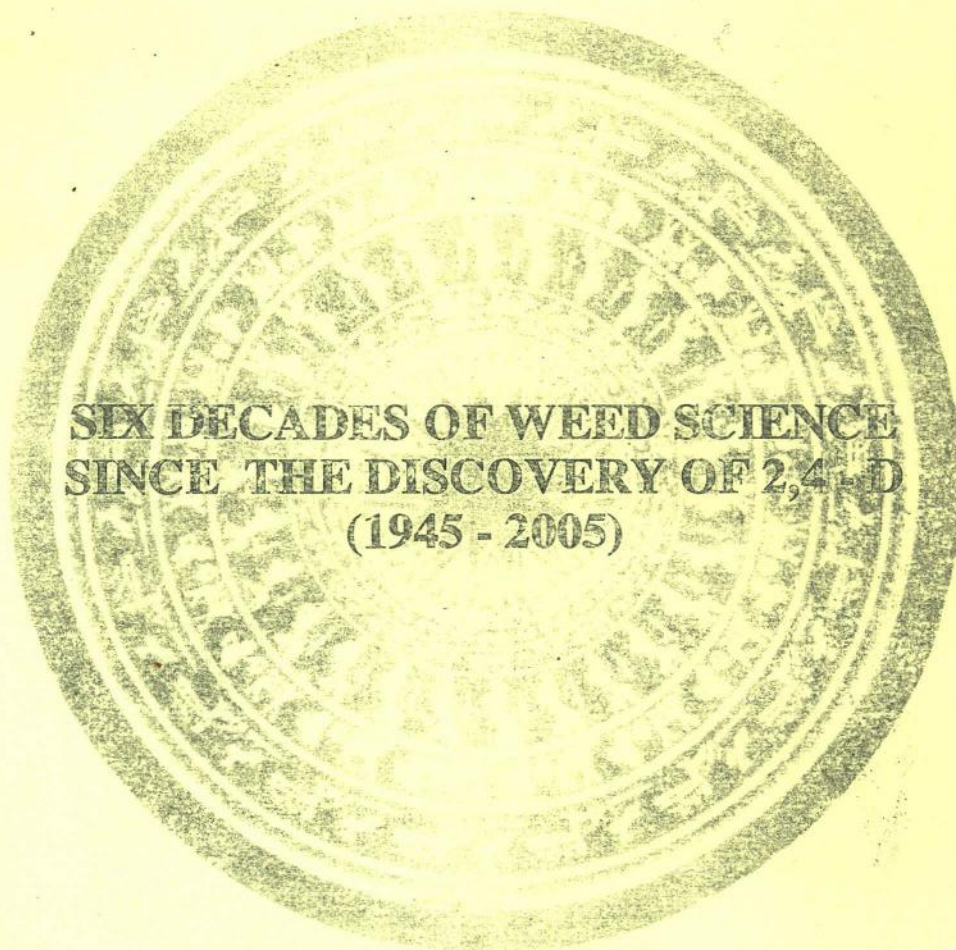


# The 20<sup>th</sup> Asian - Pacific Weed Science Society Conference



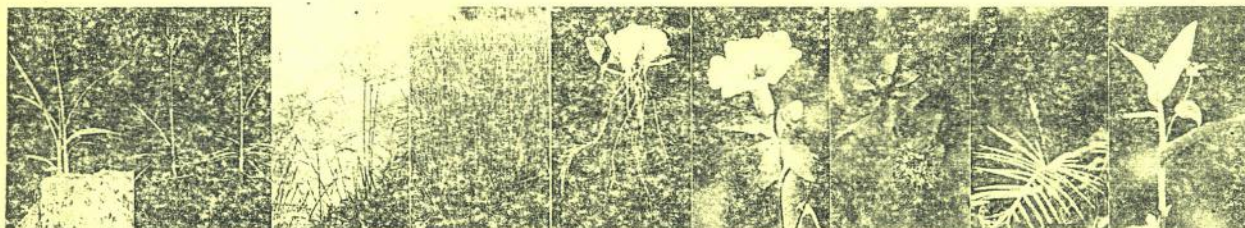
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# **PROCEEDINGS**

## **The 20<sup>th</sup> Asian - Pacific Weed Science Society Conference**

**7 – 11 November, 2005**

**Ho Chi Minh City  
Vietnam**



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## Welcome Address

Welcome to Vietnam and in particular, Ho Chi Minh City!

2005- The year commemorates the sixth decade of weed science since the discovery of 2,4-D (1945-2005). We have a privilege to host the 20<sup>th</sup> Asian – Pacific Weed Science Society Conference which is held at Rex Hotel, Ho Chi Minh City on 7-11 November 2005.

The Asian – Pacific Weed Science Society Conference 2005 will bring scientists from many countries together to discuss issues related to weed management and the environment. This is a meaningful event because the challenges should be overcome through exploitation of new technologies in couple with supporting policies. In this Conference, scientists will share the latest research findings and models to improve crop productivity by weed management aiming at sustainable agricultural development.

We warmly welcome the distinguished guests and participants of the Asian – Pacific Weed Science Society Conference 2005. We are extremely grateful to the sponsors for providing both financial and technical assistance in organizing this important event.

The fruitfulness of the Conference will be due to all efforts by participants from different countries and agencies in the world to promote cooperative actions by members of Asian – Pacific Weed Science Society. Your excellent works have been welcome to shared challenges and accomplishments.

Once again, I would like to express my deepest gratitude to all of you in providing extensive help to the Conference. I feel honored by the presence of all of you, some of whom have come from long distances and have kindly taken much trouble to enrich the Asian – Pacific Weed Science Society Conference by their valuable participation.

Thank you and have a successful Conference.

**BUI CHI BUU**  
**Director General**  
**Cuu Long Delta Rice Research Institute**  
**Codo, Cantho, Vietnam**



## Opening speech

Distinguished guests  
Ladies and gentlemen

On behalf of the Asian-Pacific Weed Science Society, I would like to express our thanks to all of you for your attendance.

It is my pleasure to announce that the weed scientists in the Asian-Pacific region and other parts of the world have had an enthusiasm in contribution for the success of our conference. One hundred and eleven papers dealing with a wide range of issues have been published in our proceedings and about 40 posters will be displayed. Participants have come from many countries including : Australia, Bangladesh, Canada, China, India, Indonesia, Japan, Korea, Malaysia, New Zealand, Pakistan, Philippines, Poland, Sri Lanka, Thailand, United Kingdom, USA, and the host country, Vietnam. We are very happy to receive special guests from the International Rice Research Institute, representatives from many national weed science societies, particularly Dr. Bernal Valverde and Dr. Anis Rahman, the President and the Vice President of the International Weed Science Society.

Since the discovery of 2,4-D sixty years ago, a large number of organic herbicides have been developed and used worldwide making a shift in agricultural production technologies from fossil-based land preparation for weed control to minimum-or zero-tillage systems using herbicides for crop production. This contributes significantly for food production to satisfy the need of huge population in the world. However, some undesirable side-effects have also occurred during the past 60 years. Those are herbicide resistant weeds, the pollution of underground water by continuous usage of one particular herbicide in one area for a long time. Particularly the misuse of herbicide as chemical weapon in Vietnam war. This has led to the serious consequences in terms of the succession of plant species, soil erosion, depletion of water resource, soil and water contamination by toxic substances. Further more, the health consequences caused by contaminated dioxins on Vietnamese people as well as Vietnamese and American veterans and their children have been proved. In this conference it is important that we should look back in the past to learn the lessons from history and discuss the future right direction for weed science to go ahead in the 21<sup>st</sup> century.

On this occasion, I would like to express our thanks to the Ministry of Agriculture and Rural Development (MARD) for their kind support that made this conference a reality. We also thank the co-organizers including Weed Science Society of Vietnam, Plant Protection Department (MARD), BASF Chemical company, Dow AgroSciences, Kumiai Chemical Industry, Monsanto, Syngenta and supporters whose contributions ensured the success of this conference.

I hope you will find the conference useful and enjoy your stay here in Vietnam.

Thank you.

**DUONG VAN CHIN**  
**President, Asian – Pacific Weed Science Society**  
**Chair, Organizing Committee 20<sup>th</sup> APWSS Conference**

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# **Plenary Session**



# Six decades of Weed Science since the discovery of 2,4-D and challenges for the 21<sup>st</sup> century

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**Abstract:** The discipline of weed science began about 1945 with the introduction of selective, synthetic herbicides, beginning with 2,4-D. The growing need for research to study the efficacy, mode of action, environmental effects, application technology, and other aspects of herbicide-driven weed management for a stream of new herbicides being introduced led to an outpouring of research funds for such studies from the herbicide industry and governments. Within a decade, the discipline of weed science was firmly established, beginning in North America. This early period of growth was followed by a “golden age of herbicides”, during which the funding for weed science research and the number weed scientists reached their historic highs. Consolidation of the herbicide industry and the introduction of transgenic, herbicide-resistant crops began a paradigm change about ten years ago. In North America, weed science research has been shifting towards weed biology and ecology, integrated weed management, and other less herbicide-driven research. There may be fewer herbicide-related tools to deal with increasing weed problems in the future. Thus, increased research in areas such biocontrol of weeds, crop allelopathy, herbicide resistance management, and precision weed management is needed.

