pulse crops - grown in western Canada (Moore, 1975; Peschken, 1980). Canada thistle is also a problematic, competitive weed in many forage crops, pastures, and conservation sites. Populations across the Prairie Provinces have increased over the last 10 years (Thomas et al., 1998) and control remains a problem. Dandelion is another increasing and emerging weed in forage crops, pastures, urban areas, and conservation sites (Moyer, 1998). Its control remains a challenge. Annual sow-thistle (*Sonchus asper* (L.) Hill and *S. oleraceus* L.) are problematic weeds in pulse, cereal, and oilseed crops due to their high relative growth rate and seed production (Lemna and Messersmith, 1990).

Our program focuses on two aspects: (1) the using of living microorganisms (fungal and bacterial plant pathogens) as bioherbicides and (2) the use of microbial natural products as biologically based herbicides. To date, the following advancements have been made:

**Cleavers:** Over 160 fungal isolates were isolated from naturally infested cleavers at various locations in Alberta, Canada. Among those isolates, 74 were pathogenic to cleavers after applying Koch's postulates. Pathogenic isolates were further assessed on weed control efficacy (virulence) using a 0 to 3 scale (0 - no symptoms, + - light infection, ++ - moderate infection, and +++ - severe infection to death). Results have shown that 47 isolates are virulent (with a 2 or 3 virulence rating) to cleavers. Virulent isolates proceeded to a crop safety test (preliminary host range test) on nine major crops (wheat, barley, oats, canola, flax, safflower, field pea, lentil, and alfalfa). To date, a fungal pathogen CL98-103 has been selected for further development as a bioherbicide for the control of cleavers. CL98-103 killed false cleavers seedlings when applied at a concentration of  $1 \times 10^7$  conidia/ml and provided with 12-16 h dew. The efficacy of CL98-103 on herbicide-resistant cleavers was identical to that on herbicide-susceptible cleavers. Host specificity testing of CL98-103 has further been expanded to a total of 35 plant species selected based on the modified centrifugal phylogenetic method, including taxonomically related plants, economically important plants known to be attacked by CL98-103, and other economically important plants in western Canada. Results have demonstrated that CL98-103 is sufficiently safe as a biocontrol agent against cleavers. Growth, sporulation, and submerged spore production of CL98-103 have been characterized. Large quantities of spores of CL98-103 can be produced in submerged liquid culture.

**Chickweed:** A total of 150 fungal isolates and 545 bacterial strains have been screened against chickweed. A fungal pathogen CW98-235 and a bacterium CW00B006C cause severe diseases on chickweed. Both the fungus and the bacterium are non-pathogenic to nine major crops including wheat, barley, oats, canola, flax, safflower, field pea, lentil, and alfalfa. Further tests have shown that the bacterium did not attack any of five turf grass species tested.

**Canada thistle, annual sow thistle, and dandelion:** Severe bacterial infected Canada thistle plants have been discovered and collected in pastures and market gardens in Mulhurst and Stony Plain, Alberta. A typical symptom of these infected plants was apical chlorosis, sometimes with dark and necrotic leaf spots. The infected plants show reduced vigor, stunted growth, less shoots, and inhibition of flowering. Severe infections cause the death of plants. A pathogenic bacterium (16 C) has been isolated from these diseased Canada thistle plants. Interestingly, this bacterium causes severe disease not only on Canada thistle, but also on annual sow thistle and dandelion. Further host range test is required to determine its potential as a bioherbicide for control of multiple weeds.

**Use of microbial products for control of weeds and diseases:** A fungal isolate isolated from diseased cleavers, produced secondary metabolites that completely killed cleavers seedlings but did not affect canola seedlings. Natural products produced by this fungal isolate must therefore have phytotoxic activities, which are selective between cleavers and canola. It was also noted during initial studies of the herbicidal activity that proliferate fungal contamination was present on control plates, yet no fungal contamination was present on plates treated with cell-free culture filtrates of this fungal isolate. This led us to test the anti-fungal activity of secondary metabolites produced by

this fungal isolate against major crop diseases. Results clearly show that cell-free culture filtrates significantly inhibit the growth of a major crop disease – blackleg (*Leptosphaeria maculans* (desm.) Ces. & de Not.). Similar results were obtained with three other major crop diseases - *Rhizoctonia solani*, *Ascochyta pisi*, and *Sclerotinia sclerotiorum*.

Fractions of secondary metabolites from fungal cultures of this isolate have been isolated. One fraction possesses the ability to kill false cleavers seedlings with selectivity between false cleavers and canola. Some fractions also showed the potential for the control of diseases such as blackleg. Further study to elucidate the chemical structures of the purified compounds will be conducted.

Hundreds of weedy fungal and bacterial isolates have been isolated each year under our bioherbicide program. This provides us opportunities to discover more novel microbial natural products that may be developed as biologically based herbicides for weed control.

## RESULTS AND DISCUSSION

Chemical pesticides have been extensively used to control weeds, plant diseases, and insects for many years. However, the growing awareness of the adverse effects of chemical pesticides on the environment has prompted global efforts to search for alternative tools. Microbes and microbial natural products have shown potential to be used as an alternative strategy or complementary tactic to chemical pesticides. However, their use is still limited, representing only a small part of the pest management market. Further study is required to expand the market potential of microbes and microbial natural products. More research on fermentation, formulation, shelf life, and field application of the living microorganisms is required to improved field performance under variable environmental conditions. Integration of microbial pest control into existing pest management strategies is also required. Further collection and evaluation of weed pathogens will assist the discovery of useful and novel microbial natural products. By increasing the quality and quantity of commercially available microbes and microbial natural products, it is hoped that the market for these pest management products can be expanded.

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## BIOLOGICAL WEED CONTROL IN CHINA: AN UPDATE REPORT

F. H. Wan, J. Y. Guo, and R. Wang

Institute of Biological Control, Chinese Academy of Agricultural Sciences,  
12 Zhong-Guan-Cun Nan-Da-Jie, Beijing 100081, P.R. China.

bcicaas@public.bta.net.cn

**Abstract:** Past and current inundative and classical weed biocontrol activities in China are reviewed. Excellent results have been achieved with native fungus, *Colletotrichum gloeosporioides* f. sp. *cuscutae*, for control of dodder and with fish to control weeds in rice paddies. The fly, *Procecidochares utilis*, introduced for control of crofton weed (*Eupatorium adenophorum*) has become common but has not reduced its host. The beetle, *Agasicles hygrophila*, is effective against alligator-weed, *Alternanthera philoxeroides*, in southern China, but needs to be released annually in some provinces. The defoliating beetle, *Zygogramma suturalis*, has failed to increase on the ragweed, *Ambrosia artemisiifolia*, but a stem-boring moth, *Epiblema strenuana*, appears promising for control of both *A. artemisiifolia* and *A. trifida* in southern China. Also, an integrated management system was developed for control these two ragweed species in northern China. The use of fish, *Ctenopharyngodon idellus* (C. & V.) and *Hypophthalmichthys molitrix* Val. to control weeds of rice was developed in Jiangsu, Zhejiang, Hubei, Sichuan & Anhui Provinces in the 1980s. As of 1985, 22 weeds including *Echinochloa* spp., *Cyperus difformis* L., *Ammannia* spp. and *Monochoria vaginalis* (Burm. fil.) Presl. were controlled on one million ha of rice. This control method has doubled net returns from rice culture with the production of 300 kg per ha of edible fish and a saving of 450 RMB per ha in herbicides. Recently, a new bio-control project of water hyacinth, *Eichhornia crassipes* Solms, was initiated by BCI, CAAS in 1995. Two weevils of *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Col.: Curculionidae) were introduced from USA and Argentina in 1995. Host specificity test showed that the beetles only damaged water hyacinth plant. The beetle could be used to control the population of water hyacinth safely and effectively. Researches on bio-herbicide against water hyacinth, and an integrated method with the beetles and low-toxic chemicals are under development. International weed bio-control collaborative projects with USA and Canada are also reviewed in this paper.

**Key Words:** Weed bio-control, Dodder, Crofton weed, Alligator-weed, Ragweed, Water hyacinth

## INTRODUCTION

The first biological weed control project in China was the successful control of dodder (*Cuscuta* spp.) in soybean fields with the native fungus *Colletotrichum gloeosporioides* f. sp. *cuscutae* (Penzig.) Penzig & Saccardo (Coelomycetes) in Shandong Province in the 1960s. In 1984, the exotic tephritid, *Procecidochares utilis* Stone (Diptera: Tephritidae), was successfully established in Yunnan Province for the control of *Eupatorium adenophorum* Sprengel (Asteraceae). The success of these two projects inspired the Institute of Biological Control, Chinese Academy of Agricultural Sciences (CAAS) to sponsor National Symposia on Biological Control of Weeds in 1985 and 1988, which subsequently resulted in a nation wide weed biocontrol program.

## WEED PROJECTS

### *Cuscuta* spp., dodder (Cuscutaceae)

Of the 11 species of dodder (*Cuscuta* spp.) present in China, three *C. maritima* Makino, *C. chinensis* Lam. and *C. australis* R. Brown cause large economic losses of soybean, vegetable, forage grass, peanut, potato and oilseed crops in many provinces (Teng, 1982). A 1979 survey of Helongjiang Province showed that almost all the province's 30,000 ha of soybean were infested with *Cuscuta* spp. resulting in yield losses of 20 to 95%.

Biocontrol of dodder was initiated in 1963 after a native fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *cuscutae*, was isolated in Jinan, Shandong Province. Studies on the biology and culture of the pathogen during 1963 to 1966 led to the development of a mycoherbicide called "Lubao 1", which was tested in soybean fields in five provinces in 1966 and by the end of the decade had being applied to 600,000 ha of soybean in 20 provinces (Liu & Zhu, 1980). Over 85% control was achieved resulting in yield increases of 30 to 50% (Gao & Gan, 1992). A granular formulation of Lubao 1 was produced commercially in two factories in Ningxia Autonomous Region in 1990s.

However, since the late 1970s, the efficacy of Lubao 1 product has decreased considerably. The herbicide's requirement for high humidity in the field and limited shelf life (2-3 months), in addition to technical problems associated with its production have resulted in a dramatic decline in its use (Li, 1985). For example, in the last 10 years, only 18,000 ha of soybean in Ningxia has been treated annually (Gao & Gan, 1992).

Genetic analysis indicates that the decline in efficacy was due to a change from diploid to monoploid phase during culture (Gao & Gan, 1992). A new strain of *C. gloeosporioides* f. sp. *cuscutae*, called Lubao 1 S<sub>22</sub>, which was isolated in 1985 has much higher infectivity and productivity than the original culture. There has been no change in its virulence over 150 generations in culture over 5 years.

Currently, Lubao 1 S<sub>22</sub> is produced by small-scale solid fermentation in two small factories and the total annual production of 10 tons does not meet the national need. It is likely that the ideal production system for Lubao 1 S<sub>22</sub> will involve many small businesses, somewhat analogous to Western fast food franchises, each supplying their immediate area using local labour and thereby adding to the economic viability of rural areas. Additional research on production and storage methods and quality control is needed.

### *Eupatorium adenophorum* Sprengel, crofton weed (Asteraceae)

This South American perennial shrub was introduced inadvertently into southwest China about 1950, and has spread rapidly, invading many habitats and displacing the native flora, particularly in Yunnan, Sichuan, Guangxi and Tibet. In Yunnan Province, 24 million ha of forest and agricultural land are infested and the weed is spreading northward at 10 km per year (He *et al.*, 1988). It is poisonous to domestic animals, especially horses. In uncultivated habitats, chemical and mechanical control are impracticable and expensive. Indeed, Yiliang County, Yunnan Province spent 140,000 RMB (\$35,000) using these control methods in 1985 with little or no success (Anonymous, 1988).

In 1984, the Kunming Institute of Ecology, Academia Sinica found a gall-forming tephritid, *Procecidochares utilis*, attacking crofton weed near the Chinese/Nepalese border. This Mexican insect had been released in India for the biocontrol of crofton weed in 1963 (Julien, 1992). Retesting of the tephritid's host range in China confirmed that it was specific to the weed (He & Chen, 1987). It was mass propagated in the laboratory and released at 30 sites in Yiliang County, Yunnan Province, and by 1987 had spread to six adjacent counties with up to 75% of crofton weeds being stem galled in an area of 100,000 ha (Chen & He, 1990). Unfortunately, the weed has not been noticeably suppressed (He *et al.*, 1988).

In 1987, a fungus native to Yunnan, *Mycovellosiella* sp. was found to cause leaves of crofton weed seedlings to wither within 35 days (Guo *et al.*, 1991).

Another fungus, *Alteranria alternata* (Fr.) Keissler, isolated from *E. adenophorum* leaves has been tested for its impact on the crofton weed in 1998 (Qiang, 1998; Qiang *et al.*, 1999). The results showed that the abstract had remarkable phytotoxicity on leaves of the crofton weed. Using the phytotoxin to develop micro-herbicide is being undertaken.

Although fungi causing leave lesions are generally not particularly effective biocontrol agents, *Mycovellosiella* sp. and *Alteranria alternata* may be more effective than the stem galling tephritid. Other biocontrol insects are being investigated. *Eupatorium riparia* Regel, which is closely related to crofton weed, has been substantially to completely controlled in Australia and Hawaii by *Oidaematophorus beneficus* Yano & Heppner (Lep.: Pterophoridae), *Procecidochares alani* Steyskal, and *Xanthaciura connexionis* Benjamin (Diptera: Tephritidae) (Julien, 1992). If these insects increase on *E. adenophorum*, integrated control may be possible with a complex of insect species, the fungus, *M. eupatorii-odorati*, and vegetation replacement with competitive grasses, forbs or trees. In developing an effective release strategy, close attention will have to be paid to the adaptability of the biocontrol agents to various climatic regions.

#### ***Alternanthera philoxeroides* (Martius) Grisebach, alligatorweed (Amaranthaceae)**

The largely aquatic alligatorweed was introduced to eastern China from South America in the 1930s. During the 1950s and 1960s, it was regarded as "excellent pig forage" and cultivated in most of southern China. However, ten years later, it was out of control, invading citrus orchards, vegetables, rice fields, fish ponds, and irrigation canals (Wang & Wang, 1988). In most areas, terrestrial infestations cause the most economic damage. In Sichuan Province alone, alligatorweed infests 350,000 ha. Herbicides provide effective control in fields, but farmers are often discouraged by the cost of the chemicals and rapid reinvasion by the weed.

The biocontrol of alligator weed was initiated in 1986 by the Institute of Biological Control, CAAS. Non-host specific insects were found in China, but a flea beetle, *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae) was introduced from Florida.

In southern China, the beetle has seven or eight generations a year and became sufficiently abundant to suppress aquatic infestations of alligator weed. Between 1988 and 1991, a total of 50,000 beetles were released at Dongting Lake, Hunan Province and reduced the alligatorweed by 90% over 1000 ha (Li & Wang, 1994). However, the beetle can not survive winter without human intervention, so it is now collected in the fall and overwintered in greenhouses at 10-15°C. The beetle completes one or two

generations in the greenhouse, and in spring 3,000 beetles per hectare are released. Release at the same site for 3 to 4 years in succession usually achieves complete control.

This capture-release method could be extended to other regions that are too cold for the beetle to survive over winter. A training program and manual for instructing farmers how to rear the beetles in winter and release them in spring will be necessary for successful use of this technology.

The pyralid moth, *Vogtia mallioi* Pastrana (Lep.: Pyralidae), previously used in Australia and New Zealand (Julien, 1992), might be a more effective agent for control of the terrestrial infestations in China.

#### ***Ambrosia artemisiifolia* L. and *A. trifida* L., ragweed (Asteraceae)**

*Ambrosia artemisiifolia* and *A. trifida*, two North American weeds that are now cosmopolitan in distribution, were probably introduced into China in the early 1930s. Both species have spread rapidly and by 1987 were present in 15 provinces, mainly along railroads and highways. *Ambrosia artemisiifolia* occurs throughout northeast, north, south and east China, infesting public parks, urban areas, residential communities, orchards, roadsides, highway shoulders and agricultural fields, while *A. trifida* is presently restricted to the northeast (Wan *et al.*, 1993). In the heavily infested areas, ragweeds are often found in dense, pure stands. The ragweeds are not yet a major agricultural problem, although dense infestations displace the native plant communities and cause economic losses in orchards and gardens. However, the primary problem is allergenic "hay-fever" caused by the pollen. In Shenyang city 1.5% of the population is seriously affected (Xia, 1983) and this incidence is likely to increase unless the weed is controlled.

Two colonies of the defoliating beetle, *Zygogramma suturalis* (F.). (Coleoptera: Chrysomelidae) were imported from Canada and the former USSR in 1987 and 1988, respectively. In quarantine, it was confirmed that the beetle was specific to *Ambrosia* spp. and that it was a promising biocontrol agent (Wan *et al.*, 1993). Between 1988 and 1991, a total of 30,000 beetles were released in Changsha (south China), Nanjing (southeast China), Tieling, Shenyang and Dandong (northeast China), but only a few beetles have been recovered in Changsha and Dandong, and none in Nanjing, Tieling and Shenyang. However, when protected from predation by a cage, large populations of the beetle were produced in Beijing. Spiders, mantids, and reduvids prey on the eggs, larvae, and even the adults and appear to be the reason for the beetle's failure to establish at field sites.

In habitats with low predator populations, *Z. suturalis* is an effective biocontrol agent for ragweed as has been the case in parts of the former USSR. Further research may identify such habitats in China.

Another insect, the stem galling tortricid, *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) was introduced from Australia in 1990. Choice and no-choice feeding and oviposition tests conducted in quarantine and in field cages showed that *E. strenuana* completed its life cycle only on the closely related weeds *A. artemisiifolia*, *A. trifida*, *Parthenium hysterophorus* L. and *Xanthium* sp. and did not attack any of 37 species of crop plants or ornamentals. Because that was concern that this species might attack sunflower, which is in the same tribe as *Ambrosia*, additional tests were conducted with 60 sunflower varieties in pure sunflower stands, mixed sunflower and ragweed, and sunflower surrounded by ragweed during 1990-1992. In a field test with

mixed sunflower and ragweed, 36 galls were found late in the season on dwarf, lodged and weak sunflower plants and five adult moths emerged from these galls. However, no eggs had been laid on the sunflower plants. The heavily infested ragweeds (10 to 20 galls per plant) had died prematurely and some immature larvae transferred to the adjacent, still-green sunflower stems and completed their development. However, in a field cage (10x12x3 m) planted with sunflower only, introduced moths laid few eggs and those larvae that hatched did not feed or form galls (Wan & Ding, 1993). Apparently sunflower is not a suitable host for this species and it is unlikely to damage sunflower in nature. The effectiveness of *E. strenuana* in field plots indicates that it is an excellent biocontrol agent and largely immune from the predators affecting *Z. suturalis* (unpublished data, Wan). Generally, slight damage to adjacent non-host plants following sudden collapse of the host plant is fairly common in weed biocontrol, but the problem disappears as the weed density is reduced (Harris, 1990). *E. strenuana* does not infest sunflowers in either North America, where it is native, nor Australia, where it is introduced (McFadyen, 1985; Julien, 1992). However, to be safe, it has been recommended that releases be restricted to the South of the Yangtze River where sunflower is not a major crop. In most parts of China, it appears that *E. strenuana* will be a more effective biocontrol agent than *Z. suturalis* for both *A. artemisiifolia* and *A. trifida*.

Risk analysis of this introduced agent has been conducted in Hunan Province during 1999-2000 and the results showed that the moth has no risk to sunflower (Ma and Wan, unpublished data, 2001).

In northeast China, control of *Ambrosia* spp. along highways and railways is being achieved by replacement planting of economic and ornamental species such as indigo-bush, *Amorpha fruticosa* L., and crown vetch, *Coronilla varia* L. *Amorpha fruticosa* has been particularly effective in reducing ragweed growth and flowering (Wan *et al.*, 1993; Guan *et al.*, 1993).

Additional research is needed to evaluate other ragweed insects being used as control agents in other countries (Kovalev *et al.*, 1989) and other native North America species (Harris & Piper, 1970; Goeden & Ricker, 1974a,b, 1975, 1976a,b,c). *Liothrips* sp., *Tarachidia candefacta* (Hubner) (Lep.: Noctuidae), *Stobaera concinna* (Stal) (Hemiptera: Delphacidae), *Euaresta* spp. (Diptera: Tephritidae), *Trigonorhinus tomentosus* (Say) (Col.: Anthribidae), *Zygogramma bicolorata* Pallister and *Z. disrupta* Rogers (Col.: Chrysomelidae) are potential candidates. Most of these species have already been screened for specificity. If they can thrive in China it will be relatively cheap to use them to enhance ragweed biocontrol in China.