**Key words:** Herbicide, herbicide resistance, herbicide-resistant crop, weed science societies.

## INTRODUCTION

In preparation for writing this short review, I realized that I was born at about the time that weed science was being developed as a separate discipline and that I have been actively involved in weed science for about half of the life of weed science as a discipline. This article will be largely oriented toward weed science in North America, which has often led the discipline of weed science with new technologies. The aspects of weed science that I consider most important will be emphasized. I will cover the first 20 to 25 years or so of the discipline of weed science when the age of herbicides was being established. My introduction to weed science was near the beginning of what I call the golden age of herbicides. This period persisted for the next 25 or 30 years. Ten years ago, transgenic, herbicide-resistant crops were introduced, which caused a paradigm change in weed science in several major crops, and has indirectly affected weed management in other crops. I will end by providing my projections of how I think weed science may evolve during the first half of this century. Finally, my insights into the history of weed science recorded here have been shaped by my background and experiences and, thus, may be quite different than the perceptions of others.

## EARLY HISTORY OF WEED SCIENCE

Before the synthetic herbicides were introduced in the 1940's, there was no separate discipline of weed science. Weed management was considered a subdiscipline of agronomy, and there were very few scientists who did full time research on weeds and their control. Before synthetic herbicides were introduced, weeds were managed with tillage, manual methods (pulling or hoeing), inorganic chemicals, and cultural strategies such as crop rotation. The discovery of 2,4-D as a plant growth regulant (Zimmerman and Hitchcock 1942) and reports of its use for selective weed control (Hamner and Tukey 1944; Marth and Mitchell 1944) started a process of discovery and



commercialization of new, synthetic herbicides that provided the impetus for weed science to become a discipline of its own. The success of 2,4-D led to the beginning of large scale herbicide discovery efforts by many chemical companies. Regulatory requirements for commercializing products were minimal.

Farmers wanted unbiased assessments of this new, burgeoning technology by university and government scientists. Chemical companies involved in herbicide discovery and sales provided university scientists with funds to carry out research on their potential and existing products. These needs and funds to meet those needs provided the rationale and resources for scientists to do all their research in weed management. A growing cadre of plant physiologists and agronomists became what we now know as weed scientists. The Weed Science Society of America (WSSA) was formed in 1956 when scientists from industry, government, and universities decided that their needs could be better met by a scientific society that dealt only with the research area on which they were spending most or all of their time. Formation of WSSA was followed throughout the world with many regional (e.g., the Asian-Pacific Weed Science Society) and national (e.g., the Japanese Weed Science Society) weed science societies to meet the needs of scientists devoted to weed-related research. Some of these societies began publishing refereed journals such as *Weeds*, later to become *Weed Science*.

New research areas such as mode of action of herbicides, uptake and translocation of herbicides, weed biology as it related to chemical weed management, agricultural engineering of herbicide application devices, and environmental toxicology of herbicides were driven by the introduction of more and more herbicides for an increasing number of crops and cropping situations. The herbicide industry invested a large amount of effort in the synthesis of new compounds and testing them for herbicide activity. Although there was some studies on other aspects of weed science, herbicide-oriented research was dominant because relatively little research money was available for any other type of research.

During the early years of weed science, adoption of herbicides for weed management grew steadily in many crops (e.g., Figure 1) until herbicides became the primary approach to weed management in almost every crop in North America by the early 1960s. Concomitantly, the cost of hand labor for weed management increased relative to the cost of herbicide-based weed management. In summary, the changing economics of weed management favored herbicide use. Herbicide domination in weed management was driven by the products of the disciplines of pesticide science and the new discipline created by herbicides, weed science.

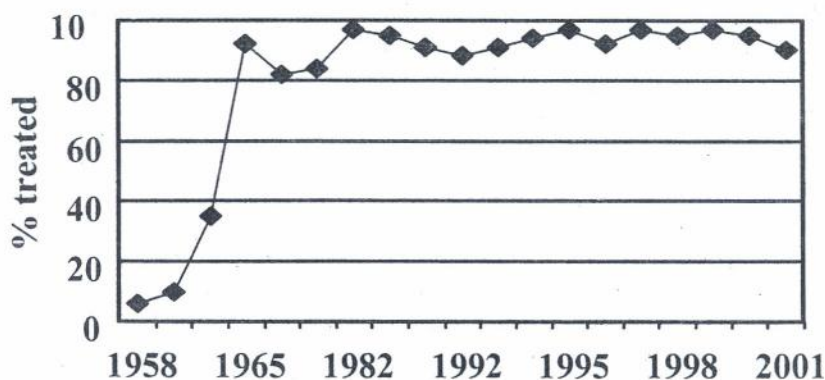


Figure 1. Changes in the use of herbicides in cotton in the USA with time.



## THE GOLDEN AGE OF HERBICIDES

After these early growth years of weed science, a period of 25 to 30 years of what I term “the golden age of herbicides” began. By the mid to late 1960s, many companies worldwide were involved in herbicide discovery and development. The herbicide market was not yet fully mature, so there were many market niches for new or improved products. The cost of discovery and regulatory approval was not prohibitive, so even small companies could profit from being involved in this process. Indeed, at my first weed science society meeting (the Southern Weed Science Society in 1976), dozens of companies were represented. In North America, weed science societies had their historically largest memberships, with most of their membership being employed by the herbicide industry. Almost all university weed scientists were assured generous funding from the herbicide industry to evaluate new herbicides and new uses for older products. USDA expanded its weed science research dramatically to meet needs of farmers. Most of the fundamental research on mode of action of herbicides was conducted by university scientists. Much of this research led to seminal discoveries in plant physiology and biochemistry, such as the discovery of the proteins involved in photosystem II of photosynthesis (e.g., Pfister et al. 1981). Some of these discoveries are reviewed by Hess (2000).

During the golden age of weed science, universities were generating many new Ph.D. weed scientists to meet the demands from universities, federal laboratories, and especially company demand for weed scientists. Sales of herbicides eventually dominated the pesticide market in the U.S., reaching a market share level of about 70% (Figure 2), where it has remained ever since. Jobs and research funding were in relative abundance, and few weed scientists could complain about their lot. The WSSA became so large, just as WSSA had been formed by scientists that left the Agronomy Society of America and the American Society of Plant Physiologists, that those whose interests were focused on weed management in aquatic situations formed the Aquatic Weed Management Society.

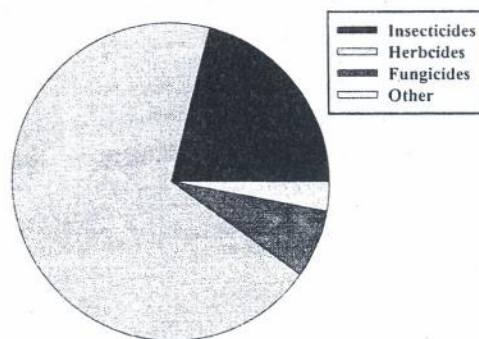


Figure 2. Pesticide use in crop protection in the U.S. in 1997.

However, during this period, the rumblings of trouble in the future were beginning to be heard. The discipline of weed science was criticized by some as being nothing more than herbicide science. However, expansion of the discipline also saw more emphasis on research on biocontrol of weeds (Templeton et al. 1986), weed biology, non-herbicide related weed physiology, and weed ecology. Nevertheless, the perception that weed science was only the technology of herbicides eventually proved detrimental to the discipline.

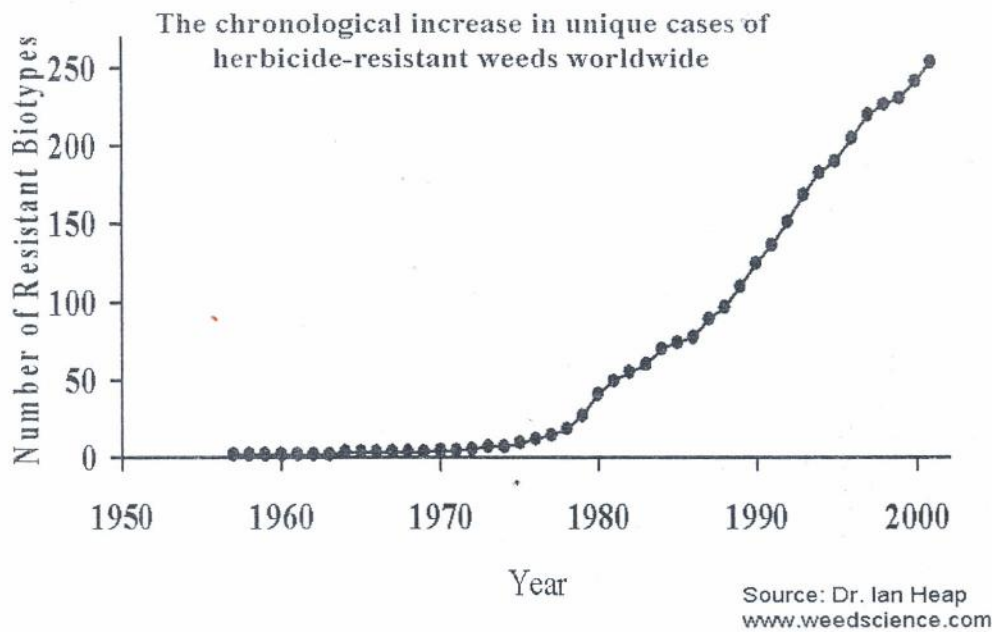
Beginning with the publication of Rachael Carson's book, *Silent Spring* (1962), concerns of the U.S. public about health and environmental risks of pesticides grew dramatically. This influenced weed science significantly in two ways: 1) there was more funding for environmental toxicology of



herbicide research, and 2) there were increased regulatory requirements for introducing new herbicides to the market. The latter influence manifested itself in increased costs for the herbicide industry, which was a contributing factor to the consolidation and attrition of this industry that began in earnest during the latter part of the golden age of herbicides.