#### ***Eichhornia crassipes* Solms, Water hyacinth (Pontederiaceae)**

Water hyacinth, *Eichhornia crassipes* Solms, a southern American aquatic weed has posed a great threat to Dianchi Lake of Kunming City, Yunnan province in Southwest China. Based on researches and achievements of the weed biocontrol with *Neochetina* weevils in over 20 countries, a biocontrol project of waterhyacinth was initiated by BCI, CAAS in 1994.

Two weevils of *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Col.: Curculionidae) were introduced from USA and Argentina in Nov. 1994. Host specificity test showed that the beetles only damaged water hyacinth plant. No damage was found on the other 45 plant species, which belong to 24 families represented local economic, ecological and ornamental plants around Kunming region.

The control effect test indicated that the adults feed on the leaves and stems of the plants, and about 60% of leaves were destroyed. More than 200 scars could be found on one leaf of the plant. The larvae of the beetles bored the stem and made some stems decay, and even the whole plant died. Compared with the health plant, the reproduction rate of the attacked plant decreased by 32% after 40 days of releasing the beetles. The biomass of the plant also decreased by 55%. It also showed that the height of the plant and the length of the root were shorter than that of the non-attack plant. The beetles occurred 2-3 generations one year in Kunming area. The egg, larva, pupa and adult could develop normally in this area.

The tests above showed the beetle could be used to control the population of water hyacinth safely and effectively. It is feasible to manage water hyacinth with biological control approaches in Dianchi Lake.

For the fast and sustainable suppression of water hyacinth, an integrated management method, combination usage of the weevils and chemicals, has been developed in the Institute of Biological Control, CAAS. It showed that the reproduction and bioassay of water hyacinth were greatly reduced when combining use of weevil and 41% Roundup at a concentration of 0.45kg/ha (Ding *et al.*, 1999). However, at the Roundup concentrations of 4.5kg/ha and 1.5 kg/ha, 50-100% water hyacinth plants died after 3-8 weeks, and all weevils also died because of the lack of food. It is suggested that the Roundup concentration should be in a suitable level so as to leave enough food for weevil development.

During 1998-2000, 12 fungus strains were tested for their virulent and host specificity. A promising fungus agent was screened out. And its biology and fermentation techniques are under study for developing into bio-herbicide in the Institute of Biological Control, CAAS.

Pathogenicity of 12 pathogens fungi strains belonging to three genera and five species, was compared in greenhouse screening test. *Alternaria eichhorniae* C416 is the most virulent. All the inoculated leaves were killed within 6 weeks, and the whole mean disease severity (DS) on leaves was 7.04. Host-range tests revealed that it is specific to the plant. Temperature and moisture were main influential factors of its infection and pathogenicity. *A. eichhorniae* C416 caused high DS and disease development rate in 30 °C. The optimal dew period was 16h. Pathogenicity was also related to light intensity. Low light intensity (1200Lux) cause 46% DS on leaves, more than that of high light intensity (3000Lux). The best initial pH of media was 7. The best carbon and nitrogen sources for mycelium growth were sugar and peptone with the optimal carbon-to-nitrogen ratio 60:1. Adding Vitamin B<sub>1</sub>, B<sub>12</sub> could improve the production of mycelia significantly. The optimal solid-phase sporulation media consisted of Maltose, NaNO<sub>3</sub>, KCl, MgSO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub> and 10mg/l IAA, with the carbon-to-nitrogen ratio of 10:1 (Zhao and Wan, unpublished data, 2001).

The infection process was observed. Penetration of mycelia occurs not only through the stomata of water hyacinth leaves, but also through the leave surface directly. The whole infection finished within 16 to 20 hours. Typical ultrastructure host responses were the dramatically change of the membrane structure, such as starch particle in chloroplasts disappeared, cytoplasmic connection grown thicker, and lots of phenolic compounds was produced by infected cells (Zhao, 2000).

All these results provided basis data and theoretic foundation for development of new bioherbicide for biocontrol of water hyacinth.

### ***Echinochloa crusgalli* (L.) Beauv., barnyardgrass (Graminae)**

*Echinochloa crusgalli* is a big problem in rice fields in China. A project funded by Ministry of Agriculture in 1995 for developing bio-herbicide has been conducted for 5 years in Chinese Agricultural University. Eight pathogenic fungi were isolated from naturally infested *E. crusgalli* var. *mitis* (Pursh) in China. Screening tests showed that *Exserohilum monoceras* provided a good control of the weed at the 1-4.5 leaf stage. It is a potential agent for developing a bio-herbicide against barnyard grass in rice fields (Chen & Ni, 1999).

Another two fungus strains, *Alternaria alternata* and *Curvularia lunata*, were also evaluated as potential agents in China National Rich Research Institute. Their conidia production characterization was conducted in laboratory. The results showed that these two strains could be developed for bio-herbicides (Huang & *et al.* 1999).

However, more researches on host-specificity and pathogenicity of the above fungus strains should be confirmed before researching on culture conditions for production.

### **Biological weed control with fish in rice fields**

The use of fish, *Ctenopharyngodon idellus* (C. & V.) and *Hypophthalmichthys molitrix* Val. to control weeds of rice was developed in Jiangsu, Zhejiang, Hubei, Sichuan & Anhui Provinces in the 1980s. As of 1985, 22 weeds including *Echinochloa* spp., *Cyperus difformis* L., *Ammannia* spp. and *Monochoria vaginalis* (Burm. fil.) Presl. were controlled on one million ha of rice (Yu & Wu, 1988). The release of 30,000 fish (10-13 cm long) per ha at the beginning of rice growing season has been reported to reduce weed populations by 90% (Yu & Wu, 1988). This control method has doubled net returns from rice culture with the production of 300 kg per ha of edible fish and a saving of 450 RMB per ha in herbicides (Wang & Rao, 1988). However, problems integrating fish culture, insecticides and agronomic practice remain.

## **INTERNATIONAL COOPERATION ON BIOLOGICAL WEED CONTROL**

China is a resource pool of natural enemies of weeds that are troublesome in North America. The Sino-American Biological Control Laboratory was established in Beijing in 1987 to seek biological control agents for control leafy spurge (*Euphorbia esula* L.), Eurasian watermilfoil (*Myriophyllum spicatum* L.), hydrilla (*Hydrilla verticillata* Bartr.), waterchestnut (*Trapa natans* L.), tamarisk (*Tamarix* spp.), *Pueraria lobata* (Willd.) Ohwi, mile-a-minute (*Polygonum perfoliatum* L.) in USA. Since then, 14 promising biocontrol insects have been collected and shipped to the USDA-ARS for screening tests.

During 1991-1997, an agreement between Canada and China was approved for cooperative research on the biocontrol of Canada thistle (*Cirsium arvense* (L.) Scop.). Several promising species found in Xinjiang, were screened and evaluated as potential bio-control agents, including a defoliating beetle, *Altica carduorum* Guer., and a stem-mining weevil *Lixus* sp. The screening tests of *A. carduorum* were undertaken in the laboratory at Lethbridge and in field studies in Xinjiang, China.

## **PROSPECTS**

Biological weed control is a recent development in China, and despite several

successes, it is still, to a large extent, an art. A more systematic scientific approach will likely accelerate progress. There is an urgent need for well-trained professionals and for increased understanding of the potential of weed biocontrol by both government and the public. In appropriate situations, biocontrol is the most economical and environmentally benign means of weed control.

Chemical herbicides will undoubtedly remain the main means of weed control for the near future. The best return on research investment is likely to be from classical biocontrol of exotic weeds. However, bioherbicides currently have better public acceptance and development of such agents has a greater likelihood of government funding. It is unrealistic for China to consider foreign exploration for biocontrol agents of exotic weeds, but in many cases suitable agents can be obtained through cooperation with other countries. China is in the fortunate position of being able to capitalize on the experiences of other countries. Some weed biocontrol agents that have been successfully used overseas have a high likelihood of success in China (Wang, 1986, 1989; Wan & Wang, 1991).

Conflicts between groups wanting weed biocontrol and those wanting to keep a high weed density for other purposes, such as bee culture, have received little attention in China. Stricter regulations for the release of weed biocontrol agents are needed in order to protect national interests and to provide guidelines for researchers.

China has just begun to explore the opportunities for weed biocontrol. Considerable progresses have been made, but there is enormous scope for further work. Increased government and public support, closer international collaboration, and most importantly, more well-trained researchers are needed.

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## EFFICACY OF *EXSEROHILUM MONOCERAS* MIXED WITH QUINCLORAC IN CONTROLLING *ECHINOCHLOA CRUS-GALLI* IN RICE

Y. Chen, H. Ni, and X. J. Li

Weed Sci. Research Lab., Dept. of Agronomy, China Agri. Univ., Beijing 100094,  
China. chygg@263.net

**Abstract:** *Echinochloa crus-galli* is one of the ten worst weeds in all over the world and hard to be control. This study focused on joint action of pathogen *Exserohilum monoceras* and chemical herbicide quinclorac and evaluate the efficacy of tank-mixed them against the weed in field condition. The results showed that there was significant synergism between the pathogen and the chemical. Under paddy field condition, the pathogen at the rate of  $7.7 \times 10^{11}$  conidia/ha only gave about 60% control for the *E. crus-galli*. However, the performance of tank-mixed *E. monoceras* with quinclorac was very good and provided around 90% control.

**Key word:** *E. monocera*, *E. crus-galli*, quinclorac, joint action, synergism, rice

### INTRODUCTION

Barnyardgrass (*Echinochloa crus-galli*) is one of the ten worst weeds in all over the world, and also is the most important weeds occurring in rice (Holm et al. 1977). Meantime, it is the troublesome weeds in soybean, cotton, vegetable, yam, some cereal crop etc.

Although various management strategies are available for the weed suppression in rice. However, highly effective and safe methods are limited. Chemical control is the major strategy for *Echinochloa* suppression in rice at present. Barnyardgrass easily develops resistance to herbicides (Ellis 1975). Intensive and repeated use over the past several decades has led to the development of herbicides-resistant barnyardgrass. The weed was reported resistant to propanil (Norsworthy et al. 1998), butachlor and thiobencarb (Huang et al. 1994, 1995).

A lot of research was done on the evaluation of the bio-efficacy of *Exserohilum monoceras*, a pathogen of barnyardgrass, in the weed control. The results showed that the pathogen had great potential as a mycoherbicide. The good efficacy of control was observed at the 1.5~2.5-leaf stage of the weed under the condition of 24-h dew period (Zhang et al. 1997; Chen and Ni 1999, 2001).

In the development of mycoherbicides, the maintenance of genetic stability and virulence is a big problem (Tisdell et al. 1984) and the requirement of long period of free moisture for infection is another problem (Boyette et al. 1993; Charudattan 1996). The simultaneous occurrence of several weed species in a field has a negative effect on both the technical and economic feasibility of bioherbicide approach. Combination of pathogens with chemical herbicides at sub-lethal dosage is one way to solve the latter two problems. Lohuis et al. (1990), Smith (1992), and Hoagland (1996) reported that mixing bioherbicide with chemical herbicides could improve efficacy.

The objectives of this study were to evaluate joint action of the pathogen and chemical herbicide quinclorac and the efficacy of the tank-mixture of them against barnyardgrass under paddy field condition.

## MATERIALS AND METHODS

### Barnyardgrass seedlings used

Barnyardgrass seeds collected from rice paddy fields in Beijing were incubated in petri dishes with moist filter paper at 35°C for 24h and then at room temperature for 24h. The germinated seeds were sown on 10-cm-diameter plastic pots and then pots were placed in a greenhouse at day/night temperature of 35/20±5°C. The soil moisture content was kept saturated for the whole experimental period.

### Preparation of conidia of *E. monoceras*

A single-conidium isolate of *E. monoceras* growing on potato dextrose agar (PDA) slants in small vials was maintained under mineral oil at 4°C as the stock culture (Tuite 1996). Small pieces of mycelium from the stock culture were aseptically transferred to petri dishes with PDA and then incubated at 28°C for 7 days. Agar plugs with mycelium (6mm diameter) from the margins of these young colonies were used as "seed" inoculum. The "seed" were placed into 250-ml Erlenmeyer flasks containing 80ml YSC liquid media containing yeast, soybean and corn flour and then the flasks were incubated culture chambers at 28°C and 180rpm/min. Mycelia were transferred to rice (husked) after 3-days liquid culture and cultured at room temperature in the dark for 3 weeks. *E. monoceras* conidium flour (EMCF) were harvested by sifting of a 150 meshes wire sifter and maintained at 4°C for use. The EMCF contained  $1.2 \times 10^8$  conidia/g.

### Greenhouse experiments

EMCF, quinclorac, and the mixture of *E. monoceras* plus quinclorac were sprayed with a pot sprayer tower at the 2.5-leaf stage of barnyardgrass seedlings. Six rates for each treatment were designed. All solutions for spray contained 0.05% Tween 20 as a wetting agent, and the spray volume was 80ml/m<sup>2</sup>. After spraying, all pots were immediately placed in growth chamber about 90% relative humidity and 28°C for 24h and then transferred to the greenhouse.

Fresh weights above ground were measured 10 days after treatment (DAT). The fresh weight data were expressed as percent reduction in biomass compared with the biomass of untreated controls.

The experiments were done twice in a randomized complete block design with 4 replications.

### Field experiments

A field experiment with randomized complete block design and 4 replications was carried out in Beijing in 2000 to evaluate the efficacy of *E. monoceras* applied alone and tank-mixed with quinclorac. Seedlings (30-day old) of rice cv. Zixiangnuo were transplanted on May 15. EMCF, 50%quinclorac, and the mixture were applied at the rates of 7700, 400, and 7900g/ha, respectively, 22 days after transplanting at the 2.5-leaf stage of barnyardgrass. The mixture was composed of 7700g EMCF and 200g 50% quinclorac WP. The spray volume was 900L/ha.

The lesions on leaves of the weed and rice were recorded 7 DAT and, the number and fresh biomass of surviving *E. crus-galli* plants were counted and weighed 14 and 60 DAT.

### Statistical analysis

For the greenhouse experiment, all percentage data were arc sine-transformed before analysis (Gomez and Gomez 1984). A four-parameter model (Finney, 1987) was used to describe the relationship between dry weight reduction (Y) and application rate (X):

$$\log_e(Y) = \log_e[(D-C)/\{1+\exp(-2(a+b\log_{10}(X)))\}+C] \quad (1)$$

where D and C denote the upper and lower limits of the dose-response curve at zero and large dose, respectively, *a* denotes the ED<sub>50</sub> and *b* the slope around ED<sub>50</sub>.

The theory value of the mixture of *E. monoceras* plus quinclorac was calculated based on additive model.

ANOVA was done for the field experiment. All data analyses were done using SPSS software.

## RESULTS AND DISCUSSION

### Greenhouse experiments

Figure 1 showed actual efficacy of the mixture was greatly higher than its theory value. According to the join action anlysis by Finney method, there was significant synergism between *E. monoceras* and quinclorac based on either ED<sub>50</sub> or ED<sub>80</sub>. The theory values of the ED<sub>50</sub> and ED<sub>80</sub> for the mixture were 1.397 g/m<sup>2</sup> and 2.77 g/m<sup>2</sup>, respectively, while the actual values of them were 0.395 g/m<sup>2</sup> and 0.65g/m<sup>2</sup>, respectively (Table 1).

Table 1. The nonlinear regression on the means of arc sine transformed percent fresh weight reduction of barnyardgrass as a function of the log (application rate) 10 days after treatment.

Treatment	Equation	R <sup>2</sup>	Dose range (g/m <sup>2</sup> )	ED <sub>50</sub> (g/m <sup>2</sup> )	ED <sub>80</sub> (g/m <sup>2</sup> )
EMCF	$y = 0.959 - 0.656 / (1 + (x/4.931)^{4.292})$	0.9847	0.0031~3.1	0.142	1.54
Quinclorac	$y = 0.959 - 0.656 / (1 + (x/4.048)^{17.517})$	0.8988	0.21~7.6 <sup>a</sup>	1.10	1.17
Actual Mixture	$y = 0.959 - 0.656 / (1 + (x/2.308)^{7.992})$	0.9173	0.15~4.93 <sup>a</sup>	0.395	0.65
Predicted Mixture	$y = 0.959 - 0.656 / (1 + (x/3.703)^{10.913})$	0.9999	0.15~4.93 <sup>a</sup>	1.397	2.77

a: the dose of quinloac was transformed (the dose × 10<sup>3</sup>) before doing the nonlinear regression.

### Field experiment

Obvious infested symptoms on barnyardgrass in all the treated plots, but no symptoms on rice 7 DAT. The severity of infestation was higher in mixture treated plots than in EMCF treated plots.

EMCF applied alone provided lower control efficacy to the weed, only 45% and 61% in terms of plant number and fresh weight reduction. Tank-mixed the pathogen

with quinclorac gave good control for barnyardgrass. Adding half dosage of quinclorac increased the control efficacy of EMCF from 60% to 90% (Table 2).

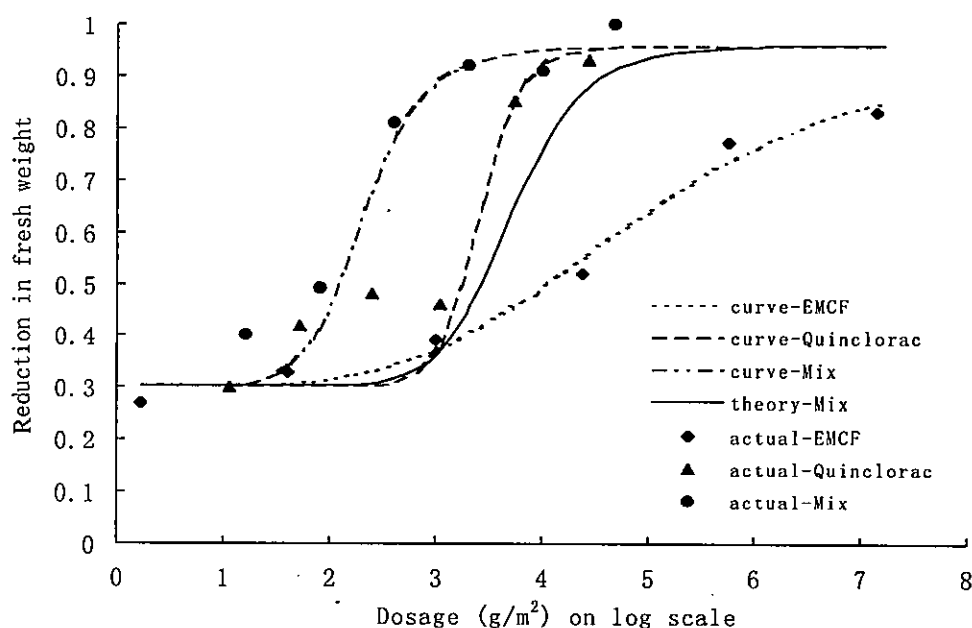


Figure 1. The relationship between biomass reduction and application rate. EMCF = *E. monoceras* conidium flour.

Table 2. Result counted of the efficacy of *E. crus-galli* control 14 and 60 days after treatment in rice

Treatment	Rate (g/ha)	14 Days After Treatment				60 Days After Treatment			
		No.	No. Reduction (%)	Fresh WT (g)	WT Reduction (%)	No.	No. Reduction (%)	Fresh WT (g)	WT Reduction (%)
EMCF	7770	35	65	161	61	68	28	2060	56
Chemical	400	14	78	121	71	17	82	997	79
Mixture	7970	31	52	40	90	14	85	557	88
Untreated		63		417		95		4667	

Mean values of one experiment with three replications per treatment. Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test ( $p=0.05$ ). EMCF = *E. monoceras* conidium flour.

The requirement of long period of free moisture for infection is generally one of the principal constraints to the development of many fungal pathogens as mycoherbicides (Boyette 1993 and Charudattan 1996). Mixing sub-dosage is a practical way to overcome this constraint. The findings from both the greenhouse and field experiments indicated adding sub-dosage of quinclorac could significantly improve *E. monoceras* efficacy against barnyardgrass.