Several other things were occurring as this “golden age” of weed science progressed. The first case of evolved resistance to a synthetic herbicide was reported about 25 years after use of the synthetic herbicides began (Ryan 1970). This was tip of an iceberg. From this date on, after a lag phase of about 5 years, there has been a linear increase in documented cases of evolved herbicide resistance worldwide (Figure 3).

Throughout this golden age of herbicides, there were no significant challenges to the herbicide paradigm of weed management. Certainly, there was a tremendous amount of research on methods to more economically and safely use herbicides and a limited amount of research on alternative methods of weed management. Many of the trends that led to the demise of the golden age of herbicides were being strongly felt by the early 1990s. The one event that closed this era quite quickly was the introduction of transgenic, herbicide-resistant crops.



THE TRANSGENIC ERA AND THE CURRENT STATUS OF WEED SCIENCE

Transgenic, herbicide-resistant crops (HRC) were first commercialized in North America in 1995 with the introduction of bromoxynil-resistant cotton and glufosinate-resistant canola (Table 1). This was followed by eight more HRCs. Some of these crops, such as glyphosate-resistant soybean and cotton (Figure 4), have eventually dominated the market where they have been available. HRCs are the most successful of all transgenic crops, reaching about 65 million hectares worldwide in 2004, representing about 80% of all transgenic crops (ISAAA, 2005) (Table 2). Although, there is almost no planting of HRCs in some regions of the world for several reasons, including political opposition to transgenic crops, they are extensively planted in Canada, the U.S., Argentina, Brazil, and a few other countries. The overwhelming majority of these HRCs have been glyphosate-resistant crops. HRCs have been reviewed in a book (Duke 1996) and a special issue of the journal Pest Management Science (2005).

Adoption of HRCs has largely been driven by reduced costs of very effective weed control and simpler weed management strategies (Dill 2005; Giannesi 2005). This adoption of glyphosate over a large percentage of major crop area of the U.S. meant that use of many of the herbicides that helped to compose the ca. 70% market share of the pesticide business (Figure 2) went down dramatically. Even though companies drastically reduced the prices of many of their herbicides in order to compete with the glyphosate/glyphosate-resistant crop combination (Nelson and Bullock 2003), their loss of market share continued. This exacerbated and accelerated the consolidation of companies involved in discovery and marketing of herbicides. Arnold Appleby (2004) has produced an excellent chronology of the consolidation of the herbicide industry.

Table 1. Herbicide-resistant crops that have been deregulated for commercial production by farmers in North America (adapted from Duke 2005).

Herbicide	Transgene	Crop	Year available
Bromoxynil	bacterial nitrilase	cotton *	1995
	bacterial nitrilase	canola*	2000
Glufosinate	<i>bar</i> gene	canola	1995
	<i>bar</i> gene	maize	1997
	<i>bar</i> gene	cotton	2004
Glyphosate	CP4 EPSPS	soybean	1996
	CP4 EPSPS + GOX	canola	1996
	CP4 EPSPS	cotton	1997
	CP4 or GA21 EPSPS	maize	1998
	CP4 EPSPS	sugarbeet**	1999

\*no longer available

\*\*never grown

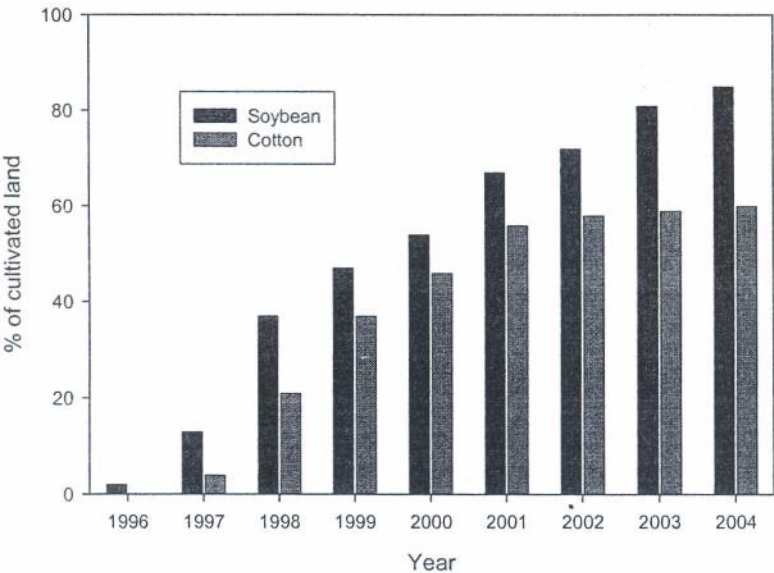


Figure 4. Adoption of glyphosate-resistant soybean and cotton in the U.S.A. by year. (Adapted from Duke, 2005)

As mentioned earlier, during the golden age of herbicides, most of the membership of weed science societies in developed countries was employed by herbicide companies. As these companies consolidated, the number of weed scientists employed by them was reduced dramatically. This led to attrition in membership in weed science societies and concern for their future. Research funding



from companies for university researchers was greatly reduced, and funding from government programs was very limited, compared to funding for other pest management disciplines. There was a view by some non-weed scientists that HRCs had solved farmers' weed problems, so that there was less need for the discipline. Another justification by some for the relatively poor funding for weed science was that there were few weed scientists that could do the type of basic research deserving of public funding. The facts that weeds usually cost farmers more to manage than other pests and that crop losses due to weed can often exceed those due to other pests did not seem sufficient reason for parity in research funding.

Table 2. Adoption of HRCs worldwide in 2004 (adapted from ISAAA 2005).

Herbicide-resistant crop	Million ha	% adoption worldwide
Soybean	48.4	60
Cotton	4.5	6
Maize	8.1	9
Canola	4.3	5

Nevertheless, industry attrition was leaving a research gap that was not being filled by public scientists. For example, the adoption of glyphosate-resistant soybeans led to a dramatic shift to reduced- and zero-tillage agriculture in the U.S. (Figure 5) and Argentina. Reduced tillage reduces soil erosion, fossil fuel use, air pollution, and water pollution (Holland 2004). Shockingly little research has been funded to evaluate the effects of the HRC-driven trend toward reduced tillage on agricultural ecosystems. The studies that have been done on the risks and environmental effects of HRCs have overwhelmingly indicated that this technology is more environmentally friendly than the technology that it replaces (Duke and Cerdeira 2005; Wauchope et al. 2003). HRCs have not been without problems. In Canada, transgene flow from herbicide-resistant canola to non-herbicide-resistant canola has been a problem for farmers that want to keep their crop transgene free. There has been transgene flow from glyphosate-resistant canola to related weedy species (Warwick et al. 2003).

Other research needs persist. Weed problems are as great as ever in crops for which there were no HRCs. Despite the rapid adoption of a few HRCs, the introduction of new HRCs slowed to a trickle around 2000 (Duke 2005). Note that only one new HRC and no new HRC transgenes have been introduced since then (Table 1). Both bromoxynil-resistant crops have been removed from the market since 2000 for economic reasons. Glyphosate-resistant sugar beets have been available, but farmers have not grown them because of concern that the confectionary and prepared food industries would not accept sugar from transgenic crops. Attempts to introduce glyphosate-resistant wheat have been abandoned for the time being because of similar concerns. The introduction of new herbicides has slowed to a trickle, largely due to the pressure from the HRCs that remain on the market, the increased cost of regulatory approval, and the fact the that the herbicide market is mature. Some valuable older herbicides are being lost from the market because they either cannot pass new regulatory requirements or the cost of reapproval is not economically attractive for these generic products. These problems, combined with increasing evolved herbicide resistance, mean that farmers may have fewer weed management tools and more weed management problems in the future.



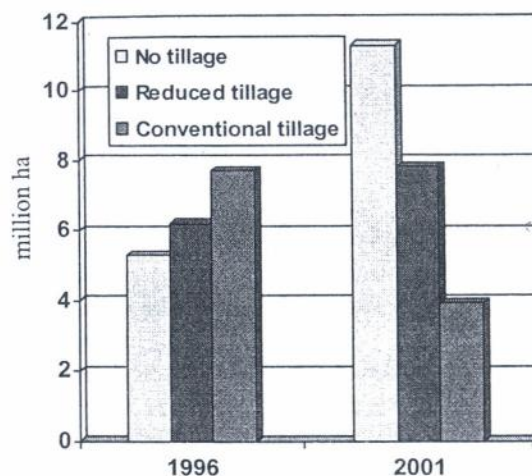


Figure 5. Soybean tillage methods by hectares farmed in the U.S.A. in 1996 and 2001. In 1996 and 2001, there were 19.2 and 23 million ha, respectively, of soybeans grown. (Drawn from American Soybean Association, 2001 data).