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## EFFECTS OF THE FUNGUS *SETOSPHAERIA ROSTRATA* ALONE AND IN COMBINATION WITH HERBICIDES ON RED SPRANGLETOP (*LEPTOCHLOA CHINENSIS* (L.) NEES) CONTROL IN DIRECT SEEDED RICE

D. V. Chin<sup>1</sup>, L. H. Man<sup>1</sup>, H. L. Thi<sup>1</sup>, B. A. Auld<sup>2</sup>, and S. D. Hetherington<sup>2</sup>

<sup>1</sup>Cuulong Delta Rice Research Institute (CLRRI), Omon, Cantho, Vietnam  
duongvanchin@hcm.vnn.vn

<sup>2</sup>NSW Agriculture, Orange Agricultural Institute, Forest Road, NSW Australia 2800

**Abstract:** *Leptochloa chinensis* is ranked as the second most important weed after *Echinochloa crus-galli* in direct seeded rice in Vietnam. In the Mekong delta where direct seeding practice is common, this weed is becoming abundant and causes concern to farmers. One of the reasons is that many rice herbicides in this country have low efficacy on this weed. Other factors affecting the high infestation of red sprangletop are the shortage of water during the onset of rainy season and secondly, remaining pockets of dry areas due to poorly levelled rice fields. An attempt was made to find out an alternative method for checking this emerging problem. The collaboration between CLRRI and the Australian Center for International Agricultural Research (ACIAR) from 1995 until now has resulted in finding out a fungus namely *Setosphaeria rostrata* which showed the high efficacy on this weed and safe to rice. Results from field experiments during the year of 1999-2000 showed that spraying of the spore concentration at 7 days after seedling transplanting (DAST) of weed had resulted in high dead plant percentage as compared to those at 14, 21 and 28 DAST. The percentages of infected leaves of *Leptochloa chinensis* sprayed at 7 DAST are 99.4; 100; 100 and 100% observed at 3, 7, 14 and 21 days after application (DAA). The application of spore concentration of *Setosphaeria rostrata* with  $5 \times 10^{11}$  spores/ha brought down weed density significantly as compared to check. The percentages of reduction were: 45.5%; 51.1% and 57.5% observed at 7, 14, and 55 DAA. Weed dry weight reduced 69% at 56 DAST. Under treatments of  $10^{12}$  spores/ha, weed population and dry matter accumulation of *Leptochloa chinensis* tend to be reduced as compared to those in the treatments of  $5 \times 10^{11}$  spores/ha but the difference is not significant. The combination of fungi *Setosphaeria rostrata* and *Cochliobolus lunatus*; for controlling *Echinochloa crus-galli*, or with bispyribac-sodium herbicide had good efficacy on both grass species of *Echinochloa crus-galli* and *Leptochloa chinensis* respectively.

**Key words:** *Setosphaeria rostrata*, *Leptochloa chinensis*, rice mycoherbicide, direct seeded rice.

### INTRODUCTION

Rice is the most important crop in Vietnam with more than 7 m.ha of sown area and 29 m.tons produced every year. There are 4.199 m.ha for rice production sharing 74.1% of arable land in the whole country. Total production in the year 1998 is 29.141 m.tons (SPH 1999). Vietnam produces 31.4 m.tons rough rice and exports 4.5 m.tons of milled rice in 1999. However, the average rice yield (4.1 tons /ha ) is still low as compared to many other countries. There are some abiotic and biotic factors

contributing for the low yield. Weeds are a major biotic factor affecting rice growth and yield compared with insects and diseases. In order to check this important pest, herbicides have been used widely in rice production by farmers in this country. High dependency on herbicides results in accumulation of herbicidal residues in the environment. Using the same compound for many years in the same field can create the new problem of herbicide resistant weeds. Following the IPM concept is one part of the policy of Vietnam government in crop production in which the non-chemical methods have been encouraged. One possibility is using biological control methods including mycoherbicides as one component of integrated weed management. Experimental results presented in this paper using fungus *S. rostrata* to control *L. chinensis* in direct seeded rice in Vietnam is part of the collaborative work between Cuulong Delta Rice Research Institute (CLRRI) Vietnam and NSW Agriculture, Orange Agricultural Institute, NSW, Australia.

## MATERIALS AND METHODS

### Isolation and characterization of fungi

Diseased leaves of *L. chinensis* were collected in and near the rice fields of Omon and Thotnot districts, Cantho province, Mekong delta in Summer-Autumn of 1997. The infected parts of weed were surface sterilized with 0.1% HgCl<sub>2</sub> and incubated on fresh Potato-Dextrose-Agar (PDA). The pathogens were isolated using standard isolation techniques. A live specimen of this fungus was sent to the International Mycological Institute in U.K. for identification. The specialists at IMI identified the said fungus as *Setosphaeria rostrata*. For long term storage, pure culture of original isolate was maintained on filter paper followed by IRRI methods at -20°C as stock culture.

### Pathogenicity testing of isolates

A single lot of seeds of *L. chinensis* collected from rice fields in Omon district was used in all experiments. Soaked seeds were broadcast randomly on mud contained in trays. Three to four days after sowing, young weed seedlings were transferred to 30-cm diameter pots, planted in lines and kept in a greenhouse. Spores were prepared from 10 to 15 day-old cultures, filtered in nylon cloth and the concentration adjusted to 10<sup>5</sup> to 10<sup>6</sup> spores per ml. (Tween 80 was added.) Weed seedlings at three- to four- leaf stage were sprayed by hand sprayer with the spore suspension at different doses depending on treatments and kept in the green house under natural conditions. In the field testing, only *L. chinensis* was maintained in the field for studying in relation to *S. rostrata*. In case of both *C. lunatus* and *S. rostrata* suspensions were sprayed, seeds of two main grasses of *E. crus-galli* and *L. chinensis* were sown in the experimental field. All other weeds were removed completely by hand.

## RESULTS AND DISCUSSION

Data from a pot experiment (Table 1) showed that older seedlings of *L. chinensis* are less susceptible to the fungus spores than younger ones. At seven days after seedling transplanting (DAST), spraying water only can cause infection on 4.3% of leaves. The reason may be due to the spores of *S. rostrata* existing naturally in the environment. Spraying at 7 DAST resulted in high infection for 21 DAA. Older seedlings had

progressively less infection with the corresponding data of 77.0%, 70.8% and 54.4% when sprayed at 14, 21 and 28 DAST.

Results from the field experiment conducted at CLRRI experimental farm during Summer-Autumn 2000, revealed that concentration of *S. rostrata* at the rate of  $5 \times 10^{11}$  - spores/ha (T3) and  $10^{12}$  spores/ha (T4) resulted in significant reduction of *L. chinensis* population observed at 7, 14 and 35 DAA. The T4 treatment with higher spore concentration was likely to be better than T3 but the difference was not significant. Two treatments of spraying fungus spores give the equal effect with hand weeding twice. Averaged weed density under treatments of spraying fungus is reduced 59% as compared to untreated check. Similarly, weed dry weights were brought down drastically and reduced 76% as compared to check. Rice yields under all treatments are higher than check (1.99 T/ha) statistically. Two treatments of spraying *S. rostrata* have the same yield with each other (4.08 T/ha and 4.17 T/ha) and lower than that of hand weeding twice (5.27 T/ha) significantly (Table 2).

Table 1. Percentage of infected leaves (%) of *L. chinensis* observed at different times after application (DAA) in pot experiment 1.

Treatments	Mean Infected leaves (%)			
	3DAA*	7DAA	14DAA	21DAA
T1) Spray water at 7DAST	5.1	5.1	3.9	3.0
T2) Spray <i>S. rostrata</i> at 7DAST(**)	99.4	100.0	100.0	100.0
T3) Spray <i>S. rostrata</i> (***) at 14DAST	82.3	81.2	75.1	68.3
T4) Spray <i>S. rostrata</i> at 21 DAST	74.2	75.1	73.8	60.0
T5) Spray <i>S. rostrata</i> at 28 DAST	56.4	58.3	52.4	50.3

\*DAA = days after application; \*\* DAST = days after seedling transplanting; \*\*\* Application rate:  $2 \times 10^7$  spores/30-cm diameter pot.

Table 2. Effects of treatments on *L. chinensis* population (No. plants/m<sup>2</sup>), weed dry weight (g/m<sup>2</sup>) and rice yield (T/ha).

Treatments	Weed population (No plants /m <sup>2</sup> )		Weed dry weight (g/m <sup>2</sup> )		Yield (T/ha)
	7DAA*	14DAA	35DAA	35DAA	
T1) Untreated check	10.2a	11.5a	17.3a	5.6a	1.99a
T2) Hand weeding twice	0.7b	0.7b	1.3b	0.7b	5.27b
T3) <i>S. rostrata</i> at $5 \times 10^{11}$ spores/ha	5.6b	5.6b	7.3b	1.7b	4.08c
T4) <i>S. rostrata</i> at $10^{12}$ spores/ha	3.4b	4.0b	6.9b	1.0b	4.17c

DAA=days after application; transformed data (square root of  $x+0.5$ ); Data in a column followed by the same letter are not different significantly by DMRT.

The pot experiment was conducted in the green house at CLRRI during Summer-Autumn of 1999 to find out the effects of *S. rostrata* alone and the fungus combination with herbicides to control *L. chinensis*. Data of this experiment are presented in Table 3 and Table 4. Two herbicides namely pyrazosulfuron-ethyl and bispyribac-sodium were chosen for this study because they are weak in controlling the target weed of *L. chinensis*, especially when weed seedlings are old. All treatments brought down the plant heights of *L. chinensis* significantly as compared to untreated check observed at 14, 21 and 45 DAA. Spraying spore concentration of *S. rostrata* at  $2.8 \times 10^{12}$  spores/ha reduced the weed plant height of 74%, 69% and 56% observed at 14, 21 and 45 DAA respectively.

The fungus was more effective than pyrazosulfuron-ethyl in terms of height reduction of the weed. There was a positive interaction between this herbicide and fungus virulence. Plant heights under herbicide alone were 36.9; 47.7 and 109 cm whereas under combined treatments, the heights were 8.4 ; 19.9 and 33.8 cm respectively. The trend was also observed in the number of tillers/plant observed at 14, 21 and 45 DAA (Table 3).

Table 3. Effects of treatments on *L. chinensis* plant height (cm) and number of tillers/plant (Pot Experiment 1).

Treatments	Plant height (cm)			No of tillers /plant		
	14DAA	21DAA	45DAA	14DAA	21DAA	45DAA
T1) Untreated check	47.8	59.2	116.7	1.38	2.09	2.59
T2) <i>S. rostrata</i> at $2 \times 10^7$ spores/pot*	12.4	18.2	51.4	0.48	0.87	1.51
T3) Pyrazosulfuron at 25g.a.i./ha	36.9	47.7	109.0	1.11	1.19	1.97
T4) Bispyribac at 20g.a.i./ha	0.0	0.0	0.0	0.00	0.00	0.00
T5) Pyrazosulfuron + <i>S. rostrata</i>	8.4	19.9	33.8	0.38	0.41	0.74
T6) Bispyribac + <i>S. rostrata</i>	0.0	0.0	0.0	0.00	0.00	0.00
LSD(0.05)	0.5	0.9	0.9	0.09	0.08	0.14

\*  $2 \times 10^7$  spores/30-cm diameter pot =  $2.8 \times 10^{12}$  spores /ha

Weed mortality and dry weights as affected by treatments are shown in table 4. At 7DAA, results showed that *S. rostrata* was effective in controlling *L. chinensis* with 75.6% killed plants. The herbicide pyrazosulfuron-ethyl is weak on this weed with only 11.1% whereas bispyribac-sodium performed better with 58.6%. However, the interactive effect between pyrazosulfuron-ethyl with the fungus was very clear being 6.9 times higher in terms of killed plants under combined treatments (11.1% and 76.7%). This trend was also observed in the case of the combination between bispyribac-sodium and *S. rostrata* at this early stage. At 14 DAA, the fungus remained effective with a high killed percentage of 77.9%. Bispyribac-sodium alone as well as herbicide combination control *L. chinensis* sufficiently. The interaction between *S. rostrata* and pyrazosulfuron-ethyl continued to exist with 82.5% dead plants under the combined treatment as compared to that of herbicide alone (29.4%). The trend was similar in the observation at 21 DAA. All treatments reduced *L. chinensis* dry weight significantly as compared to the untreated check. The fungus *S. rostrata* reduced weed dry weight to 16.7 g/pot (78% reduction) as compared to 77.4 g/pot under check. Both treatments of bispyribac-sodium and *S. rostrata* performed very well in terms of weed dry weight reduction. *S. rostrata* interacted with the herbicide pyrazosulfuron-ethyl to check successfully the dry matter accumulation by the weed. It reached only 9.3 g/pot under combined treatment as compared to 45.6 g/pot in herbicide treatment alone.

A field experiment conducted during Winter-Spring of 1999-2000 with artificial sowing seeds of two main grasses namely *Echinochloa crus-galli* and *L. chinensis* was carried out at CLRRRI. The objective of this experiment is to find out the effects of fungi, herbicides, fungus + herbicide and fungus + fungus combinations on grass control in direct seeded rice field (Table 5). *E. crus-galli* was not affected by the spore suspension of the fungus *S. rostrata* and therefore the dry matter accumulation by the weed observed at 56 DAS (days after sowing) was equal to that under untreated check statistically. The rest of the treatments including herbicide alone, or the combination of herbicide and fungus, except two fungi combinations, reduced the population of *E. crus-*

*galli* significantly as compared to check. The fungus *Cochliobolus lunatus* cannot control *E. crus-galli* successfully as compared with the tested herbicides. All treatments were effective in checking the population and dry weight of *L. chinensis*. Rice yields under all treatments were significantly higher than that of untreated check. The highest yields were under hand weeding twice and bispyribac-sodium treatments (6.29 T/ha and 5.73 T/ha respectively). The fungus *S. rostrata* cannot control *E. crus-galli* and therefore this weed competed strongly with rice resulting in lowered yield (4.20 T/ha) compared with other treatments. Yields in combined treatments with fungus and herbicide as well as fungus and fungus were not significantly different.

Table 4. Effects of treatments on killed plant percentage (%) and dry matter accumulation by weed (Pot Experiment 2).

Treatments	Killed weed percentage(%)			Weed dry weight(g/pot)
	7DAA	14DAA	21DAA	
T1) Untreated check	0.0	0.0	0.0	77.4
T2) <i>S.rostrata</i> @2x10 <sup>7</sup> spores/pot(*)	75.6	77.9	78.3	16.7
T3) Pyrazosulfuron@25g.a.i./ha	11.1	29.4	30.0	45.6
T4) Bispyribac@20g.a.i./ha	58.6	100.0	100.0	0.0
T5) Pyrazosulfuron + <i>S.rostrata</i>	76.7	82.5	83.9	9.3
T6) Bispyribac + <i>S.rostrata</i>	98.3	100.0	100.0	0.0
LSD(0.05)	5.8	4.3	4.2	0.8

\* 2x10<sup>7</sup> spores/30-cm diameter pot =2.8x10<sup>12</sup> spores/ha.

Table 5. Effects of treatments applied at 10 days after sowing (DAS) on weed population (No. plants/m<sup>2</sup>), weed dry weight (g/m<sup>2</sup>) and rice yield (T/ha).

Treatments	Population (plants /m <sup>2</sup> )		Dry weight(g/m <sup>2</sup> )		Yield (T/ha)
	ECHCG	LEPCH	ECHCG	LEPCH	
T1) Untreated check	19.1a	10.2a	14.4a	3.3a	1.41a
T2) Hand weeding twice	0.7c	0.7b	0.7b	0.7b	6.29b
T3) <i>S. rostrata</i> @ 4.5 x 10 <sup>10</sup> spores/ha	14.1a	1.2b	10.0a	1.1b	4.20d
T4) Bispyribac @ 20g.a.i./ha	0.9c	0.7b	0.9b	0.7b	5.73b
T5) Bispyribac + <i>S.rostrata</i>	0.7c	1.9b	0.7b	1.8b	5.31c
T6) Pyrazosulfuron @ 25g.a.i./ha	1.2c	1.3b	0.9b	0.9b	4.96c
T7) Pyrazosulfuron + <i>S.rostrata</i>	0.7c	1.1b	1.0b	0.9b	5.13c
T8) <i>C. lunatus</i> + <i>S.rostrata</i>	3.8b	2.6b	2.6b	1.3b	5.20c

ECHCG = *Echinochloa crus-galli*;LEPCH = *Leptochloa chinensis*.

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## THE DEVELOPMENT OF A BIOHERBICIDE FOR *ECHINOCHLOA CRUS-GALLI* BASED ON THE FUNGAL PATHOGEN *EXSEROHILUM MONOCERAS* IN VIETNAM

S. D. Hetherington<sup>1</sup>, N. V. Tuat<sup>2</sup>, B. A. Auld<sup>1</sup>, and H. M. Thanh<sup>2</sup>

<sup>1</sup>Orange Agricultural Institute, NSW Agriculture, Forest Road, Orange, Australia  
shane.hetherington@agric.nsw.gov.au

<sup>2</sup>National Institute of Plant Protection, Chem, Tu-Liem, Hanoi, Vietnam

**Abstract:** In 1995 a study was initiated to determine the most suitable system for development of bioherbicides in Vietnam. A weed survey of the Red River Delta identified *Echinochloa crus-galli* as the most common grass weed of irrigated rice cultivation. A large number of indigenous fungal pathogens were isolated following a subsequent survey of diseased plants. The most virulent and selective fungal species was identified by the International Mycological Institute as *Exserohilum monoceras*. A single isolate of this fungus, given the accession number 85.1, was chosen for further study. Infection with this fungus initially results in spreading chlorotic lesions which coalesce to form areas of general necrosis. Given suitable conditions 100% weed mortality is achieved following inoculation with a fungal suspension at a concentration of 10<sup>6</sup> spores/ml. The most commonly grown cultivars of rice in Vietnam were challenged by isolate 85.1 and symptoms were restricted to hypersensitive flecking; these symptoms carried no morphological penalty. While laboratory and glasshouse testing of this fungus have relied on inocula produced on green bean agar, experiments are currently underway to determine the viability of growing *E. monoceras* on agricultural by-products such as rice husks and groundnut pods. It is hoped that a "cottage industry" can be developed allowing farmers to produce their own weed control product from widely distributed "seed" cultures. Field testing of *E. monoceras* is currently being conducted in north Vietnam.

**Key words:** Biological herbicide, field testing, mass-production.

### INTRODUCTION

Weeds impose a significant burden upon rice production in Vietnam. Reduction in crop yield due to weeds may reach 40% significantly decreasing the profit available from a crop which constitutes 35% of the countries GDP.

A survey identified *Echinochloa crus-galli* (barnyard grass, co long vuc) as the most common grass weed associated with irrigated rice cultivation in the Red River Delta (Tan *et al.* 2000). This weed is also significant in other crops of importance such as corn, soybean, citrus and tea (Holm *et al.* 1977).

Traditionally control of *E. crus-galli* in rice has involved hand weeding, typically involving women. Recent increases in the area and intensity of rice cultivation have removed women from their traditional role as homemaker for longer periods of time reducing the viability of this control option. Increasingly weed control involves the use of selective herbicides combined with broadcast rice cultivation. While reducing labour input, the use of herbicides poses a number of risks. Herbicide contamination of paddy water and mud may create human health risks given the multiple use nature of these systems. Paddies are used for aquaculture, household cleaning water and the gathering

of various "wild" foods (salad greens, frogs, snails). Additionally, *E. crus-galli* is thought to be the most likely tropical weed to develop herbicide resistance (Evans 1991) and butachlor and thibencarb resistant populations are widespread in Southern China (Huang and Gressel 1997). It is likely that resistant populations also exist in Vietnam but no surveys have been undertaken.

An alternative control approach may be to use inundative doses of propagules from indigenous fungal pathogens (the bioherbicide approach) to control this weed. Our group has identified *Exserohilum monoceras* as the most virulent and selective fungal pathogen infecting *E. crus-galli* in northern Vietnam (Tuat et al. 1999). In greenhouse trials 100% mortality of a number of morphotypes of *E. crus-galli* were recorded 10-15 days after inoculation with fungal suspensions at concentrations of  $10^6$  spores/ml. Symptoms on the most widely planted cultivars of rice in Vietnam were limited to hypersensitive flecking. This flecking carried no morphological penalty.