Weed scientists have recognized this dilemma. Quality research in the areas of precision agriculture as it relates to weed management, weed biocontrol, integrated weed and pest management, and basic weed biology and ecology is being conducted, and there are strong efforts by weed science societies find ways to increase funding for these research areas. Non-herbicide-oriented molecular biology approaches to weed science and weed management problems are in their infancy (Basu et al. 2003; Duke 2003).

During the current era of weed science, awareness of the incredible ecological damage to native ecosystems caused by invasive, exotic plant species has become clear. Other disciplines have studied the phenomenon, but have offered few realistic solutions. This was recognized as a large problem for which weed scientists have the appropriate expertise for solving. First, in Australia, and then in the U.S., this aspect of weed science has become a growing emphasis for weed science research during the current era of weed science.

### CHALLENGES FOR THE 21<sup>st</sup> CENTURY

Weeds remain the most costly pest to crop production, yet weed science is the most poorly funded discipline of the three major pest sciences. In the developed world, and increasingly in the developing world, herbicides are by far the dominant technology for managing weeds of major crops. The large markets for herbicides in major crops have made it profitable for companies to develop both herbicides and HRC varieties for these crops. However, as discussed above, the cost of introducing herbicides has increased dramatically, and the risk and cost of development of HRCs has inhibited the introduction of new examples of this technology (Devine 2005). Weeds are evolving resistance to glyphosate in glyphosate-resistant crops (Owen and Zelaya 2005). Thus, the clear advantage of the existing HRCs may diminish within a few years. The monstrous ecological damage to native habitats done by exotic, invasive weeds continues virtually unabated.

Clearly, there is a huge and growing need for weed science research to solve the current and evolving weed management problems. There is little or no economic incentive for much of this research to be funded by the private sector. Thus, will be left to university and government weed scientists to solve these problems. Better communication among the international weed science community would be helpful in leveraging resources and finding solutions. Considering the

tremendous research needs, weed science as a discipline should have a busy future in this century, although I expect that it will drift away from its herbicide-driven orientation that brought it into being.

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**Abstract:** The discovery of 2,4-D in the early 1940's and its commercial release after World War II, as well as its rapid acceptance by farmers has revolutionized selective weed control worldwide. Its launching has also accelerated weed management by the use of herbicides. Even six decades after its introduction, 2,4-D still continues to be the most commonly and widely used herbicide all over the world as it provides relatively inexpensive, extremely effective and selective post-emergence control of broadleaf weeds in grass crops, non-crop lands and aquatic weeds. It is also the most thoroughly researched herbicide in the world, in terms of human health, effect on non-target organisms and environmental safety. Though no exact figure has been placed on the economical benefits provided to world agriculture by the introduction of this herbicide, which started the herbicide revolution in developed countries, the estimated value to humankind from reduced labor requirements alone is in the billions of dollars annually. The paper traces the history of events that led to the discovery and development of 2,4-D and the subsequent research efforts in developing the chemical into a herbicide.

**Key words:** 2,4-D, World War II, Vietnam War, Agent Orange

## INTRODUCTION

The use of 2,4-D as a herbicide after World War II heralded the beginning of modern weed science and revolutionized agriculture in developed countries. After 50 years of use, 2,4-D is still the third most widely used herbicide in the United States and Canada, and the most used herbicide worldwide. It is registered for broadleaf weed control in about 65 crops and many non-crop situations. Its major uses in agriculture are on wheat, rice, corn, sorghum, small grains, sugarcane low-till soybean, rangeland and pasture. It is also used on rights-off-way, roadsides, non-crop areas, forestry, lawn and turf care and on aquatic weeds offering effective weed control options at very affordable cost. The research leading to the discovery and subsequent use of 2,4-D as herbicide during those turbulent years of World War II is truly interesting. It is an illustration of how sound fundamental research may lead to the solution of a practical problem. It is a tribute to all those great scientists who worked with great dedication and sincerity.

Basic research that led to 2,4-D

Botanists have long been intrigued with plant shoot and root growth and the mechanisms causing plants to respond to stimuli. Went and Thimann (1937) have discussed in detail the observations made by eminent botanists of the erstwhile era. Darwin in 1880 reported that in phototropic response plants. Boysen-Jensen from Denmark in 1911 observed that when an oat coleoptile exposed to directional light was excised and re-attached to unexposed oat tips, the bending still occurred. He concluded that the influence from coleoptile tips was thus chemical not physical. Went from the Netherlands in 1926 collected the chemical from oat coleoptile tips and found it to be an active plant growth regulator. Kögl and Haagen-Smit from the Netherlands in 1934 reported the isolation of indoleacetic acid (IAA) from plants and identified it as the principal naturally occurring hormone (later called an auxin) in plants. Because IAA was unstable outside of plants, researchers began synthesizing and investigating the effect of IAA derivatives and homologs on plant growth activity. Zimmerman and Wilcoxson (1935) reported the discovery of phenylacetic acid and naphthylacetic acid, which affected plants by preventing premature fruit drop, inducing rooting, accelerating fruit ripening, and causing seedless tomatoes. Pokorny (1941) from the United States



synthesized 2,4-D and 2,4,5-T while looking in vain for a fungicide. Zimmerman and Hitchcock (1942) reported that 2,4-D was 300 times more potent than indolebutyric acid, the major plant growth regulator at that time, for inducing seedless tomatoes.

The discovery of 2,4-D could be considered more fortuitous than systematic. Went stated that, "When I worked 25 years ago with the growth hormone (2,4-D), I had many wild ideas about what it might do once it was available in large quantities, but I never dreamed that it would lead to the development of weed killers. This is an excellent example, how fundamental research may lead to the solution of very practical problems." (Andersen 1991). Therefore, basic research on plant growth regulators during the 1930's and earlier facilitated the development of selective phenoxy herbicides particularly 2,4-D in the 1940's. Thus, the discipline of weed science evolved from the field of economic botany or more specifically the study of plant growth regulators.

#### Military interest in 2,4-D

Interest in 2,4-D within the scientific community seemed to lose momentum during World War II when both United States and England scientists initiated secret biological warfare research on plant growth regulators with the objective of destroying enemy crops. Kraus at the University of Chicago, USA had observed since 1936 that certain growth regulators were phytotoxic and in 1941, he proposed that they might work as herbicides (Peterson 1967). Secretary of War H. L. Stimson on consultation with Kraus and other prominent scientists initiated biological warfare research with special emphasis on crop destroying chemicals in 1942 at Camp Detrick (later renamed Fort Detrick) in Frederick, Maryland. In March 1943, the United States Army paid the University of Chicago \$3500 for herbicide research completed by Kraus (Peterson 1967). In January 1944, research at Camp Detrick was accelerated on crop destroying herbicides (USDA-NAPIAP 1996).

While laboratory facilities were under construction at Camp Detrick, E. J. Kraus at the University of Chicago and J. W. Mitchell with the USDA at Beltsville, Maryland, were supported by contract to continue studies of the herbicidal effects of plant growth regulators. Somewhat later, a synthesis program was commenced under contract with A. S. Newman at Ohio State University.

In 1944, the biological warfare effort at Camp Detrick was stepped up by the Special Projects Division of the Chemical Warfare Service, U.S. Army under a heavy cloak of secrecy. Herbicide research with crop destruction as the objective was vigorously pursued under the direction of A.G. Norman by a team of about a dozen scientists drawn from other assignments in the services. The research program was broadly conceived to throw light on the nature of plant responses, plant and herbicide specificities, environmental effects, effective rates of application, spray volumes and droplet size, co-agents and carriers, etc. Although attention was soon centered on halogen-substituted aryloxy acids, particularly the phenoxyacetics, many new compounds and derivatives were synthesized and screened for growth regulating properties. Sufficient amounts of the more active compounds, such as 2,4-D, 2,4,5-T and others, were procured for field trials, which included aerial application. Attention was also given to forest defoliants for the purpose of reducing cover of enemy defense positions in the expected assault on Japan.

All the research conducted at Camp Detrick was kept under military secrecy until the end of World War II. The entire June 1946 (Vol. 107) issue of the *Botanical Gazette* consisted of papers from Camp Detrick scientists. These papers reported well-designed experimental observations likely to be of general interest. Much additional information was obtained, but not all in the rigorous experimental detail justifying journal publication. Additional papers appeared later in the *Agronomy Journal*, *American Journal of Botany*, *Science*, *Weeds*, and other journals. The research at Camp Detrick formed a major part of the beginning of modern weed science and represented a significant part of the foundation knowledge on weed control with herbicides.



Among the accomplishments of the Camp Detrick scientists were the development of methods for evaluating over 1000 chemical compounds for their herbicidal properties, determining histological effects of 2,4-D and 2,4,5-T, cytological effects of protham, demonstration that weeds can be controlled with ultra low-volume sprays, investigating the importance of carriers for 2,4-D (solvents, surfactants, granules, etc.), defining the selective action of sprays on broadleaf plants, identifying the herbicidal effects of soil and water applications, and determining the dosages required.