Deployment of this product requires extensive field testing and the development of a suitable mass-production system. As *E. monoceras* does not produce infective propagules in liquid culture (Zhang and Watson 1997), a solid-substrate mass production system is required. This paper reports on experiments to determine the suitability of a number of agricultural by-products as suitable substrates for *E. monoceras* growth. It also presents results from preliminary field experiments conducted at the National Institute of Plant Protection (NIPP), Hanoi.

## MATERIALS AND METHODS

### Mass production

During preliminary experiments 27 agricultural substances were inoculated with an *E. monoceras* conidial suspension. The greatest sporulation occurred on maize seed, rice seed, groundnut bark, rice husk and maize leaf. These substances were chosen for inclusion in a more rigorous experiment to determine their suitability as a conidial production medium.

A sterile 20g sample of each substance was aseptically placed into a 250 ml erlenmeyer flask and 30 ml of sterile water added. Ten ml of an *E. monoceras* spore suspension [ $10^6$  conidia/ml] was added to the flask and it was sealed. Treatments were replicated ten times. Three weeks after inoculation 50 ml of sterile water was added under aseptic conditions, the substance agitated to obtain an even suspension, the liquid removed and the suspension's spore concentration determined using a haemocytometer. The flask was again sealed aseptically and after another week (4 weeks after the original inoculation) the spore production was assessed as above.

### Field experiments

In autumn 2000 (October-November) fifteen  $1\text{m}^2$  plots were established at NIPP and flooded to a depth of approximately 3cm. Each of these plots were directly sown with 15g of rice seed (cv CR203) and 15g of *E. crus-galli* seed. When plants had reached the two-leaf stage plots were inoculated with conidial suspensions at various concentrations. Each plot received 150ml of a water-based conidial suspension amended with 0.1% Tween 20<sup>®</sup>. Suspensions were at concentrations of 0,  $10^4$ ,  $10^5$  and,  $10^6$  conidia / ml. A fifth treatment was initially sprayed with 150 ml of a suspension of  $10^6$  conidia / ml and sprayed again five days later with another suspension at the same volume and concentration. Treatment layout was randomised and replicated three times.

The disease incidence, severity and the mortality of the weed were recorded 5, 10 and 15 days after the initial inoculation. At each assessment five points/plot were randomly chosen and twenty plants assessed from each of these points. Disease severity was measured on a 0-5 scale where 0 = no disease; 1 = 1-5% leaf area infected; 2 = 6-10% leaf area infected; 3 = 11-25% leaf area infected; 4 = 26-50% leaf area infected; and 5 = >50% leaf area infected. Plants were harvested 30 days after the initial inoculation and biomass measured after drying in an oven at 60°C overnight. The effect of the various treatments on disease severity and biomass were analysed using the analysis of variance procedure of the Genstat Statistical Package (Genstat, Rothamsted, UK).

## RESULTS AND DISCUSSION

### Mass Production

By far the greatest conidial production was achieved on rice husks, while rice seed was another substrate upon which reasonable numbers of conidia formed. Following an initial harvest of conidia 3 weeks after inoculation a second harvest was possible on rice husks a week later (4 weeks after the initial inoculation) (Figure1).

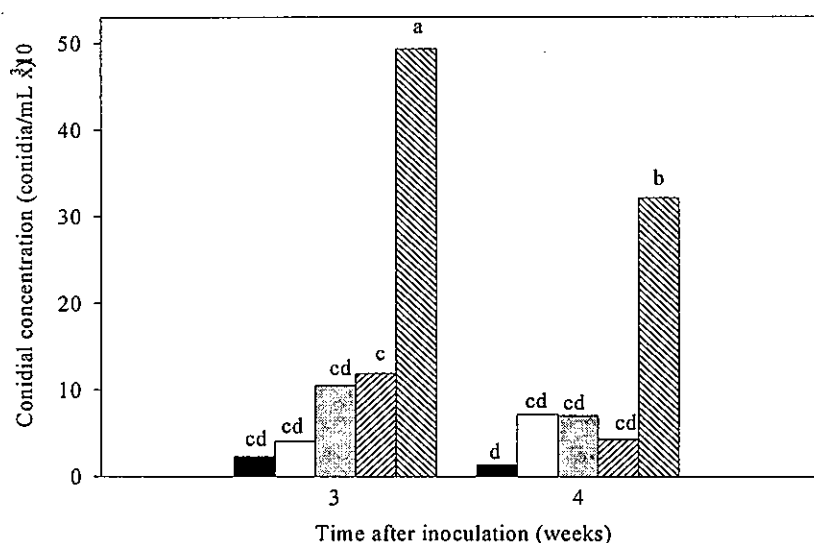


Figure 1. Conidial production of *E. monoceras* on various agricultural substrates. Maize leaf ■, maize seed □, groundnut bark ▨, rice seed ▤, rice husk ▩; lettering above bars indicates statistical similarity ( $p < 0.05$ ).

Rice husks are a by-product of polishing brown rice to obtain white rice. These husks are commonly burnt or fed to stock. The growth and sporulation of *E. monoceras* upon this commodity will provide farmers an opportunity to produce their own weed control organism at very low cost. It is anticipated that an initial starter culture could be distributed to farmers who could produce their own product using techniques taught to them by agricultural extension workers. The ability of the fungus to produce a second conidial yield from the same substrate would reduce labour input. These results contrast with those of Zhang and Watson (1997) who found that highest levels of *E. monoceras*



conidial production occurred on maize leaves and that rice substrates produced less than  $10^3$  conidia  $g^{-1}$ .

### Field Experiments

*E. monoceras* is an indigenous pathogen and infection of plots inoculated with water amended with tween 20<sup>®</sup> was due to levels of background inoculum. This level of inoculum was insufficient to effect the vigour of the weed. Inoculation with a dilute suspension of conidia ( $10^4$  conidia / ml) resulted in a significantly higher disease severity than controls (Figure 2) which translated to a reduction in biomass of 36.7% 30 days after inoculation (Figure 3).

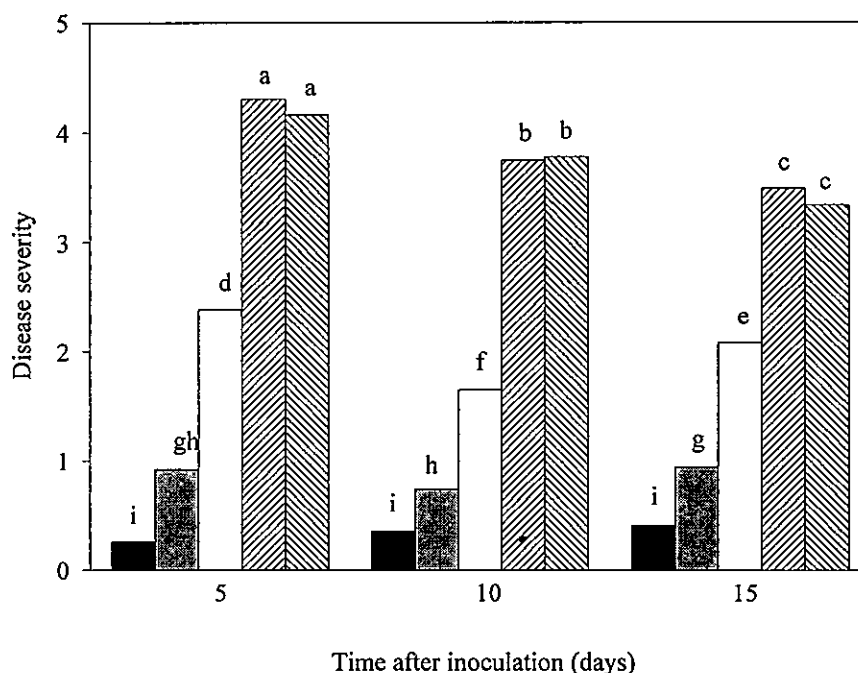


Figure 2. Severity of Disease Recorded on *E. crus-galli* Seedlings 5, 10 and 15 Days after Inoculation with *E. monoceras* Conidial Suspensions at Concentrations of 0  $\blacksquare$ ,  $10^4$   $\square$ ,  $10^5$   $\square$ , and  $10^6$   $\square$  conidia / ml. A further treatment was initially sprayed with a conidial suspension at a concentration of  $10^6$  conidia / ml and subsequently sprayed with a suspension of equal volume and concentration 5 days later  $\square$ . Disease severity is rated as 0 = no disease; 1 = 1-5% leaf area necrotic; 2 = 6-10% leaf area necrotic; 3 = 11-25% leaf area necrotic; 4 = 26-50% leaf area necrotic; and 5 = > 50% leaf area necrotic. Lettering above bars indicates statistical similarity ( $p < 0.05$ ).

Weed biomass was reduced by 56.5% when inoculum concentration was increased to  $10^6$  conidia / ml. A second, delayed application of a conidial suspension at a concentration of  $10^6$  conidia / ml did not increase disease severity or reduce weed biomass when compared to a single application at the same concentration. No weed mortality was recorded. Disease severity was lower after 15 days than recorded after 5 days. It is thought that this was due to vegetative recovery by the weed.

Irrigated rice is not grown during autumn in northern Vietnam and these experiments are considered preliminary to a series of experiments to be carried out during the summer and spring seasons of 2001 and 2002. Nevertheless the results are encouraging. High levels of in-field infection of *E. crus-galli* by *E. monoceras* were recorded and these resulted in significant decreases in weed biomass. A lack of post-infection disease development leading to general necrosis and blighting prevented mortality. It is thought that the lack of post-infection disease development was due to the low temperatures and humidity experienced during the experiment. During the rice growing season temperatures and humidity are high and mortality is expected. Controlled environment experiments are being carried out at NIPP to determine the relationship between temperature and disease severity. Given what we believe to be sub-optimal environmental conditions preliminary results are encouraging.

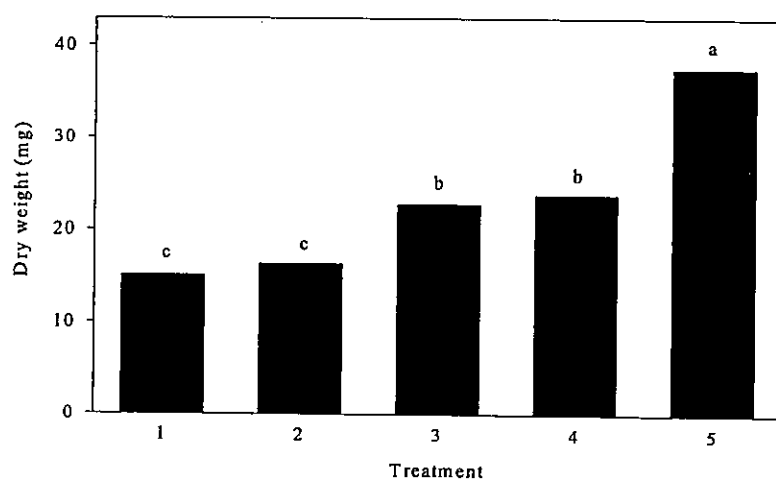


Figure 3. Weed biomass following inoculation of field plots with a spore suspension of *E. monoceras* at various concentrations. Treatment 1 =  $1 \times 10^6$  conidia / ml (sprayed twice at 5 day interval); Treatment 2 =  $10^6$  conidia / ml; Treatment 3 =  $10^5$  conidia / ml; Treatment 4 =  $10^4$  conidia / ml; Treatment 5 = water + tween 20 control.

#### ACKNOWLEDGEMENTS

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## ISOLATION OF HOST SPECIFIC FUNGAL ISOLATE YK-201 TO BULRUSH (*SCIRPUS HOTARUI* OHWI) AND WEEDING EFFECT OF THE PLANTS CAUSED BY NATURAL INFECTION IN PADDY FIELD

Y. K. Hong, S. B. Song, J. B. Hwang, D. B. Shin, and D. C. Lee  
National Yeongnam Agricultural Experiment Station, Miryang 627-130, Korea.  
hongyk@rda.go.kr

**Abstract:** Brown stem blight of bulrush (*Scirpus hotarui* Ohwi), caused by isolate YK-201 (*Alternaria* sp.), were observed at naturally occurred in rice paddy field, is first reported in Korea. Typical symptom on stem having watersoaked brown blight were formed and which severely affected the seed malformation. Among the isolated fungi YK201 was the most virulence to bulrush plant in greenhouse test. The fungus grew well at 25-28°C and produced black pigment on PDA at 15 days. It was the very interesting event that diseased plants were severely suppressed of seed forming caused by this host specific fungal organism. Therefore, we conclude that the fungus may have a potential as a biological control agent of bulrush in rice paddy field.

**Keywords:** Brown stem blight, *Scirpus hotarui* Ohwi, biological control

### INTRODUCTION

A summer annual, *Scirpus hotarui* that grows in rice paddies, ditches throughout Korea. The weed produces mainly by seed, but the stem base thickened like tuber may over-winter and buds sprout from the stock next year. It grows in May to October and flowers in July to August.

Formation of abundant seeds and their irregular sprouting habits of the plant make it difficult to control even by herbicides. In rice field at Milyang, Korea in 2000, diseased *Scirpus hotarui* plants were found in which the brown stem blight symptoms were first observed in August and were rapidly developed during August to September. The infected plants were blight and eventually completely killed. The casual fungus was isolated from the lesion of seeds and identified.

To develop a successful biological control system of weeds, the selection of promising biological control agent is the most important factor (Danial et al 1973; Tebeest 1988; Walker 1981a; Weidemann 1988). The agent should be highly pathogenic to the target weed, but not pathogenic to other crop plants. In addition, its application technology including inoculum concentration, and frequency and time of application have to be developed to enhance weeding efficacy (Hannush and Boland 1996; Martinez and Anderson 1957; Tebeest and Templeton 1988). Therefore, the purposes of this research were to elucidate of the fungus and to develop a successful biological control system of *Scirpus hotarui* using this fungus.

### MATERIALS AND METHODS

#### Isolation

Plants showing typical symptoms with lesions were collected from rice fields during the summer of 2000. Diseased stems were cut into 2~3mm pieces and surface-sterilized by submerging them in 2 % sodium hypochloride for 1 min. and 70 % ethyl

alcohol for 1 minute, then washing with sterilized water. The sterilized pieces were transferred aseptically to water agar. Hyphal tips of the colonies formed were transferred to acidified potato dextrose agar (PDA, pH 5.5) and cultured for 10 days at 28 C. A mycelial disc (d-5-mm) of the culture was preserved for future use in a -70 C deep freezer in Cryovial, containing 1:1 mixture of 1.5 ml of 40 % glycerol and 10 % skim milk (Hong et al 1996).

#### Preparation of conidial inoculum

Aerial mycelia on 15-day-old PDA culture were physically removed with a spatula, and kept them in the growth chamber at 28 C for 48 hr under a 4,500 lux fluorescent lamp. Conidia produced on PDA were collected by washing them in 20 ml of distilled water. The concentration was adjusted prior to use by a hemacytometer.

#### Raising test plants

*Scirpus hotarui* seeds were collected by digging from rice paddy fields after harvest. The soil containing seeds were dried several days in the shade, and put in plastic pot (15cm diameter). For sprouting the pot was soaked in water, and placed at 20-28 C for 25 days in greenhouse bed. When shoots were grown up to 20-25 cm, the plants were inoculated with the conidial suspension.

#### Pathogenicity test

Pathogenicity test was conducted with 5 isolates which were chosen among 35 isolates collected from rice fields by their cultural and morphological characteristics. Twenty five days of plants in plastic pot were inoculated with three replications (=pots) per isolate. Plant mortality, i.e percentage of dead plants and inhibit ratio of seed formation as degree of infection were examined 30 days later.

#### Host range test

Four species of Ghaminaea family and 6 species of Cyperaceae were grown in plastic pots (21X17cm) filled with rice paddy soil. At the 3 to 5 leaf stages, they were inoculated with conidial inoculum of isolate YK201. The possible symptom development was examined for 15 days after inoculation.

## RESULTS AND DISCUSSION

#### Hyphal morphology and conidia

On PDA, colonies appeared grayish black to black with dense mycelia. Sometimes, mycelium was septate, hyaline to pale brown, and developed small dense felty colonies. Conidial production was most abundant at 28 C and with RH 60-80. Illumination of 3,000 to 7,500 lux was effective in stimulating conidial production of the fungus on PDA. Light intensity affected sporulation more significantly the temperature did (Fig. 1).

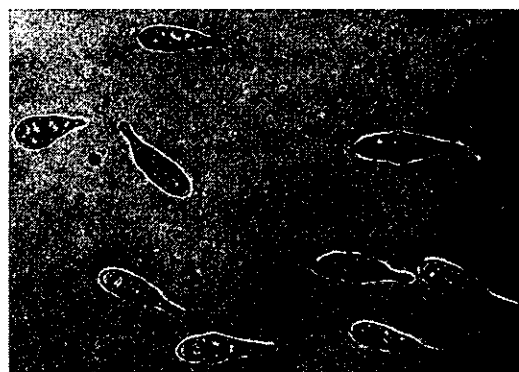


Figure 1. The conidia of the isolate d fungus with 2or 3 celled septae

### Disease symptoms

Symptoms first appeared on stems of *Scirpus hotarui* 10 to 12 days after inoculation in greenhouse conditions. Dark brown lesions, initially pinpointed enlarged later to become reddish brown. The lesions rapidly elongated, expanded around the stem, and girdled the stem completely within the next 20 to 25 days, which led to ultimate death of the plant.

### Pathogenicity of isolate YK 201

All 5 isolates tested developed diseases on the *Scirpus hotarui* seedlings, similar to those observed in field conditions (Table 1, Fig. 2, 4). The infected shoots were ultimately killed within 20 to 25 days and severely inhibited seeding.

Table 1. Suppression effect of host specific isolate YK-201 on seeding of *Scirpus juncoides* Roxb. var. *hotarui* Ohwi

Degree of infection*	Fresh Weight (g/5 plants)	Seeding amount (g/5 plants)
Normal	146	10.1
1/3	126	7.9
2/3	111	3.4
3/3	78	2.9

\* Degree of infection was classified as follows; Normal: Uninfected normal plants, 1/3: one of three seed was malformed, 2/3: two of three seeds were malformed, 3/3: all three seeds were malformed or no seeds

Table 2. Host range of host specific isolate YK-201 to Graminae and Cyperaceae families

Family	Host	Infection*
Graminae	<i>Echinochloa crusgalli</i> Beauv. Var. <i>Oryzicola</i> Ohwi	-
	<i>Echinochloa crusgalli</i> Beauv. Var. <i>Caudata</i> Kitagawa	-
	<i>Echinochloa crusgalli</i> Beauv. Var. <i>Crusgalli</i> .	-
	<i>Leersia japonica</i> Makino	-
Cyperaceae	<i>Cyperus amuricus</i> Maxim.	-
	<i>Cyperus difformis</i> L.	-
	<i>Cyperus iria</i> L.	-
	<i>Cyperus serotinus</i> Rottb.	-
	<i>Eleocharis kuroguwai</i> Ohwi	-
	<i>Scirpus hotarui</i> Ohwi	+

\* +: Infected, -: Uninfected

Seeding inhibition rate was up to 85% (Fig. 3). The infection rate i.e weeding efficacy of the fungal isolates varied from 75.4 % to 82.5%, and the isolate YK 201 was the most virulent

### Host Range of YK 201

Four species of Graminae family and 5 species of Cyperaceae tested in host range experiments were not infected by YK 201 except *Scirpus hotarui* (Table 2) There were no differences in number, size and vigor of the plants between the inoculated and the

uninoculated. In contrast, *Scirpus hotarui* plants were heavily infected, and infections was evident with production of blackish brown lesion 20-25 days after inoculation.

In the studies of biological control of weeds with pathogens, in general, most of weed species are hosts for many pathogens (Hong and Grogan 1975; Boyette 1991; Conway 1976; Walker 1981b; Walker and Boyette 1985; Wapshire 1974; Weidemann 1988). TeBeest (1988) has recently discovered that *C. gloeosporioides* f. sp. *aeschynomene*, the incitant of anthracnose on northern jointvetch (*Aeschynomene virginica*) has much broader host range than that originally reported by Daniel et al. (1973). Daniel suggested that one of the highly desirable characters for biological weed control agents must be genetically stable and specific to the target weed.

According to the present study, *E. nematosporus* has some definite advantages as a potential biological control agent; 1) *E. nematosporus* occurs naturally in rice fields in Korea, 2) the fungus is highly pathogenic and specific to water chestnut, and 3) it kills stems, and inhibits underground tuber formation (Hong et al. 1996, 1997, 1991, 1992).