### 2,4-D as a biological warfare weapon

The development of organic herbicides was greatly facilitated during World War II because of their military potential as biological warfare weapons (Peterson 1967). After the war the Camp Detrick program was broadened to encompass various types of growth responses in plants. Synthesis and screening were continued and improved procedures for assessing herbicidal activity were devised. Camp Detrick research was subsequently supplemented by contracts with universities, and research was initiated on abscission and the gibberellins. Leaf abscission and the testing of defoliant for woody species were pursued during 1961-72 under the direction of C. E. Minarik, which led to the military use of Agent Orange in Vietnam (Davis 1979). All biocidal research activities were terminated in 1972, following a declaration by President Richard Nixon that the United States would no longer conduct biological warfare research or develop biological weapons.

### Agent Orange

Phenoxy herbicides earned notoriety and adverse publicity when Agent Orange was used by the US military during the second Indochina war as a forest defoliant to prevent guerilla fighters from hiding. Agent Orange is a roughly 1:1 mixture of 2,4-D and 2,4,5-T and as it came in drums with orange stripes, it was called Agent Orange. In Vietnam, military applications were made at the rate of three gallons per acre containing approximately 12 pounds of 2,4-D and 13.8 pounds of 2,4,5-T, which were at least six to 25 times the rate used in agriculture. Operation Hades, later changed to Operation Ranch Hand, sprayed about 19 million gallons of defoliant on about 6 million acres of forest in Vietnam between 1962 to 1966. Besides Agent Orange, Agent Blue (cacodylic acid), Agent White (4:1 mixture of 2,4-D and picloram), malathion and others were also employed in the war.

The extensive and indiscriminate use of Agent Orange caused untold misery and suffering to the Vietnamese people and US war veterans. The Agent Orange was found to contain toxic dioxin contaminant which was responsible for all the adverse effects. The particular dioxins present in Agent Orange was 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD, which is highly toxic and persistent in the environment for years. Millions of Vietnamese people were exposed to Agent Orange during the war. It is estimated that today approximately a million people have disabilities or other health problems associated with Agent Orange.

### Commercial development of 2,4-D as a herbicide

Scientists in the United States (Marth and Mitchell 1944; Zimmerman and Hitchcock 1942) and England (Blackman 1945; Slade et al. 1945) continued to do limited research with 2,4-D during World War II. Research in England stressed the development of MCPA, a herbicide similar to 2,4-D. MCPA was favored in England because of a plentiful supply of cresol extracted from coal and used to make MCPA versus a plentiful supply of phenol from oil refineries in the United States, which was used to make 2,4-D.

In June 1944, Mitchell and Hamner with the United States Department of Agriculture (USDA) Bureau of Plant Industry at Beltsville, Maryland made the first public announcement of using 2,4-D as a herbicide that exhibited differential weed kill (Marth and Mitchell 1944). Hamner and Tukey



(1944) caused considerable public interest when they reported in 1944 that within 10 days after spraying field bindweed with 2,4-D, the weeds died. English researchers had worked with MCPA, 2,4-D, and other plant growth regulators during the early 1940's but delayed publishing their results until after World War II (Blackman 1945; Slade et al. 1945).

Marth and Mitchell (1944) sprayed 2,4-D on a lawn infested with dandelions at Beltsville, Maryland and achieved selective broadleaf weed control with no injury to the lawn grasses. Mitchell et al. (1944) then conducted additional studies on a golf course and reported selective broadleaf weed control in turf grass.

The popular abbreviation, 2,4-D, first appeared in the literature in 1945 during the second annual North Central Weed Control Conference (NCWCC) meeting at St. Paul, Minnesota (Timmons 1945). The data from 30 cooperators with 140 experiments conducted in the United States and 36 experiments conducted in Canada were reported.

The original patent of 2,4-D and related compounds (U.S. Patent Number 2,322,761) was as plant growth regulators by John F. Lontz and assigned to E.I. du Pont de Nemours and Company dated June 29, 1943 (Peterson 1967). Franklin D. Jones with the American Chemical Paint Company (ACPC) filed on March 20, 1944 and received use patent 2,390,941 in December 1945 for 2,4-D as a herbicide. In June 1945, ACPC marketed 2,4-D under the brand name 'Weedone', which was the first selective, systemic herbicide produced and sold on a commercial scale. In 1945, *Weedone* sold poorly but it picked up the following year with 150,000 acres being treated. The cost fell from \$3.00 per pound in 1945 to \$0.50 per pound in 1950. The production rose from 917,000 pounds in 1945 to 5,466,000 pounds in 1946 and 14, 36 and 54 million pounds in 1950, 1960 and 1964 respectively (Peterson 1967).

#### Initial toxicity studies with 2,4-D

Recognizing the importance of 2,4-D, the USDA ordered human toxicity studies in 1945 and all of these proved negative. Mitchell et al. (1946) reported that treating pastures with twice the normal use rates of 2,4-D produced no toxic effects in sheep and cows grazing on them, and feeding a cow 5.5 g of pure 2,4-D per day for 3 months produced no ill effects to the cow or her calf fed entirely on milk from that cow. Kraus even announced that he had personally eaten one-half gram of 2,4-D per day for 3 weeks with absolutely no ill effects (Kephart 1945).

#### Impact of 2,4-D on weed science discipline

The phenoxy herbicides were so effective and economical for selective control of broad leaved weeds in cereals that they put selective weed control in the "public spotlight" worldwide. The phenoxy herbicides stimulated chemical industry involvement in herbicide development because industry personnel recognized the weed control need in agriculture and non-crop land and the profit potential of this technology. The spectacular results with 2,4-D stimulated an industry-wide search for additional herbicides, which ushered in the herbicide era that has lasted for six decades. The industry rapidly expanded personnel in herbicide development, manufacture, and sales in order to exploit the market potential of herbicides that increased rapidly since 1945. The phenoxy herbicides had such an impact on weed technology that most weed scientists became engrossed in chemical weed control research and education endeavors.

2,4-D raised weed management to a higher level, and producers accepted this new technology rapidly and with enthusiasm, because they were able to achieve better weed control with less labor and expense (Timmons 1970). Phenoxy herbicides particularly 2,4-D provided effective, economical, and selective broadleaf weed control in small grains, corn, sorghum, pastureland, rangeland, and turf grass.



Farmers began expecting selective herbicides for numerous other cropping systems, and the agricultural chemical industry responded positively because of the profit potential. Herbicides such as 2,4-D and MCPA established the concept of post-emergence, selective weed control; chloramben and atrazine established pre-emergence, selective weed control; and EPTC and trifluralin established pre-plant soil incorporated, selective weed control. Examples of changes in herbicide technology over the past six decades are inorganic to organic herbicides, non-selective to selective herbicides, post-emergence to soil-applied (some incorporated) herbicides and now back again, mechanical to more chemical control of weeds, single herbicides to mixtures and even multiple applications, formulated herbicides used alone to utilizing various herbicide additives, and crops bred or transformed for tolerance to specific herbicides rather than herbicides being screened for crop tolerance (Burnside 1993).

#### Impact of 2,4-D on public health and environment

Detailed discussion on the effect of 2,4-D on public health and environmental quality is outside the scope of this paper. However, the review of 2,4-D epidemiology and toxicology data packages by the U.S. Department of Agriculture has concluded that after 50 years of extensive use, "The phenoxy herbicides are low in toxicity to humans and animals. No scientifically documented health risks, either acute or chronic, exist from the approved uses of the phenoxy herbicides." (USDA-NAPIAP 1996). Typically, the general public is not exposed to unsafe levels of the phenoxy herbicides. The report further states that switching to alternative herbicides does not guarantee increased safety to human health as compared to the enviable human safety record achieved with the phenoxy herbicides. Recent reviews by regulatory agencies such as the WHO, U.S. EPA and the European Commission agree that 2,4-D is not an animal carcinogen, mutagen or teratogen.

#### Economic impact of 2,4-D

Even after its first use in 1945, 2,4-D still remains as one of the most widely used herbicides offering effective and weed control in several crop and non-crop situations worldwide. However, the agrochemicals are subjected to close scrutiny for their alleged negative impact on public health and environment. Several countries are phasing out or re-registering pesticides after thorough scientific review of their relative risks and benefits to the society. A case study made by the U.S. Department of Agriculture with its National Agricultural Pesticide Impact Assessment Program (NAPIAP) has brought to the fore the impact of banning phenoxy herbicides on US agriculture (NAPIAP Report 1995). The net societal loss (production and consumer costs) of banning the 2,4-D alone would be \$1.68 billion annually. The value for all phenoxy herbicides would be \$2.559 billion annually. Further it would impact the aesthetic value and the quality of surroundings, water bodies. Controlling the development of herbicide resistance in weeds would be more difficult because the phenoxy herbicides have shown little potential for development of weed resistance, and they are widely used with other herbicides to expand the weed control spectrum. The industry would lose \$171 million in annual retail sales of phenoxy herbicides, but could compete for the new market created for alternative herbicides worth \$914 million. Minor crops would suffer more production losses these low-acreage, high-value crops do not often justify the cost of registering alternative herbicides.