In Korea, we first report that the casual agent of stem blight on *Scirpus hotarui* in nature was isolated. We first report the significant natural incidence of this disease on the plant. When the spore suspension of isolate YK201 was inoculated on upper stems of *Scirpus hotarui*, the plant was readily infected, showing the symptoms as observed in fields.

All 5 isolates were pathogenic to *Scirpus hotarui* seedlings in inoculation test. Based on our experiment, there were no differences in pathogenicity among the isolates that



Figure 2. Suppressing effect of seeding of bulrush severely infected by the fungus in early stage

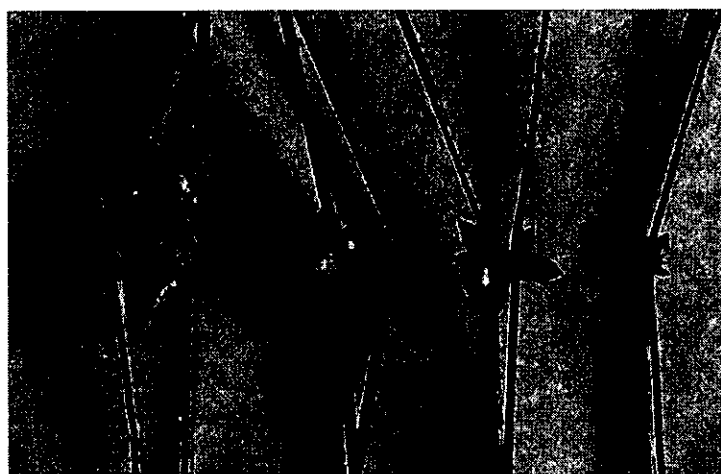


Figure 3. Suppressing effect of seeding of bulrush severely infected by the fungus at late season; A: uninfected, B: 1/3 malformation, C: 2/3 malformation, D: 3/3 malformation

can be differentiated by the morphological characters and collecting sites

All tested weed plants other than *Scirpus hotarui*, were immune to infection by the isolate YK 201 when inoculated with the conidial suspension. The result obtained in this study and elsewhere indicated that the fungus seemed specifically pathogenic to *Scirpus hotarui*.

Although additional studies must be conducted to give full confidence to isolate

YK 201 as a biological control agent of water chestnut, we should mention some definite advantages of the fungus as a biological control agents; YK 201 occurs naturally in rice fields in Korea, 1) is highly pathogenic and specific to *Scirpus hotarui*, 2) affects seeds formation as well as stems, and 3) easily produces spores in mass. Therefore we concluded that YK 201 isolate has potential biological control agents of *Scirpus hotarui* in Korea.

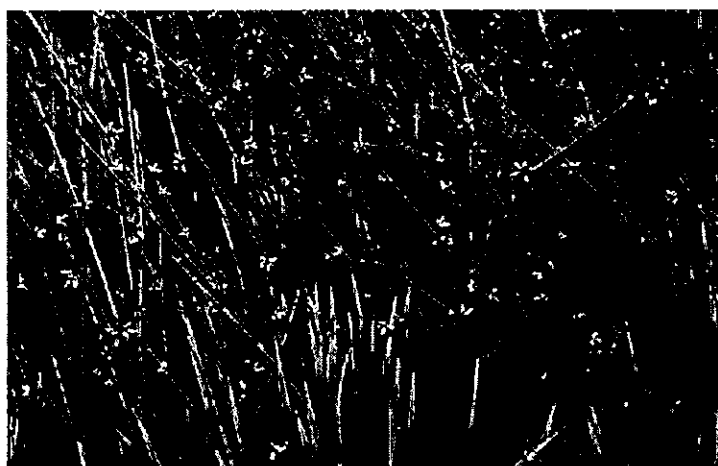


Figure 4. Naturally infected stems caused by the isolate YK-201 in late September in 2000

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## EFFICACY OF PATHOGENIC FUNGI AND COMBINATION USE WITH THE HERBICIDE TO CONTROL *ECHINOCHLOA CRUS-GALLI* IN RICE FIELDS

S. W. Huang<sup>1</sup>, L. Q. Yu<sup>1</sup>, G. F. Duan<sup>1</sup>, and A. K. Watson<sup>2</sup>

<sup>1</sup>China National Rice Research Institute CNRRI, Hangzhou 310006, China.

swhuang@mail.hz.zj.cn

<sup>2</sup>International Rice Research Institute IRRI, P.O. Box 933, Manila 1099, Philippines

**Abstract:** *Exserohilum monoceras* (EM) and *Drechslera monoceras* (DM) were two potential promise pathogenic fungi for biological control of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.). Most barnyardgrass seedlings at 1.0~5.0-leaf stage were killed by spraying *E. monoceras* and *D. monoceras* suspension respectively at dosage of  $5.0 \times 10^7$  conidia  $m^{-2}$  with 24h dew exposure period. Treatments of *E. monoceras* at  $5.4 \times 10^6$  and *D. monoceras* at  $5.9 \times 10^6$  conidia  $M^{-2}$  reached 66.1% and 64.4% dry weight reduction of barnyardgrass respectively. Combination use of *E. monoceras* and *D. monoceras* with Quinclorac of 5.6mg a.i.  $m^{-2}$  improved a better control of barnyardgrass and reduced the plant height and dry weight of rice seedlings significantly.

**Key words:** Barnyardgrass (*Echinochloa crus-galli*), pathogenic fungi, biological control

### INTRODUCTION

With the wide-range application of chemical herbicides, more and more herbicide-resistant weeds appeared (Heap, 1998). Barnyardgrass (*E. crus-galli*), a major troublesome weed in paddy fields (Su, 1996), also showed resistance to some herbicides (Huang, 1993). Dosage of herbicides for controlling herbicide-resistant weeds is several or several-hundred times of that for the sensitive biotype weeds (Li, 1999).

Weed biological control of herbicide-resistant maybe an alternative approach. *Exserohilum monoceras* (EM) and *Drechslera monoceras* (DM) show higher pathogenicity to barnyardgrass and safety to rice (Zhang, 1996; Huang, 2000). Joint usage of pathogens with the sub-lethal dosage of chemical herbicide maybe a good way to gain better weed control efficacy and to reduce herbicide dosage application.

### MATERIALS AND METHODS

EM, DM and *Curvularia lunata* (CL) pathogenic fungi: Isolated from natural infected leaves of barnyardgrass. The fungi were maintained on half-strength PDA slants in test tubes at 4°C.

Chemical herbicide: 50% quinclorac (Qu) wet powder (WP).

Small pieces of mycelium from the stock culture of each fungus were aseptically transferred to fresh PDA media. Plates were incubated in the dark at 28°C for 7 days. 3 pieces of agar plugs (9 mm diameter) from the young colonies were transferred to the seeds substrates of barnyardgrass (Huang, 1999). The substrates were incubated in the dark at  $27 \pm 1^\circ C$ . Conidia were harvested 15 days after incubation by stirring and washing the substrates with distilled water. Suspensions were filtered through a layer of cheesecloth and conidial concentrations were determined with a hemacytometer.

Seeds of barnyardgrass and rice cultivar were incubated in petri dishes on moistened cheesecloth at room temperature for 48h. Ten germinated seeds (coleoptile and radicle just emerged) of barnyardgrass and rice were seeded in 25cm length  $\times$  10cm width  $\times$  12cm depth plastic box filled with saturated soil. Seeded boxes were placed in greenhouses for cultivation at 34/23 $\pm$ 2°C day/night temperatures with natural light.

Pathogenicity test of EM and DM: Barnyardgrass seedlings at 1.5~2.0, 3.0~3.5, and 4.5~5.0-leaf stage were inoculated by spraying 50 ml m<sup>-2</sup> of EM and DM suspension of 1.0 $\times$ 10<sup>6</sup> conidia per ml respectively. After treatment, boxes were placed in a chamber with 95% relative humidity (RH) at 25°C for 24h without light. Subsequently, boxes were moved to greenhouses at temperature of 34/23 $\pm$ 2°C day/night and 85% to 95% RH for 48h. The disease reactions of barnyardgrass and rice to each treatment were recorded in the 3rd and 7th day after inoculation (DAI).

Seedlings at 3.0~3.5-leaf stage were sprayed 45ml conidia suspension of EM, DM and CL separately and combination use with sub-lethal dosage of Qu with 0.05% Tween 20. All experiments were a randomized complete block design with three replications. The control treatment was sprayed with distilled water containing only Tween 20.

The treatments were designed as follows:

1. Spraying EM suspension 45ml with the dosage of 5.4 $\times$ 10<sup>6</sup> conidia m<sup>-2</sup> only;
2. Spraying DM suspension 45ml with the dosage of 5.9 $\times$ 10<sup>6</sup> conidia m<sup>-2</sup> only;
3. Spraying CL suspension 45ml with the dosage of 3.5 $\times$ 10<sup>8</sup> conidia m<sup>-2</sup> only;
4. Spraying 45ml EM suspension with 5.4 $\times$ 10<sup>6</sup> conidia + 5.6mg a.i. Qu m<sup>-2</sup>;
5. Spraying 45ml DM suspension with 5.9 $\times$ 10<sup>6</sup> conidia + 5.6mg a.i. Qu WP m<sup>-2</sup>;
6. Spraying 45ml CL suspension with 3.5 $\times$ 10<sup>8</sup> conidia + 5.6mg a.i. Qu m<sup>-2</sup>;
7. Spraying 45ml 5.6mg a.i. Qu m<sup>-2</sup> only (recommended application dosage of QWP is 22.5mg a.i. m<sup>-2</sup>);
8. Spraying 0.05% Tween 20 water solution of 45ml m<sup>-2</sup> only (CK).

## RESULTS AND DISCUSSION

The disease incidence (DI) of EM and DM at different leaf stages of barnyardgrass was over 90%. EM and DM caused seedling mortality in a range of 70-85%, and reduced dry weight at 37 to 65% (Table1).

Table1. Pathogenicity of pathogenic fungi on barnyardgrass.

Treatments ( $\times 10^7$ conidia/ M <sup>-2</sup> )	1.5~2.0-leaf			3.0~3.5-leaf			4.5~5.0-leaf		
	DI <sup>1</sup> (%)	M (%)	R DW <sup>2</sup> (%)	DI (%)	MR (%)	CEDW (%)	DI (%)	MR (%)	CEDW (%)
<i>E. monoceras</i> 5.0	91.8b	85.4b	48.4a	99.6a	81.5a	65.1a	95.4b	77.2a	57.1a
<i>D. monoceras</i> 5.0	100a	75.2a	40.8b	99.7a	73.4b	41.7b	97.5a	70.7b	37.8b
<i>C. lunata</i> 35	25.3c	0c	5.6c	21.5b	0c	11.7c	15.8c	0c	9.8c
Tween 20 water solution <sup>1</sup>	4.3d	0c	3.3d	5.9c	0c	4.8d	5.8d	0c	5.2d

<sup>1</sup> DI = disease incidence; M = mortality; CEDW = reduction of dry weight; <sup>2</sup> Values followed by different letters in the same column are significantly different at 5% level.

A lot of water soaked lesions occurred on barnyardgrass leaves treated by EM and DM conidia suspension in 3 days after inoculation (DAI). No disease lesions occurred on rice seedlings. The old leaves of barnyardgrass inoculated with EM and DM were withered, but the young leaves were normal. Compared with the untreated plants, barnyardgrass plant height treated by EM and DM decreased by 22.5% and 23.2%, dry weight decreased 61.0% and 64.4%, respectively. CL had no infection on barnyardgrass seedlings (Table 2).

Table 2. Control efficacy of conidia suspension of pathogenic fungi on barnyardgrass

Treatments ( $\times 10^6$ conidia/ $M^{-2}$ )	Plant height (cm)	Dry weight Reduction (%)	Mortality (%)
<i>E. monoceras</i> 5.4	23.1c <sup>1</sup>	66.1a	56.4a
<i>D. monoceras</i> 5.9	23.2c	64.4a	51.8a
<i>C. lunata</i> 350	33.1a	5.1b	0b
Tween 20 (CK)	30.2b	5.9b <sup>2</sup>	0b

<sup>1</sup> Values in each column followed the same letter are not significantly different at 5% level.

The conidial suspensions of *E. monoceras* and *D. monoceras* whether single use or combination apply with the herbicide reduced the plant height and dry weight of rice seedlings significantly, compared with Quinclorac and 0.05% Tween 20 treatments. It seems that the sub-lethal dosage of Quinclorac can improve the growth of rice seedling (Table 3).

Table 3. Control efficacy of combination use of pathogen and chemicals on barnyardgrass

Treatments ( $\times 10^6$ conidia/ $M^{-2}$ )	3DAT <sup>1</sup> Injury	7 DAT	
		Plant height (cm)	Dry weight of plant (g)
EM 5.4	-	17.7c <sup>2</sup>	2.1c <sup>2</sup>
DM 5.9	-	18.0c	1.9d
CL 350	-	18.6b	1.9d
EM + Qu 5.4+5.6mg a.i./ $m^2$	-	18.5b	1.8d
DM + Qu 5.9+5.6mg a.i./ $m^2$	-	18.6b	1.8d
CL + Qu 350+5.6mg a.i./ $m^2$	-	20.2a	2.8a
Qu 5.6mg a.i./ $m^2$	-	19.9a	2.9a
Tween 20 (CK)	-	18.4b	2.3b

<sup>1</sup> DAT: Days after treatments; -: no injury; <sup>2</sup> Values in the same column followed by different letter are significantly different at 5% level.

Many acute water soaked lesions occurred on the leaves of barnyardgrass treated by EM, DM in combination with sub-lethal dosage of QWP. Barnyardgrass seedlings turned yellowish treated by CL + 5.6mg a.i. Qu  $m^{-2}$ . The young leaves turned yellowish and the old leaves still kept green only spraying by 5.6mg a.i. Qu  $m^{-2}$ .

100% barnyardgrass seedlings were dead when treated with EM, DM in combination with sub-lethal dosage of Qu in 7DAI. The average plant height decreased by 64.9% and 63.6%, and the dry weight reduced 98.3% and 98.3%, respectively, compared with CK (Fig 1).

The same experiments carried out under the natural conditions in June and July in Hangzhou, Zhejiang provinceshowed that the control efficacy was significantly affected by temperature and humidity.



Figure 1. Dry weight reduction rate of barnyardgrass after treated with conidia suspension alone and conidia plus Quinclorac. EM-*E. monoceras* in  $5.4 \times 10^8$  conidia/m<sup>2</sup>; DM-*D. monoceras* in  $5.9 \times 10^6$  conidia/m<sup>2</sup>; CL-*C. lunata* in  $3.5 \times 10^8$  conidia/m<sup>2</sup>

The management of weed populations requires an integrated system approach that employs chemical, cultural, mechanical, biological, ecological, and bioenvironmental methods.

However biological control, especially with plant pathogens, would offer some solution to some of herbicide side-effects. Biological controls will not, however, solve all weed problems. Some constraints include unfavorable temperature and humidity, resistance factors, spatial continuity of hosts, host geographic range and distribution, diversity of host genetic base, and age and vigor of host plants.

Plant pathogens in combination with a sub-lethal dosage of herbicide maybe an alternative approach of improving weed control efficacy while reducing the herbicide dosage application. Further studies should be done to select the optimal fungus-herbicide mixture and ingredients, to determine the optimal application stage and optimal approach.

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## HOST PREFERENCE OF *RHINOCYLLUS CONICUS* ON *CIRSIUM ARVENSE* AND *C. FLOODMANII*

B. H. Li

Institute of Cereal and Oil Crops, Hebei Academy of Agricultural Sciences,  
Shijiazhuang, 050031, P. R. China. Libh@china.com

**Abstract:** Field surveys were made on the seed heads of *Cirsium arvense* and *Cirsium floodmanii* to examine egg, larvae, pupae and adult numbers of *Rhinocyllus conicus* in early and late seasons. Results showed that *R. Conicus* preferred to feed on *C. floodmanii* and caused more seed reduction.

**Key Words:** *Rhinocyllus conicus*, *Cirsium arvense*, *Cirsium floodmanii*

### INTRODUCTION

Canada thistle (*Cirsium arvense*) spreads rapidly by horizontal roots which give rise to aerial shoots. It is widely distributed in Canada, North Africa, Asia and southeastern Australia. It damages wheat, corn, peas, beans, sugar beet, potatoes, and also common in pastures and ranges. It is reported that 2 thistle shoots per 0.84m<sup>2</sup> reduced wheat yield by 15%; 12 shoots, by 35%; 25 shoots, by 60% in Montana (Hodgson, 1968). The weed also harbors insects (bean aphid, stalk borer) and it is an alternate host for some pathogenic organisms. Floodman thistle (*C. floodmanii*) is a "gentle" grass and regarded as a useful species in Canada. It is a native plant and should not be a weed target in some conditions.

*Rhinocyllus conicus*, a bio-control agent against Canada thistle, was released in Canada in 1968 and introduced into USA in 1969. It overwinters in the adult stage, and becomes active in mid to late April. Each female lays about 100 to 200 eggs. Eggs are laid on the bracts of developing buds and hatch in about 6 days. Newly hatched larvae feed through the bracts into the buds. The larvae feed on the receptacle and prevent the production of viable seeds. They complete development in 4-6 weeks and turn into pupae in the thistle heads. The new adult emerges in 7-10 days. These new adults do not stay on the plant for long. They seek shelter and pass summer in hiding, and then hibernate in winter. They re-emerge in the next spring to lay eggs before dying. The weevil was used to control Canada thistle, however, we found it was more favorite Floodman thistle than Canada thistle. This might destroy this desirable thistle species in Canada. The objective of this study is to make sure if the weevil prefers Floodman thistle or not and the degrees.

### MATERIALS AND METHODS

#### Field survey of *Rhinocyllus conicus* eggs on thistle plants in early season

Surveys were made on *Cirsium arvense* and *C. floodmanii* at Stavly farm, where the colony of *R. conicus* had been build up few years ago, on July 7, 1999. Fifteen plants of each thistle species were examined for eggs of *Rhinocyllus conicus* on the bracts, and also plant buds and heights were counted and measured.

### Field survey on larvae, pupae and adults of *Rhinocyllus conicu* in late season

Field survey was made at Stavly farm on Sep. 7, 1999. Five hundred and thirty-six plants of the two thistle species were dissected for pupae, larvae and adults of *Rhinocyllus conicu*. And also young and mature flower heads were measured for width, length and wet weight and seeds were counted. Then the plants and seeds were labeled and dried for 14 days in the drying room.

## RESULTS AND DISCUSSION

### Eggs on the plants in early season

Even though Canada thistle was taller and had more buds than that of Floodman thistle in the early season, more *Rhinocyllus conicu* eggs were founded on Floodman thistle than that on Canada thistle (Table 1). There were 13.73 eggs per head on Floodman thistle and only 2.33 eggs on Canada thistle.

Table 1. Eggs on two thistle species in the early season

Species	Height (cm)	Buds	Eggs of <i>Rhinocyllus conicu</i>
Canada thistle	31.28	6.33	2.33
Floodman thistle	27.23	2.33	13.73

### Larvae, pupae and adults on plants in late season

Dissection of thistle plants showed that an average of 3 weevils was accounted on new floodman thistle and 3.77 on old floodman thistle, respectively. Only 1.17 weevil was found on old Canada thistle plants, no weevil was found in the new Canada thistle heads. 51.4% of new floodman thistle heads and 93.55% of old floodman thistle heads were infested, while only 6.45% of old Canada thistle heads were infested. (Fig.1). These results showed that *Rhinocyllus conicu* preferred to feed on floodman thistle.