Similarly, an assessment made in Canada in 1988 concluded that the net benefits of 2,4-D in Canada totaled a third of a billion dollars annually (Industry Task Force II on 2,4-D Research Data Web Site). A worldwide study of the benefits of 2,4-D measured in terms of increased food production and lower food prices has never been done, although those benefits are known to be enormous. 2,4-D has for the past fifty years, been a major tool in the continuing fight to reduce world hunger



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**Abstract:** Research on plant growth regulator started in the 1920s, with an accidental success in the utilization of 2,4-D as an organic selective herbicide. In the subsequent decades of 1930s and 1940s, scientists used 2,4-D for studying plant growth regulator or as a fungicide. In the mean time, American and British military scientists were more interested in turning 2,4-D into a chemical which can be used to destroy crops, vegetation and forests in Japan. However, their ambition was not realized until the end of the Second World War. The U.S. military used herbicides in large scale as chemical weapon for the first time in Vietnam during the war. From 1962 to 1971, American military had sprayed approximately 77 million liters of defoliant containing mainly 2,4-D, 2,4,5-T, and picloram onto Vietnamese soil. The most dangerous contaminant in those herbicides is dioxin. Herbicides destroyed forests, crops, vegetation causing soil erosion, depletion of water resource and environmental pollution. The contaminated dioxins have caused direct and long term effects on human health in both Vietnamese people and foreign veterans.

**Key words:** 2,4-D, 2,4,5-T, Agent Orange, Vietnam war, defoliant, birth defect.

## THE DISCOVERY OF 2,4-D

At the end of the 19<sup>th</sup> century, while sodium chloride salt and ash were used to control weeds along roadsides, an inorganic selective herbicide was discovered by chance in France. Few French farmers sprayed a Bordeaux solution to control downy mildew disease in their grape plantation and they observed that some drifted drops of the solution could kill broadleaved weeds in the ground. Later the component of copper sulphate in the Bordeaux solution was found to be the agent killing weeds. Research experiments done in France, Germany, and the United States led to the conclusion that  $\text{CuSO}_4$  which can be used as an inorganic selective herbicide for controlling weeds in wheat, barley, oat. The popular herbicides used during 1896-1910 were: acid sulphuric, iron sulphate, copper nitrate.... From 1930-1940, both inorganic and organic selective herbicides were used. Boron compound, ammonium sulphate, sodium chlorate, carbon bisulphide, sodium arsenite, dinitrophenols were used in a limited areas of America and Europe.

A real breakthrough in selective chemical weed control was achieved in 1945 with the announcement of simultaneous discovery of 2,4-D and MCPA in the USA and England, respectively. In 1935, in the US, Zimmerman and Wilcoxson reported that phenylacetic acid and naphthyl acetic acid preventing premature fruit drop, inducing rooting, accelerating fruit ripening and causing seedless tomatoes. In 1941 in England, while conducting pot experiments on the effects of naphthyl acetic acid (NAA) as plant growth regulator on wheat, W.G. Templeman found by chance that NAA killed a few wild mustard plants (*Brassica kaber*) growing as weeds in wheat pots. This induced him and W.A. Sexton at the Jealotts Hill Research Station to search for some more potent growth regulators than NAA for broadleaf weed control in small grain. Their elaborate trials finally yielded 2,4-D and MCPA as potent growth regulator type of herbicides, the scientists were looking for (Gupta, 2000). In 1941 in the US, Pokorny for the first time synthesized 2,4-dichloroacetic acid (2,4-D) and 2,4,5-trichloroacetic acid (2,4,5-T). His intention was to study the effect of 2,4-D and 2,4,5-T on fungal diseases to find out the new fungicides (Pokorny, 1941). In 1942, Zimmerman and Hichkock concluded that 2,4-D was an effective plant growth regulator on the production of seedless tomatoes. The potent effects of its salts on plant growth were first



described by both of them (Zimmerman and Hitchcock, 1942). In the community of civilian scientists, the discovery of 2,4-D as herbicide for crop protection was fortuitous. The credit for introducing 2,4-D as a herbicide goes to Marth and Mitchell of USA who in 1944 reported selective weed control by 2,4-D in bluegrass lawn (Marth and Mitchell, 1944) and Hamner and Tukey also of USA who in the same year used 2,4-D successfully in field weed control (Hamner and Tukey, 1944). Thus, demonstration of herbicidal properties of 2,4-D in field weed control represented a technological breakthrough that heralded the modern era of weed control.

Although based on the same research results on plant growth regulators but the military scientists did research with different goal. During the time of World War II, USA and England had a common project on biological chemical warfare from plant growth regulators to kill crops for the depletion of enemy foods. In 1936 at Chicago University, Kraus observed that some plant regulators were toxic to plants. He suggested to study them in details to kill vegetation. Mr. H.L. Stimson, Secretary of Military Affairs at that time, discussed with Kraus and other scientists to form a project aiming to find out a chemical for destroying crops. This project was initiated and functioned in 1942 at Detrick Station, Maryland State. The main objective of this project was to find out a chemical which could kill rice and other crops to deplete the food reserve of Japan. In 1944, this project on chemical weapons succeeded. A substantial amount of 2,4-D and 2,4,5-T were synthesized and sprayed in large scale for testing, even by airplanes. The other intention was the use of those herbicides as defoliants to cause leaf drop in forests to clear the defence places of Japanese soldiers. However, after the discussion between the US President, Dwight Roosevelt and General William D. Leahy, Chief Representative in the White House, they decided not to use this chemical warfare in the war against Japan.

### **Large scale spraying of 2,4-D and 2,4,5-T in Vietnam**

At the end of the 1950's, after the success of England in the utilization of 2,4,5-T for crop destroying in Malaya (Department of Defense, 1970), the US Department of Defense (DoD) assigned the Advanced Research Project Agency to conduct research and development of herbicides for military use. A large-scale testing on Agent Purple was carried out successfully at Drum Station (New York State) in 1959 and this model was applied in Vietnam some years later (Wright Air Development Center, 1951). On November 20, 1961, the US President, John F. Kennedy approved a plan in which American military was to carry out the defoliant activities on Vietnam forests (NSAM, 1961). This decision was enthusiastically supported by the then President of the South Vietnam, Ngo Dinh Diem. American politicians and military service men argued that Agent Orange was used because they could clear the forests, exposing the hiding places of Vietnamese revolutionary forces so that American soldiers could cut the supplying source from the North through Truong Son trail by heavy aerial bombardment. Those chemicals were transported to the South Vietnam from August to December 1961.

Before application in Vietnam, US military scientists knew very well that Agent Orange was a very toxic substance. Dr. Jame R. Clary, who worked at the Chemical Weapons Branch of the Airforce Armament Development Laboratory, Florida, wrote a confirmed letter to one Member of Parliament, Mr. Tom Daschle on 9 September 1998. In that letter, he stated: *"When we (military scientists) initiated the herbicide program in the 1960's, we were aware of the potential for damage due to dioxin contaminating in the herbicides. We were even aware that the military formulation had a higher dioxin concentration than the civilian version due to the lower cost and speed of manufacture. However, because the material was to be used on the enemy, none of us were overly concerned. We never considered a scenario in which our own personnel would become contaminated with the herbicide. And, if we had, we would have expected our own government to give assistance to veterans so contaminated"*. In Vietnam, the chemicals were transferred to the government of the South Vietnam at the time in color coded drum (each with 250 liters). There



were Agent Orange (50% 2,4-D+ 50% 2,4,5-T), Agent White ( 80% 2,4-D + 20% pichloram), Agent Blue (cacodylic acid), Agent Pink, Agent Green, and Agent Purple. Most of the chemicals had the major compound, namely 2,4,5-T.

The program of spraying defoliants in Vietnam was named << Operation Trail Dust>>. There were many operations under this program, among those; the <<Operation Ranch Hand>> was the largest and logest campaign, with 95% of the chemicals being sprayed under this operation. Since 1962, US Airforce began to spray herbicides in large scale in the Southern and Central parts of South Vietnam. Majority (90%) of Agent Orange was aerial blanket sprayed by C123 airplanes and the rest (10%) by helicopters, trucks and hand moved devices. This operation was ordered to terminate by President Richard Nixon in July 1971 (Memo, 1971) after the resolution of the General Assembly of the United Nations declaring that Operation Ranch Hand in Vietnam was illegal. American destroyed 14% of forest areas in the South Vietnam in 9 years.

### **Consequences of herbicides and contaminated dioxins**

Vegetation including forests in South Vietnam was severely affected by defoliants. High value dicot perennial huge plants were destroyed and replaced by less value monocot plants such as bamboos, grasses, etc. Vegetation was cleared and therefore soil erosion occurred, water reservation was reduced and depleted, soil and water were polluted by toxic substances. A large number of economic plants and crops were destroyed or damaged due to direct effects or by drift movements of herbicides.

Dioxin is a group of many molecules including 75 polychlorinated dibenzo-p-dioxins and 135 polychlorinated dibenzo-p-furans. Among them, the most toxic molecule is 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (2, 3, 7, 8 TCDD). Dioxins appear anywhere in our planet, in the air, water, soil, organic waste... Dioxins are also traced in the by-products of industry, in the furnace of hospital waste burning. Forest fires also create dioxins. The amount of dioxins produced in a large forest fire ranges from few grams to several kilograms of dioxins. Dioxin crystal is a non-colored or white colored substance. It melts at 295°C and degrades at 500°C. Its half life is 6 to 10 years. Dioxin is a lipophilic substance and it is difficult to dissolve in water but it has a close linkage with lipid. In rivers and ponds, lakes, etc dioxins usually exist in deposited wastes and other organic substances. Popular human foods such as meat, vegetable, sea foods (fishes, shrimps, squids...) also contain a little amount of dioxins. Herbicides used by American in Vietnam War were contaminated with a substantial dioxins during the process of manufacturing. The amount of dioxins varied from herbicides to herbicides. Agent Pink contains 45 ppm while Agent Orange 13 ppm.