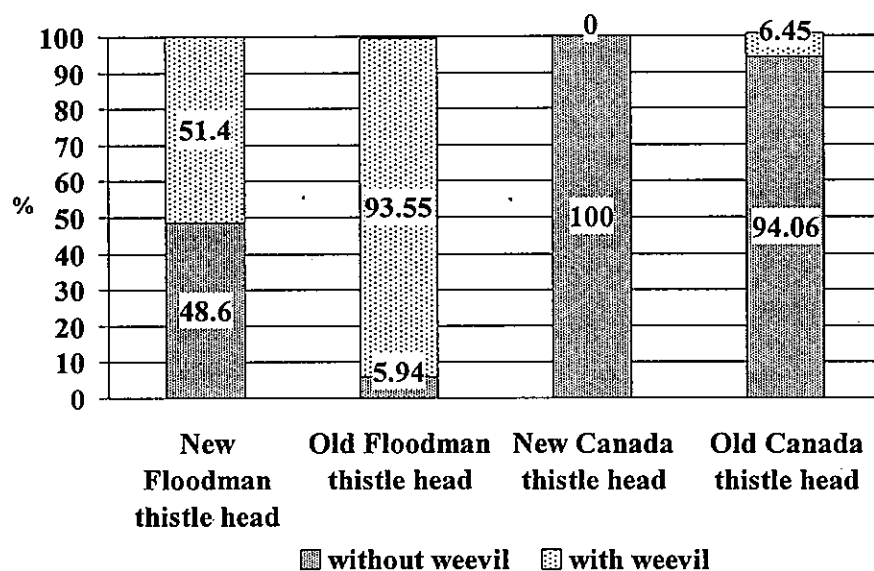


Figure 1. Percentage of thistle heads with or without weevil

Table 2. *Rhinocyllus conicu* founded on different thistle flower heads in late season

Flower heads	Seed No.	No. of <i>Rhinocyllus conicu</i>			
		Larvae	Pupae	Adults	Total
New Floodman thistle heads	97.22	2.11	0.93	0	3.04
Old Floodman thistle heads	25.97	0.20	3.13	0.44	3.77
Old Canada thistle heads	46.67	0.50	0.67	0	1.17

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## EVALUATION OF *ALTICA CIRSIOLA* AS A BIOCONTROL AGENT AGAINST *CEPHALONOPLOS SEGETUM*

H. Y. Pan, S. H. Wang, J. H. Xi, and X. M. Wang

Department of Agronomy, Changchun University of Agriculture and Animal Sciences,  
Changchun 130062, P.R China

**Abstract:** Host range tests showed *Altica cirsiola* is an oilgophagous insect, fed on *Cephalonoplos segetum*, *Cephalonoplos setosum*, *Cirsium maackii*, *Cirsium pendulum*, *Cirsium vlassonianum*, *Taraxacum ohwianum* and *Fragaria ananass*. However, it preferred to feed on *Cephalonoplos segetum*, developmental period was shorter, survival rate and oviposition capacity were higher than that on other tested plants. These results indicated that *Cephalonoplos segetum* is favorite host for *Altica cirsiola*.

**Key words:** *Altica cirsiola*, biocontrol, weed, host

### INTRODUCTION

*Altica cirsiola*, a leaf feeder of *Cirsium* and *Cephalonoplos* spp., has drawn attentions by weed experts recently. Field survey shows that *Altica cirsiola* is a dominant species to feed on thistle plants and distributes widely in the northeastern part of China. However, there are not much work be done on its host ranges and potential as a bio-control agent against thistles in China. This paper dealt with its host specificity and bionomics.

### MATERIALS AND METHODS

#### Host Specificity of No-choice Test

In no-choice test, 29 plant species (Table 1) representing 10 families were used for testing host range of *Altica cirsiola* larvae, each plant species with 5 replicates. Fresh leaves were cut to feed *A. cirsiola* larvae in Petri-dishes in laboratory, Leaves were changed every 1~2 days, Feeding and larval development were recorded each day.

#### Host Specificity of Multi-choice Test

Thirty-five medium-size potted plants were randomly put in a greenhouse. 200 adults were released on test plants. Oviposition selection and feeding were recorded every day.

#### Oviposition of *Altica cirsiola* on selected host plants

Selected plants (Table 2) grown in boxes (40cmX40cmX80cm) were used for observing *A. cirsiola* oviposition in cages, 10 adults were incubated in cages, each plant species with 3 replicates.

All above laboratory tests were conducted under temperature  $25 \pm 0.5^{\circ} \text{C}$ , 16L:8D and RH 70%.

### RESULTS AND DISCUSSION

#### Host specificity of *Altica cirsiola*

For all 29 tested plant species, *Altica cirsiicola* only feeds on *Cephalonoplos segetum*, *Cephalonoplos setosum*, *Cirsium maackii*, *Cirsium pendulum*, *Cirsium vlassonianum*, *Taraxacum ohwianum* and *Fragaria ananassa*, but not any feeding on other tested species in both non-choice and multi-choice tests (Table 1). All of these plants fed by *A. cirsiicola* are weeds and not much economic and ecological values in China. The larvae fed on those 7 species developed to adults, however, the larvae feeding on *Cephalonoplos segetum* developed faster, survival rate was much higher, and adults laid more eggs than those feeding on other 6 species. These results showed that *A. cirsiicola* is an oligophagous insect, and could be used as a bio-control agent to control *Cephalonoplos segetum*.

Table 1 Host specificity of *Altica cirsiicola* in no-choice and multi-choice tests

Plant species tested	No-Choice test		Multi-choice test	
	Feeding	Develop to adults	Feeding	Develop to adults
<i>Cephalonoplos segetum</i>	+++	Y	+++	Y
<i>Cephalonoplos setosum</i>	+++	Y	+++	Y
<i>Cirsium maackii</i>	+++	Y	+++	Y
<i>Cirsium pendulum</i>	+++	Y	+++	Y
<i>Cirsium vlassonianum</i>	+++	Y	+++	Y
<i>Stevia vebaudiana</i>	-	N	-	N
<i>Chrysanthemum carunatum</i>	-	N	-	N
<i>Lactuca sativa</i>	-	N	-	N
<i>Taraxacum ohwianum</i>	-	N	-	N
<i>Xanthium sibiricum</i>	+++	Y	+++	Y
<i>Bidens bipinnata</i>	-	N	-	N
<i>Fragaria ananassa</i>	+++	Y	+++	Y
<i>P. Armeniaca</i>	-	N	-	N
<i>P. Salicina</i>	-	N	-	N
<i>Pyrus bretschneideri</i>	-	N	-	N
<i>Malus spectabilis</i>	-	N	-	N
<i>Glycine max</i>	-	N	-	N
<i>Phaseolus vulgaris</i>	-	N	-	N
<i>Vigna sinensis</i>	-	N	-	N
<i>Triticum aestivus</i>	-	N	-	N
<i>Zea mays</i>	-	N	-	N
<i>Solanum mulongena</i>	-	N	-	N
<i>Lycopersicon esulentum</i>	-	N	-	N
<i>Cucumis sativus</i>	-	N	-	N
<i>Apiumgraveolens</i>	-	N	-	N
<i>Amaranthus tricolor</i>	-	N	-	N
<i>Beta vulgaris</i>	-	N	-	N
<i>Spinacia oleracea</i>	-	N	-	N
<i>Brassica chinensis</i>	-	N	-	N

+++ means feeding, and -, no-feeding. "Y" means larvae developed to adults and "N" means not.

#### Oviposition selection on tested plants

Oviposition selection tests were made on 6 selected plant species (Table 2). The

results showed that *Altica cirsiicola* laid more eggs on *Cephalonoplos segetum* than that on other 5 species, no adults and eggs were found on *Helianthus annuus*, which an economic plant in northern China. A total of 2910 eggs was recorded on *Cephalonoplos segetum* during the testing period, while as only 1346 on *C. setosum*, 1320 on *Cirsium maackii*, 1085 on *C. pendulum*, and 246 on *C. vlassonianum*, respectively. These results indicated that *Cephalonoplos segetum* is favorite host for *Altica cirsiicola*.

Table 2 Oviposition selection of *Altica cirsiicola* on selected plant species

Plants tested	Adults found on plants (No./plants)			Total	Total No. of egg
	Rep.1	2	3		
<i>Cephalonoplos segetum</i>	18	25	22	65	2910
<i>C. setosum</i>	10	16	9	35	1346
<i>Cirsium maackii</i>	15	12	11	38	1320
<i>C. pendulum</i>	1	5	15	21	1085
<i>C. vlassonianum</i>	4	2	0	6	246
<i>Helianthus annuus</i>	0	0	0	0	0

#### Development of *Altica cirsiicola* on different plant species

Developmental periods of *Altica cirsiicola* were observed in greenhouses in cages on selected plants. *Altica cirsiicola* fed on *Cephalonoplos segetum* only needed 27 days from egg developing to pupa stage, while only 34.2 days on *Cirsium pendulum* (Table 3). The results showed again that *Altica cirsiicola* more like to feed on *Cephalonoplos segetum* than that fed on *C. setosum*, *Cirsium maackii* and *C. pendulum*.

Table 3 Development of *Altica cirsiicola* on selected plants

Plants tested	Developmental periods (days)			
	Egg	Larva	Pupa	Total period
<i>Cephalonoplos segetum</i>	5.4	12.3	9.5	27.2
<i>C. setosum</i>	6.0	15.5	10.2	31.7
<i>Cirsium maackii</i>	5.1	15.0	12.0	32.1
<i>C. pendulum</i>	6.7	16.9	10.6	34.2

#### Bionomics of *Altica cirsiicola*

Results of field systematic investigation showed that *Altica cirsiicola* has one to two generations each year. Population peak occurred on May. Adults like to eat tender leaves and buds of plants. Eggs are laid on underside of the host plant leaves. Larvae feed leaf pulp, leaving only leaf surface. This makes leaves become withered and curled. Larvae pupate in soil.

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## EVALUATION OF MYCELIUM VIRULENCE OF *ALTERNARIA ALTERNATA* TO *EUPATORIUM ADENOPHORUM*

S. Qiang<sup>1</sup>, B. A. Summerell<sup>2</sup>, and Y. H. Li<sup>1</sup>

<sup>1</sup> Weed Research Laboratory, Nanjing Agricultural University, Nanjing 210095, P.R. China.  
wrl@njau.edu.cn

<sup>2</sup> The Royal Botanic Gardens, Sydney NSW 2000, Australia

**Abstract:** The factors influencing the development of disease by mycelium of *Alternaria alternata* (Fr.) Keissler to crofton weed (*Eupatorium adenophorum* Spreng.) were studied. The development of disease was significantly affected by those factors: concentration of mycelial fragments, plant age, humidity, temperature, and the temperature and duration length during storage.  $3.5 \times 10^6$  mycelial fragments  $\text{ml}^{-1}$ , the optimum concentration, could kill all inoculated plants within two weeks. The younger plant might be suitable for treatment while the age of plant with four pairs of leaves was the optimum stage. The optimum dew period and temperature were 14-18hrs and 18-25°C separately. The storage of mycelium in water at low or room temperature was unfavorable for preserving mycelial virulence.

**Key words:** *Eupatorium adenophorum*, *Alternaria alternata*, mycoherbicide, crofton weed, mycelium

### INTRODUCTION

Crofton weed (*Eupatorium adenophorum* Spreng.) has been resulting in an important harmful effect on the development of agriculture, forestry and livestock husbandry in coastal areas of Australia, southwestern China, and many other tropic and subtropical areas of the world (Auld, 1969, 1981; Fuller, 1981; Mari'n, Negrillo et al., 1983; O'Sullivan, 1985; Zhao & Ma, 1986; Morris, 1989). And its infestation scope is still expanding in China (Xiang, 1991). Thus, control of this weed by different strategies including mechanical, chemical and classical biological control technology have been attempted (Bess & Haramoto, 1959; Hoy, 1960; Wilson, 1960; Dodd, 1961; Bennett & Staden, 1986; Morris, 1989 and 1991; Kluge, 1991; Rahman & Agalwal, 1991; Zhang et al., 1988). However, the object of effective and economical control for the weed in various environments has not been reached. Since 1960's, the fungus *Phaeoramularia* sp. has been employed as a classic biocontrol agent in Australia and South Africa (Dodd 1961, Liu & Guo 1988, Morris 1989, Guo et al., 1992; and Wang & Summerell 1995 unpublished). Although this might play a role in slowing down the speed of its spread (Yang & Guo, 1991), it seems that because of the low level and long duration of the disease development the fungus was insufficient to control the weed if *Phaeoramularia* sp. was attempted as a mycoherbicide (Wang & Summerell 1995 unpublished).

The pathogenicity of conidium and mycelium of *Alternaria alternata* (Fr.) Keissler to crofton weed (*Eupatorium adenophorum*) was compared (Qiang & Summerell, 1999). The

results showed that the mycelium would be very virulent to the weed because of quickness, high level and short duration of disease development. The mycelium of Isolate 501 of this fungus might have potential to be developed as a mycoherbicide for crofton weed. This study aimed to evaluate the feasibility of the development of the mycelium as a mycoherbicide for the weed further.

## MATERIALS AND METHODS

### Production of inoculum

A 0.5cm-piece of agar culture of *Alternaria alternata* was inoculated in a sterilized flask contained 167ml liquid medium. The liquid medium was consisted of soybean flour(15g), corn flour(15g), sucrose(30g) and  $\text{CaCO}_3$ (3g) per litre. The flask was kept in an orbital incubator with rotary shaker (110rpm) in the dark at 25C for 3 days. The liquid cultures were homogenized in a blender for 20min. The homogenized mycelial fragment suspension was ready for inoculation of plants after counting with a haemocytometre.

### Culture of plants

The fruits of crofton weed were put into a piece of filterpaper in a petri-dish, and then 10ml sterile water was added. The germination of the seeds was induced in 12/12hrs light/dark at 25°C for 5 days. The little seedlings were transplanted into a pot with a mixture of soil till they grew up to 4cm high for about 6 weeks. Each of the bigger seedlings was planted in a pot. After 10 days or more the potted-plants with 4 pairs of leaves were ready for inoculation.

### Factors affecting the virulence of mycelia

**Concentration of mycelial fragments:** Mycelial fragment suspension was diluted to such concentration as  $0.75 \times 10^6$ ,  $1.2 \times 10^6$ ,  $2.0 \times 10^6$ ,  $2.5 \times 10^6$ ,  $3.2 \times 10^6$  and  $3.9 \times 10^6$  for inoculation. Two-month old potted-plants with 4 pairs of leaves were inoculated with the above 5 different concentrations of mycelial suspension respectively. Each of the treatments was subject to 7 replicates. Three controls were sprayed with water.

**Plant age:** The different age potted plants with 4, 5, 6 and 7-8 pairs of leaves from 2 to 3 months old were inoculated with the same concentration of mycelial fragment suspension respectively, each age with 7 potted plants. All plants were placed in a humidity chamber for 18hrs and then kept in a glasshouse at 18/25°C (night/light).

**Humidity:** Same concentration of mycelial fragment suspension was inoculated onto 2-month old potted-plants with 4 pairs of leaves. The inoculated plants were placed in a plastic humidity chamber for 6, 10, 14, 18, 22, 26hrs, respectively. Each treatment was subject 7 replicates.

**Temperature:** Same concentration of mycelial fragment suspension was inoculated onto 2-month old potted-plants with 4 pairs of leaves. The inoculated plants were placed in a plastic humidity chamber for 18hrs and kept in a glasshouse under the high (18-25°C), medium (15-22°C), and low (12-18°C) temperatures. Seven replicates were subject to be treated at each temperature.

**Mycelium Storage Temperature and Duration:** Original, double-fold dilution and five-fold dilution mycelial fragment suspensions were sprayed onto seven, seven and three 2-month old potted-plants with 4 pairs of leaves respectively. Mycelia cultured at same time were stored at the room (18-22°C) and low temperature (4°C). The stored mycelia were subject to be homogenized in a blender for 20min just before initiation of inoculation every time. The treatment was done once a week till 4 weeks afterward. If it was different, the concentration of the mycelial fragment suspension was adjusted with water so that each treatment was ensured at the same concentration condition.

All plants were placed in a humidity chamber for 18hrs and then kept in glasshouses at 18/25°C (night/light).

### Survey of the disease symptom and statistics

The plants were examined at intervals of one week (except the beginning time of 2days) for disease symptoms. The disease severity of stems and leaves was recorded in a disease standard (Table 1).

Table 1. The standard of the disease severity of leaves and stems

Disease scale	Percentage of sum of number of dead and lesion leaves	The extent of stem injury
4	150-200%	Entire plant
3	100-149%	50 – 100% stem, but root still alive
2	55-99%	25- 49%
1	1-49%	Top bud death to 24%
0	0	Health

The means of the disease severity scales of stems and leaves were counted as the disease ratings. Analysis of variance in two-way classification and Q-method of multiple comparison were employed to compare the difference between the treatments with the software SPSS and Excel.

## RESULTS AND DISCUSSION

### Effect of concentration of mycelial fragment suspension on the development of disease

Mycelia caused light disease at the concentration of  $0.75 \times 10^6$ . However, with mycelial fragment suspension concentration increasing, disease severity and the speed of the disease development increased (Fig.1 & Tab.2). For example, reaching the disease ratings 2.429 took 2 days at  $2.5 \times 10^6$ , but 2 weeks at  $2.0 \times 10^6$  and 4weeks at  $1.75 \times 10^6$ . The higher the concentration of mycelial fragment suspension, the quicker the speed that mycelia killed the plants and the higher the proportion of dead plants (Tab.2). After initiation of inoculation for 1 week,  $3.8 \times 10^6$  mycelial fragments per ml had killed all tested plants,  $3.2 \times 10^6$  had down six ones and the others had not yet. After 6 weeks, the number of

dead plants had reached 7, 6, 4, 2 and 1 at the treatment of  $3.2 \times 10^6$ ,  $2.5 \times 10^6$ ,  $2.0 \times 10^6$ ,  $1.75 \times 10^6$  and  $0.75 \times 10^6$  respectively.

Table 2. The means of the disease ratings recorded at seven different times\*.

Date (days)	Concentration of mycelial fragment suspension( $\times 10^6 \text{ ml}^{-1}$ )					
	0.75	1.75	2.0	2.5	3.2	3.8
2	1.143a	1.857b	2.071bc	2.429c	3.786d	3.571d
7	1.286a	2.071b	2.071b	3.071c	3.786d	4.000d
14	1.214a	2.286b	2.429b	3.375cd	3.857cd	4.000d
21	1.143a	2.357b	2.500b	3.571c	3.857c	4.000c
28	1.214a	2.429b	3.071b	3.714cd	4.000d	4.000d
35	1.286a	2.357b	3.143c	3.714cd	4.000d	4.000d
42	1.571a	2.286b	3.143c	3.786d	4.000d	4.000d
Total means	1.265a	2.235b	2.633b	3.387c	3.898d	3.939d

\*The mean values followed by different letter in each row are markedly different ( $p < 0.01$ ).

Concentration of mycelial fragments directly influenced the virulence of mycelia. The higher the concentration of mycelial fragments, the more virulent the mycelia. The huge number of mycelial fragments might damage plant's resistance to the pathogen. Phytotoxin in the residue of culture liquid might play a similar role like that (Qiang et al., 1998). Although only  $2.0 \times 10^6$  could cause plant serious diseases, in the view of absolute control of this weed it seemed that  $3.5 \times 10^6$  mycelial fragments  $\text{ml}^{-1}$  might be the optimum concentration between  $3.0 \times 10^6$  and  $3.8 \times 10^6$ .

#### Effect of plant age on the development of disease

The plants with less than six pairs of leaves did not significantly influence the development of disease because there was no difference in disease ratings between those plants with four, five and six pairs of leaves ( $p > 0.05$ ). However, there was marked effect of seven-couple-leaf plant on the development of disease caused by mycelial fragments. Seven couple-leaf plant was much more resistant to mycelium than other younger ones ( $p < 0.01$ ) (Fig.2).

The results showed that six-couple-leaf plants or younger were more sensitive than older ones. However, plants with seven pairs of leaves or more grew branch system like mature plants in morphology, increasing resistance to the disease.

#### Effect of dew priod on the development of disease

The optimum dew duration was about 18hrs, and the inoculated plants kept for 14hr dew period caught severe disease and died 85%(Fig.3, Fig.4). The dew period roughly equaled the time it took mycelial fragment to infect the weed leaf (Qiang & Summerell, 1999). 14hr-dew period might sometimes take place in natural condition.

#### Effect of temperature on the development of disease

There was marked difference in mycelial virulence at three different temperature treatments (Fig.3). At  $18-25^\circ\text{C}$ , mycelia caused the most serious disease and all treated

plants to die after two weeks. With the temperature falling, the virulence of mycelia decreased obviously ( $p < 0.01$ ). At 12-18°C, mycelia only caused medium disease and three treated plants to die after six weeks. Under the temperature of 15-22°C, the virulence of mycelia just lay between the both of them (Fig.5).

Of three temperature treatments, the disease developed more seriously at 18-25°C than at the other two ones. Since mycelia caused all treated plants to die at 18-25°C only within two weeks, the temperature might be the lowest optimum temperature. This study was not conducted separately about whether dew period or post dew period temperature influenced on the development of disease. However, according to the analysis of the development process of disease, it is reasonable that the temperature during dew period and after dew period for over one day would be the most important for infection (Qiang & Summerell, 1999).