The total contaminated dioxins sprayed in Vietnam was estimated at 366 kilograms approximately. Vietnamese people who lived in the sprayed areas were heavily affected by dioxins. However, the research results on this mass of people are very limited. Some major factors affecting this situation are: (i) The government of Vietnam does not have sufficient fund to carry out this costly research (the cost to analyse one sample of dioxin is about USD 1000), (ii) the US government is not willing to do this kind of research, (iii) About 40 years after affecting, many severely affected people were dead already. However, there are some valuable results have been reported in Italy, USA, Australia, Vietnam.. Recently, some research findings on this issue have been released.

On 10 July 1976, the Givaudan Company locating in the Seveso region of Italy was fired and exploded. This company manufactures one kind of herbicide namely 2,4,5-trichlorophenol (TCP). This explosion released an approximate amount of 30 kg dioxins. The primary analysis showed that the concentration of dioxins in the soil was 51.3 ppt. The government of Italy decided to form a group of scientists and doctors to study the effects of dioxins on people of Seveso and surrounding areas. After 20 years of research on 328 boys and 364 girls born from 239 fathers and 296 mothers,



they concluded that high dioxin concentration in the fathers' blood ranging from 61 ppt to 117 ppt, the birth rate of boy would be 47%. Similarly, the rate was only 40% if the concentration was more than 118 ppt. The affected youth also had the low male birth rate at 40% (Paolo Mocarelli et al, 2000). In 1996, 20 years after the explosion, 62 affected people and 58 normal ones were participated in a program to study the effects of dioxins on human immune systems. Many kinds of antibody were analysed to compare between the two groups. Results revealed that the affected groups had lower antibody IgG ( $1142 \text{ mg dL}^{-1}$ ) as compared to the normal ones ( $1403 \text{ mg dL}^{-1}$ ). However, there was no significant difference in terms of other antibodies such as IgM, IgA, C3 and C4. There was a significant correlation between dioxin affected people with the cancer diseases such as non-Hodgkin Lymphoma. Settlers in polluted areas of Seveso had a 30% higher rate of cancer infection as compared to the people in dioxin free areas. In women, the percentage of suffered people by multiple myeloma disease was 3,7 times higher than the normal women. However in case of men, there is no difference among the two groups.

Many research works have been carried out on American and Australian veterans, workers in herbicide factories in the US. In January 2003, the Institute of Medicine (part of the National Academy of Science of the United States) after evaluating of six studies related to the effects of dioxins on human health, declared that there were sufficient evidence to conclude that dioxin was causally associated with chronic lymphocytic leukemia. Another study on Operation Ranch Hand veterans found that dioxin concentration in blood among these veterans was still high. In addition, the risk of diabetes mellitus and glucose levels in these veterans were also significantly higher than veterans who did not involve in the Operation (Henriksen et al, 1997). Workers in companies producing pesticide had higher risk of soft tissue sarcoma than normal individuals who were not exposed to herbicides. US veterans with high dioxin concentrations in blood had 3.7 times higher risk of lung cancer than average (Michalak et al, 2000). Children of affected veterans (eg high dioxin levels) had 2.7 times higher risk of acute myelogenous (Buckley et al, 1989). Recently, the US Department of Veterans Affairs has agreed to compensate to any Vietnam veterans who has chloracne, non-Hodgkin lymphoma, soft tissue sarcoma, Hodgkin lymphoma, multiple myeloma, respiration system cancer, prostate cancer, porphyria cutanea tarda. In addition, their children with spina bifida are also compensated.

According to guideline of the World Health Organization (WHO), the acceptable limit of dioxins in human body is 5-15 parts per American trillion (5-15 per 1,000,000,000,000 = 5-15 ppt). In Vietnamese women who live in affected areas or who were exposed to Agent Orange during the war, the dioxin concentrations in milk were very high: 946 ppt (the highest one was 1,832 ppt). However, it appears that the levels have reduced over time. The corresponding data in 1973 was 226 ppt (n=3) and in 1988 it was 6 ppt (n=3). In 1991-1992, a large research project involving 2,720 Vietnamese individuals concluded that the dioxin concentration in blood in Northern, Central and Southern residents were 2.2 ppt, 13.2 ppt, and 12.9 ppt, respectively, consistently with the pattern and intensity of defoliant spray during the war. In the Southern region, some areas with high levels of dioxin were: Da Nang (19 ppt), Bien Hoa (28 ppt), Song be (32 ppt), Tra Noc, Can Tho (33 ppt).

In 2003, American and Vietnamese researchers analysed dioxin concentration in food samples in Bien Hoa and found that duck fat had the highest TCDD concentration with 304 ppt (the average of 2 samples). The dioxin concentration was also high in frog (80 ppt, n=1), fish (13.2 ppt, n=5), but was lower in chicken (5.6 ppt, n=4), or pigs (meat, n=2) and cow meat (0.08 ppt, n=2) (Schechter et al, 2003).

Prof Nguyen van Tuan has reviewed several studies on the association between Agent Orange / dioxins and birth defects. Among the 313 papers in Medline, he found 9 studies that met his criteria (eg research on human, clear definition on the effect on human health, remove the duplicate papers, etc) and 13 conference abstracts. Based on these studies, he performed a meta-analysis and found



that the risk of birth defects in Vietnamese parents was higher than that in American parents. Vietnamese parents affected by or exposed to Agent Orange were 3.6 times more likely to have children with birth defects (Nguyen Van Tuan, 2003).

## SUMMARY AND CONCLUSION

One of the most significant "legacy" of the war is the lasting environmental and health consequences of Agent Orange and herbicides used by the American military more than 30 years ago. Although the war was over three decades ago, millions of victims, including Vietnamese civilians and ex-servicemen in the American and Vietnamese military are still suffered from being exposed to the chemical. As many as 4.8 million individuals in 25,500 villages have been affected, among whom many have suffered from numerous diseases in three generations. Some babies were born without eyes or arms, or were missing internal organs.

The effect on human health is, nevertheless, only one aspect of the problem, the destruction of environment due to the uncontrolled use of chemicals during the war is another major aspect of the war. It has been estimated that more than 2.6 million ha. of land have been damaged or affected. Although the war has been over for 30 years, the concentration of dioxin found in soils in those affected areas is still considerably high.

During the past 40 years or so, an enormous amount of scientific data has been generated from numerous epidemiological, clinical and basic studies on the effects of Agent Orange. These data have clearly indicated that exposure to dioxin or Agent Orange is causally related to a number of cancer diseases, diabetes, spinal bifida, and possibly birth defects. These data have also formed the basis for the US government to make decision of compensation to their ex-servicemen. However, none of the Vietnamese victims has received any help from the US government or her allies during the war.

Recently, the Vietnamese Association for Victims of Agent Orange/Dioxin (VAVA) filed a suit in a New York court against the companies that produced Agent Orange during the war. On March 10, 2005 Judge Jack Weinstein ruled against VAVA and took the side of the representatives of the Departments of State and Justice, declaring that the plaintiff's case had no merit and denied their suit. Thus, despite the arguments advanced by the American veterans win in court, their arguments did not play in favour of Vietnamese civilians! It is perhaps time that the world and the US government should realise that there is a large group of victims in Vietnam (much larger than the US victims) who are still terribly suffered from the chemicals used by the US military during the war. They, like the US ex-servicemen, also want justice to be done.

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## An overview of herbicide resistance of sulfonylureas in Japan and Asia

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**Abstract:** Resistance to sulfonylureas (SU) has developed in nine annual weeds and three perennial weeds found in rice cultivated areas in Japan. SU-resistant biotypes in five annual weeds, *Monochoria korsakowii*, *M. vaginalis*, *Lindernia dubia*, *Rotala indica* and *Cyperus difformis*, and three perennial weeds, *Scirpus planiculmis*, *Scirpus juncooides*, and *Sagittaria pigmaea*, at Seocheon as of 2004 have been confirmed in paddy fields in Korea, as well as in several species in South-East Asia. For some of the resistant biotypes, the herbicide dose for 50% mortality was found to be 100 to 1000 times higher than for the susceptible biotypes. Resistant *Lindernia* spp., *Elatine triandra*, *M. vaginalis* and *S. juncooides* var. *ohwianus* are widely distributed across Japan. These resistant biotypes are found not only in paddy fields of the northern regions but also at Mie, Kyoto (Kinki area), Ibaraki, Saitama, Chiba (Kanto area) and Fukuoka (Kyusyu area) Prefectures in Japan. Also, SU-resistant species have been mainly identified in the western and southern areas practicing extensively in flooded direct-seeded rice in Korea. We would like to discuss how to manage the resistant biotypes in rice fields of Asia.