### Effect of temperature and duration during storage of mycelia on the development of disease

Under mycelia storing at low temperature (4°C), their virulence did not markedly change within first one week ( $p > 0.05$ ). However, the mycelia, subject to be stored for two weeks, were significantly much less virulent than those treated before ( $p < 0.01$ ). After two weeks, mycelial virulence markedly decreased further ( $p < 0.01$ )(Fig.6).

When mycelia were stored at room temperature (18-25°C), their virulence markedly and quickly decreased with prolonging the duration of storage ( $p < 0.05$ )(Fig.6).

Although leaf was main parts infected by mycelium, mycelium infected petiole and stem too and caused them to ulcerate and break. That was one of main reasons that mycelium caused plants to die quickly.

The results of all inoculation treatments showed that the death rate of treated plants closely related to the severity of the early infection of plants. The more serious the infection, the higher the death rate of treated plants. After three weeks, the disease never developed further, in contrary, with the time prolonging the disease severity might sometimes drop because plants grew and repaired (Tab.3). Therefore, an appropriately environmental condition at first two days was a key factor for the development of disease.

Table 3. Means of disease ratings of various treatments according to survey time sequence statistics\*

Date (day)	Concentration	Plant age	Temperature	Storage	
				At low temp	At room temp.
2	2.476a	3.071a	2.405a	2.112a	1.765a
7	2.714ab	3.143a	2.619ab	2.529ab	2.229ab
14	2.857abc	3.821b	3.000ab	2.741ab	2.476b
21	2.905bc	3.875b	3.214b	2.800b	2.500b
28	3.071bc	3.875b	3.333b	2.900b	2.565b
35	3.083bc	3.875b	3.405b	2.959b	2.559b
42	3.131c	3.875b	3.405b	3.012b	2.547b
D.R.(%)**	64.29	89.29	66.67	56.47	32.94

The mean values followed by different letter in each column are significantly different ( $p < 0.05$ ); \*\*D.R.= the



death rate of the treated plants after 42 days

Although mycelia were stored at low temperature and could maintain their virulence within one week, at low or room temperature the mycelia stored in water lost virulence very quickly. Obviously, it might not be practicable that mycelia were kept in this way. If mycelia of *A. alternata* could be developed as a mycoherbicide for crofton weed, it might be necessary to find a way that mycelia should be practically stored in stable vitality till application. Rotem (1994) reported that in the survival of *Alternaria* species, mycelium always and consistently survived longer and better than spores. And it proved that mycelium of *Alternaria* survived the best at RH 14-38% (Hogg 1966, Rotem 1968 and 1994). Therefore, drying mycelium might be a way for the storage of mycelium in good condition.

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APPLICATION PERIOD OF *DRECHSLERA MONOCERAS*, PLANT  
PATHOGEN FOR THE MYCOHERBICIDE, TO CONTROL *ECHINOCHLOA*  
*ORYZICOLA* VASING IN RICE FIELDS OF TOHOKU, JAPAN

H. Watanabe, A. Uchino, and M. Tachibana

Tohoku National Agricultural Experiment Station, Yotsuya, Omagari, Akita 014-0102  
Japan

① **Abstract:** MTB-951 and JMB-98 are the potential mycoherbicides using *Drechslera monoceras* (Syn. *Exserohilum monoceras*) fungal plant pathogen isolated from diseased annual *Echinochloa* species in Japan. Duration of their viability is not very long in water and the control efficacy is influenced by many of environmental and biological factors, e.g. temperature, water depth, inoculum density, plant leaf stage and growing vigor of the weed. To investigate the optimum application period to control *Echinochloa oryzicola* Vasing in rice fields, we conducted field and pot experiments for four years since 1997 at Tohoku National Agricultural Experiment Station located in Omagari city of Akita Prefecture, the cool temperate region of Japan. The weed emerged over a long period due to the low temperature, less than 20% of the weed plants had emerged before 1-leaf stage and 40 or 60% had emerged before 2-leaf stage in the experimental field. The control effect was lower when the mycoherbicide was applied at earlier than 1-leaf stage of the weed since more than 80% of the plants emerged and grew after the pathogenicity and the herbicidal activity had decreased. Later application at more than 2.5-leaf stage also failed to control the vigorous *Echinochloa* plants under flooded condition at 8 or 10 cm water depth. ② Conclusively the highest control effect was obtained when the mycoherbicide was treated at 2-leaf stage of *E. oryzicola* plants that was coincident with the peak of the emergence, where the dry weight of the weed reduced by 85 or 95% during one month after the application in heavily infested rice fields of this region.

**Key words:** Biological control, mycoherbicide, *Drechslera monoceras*, *Echinochloa oryzicola* Vasing,

## INTRODUCTION

*Echinochloa oryzicola* Vasing is the most noxious grassy weeds in rice fields of Tohoku area, cool temperate region of Japan. Area of its infestation is estimated more than 90% of total rice area, although many kinds of grass-killer herbicides have been developed and used to control the weed. Long duration of its emergence and slow rice growth due to low temperature make it difficult to control the weed in this region. Sequential application of grass-killer herbicides is often necessary to control it satisfactorily. Although herbicides have contributed to stable rice production, reducing weeding cost and labor saving for Japanese rice farmers and are still essential for global rice production, other options for weed control are expected to develop for reducing chemical use in arable lands.

Several biological control methods using microorganisms has been studied and some have successfully developed as commercial microbial herbicides such as "Devine" for stranglervine in citriculture (Ridings, 1986), "Collego" for northern jointvetch (Daniel et

al., 1973) and "Bio Mal" for round-leaved mallow (Mortensen, 1988) in upland fields, and "Camperico" for annual bluegrass in lawn green (Imaizumi et al., 1999a, Imaizumi et al., 1999b). To develop microbial herbicides to control water chestnut (*Eleocharis kuroguwai*), a noxious perennial rice weed in Japan, herbicidal activity of *Epicoccossorus nematosorus*, *Dendryphiella* sp. and *Nimbia scirpicola* were studied (Suzuki, 1991), but not successfully developed for their practical use. To control annual *Echinochloa* species Mitsui Chemicals Inc. and Japan Tobacco Inc. developed potential mycoherbicides, MTB-951 and JMB-98, using *Drechslera monoceras* (Syn. *Exserohilum monoceras*), fungal plant pathogen isolated from diseased annual *Echinochloa* species in Japan, and the herbicidal activity has been evaluated under various condition (Tsukamoto et al., 1997, Tsukamoto et al., 1998, Yamaguchi et al., 1998, Mihashi, 2001). Duration of their viability is not very long in water and the control efficacy is influenced by many of environmental and biological factors, e.g. temperature, water depth, inoculum density, plant leaf stage and growing vigor of the weed. In this study field and pot experiments were conducted to investigate the effect of application period on its herbicidal efficacy against *E. oryzicola* in rice fields of the cool temperate region.

## MATERIALS AND METHODS

### Field experiments

In 1997 we cultivated rice by direct seeding method; pre-germinated seeds of rice cultivar 'Fukuhibiki' were broadcasted on puddled soil surface under flooded condition on 8 May in an experimental rice field of Tohoku National Experiment Station located at Omagari-city of Akita Prefecture. Dust-granule mixture of MTB-951 (formulated by Mitsui Chemicals Inc.) was treated at four days and eleven days after rice seeding (DAS) at inoculum density of  $1 \times 10^7$ ,  $3 \times 10^7$  and  $10 \times 10^7$  conidia/m<sup>2</sup>. The herbicidal activity against *E. oryzicola* was evaluated in two weeks after the application.

In 1998 and 1999 we cultivated rice by transplanting method; young seedlings (3.5-leaf stage) of rice cultivar 'Akitakomachi' were transplanted on 17 or 18 May in the experimental field. In 1998 the JMB-98 flowable (formulated by cooperation of Mitsui Chemicals Inc. and Japan Tobacco Inc. from the same isolate of MTB-951) was applied at 7 and 14 days after rice transplanting (DAT) at inoculum density of  $3 \times 10^7$  and  $9 \times 10^7$  conidia/m<sup>2</sup>. Its herbicidal activity against *E. oryzicola* was estimated in two weeks after application. In 1999 oil solution of MTB-951 was applied at inoculum density of  $3 \times 10^7$  conidia/m<sup>2</sup> at 11 DAT. Water depth was kept at 8 or 10cm for one month after application.

### Pot experiments

In 1999, seedlings (3.1-leaf stage) of rice cultivar 'Akitakomachi' were transplanted in 0.25 m<sup>2</sup> concrete pots (0.5m x 0.5m) at the density of six hills/pot (three plants/hill) on 28 May. Oil solution of MTB-951 was applied at inoculum density of  $3 \times 10^7$  and  $6 \times 10^7$  conidia/m<sup>2</sup> at 6 and 13 DAT. Its herbicidal activity was estimated at 30 days after the application.

In 2000, seedlings (3.5-leaf stage) of 'Akitakomachi' were transplanted in 0.25 m<sup>2</sup> concrete pots (0.5m x 0.5m) at the density of eight hills/pot (three plants/hill) on 17 May. Oil solution of MTB-951 was applied at inoculum density of  $3 \times 10^7$  conidia/m<sup>2</sup> at 5, 8 and 13 DAT. Its herbicidal activity was estimated at 30 days after the application.

## RESULTS AND DISCUSSION

### Leaf stage of *E. oryzicola* at the mycoherbicide application

Table 1 shows leaf stage of *E. oryzicola* plants at the mycoherbicide application. Early growth of the weed was varied among years; it was slower in 1997 than in 1998 and 1999. This was due to the low temperature during early growing stage in 1997. The mycoherbicide was applied at coleoptile stage or 1.0-leaf stage of the *Echinochloa* seedlings in 1997, while the older seedlings were treated in other years, 1.5- or 2.8-leaf stage in 1998, 1.5- or 2.0-leaf stage in 1999 and 1.0- or 2.5-leaf stage in 2000.

**Table 1. Leaf stage of *Echinochloa oryzicola* at the mycoherbicide application**

Year	Experimental condition*	Rice seeding or transplanting	Application period		Mean air temperature*** (°C)
			Date	Leaf stage** of <i>E. oryzicola</i>	
1997	Field (DS)	8 May	4 DAS	Coleoptile	13.2
			11 DAS	1.0 L	15.2
1998	Field (TP)	18 May	7 DAT	1.5 L	16.9
			14 DAT	2.8 L	17.5
1999	Field (TP)	17 May	11 DAT	2.0 L	16.8
	Pot (TP)	28 May	6 DAT	1.5 L	17.9
2000	Pot (TP)	17 May	13 DAT	2.0 L	17.7
			5 DAT	1.0 L	16.7
			8 DAT	2.0 L	17.0
			13 DAT	2.5 L	18.0

\* DS means direct seeding and TP means transplanting; \*\* Leaf stage of the biggest (the earliest growing) *Echinochloa*; \*\*\* Means of daily air temperature before the mycoherbicide application.

### Emergence of *E. oryzicola*

In the field experiments, *E. oryzicola* emerged over a long period due to low temperature (Figure 1). In 1997, a very few plants had emerged before the earlier application period (4 DAS) and only 12% of total number of emergence had emerged before the later application (11 DAS). It means that more than 88% of the plants emerged after the mycoherbicide application. *Echinochloa* plants of 15% emerged before 1.5-leaf stage application (7 DAT) and 55% emerged before 2.8-leaf stage (14 DAT) in 1998, and 25% emerged before 2-leaf stage (11 DAT) in 1999. The emergence pattern indicated that application period of 2- or 2.5-leaf stage (10 or 15 DAT) was coincident with the peak of the weed emergence in the rice field in 1998 and 1999.

In the pot experiments, the weed emerged and grew more rapidly compared to the rice field condition. *Echinochloa* plants of 30% had emerged before 1.5-leaf stage (6 DAT) of the weed and 45% or 85% had emerged before 2- or 2.5-leaf stage (13 DAT). In the concrete pots the application period of the 1.5- or 2-leaf stage (5 or 10 DAT) was coincident with the peak the weed emergence.

### Effect of inoculum density on the herbicidal activity

Herbicidal activity was evaluated at two weeks or one month after the mycoherbicide application. It varied depending on inoculum density (Table 2). Higher herbicidal activity was obtained at high-density inoculation of more than  $6 \times 10^7$  conidia/m<sup>2</sup> where the weed dry weight reduced by more than 95% both in field and pot experiment. Dry weight of the weed (ratio to untreated control) ranged between 3% and 26% when the mycoherbicide applied at medium inoculum density of  $3 \times 10^7$  conidia/m<sup>2</sup>, and it ranged between 19%

and 42% at the low-density inoculation of  $1 \times 10^7$  conidia/m<sup>2</sup>.

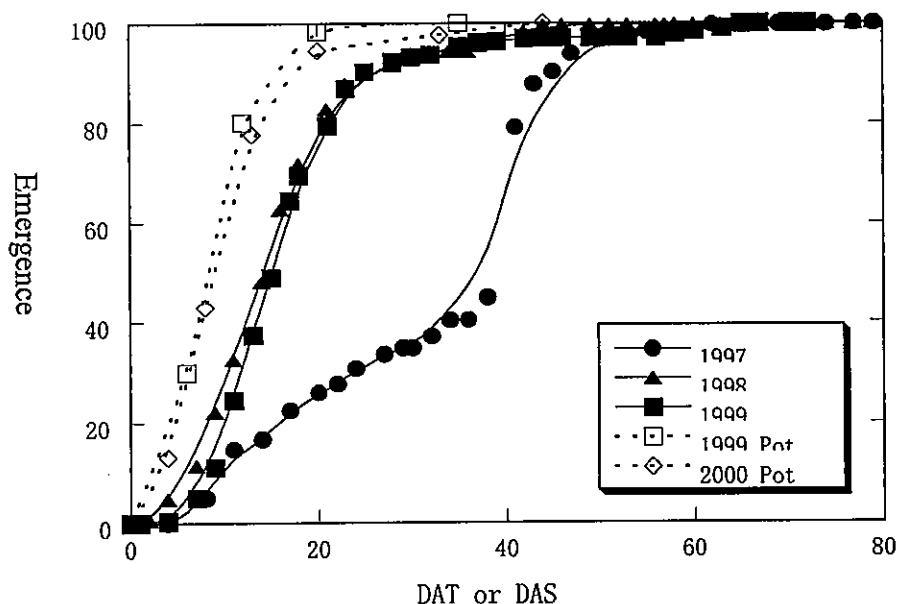


Figure 1. Emergence of *Echinochloa oryzicola* Vasing in the rice field and pot

Table 2. Inoculum density of the mycoherbicide and its herbicidal activity

Inoculum density (conidia/m <sup>2</sup> )	Year (Exp.)	DW (%) of <i>E. oryzicola</i> *			
		Mean $\pm$ SD	Min.	Max.	
Low	$1 \times 10^7$ 1997 Field	$31 \pm 16$	19	42	
	$3 \times 10^7$ 1997 Field	$8 \pm 4$	5	11	
	$3 \times 10^7$ 1998 Field	$10 \pm 9$	3	16	
Medium	$3 \times 10^7$ 1999 Field	17	-	-	
	$3 \times 10^7$ 1999 Pot	$6 \pm 4$	5	5	
	$3 \times 10^7$ 2000 Pot	$12 \pm 12$	3	26	
	$6 \times 10^7$ 1999 Pot	$2 \pm 1$	2	2	
High	$9 \times 10^7$ 1998 Field	2	-	-	
	$10 \times 10^7$ 1997 Field	$4 \pm 1$	3	5	

\* Ratio to untreated control

#### Effect of application period on the herbicidal activity

Control effect of the mycoherbicide on *E. oryzicola* was higher when it was applied later than 1.0- or 1.5-leaf stage of the weed. Application at 2.0- or 2.5-leaf stage obtained better control effect (Table 3). It was, however, reported that 1.0- or 1.5-leaf stage of *Echinochloa* species was the most susceptible to the mycoherbicide, but older or younger plants were more tolerant (Zhang and Watson, 1997). It means that the optimum application timing in rice fields is influenced not only by susceptibility of *Echinochloa* seedlings but also by other factors such as water management and emergence pattern of the weed, since duration of the pathogen viability and its residual effect is not very long in water and submergence of the inoculated leaves promoted lesion development leading

high herbicidal activity (Tsukamoto et al., 1998).

Table 3. Application period of the mycoherbicide ( $3 \times 10^7$  conidia/m<sup>2</sup>) and its control efficacy

Year (Exp.)*	Mycoherbicide	Application period		DW (%) of <i>E. oryzicola</i> **
		DAS or DAT	Leaf stage of <i>E. oryzicola</i>	
1997 Field	MTB-951	4	Coleoptile	11
(DS)		11	1.0 L	5
1998 Field	JMB-98	7	1.5 L	16
(TP)		14	2.8 L	3
1999 Pot	MTB-951	6	1.5 L	5
(TP)		13	2.0 L	6
2000 Pot	MTB-951	5	1.0 L	26
(TP)		8	2.0 L	7
		13	2.5 L	3

\* DS means direct seeding and TP means transplanting condition; \*\* Ratio to untreated control

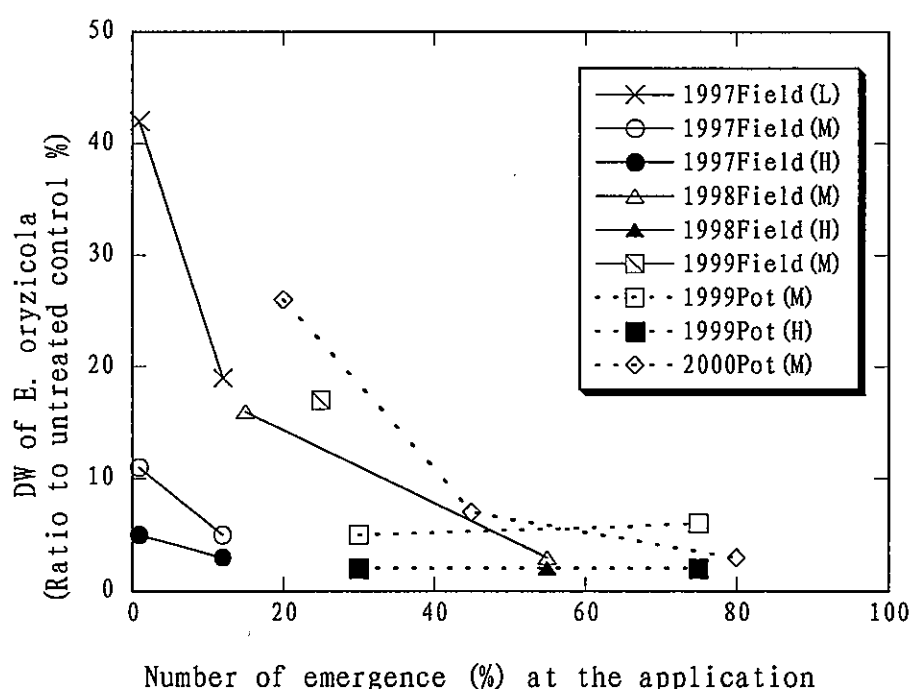


Figure 2. Number of emergence before the mycoherbicide application and its control effect on *Echinochloa oryzicola*. (L) Low-density inoculation ( $1 \times 10^7$  conidia/m<sup>2</sup>); (M) Medium-density inoculation ( $3 \times 10^7$  conidia/m<sup>2</sup>); (H) High-density inoculation (higher than  $6 \times 10^7$  conidia/m<sup>2</sup>).

Figure 2 shows the relationship between degree of *Echinochloa* emergence at the application period and control effect of the mycoherbicide. Control effect was influenced by degree of the emergence at application; it was lower when less than 20% of total emergence had emerged before application. Stable control effect was obtained when the mycoherbicide of medium inoculum density was applied after 30% of the total

emergence had emerged. The results indicated that the control effect was lower when they were applied at earlier than one-leaf stage of the weed since more than 70 or 80 % of the plants emerged and grew after their pathogenicity and herbicidal activity had decreased. We observed that later application at more than 2.5-leaf stage also failed to control the vigorous *Echinochloa* plants under flooded condition at 8 or 10 cm water depth. Conclusively the highest control effect was obtained when the mycoherbicides were treated at 2-leaf stage of *E. oryzicola* plants, where the dry weight of the weed reduced by 85 or 95% during one month after the application in heavily infested rice fields of this region.

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## EVALUATION OF *ALTERNARIA EICHHORNIAE* C416 AS A BIO-HERBICIDE AGENT AGAINST WATER HYACINTH

G. G. Zhao, F. H. Wan, and M. Xie

Institute of Biological Control, Chinese Academy of Agricultural Sciences, Beijing  
100081, China. bcicaas@public.bta.net.cn

**Abstract:** Pathogenicity of twelve fungi isolates, representing three genera and five species respectively, was compared by surveying disease severity (DS) on water hyacinth leaves with mycelial inoculum in greenhouses. *Alternaria eichhorniae* C416, the most virulent, caused a severe disease, characterized by large leaf lesions. After inoculated with *A. eichhorniae* C416, all leaves were killed within 6 weeks, and the whole mean DS was 7. Higher level of DS was yielded in 30°C. Exposure of inoculated leaves to at least 16h of dew resulted in a high level of disease. Optimum inoculum density was 25% mycelial wet weight. None of 14 economically important plants and cultivars evaluated in host range tests was susceptible to *A. eichhorniae* C416. These results showed that the fungal strain, *Alternaria eichhorniae* C416 has potential for further development as a bio-herbicide against water hyacinth.

**Key words:** water hyacinth bio-herbicide, *Alternaria eichhorniae*, pathogenicity

### INTRODUCTION

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Pontederiaceae) is one of the world's worst aquatic weeds (Holm et al., 1977). In China, it distributes in southern and eastern parts, especially in Sichun, Yunnan, Hubei, Hunan, Jiangsu, Fujian, Zhejiang and Henan provinces, and cause severely economic and ecological damages (Diao, 1989).

Mechanical and chemical control are commonly used to control this weed in China, however, they are not completely satisfied to achieve a better management, not only their efficacy, but also causing several problems, polluting water, higher control-costs, and huge labor-inputs. Attempts to develop desirable bio-herbicide against water hyacinth are underway to assist in its control (Charudattan, 1990). Freeman and Charudattan (1984) have developed *Cercospora rodmanii* Conway as a bio-herbicide in USA. A virulent Egyptian isolate named Ae5, *Alternaria eichhorniae*, has been evaluated as a bio-herbicide agent (Shabana et al, 1995a,b; 1997a,b).

In this study, twelve fungal isolates were screened for the possibility to develop a bio-herbicide against water hyacinth. Detailed researches on the most virulent isolate *Alternaria eichhorniae*, named C416, were conducted on its host-specificity, infectivity and pathogenicity in greenhouse trials.

### MATERIALS AND METHODS

#### Fungal isolates

Nine fungal pathogens collected from Africa and America, and three native isolates (Table 1) were used in screening tests. All of the isolates were cultured on fresh potato dextrose broth (FPDB; potato extract, 20g D-glucose, and water to make 1 liter).

Table 1. Isolates used in screening test

Fungal isolate	Isolates number	Collector	Collect area and year
<i>Cercospora piaropi</i>	C114	Cilliers, C. J.	Hectorspruit, Gauteng, South Africa, 1987
<i>Cercospora piaropi</i>	C387	Morris, M. J.	Delmiro Gouveira, Brazil, 1995
<i>Cercospora piaropi</i>	C400	Hill, M.	Kafue River, Zambia, 1996
<i>Cercospora rodmanii</i>	C143	Conway, K. E	Rodman Reservoir, Florida, USA., 1973
<i>Alternaria eichhorniae</i>	C416	Cillers, C. J.	Lake Chivero, Harare, Zimbabwe, 1996
<i>Alternaria eichhorniae</i>	C417	Cillers, C.J.	Tano Lagoon, Malawi, 1996
<i>Acremonium zonatum</i>	C389	Morris, M. J.	Delmiro Gouveira, Brazil, 1995
<i>Acremonium zonatum</i>	C401	Cillers & M.Hill	Enseleni River, Kwazulu-Natal, 1996
<i>Acremonium zonatum</i>	C415	Hill, M.	Kafue River, Zambia, 1996
<i>Alternaria sp. (1).</i>	BJIS1	Guo-gang Zhao	Beijing, China, 1998
<i>Alternaria sp. (2).</i>	BJIS2	Guo-gang Zhao	Beijing, China, 1998
<i>Alternaria sp. (3).</i>	BJIS3	Guo-gang Zhao	Beijing, China, 1998

### Comparison of pathogenicity of isolates

Isolates were cultured for 4d in a reciprocating shaker at 28°C and 200r/min. Mycelial mass separated by filtration with cheese cloth and blended in a mechanic blender with an equal weight : volume of sterile water for 20s and produced 60~120 µm mycelial fragments. Mycelial suspensions were prepared by diluting mycelia fragments homogenate 1:2(v/v) with sterile water and supplementing with 5% Tween80 as a wetting agent. Before inoculation, the plant leaves were washed by sterile water. Mycelia were inoculated on the plant foliage with a sterile brush until suspensions dropped from leaves. Plants inoculated with 5% Tween80 without fungal fragments as a control. Each treatment has 5 replications, and inoculated and control plants were covered with plastic bags for 2 days to maintain a high relative humidity. 12d after inoculation, the plants were rated for disease incidence (DI) and disease severity (DS). DS was determined for each leaf with pictorial disease scales of Freeman and Charudattan's (1984). Values for individual leaves were summed and averaged to derive DS for a whole plant. Tests were conducted in greenhouses in Institute of Biological Control, CAAS, Beijing, China.

### Relationship between inoculum density and disease severity

Mycelial fragments of *A. eichhorniae* C416 were obtained as described. The mycelium were used at six inoculum density levels: 3.125, 6.25, 12.5, 25, 50 and 75% (wet w/v) with 5% Tween80 as a wetting agent. The plants were inoculated and covered with plastic bags as described above. There are four replications per inoculum density. A fungus-free control was included as comparison. The plates were placed in greenhouses at 18/25°C (night/day). 12d after inoculation, the plants were rated for disease severity (DS).

### Effect of temperature on the infectivity

Inoculum density level of *A. eichhorniae* C416 mycelium was 25% (wet w/v) with 5% Tween80 as a wetting agent. The plants were inoculated and covered with plastic bags as described and placed in a constant-temperature chamber (75% relative humidity, 3000Lux) at 20, 25, 30 ± 0.5°C respectively. There are three replications per treatment.

12d after inoculation, the DS were rated.

#### **Effect of light intensity on the infectivity**

The plants were inoculated with an aqueous 25% (wet w/v) suspension of blended mycelium of *A. eichhorniae* C416 plus 0.5% Tween80 and covered with plastic bags as above. A fungus-free control was included as comparison. Inoculated and control plants were placed in a constant-temperature chamber (75% relative humidity,  $25 \pm 0.5^\circ\text{C}$ ) exposure to 1200Lux, 3000Lux light intensity respectively. There are three replications per treatment. 12d after inoculation, the DS were recorded.

#### **Optimum dew duration conducive for infectivity**

Water hyacinth plants were inoculated with an aqueous 25% (wet w/v) suspension of blended mycelium of *A. eichhorniae* C416 plus 0.5% Tween80. The inoculated plants were covered with plastic bags for 0, 8h, 16h, 24h, 32h and 48h to maintain relative high humidity respectively and placed in a constant-temperature chamber (75% relative humidity,  $25 \pm 0.5^\circ\text{C}$ , 3000Lux). The DI and DS were rated 12d later. Greenhouse trial for evaluating *A. eichhorniae* C416 as bioherbicide agent for water hyacinth.

Fifty plants were inoculated with a 25% (wet w/v) suspension of mycelium fragments of *A. eichhorniae* C416 with 0.5% Tween80 and covered plastic clothes for 48h in a greenhouse ( $25 \pm 5^\circ\text{C}$ ; about 50% relative humidity) at Institute of Biological Control, CAAS, Beijing, China. DI and DS were recorded once every week.

#### **Host range**

Host range test was conducted in the greenhouse as above. 14 plant species were inoculated 25% (wet w/v) mycelial suspension with 0.5% Tween80 and covered plastic bags for 48h. Each plant species has three replications. The test repeated three times.

#### **Statistic Analyses**

The experiments were repeated once and the data were analyzed with the SPSS (Statistical Package of Social Science). All multiple comparisons were first subjected to analysis of variance (ANOVA). Significant differences among treatment means were determined with Duncan's new multiple range.

The data on the relationship between inoculum density and disease severity were analyzed by fitting the following nonlinear least-squares regression equation of SPSS.

## **RESULTS AND DISCUSSION**

#### **Pathogenicity comparison of pathogenicity of isolates**

All the isolates were pathogenic to water hyacinth (Table 2). The most virulent isolates were *A. eichhorniae* C416 and *Cercospora rodmanii* C143.

The symptoms appeared on water hyacinth leaves in 3 days after inoculation with *A. eichhorniae* C416 and *Cercospora rodmanii* C143, and after 4 to 5 days with the other isolates except BJIS1, BJIS2, BJIS3. Plants inoculated with the BJ isolates displayed symptoms 7 days after inoculation. The characteristic symptoms caused by isolates C143, C416, C417 and C387 were big irregular lesions with faint yellow margins, and some leaves damaged severely by them were death in 25 days. The main symptom of isolates C400 was white irregular lesions. Round or oval shape lesions appeared on foliage after inoculation with C389, C401, and C415. Infectivity of BJ isolates and

C114 caused black small points on leaves. The inoculated fungi were re-isolated from diseased leaves, confirming their pathogenicity according to Koch's postulates.

Table 2. Comparison of disease severity on water hyacinth

Isolate number	Disease severity
C114	2.48±0.34 d
C387	2.26±0.42 d
C400	2.50±0.34cd
C143	3.14±1.40 b
C416	3.78±1.48 a
C417	2.94±0.78 bc
C389	2.18±0.33 d
C401	2.16±0.27 d
C415	1.64±0.27 e
BJIS1	0.96±0.38 fg
BJIS2	0.48±0.24 g
BJIS3	0.40±0.16 g
Control	0

Values within column followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05)

#### Effect of inoculum density on the infectivity of *A. eichhorniae* C416

Disease severity of the whole plant caused by C416 was promoted with increasing of mycelial inoculum density 12 days after inoculation. Inoculation with 3.125%, 6.25%, 12.5%, 25%, 50% and 75% mycelial suspensions (wet w/v) caused disease severity levels of 1.61, 4.27, 4.43, 6.21, 6.42 and 6.63, respectively. There was significant difference between DS caused by inoculum density levels of above 25% (including 25%) and of below. According to non-parameter linear regression of SPSS, the relationship of inoculum density and disease severity conformed to typical S-model curve (Fig.1). The optimum inoculum density was 25% (wet w/v) deduced from the curve.

#### Effect of temperature on the infectivity of *A. eichhorniae* C416

Disease severity of whole plant and origin leaves was influenced by different temperature treatments (Table 3). The highest disease severities of whole plant and origin leaves were 5.93 and 7.36 respectively, occurred in 30°C. It was significantly higher than that in 20°C. However, there is not a significant difference between 25°C and 30°C because of higher new leave ratio in 30°C.

Table 3. Influence of temperature on the pathogenicity of mycelial inoculum of *A. eichhorniae* C416

Temperature (°C)	Disease severity of whole plant	Disease severity of origin leaves	Leave death(%)	New leave(%)	Disease severity of new leaves
20	5.02±0.23b	5.76±1.63b	4.17	13.89	1.00±0.00a
25	5.32±0.19ab	6.06±1.75ab	4.17	13.89	1.00±0.00a
30	5.93±0.68a	7.36±1.97a	8.47	24.87	0.80±0.45a

Values within column followed by the same letter are not significantly different.

### Effect of light intensity on the infectivity of *A. eichhorniae* C416

The development of disease severity was influenced by light intensity (Fig.2). Under weak light intensity (1200Lux), disease severity of whole plant and origin leaves were 6.08 and 6.58, while they were both 3.06 under strong light intensity in the fourth day after inoculation. The level of disease severity was significantly higher than that under strong light intensity (3000Lux). In the following days, the curve of disease severity under weak light intensity was paralleled with that under strong light intensity. Disease severity of new leaves maintained a low level all the time. On the basis of the result, weak light was an important factor for the infectivity and disease promotion.

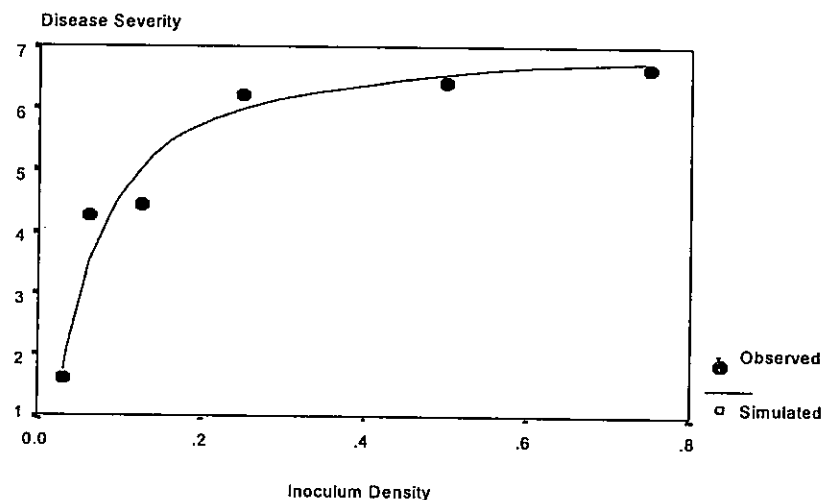


Figure 1. Relationships between inoculum density (ID) of *A. eichhorniae* C416 and disease severity (DS). ID is represented by the percentage of blended mycelium (wet w/v) in water. DS, recorded 12 days after inoculation, is assessed on a pictorial scale of 0-9 corresponding to 0 to 90% disease.

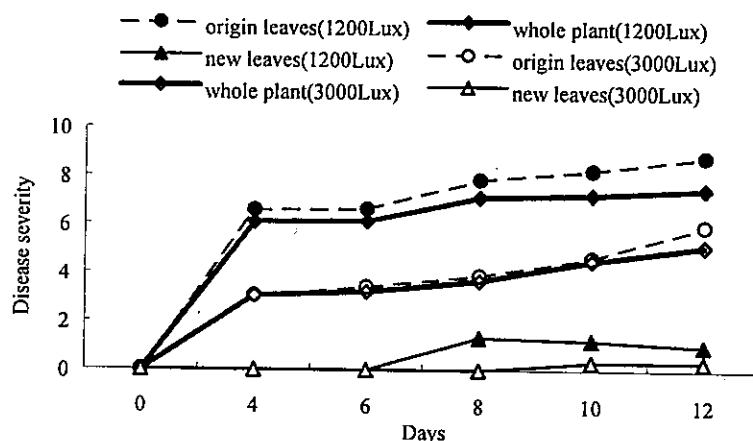


Figure 2. Disease development under different light intensities.

### Optimum dew duration for the infectivity

Disease severity was promoted with increasing the time of exposure to dew (Fig.3).

There was significant difference between exposure to dew for 16h and below it. Exposure to dew for 16h, disease severity was 6.23, while those were 0.45 and 2.41 respectively for 0 and 8h. Above 16h, disease severity has no significant difference between treatments.

### Greenhouse trial for evaluating *A. eichhorniae* C416 as bioherbicide agent for water hyacinth

The result of greenhouse trial (Fig.4) indicated that disease development in the first week was very quick, and disease severity of the whole plant and origin leaves were 2.97 and 3.80, respectively; From the second week to fourth week, disease severity increased slowly. In the fifth week, disease spread fast, and disease severity of whole plant and origin leaves were 6.29 and 8.69 individually; Most origin leaves were dead in the sixth week, while some of the whole plant sunk into water. Disease of new leaves developed slowly and influenced disease severity of whole plant. Control plants were not infected until four weeks later, which may be caused by conidia produced by the inoculated mycelia.

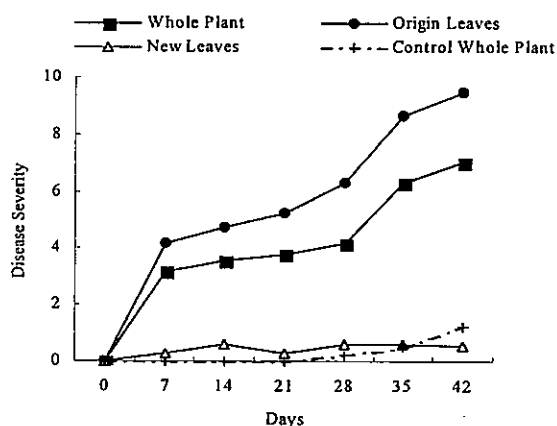


Figure 3. Disease development in greenhouse trial.

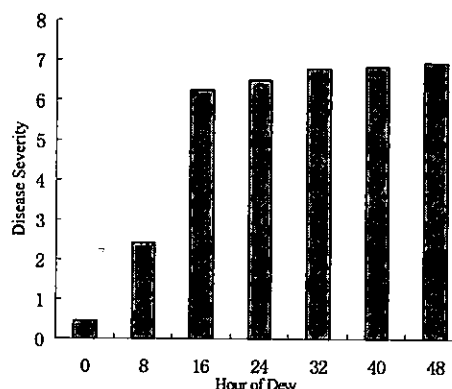


Figure 4. Effect of exposure to dew on disease severity.

### Host range test

None of 14 plant species and crop varieties was susceptible to *A. eichhorniae* C416; only water hyacinth used as a control was susceptible (Table 4). Therefore, *A. eichhorniae* C416 appears to be a safe biological control agent for water hyacinth.

*Alternaria eichhorniae* C416 f water hyacinth and showed high pathogenicity to water hyacinth in the greenhouse trials, especially on origin leaves. However, infection on newly grown leaves was low. That could be related with the rapid growth rate of water hyacinth. Perkins (1973) reported that water hyacinth produced a new plant every 5 or 6 days under proper circumstances. The other reason was shortage of media, such as insects or wind, to widespread disease in the greenhouse. Therefore, the application time, methods and epidemic factors should be considered in the fields.

Effects of temperature and light intensity on infection showed that high temperature (30 °C) and weak light intensity caused higher DS levels. These results were coincided with that long period cloudy or foggy weathers were conducive to development of disease in field trials (Conway et al. 1979).

Table 4. Non-target plants screened and proved to be immune to *A. eichhorniae* C416

Common name	Species	Infected
Rice	<i>Oryza sativa</i> L.	No
Wheat	<i>Triticum aestivum</i> L.	No
Tobacco	<i>Nicotiana tabacum</i> L.	No
Maize	<i>Zea mays</i> L.	No
Sunflower	<i>Helianthus annuus</i> L.	No
Chrysanthemum	<i>Dendranthema morifolium</i> (Ramat.) Tzvel	No
Eggplant	<i>Solanum melongena</i> L.	No
Tomato	<i>Lycopersicon esculentum</i> Mill	No
Beam	<i>Glycine max</i> (L.) Merr.	No
Rose	<i>Rosa rugosa</i> Thumb.	No
Apple	<i>Malus pumila</i> Mill	No
Potato	<i>Solanum tuberosum</i> L.	No
Cotton	<i>Gossypium hirsutum</i> Linn.	No
Weed	<i>Humulus scandens</i> (Lour.) Merr.	No
Water hyacinth	<i>Eichhornia crassipes</i> (Mart.) Solms	No

*Alternaria eichhorniae* was reported to have a narrow host range (Nag Raj and Ponnappa, 1970; Rakvidhyasastra et al., 1978) ; Shabana et al. (1995a) tested 97 economically important, non-target plant species and cultivars to be immune to *A. eichhorniae* Ae5 . Though only some important cultivars plants, and a worse weed in China were tested in our host range test , the results confirmed this pathogen, represented by *A. eichhorniae* C416, is specific. Therefore, *A. eichhorniae* C416 was a suitable bio-control agent for control of water hyacinth. However, Nag Raj and Ponnappa (1970) suggested that further information was needed about the pathogen's host range, the nature of its phytotoxins, and the factors governing toxin production. Some reports indicated that this pathogen produces a group of phytotoxic pigment related to disease caused by *A. eichhorniae* (Stevens et al., 1979; Marity and Samaddar, 1977; Charudattan and Rao, 1982; Robeson et al., 1984). Whether these toxins might have environmental or human health consequences will still need to be addressed during the development of this fungus as a bio-herbicide.

On the basis of the test results, we conclude that *A. eichhorniae* C416 can be used as a bio-control agent to develop bio-herbicide against water hyacinth.

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