**Key words:** Herbicide resistance, sulfonylurea, ALS, paddy field, Asia, Japan, Korea

### INTRODUCTION

Rice is grown on 153.3 million ha worldwide, with 90.3% in Asia in 2004 (IRRI home page). The rice herbicide market is heavily concentrated in Japan and Korea, representing almost half of the total global rice herbicide sales revenue (Allison 1999). In the early 1980s, K. Itoh with Dr. Y. Watanabe started to study paraquat-resistant *Erigeron philadelphicus* L., a perennial compositae weed in mulberry fields in Saitama Prefecture in Japan. After that, some compositae and the other weed families resistant to paraquat in fruit tree, mulberry, vegetable and tea fields and paddy levees in Japan and Malaysia were found (Itoh & Matsunaka 1990, Itoh 1998).

Sulfonylurea (SU) herbicides have been used widely in major cereal-growing areas to control or suppress broadleaf weeds and sedges since their introduction in the early 1980s. With the repeated use of the same SU-based herbicides, several weed species have developed resistance to SU herbicides. The first two confirmed cases of SU resistance following selection by chlorsulfuron were reported in *Kochia scoparia* L. and *Lactuca serriola* L. Since then, resistance to acetolactate synthase (ALS) inhibitors including SU compounds has been reported in 80 additional weed species. At least a resistant biotype of a species or more of these species has been confirmed in the USA, Canada, as well as in Australia, Denmark, UK and Israel. Most of SU-resistant weeds were collected from fields where winter wheat was grown and chlorsulfuron or chlorsulfuron plus metsulfuronmethyl had been applied for 3 to 5 years.

From 1992, SU-resistant paddy weeds have been also collected from rice fields where bensulfuron-methyl (BSM) was applied in California, USA and Australia on *Cyperus difformis* L. and *Sagittaria montevidensis* L. subsp. *calycina* (Saari et al. 1994).



## 1. Finding the Sulfonylurea Resistant Weeds in Asia

BSM and pyrazosulfuron-ethyl (PSE) were used from 1989 in one shot treatment herbicides like BSM/mefenacet, BSM/esprocarb, BSM/dimepiperate and PSE/mefenacet in Japan. In 1995, *Monochoria korsakowii* Regel et Maack, a Pontederiaceae paddy weed, was first discovered resistant to SU herbicides. This was first observed in Hokkaido Prefecture Japan (Kohara et al. 1996, Wang et al. 1999). A resistant biotype of the same species to sulfonylureas was first discovered in 1998 in reclaimed rice fields of the west coastal area of Korea that had received these herbicides for 9 years (Park et al. 1999). In the spring of 1996, SU-resistant *Lindernia micrantha* D. Don., an annual paddy Scrophulariaceae weed was observed in Yoshijima, Kawanishi Town, as well as SU-resistant *L. dubia* Pennell subsp. *dubia*, in Naka-yoshide, Yuza Town Yamagata Prefecture. Also, we found resistant *L. dubia* subsp. *major* Pennell in some paddy fields at Akita and Miyagi Prefectures. The origin of both subspecies *L. dubia* is North America. *L. procumbens* (Krock.) Philcox. and *Limnophila sessiliflora* Blume also failed to be controlled not only by SU-herbicides but also by barnyard grass-killers in the Tohoku and Hokuriku districts (Itoh et al. 1997; Seito et al. 1997; Wang et al. 1997).

*Scirpus juncoides* Roxb. var. *ohwianus* T. Koyama, a serious perennial weed in Hokkaido and Miyagi Prefectures, was found resistant to SU herbicides (Kohara et al. 1999; Yoshida et al. 1999). The fields where resistance was observed are characterized as follows: (1) herbicides were properly used, (2) other weeds were completely controlled by herbicides, (3) severe infestation of *S. juncoides* had occurred for more than two years, (4) but the *S. juncoides* had been completely controlled a few years before, (5) and SU herbicides were used for more than five years (Kohara et al. 1999).

*Rotala indica* Koehne in Akita Prefecture, *Elatine triandura* Schk. in Saitama Prefecture (Hata et al. 1998), *Monochoria vaginalis* (Burm. f.) Kunth. in Akita and Ibaraki Prefectures (Koarai and Morita 2000), *Scirpus wallichii* Nees., a perennial weed in Iwate Prefecture (Hashimoto et al. 2002), and *Sagittaria trifolia* L., a perennial weed, found at a paddy field in Omagari, Akita (Uchino and Watanabe 2002) were confirmed in Japan. Only *S. trifolia* showed resistance to BSM, while the other biotypes showed SU resistance. Blancaver et al. (2001 and 2002) showed cross-resistance in the SUs in *Rotala indica*, but had no resistance to bispiribac sodium and imazamox, the ALS herbicides.

The widespread and diverse SU-resistance problem has been found in Korea, where SU herbicides such as bensulfuron-methyl and pyrazosulfuron-ethyl have been used continuously since 1989. SU-resistant weeds in five annual weeds, *Monochoria korsakowii* (Park et al. 1999, 2003), *Monochoria vaginalis* (Kuk et al. 2003, Park et al. 2002), *Lindernia dubia* (Park et al. 2001), *Rotala indica* (Kuk et al. 2002) and *Cyperus difformis* (Im et al. 2003), and three perennial weeds, *Scirpus juncoides* (Im et al. 2003), *Scirpus planiculmis* (Park 2004) and *Sagittaria pigmaea* (Im et al. 2005) as of 2004 have confirmed in paddy fields in Korea. The *M. vaginalis*, *S. juncoides* and *C. difformis* of SU herbicides resistant weeds have been rapidly spreading (Park et al. 2001, 2003). These SU-resistant weeds have been identified mainly in the western and southern areas in flooded direct-seeded rice is practiced extensively. However, many farmers complaint on the control effect of SU herbicides. The herbicide concentrations required for GR<sub>50</sub> for SU herbicide resistant biotypes ranged from several ten-fold to several hundred-fold higher than those of the susceptible biotypes (Park et al. 2003). These ten resistant annual and five perennial species are spreading and causing problems in Japan and Korea now.

A biotype of *Lomnocharis flava* (L.) Buchenau, which is multiple resistant to 2, 4-D and BSM, was found in Peninsular Malaysia (Nakayama et al., 1999). A 2, 4-D resistant strain of the species from MARDI Seberang Perai field in Malaysia showed an approximately 30 times difference in I<sub>50</sub> value



compared with the susceptible biotype. For bensulfuronmethyl, the difference was more than 28 times. In Malaysia, *Limnophila erecta* (Scrophulariaceae family) first evolved multiple resistance in 2002 and infested rice (Azmi et al. 2003). These particular biotypes are known to have resistance to 2,4-D, bensulfuron-methyl, cinosulfuron, etc. Also, *Sagittaria guyanensis* (Alismataceae family) first evolved resistance to SUs in 2000 and infested rice. *Bacopa routundifolia* (Michx.) Wettst., from southern USA in paddy fields of the Seberang Perai and the Sekinchan in Malaysia had a high probability of occurrence of SU resistant biotypes (Valverde and Itoh 2001). Resistant biotypes of *Solanum phoeinocarpum* and *Bidens pilosa* to metsulfuron-methyl reported from China (Wang et al. 1999).

## 2. Distribution of Sulfonylurea Resistant Weeds

A field survey and questionnaire filled by farmers were conducted in the rice growing season, during mid-June of 1996 in Yuza Town, Yamagata Prefecture, Northern Japan, where sulfonylurea resistant biotypes of *Lindernia* weeds (mostly *L. dubia* var. *dubia*) had vigorously infested. The weeds were observed in 229 (34.1%) of total 671 patches (189 farmers, 250 ha), two to three weeks after herbicide treatments. The differences in distribution patterns of infested paddy fields were based on the weed control method that had been adopted by individual farmers. The method involves the consecutive use of one-shot treatment herbicides including SUs, ex. BSM, and PSE. The herbicides had intensified the infestation of the resistant biotypes of SUs (Itoh et al. 1997).

From the agricultural background of rice cultivation in the surveyed area, the infestation of *Lindernia* spp. in rice fields of Yuza Town was due to: (1) a consecutive use of one-shot treatment herbicides; (2) prolonged water seeded rice cultivation, (3) presence of vacant spots in paddy fields for rice over production, (4) seed dispersal through agricultural machineries, and (5) soil movement when grand consolidation. The SU resistant *L. micrantha* observed in the paddy fields at Nangai, Akita, Kawanishi, Yamagata, Kazo, Saitama, and Ohmiya, Kyoto in the separate districts in central and northern Japan was the same as that of Yuza Town (Itoh et al. 1999).

## 3. Herbicidal Selection Pressure

A pot experiment was conducted simultaneously with the rice growing season. The resistant seeds were collected from *L. micrantha* that had survived SU-based herbicide treatments in a paddy field at Yoshijima, Kawanishi Town, Yamagata Prefecture and the susceptible seeds were collected from an untreated area in Omagari City, Akita Prefecture in 1996. 1/5,000 a pots filled with gray lowland soil were set in vinyl house and 50-100 seeds were sown under the submerged condition with 3 to 5 cm water depth. Four SU herbicides with 6-7 doses each were applied during the 1-leaf stage of the seedlings.

The resistant biotype of *L. micrantha* exhibited a high level of resistance to BSM, and the biotype showed cross resistance to PSE, imazosulfuron and ethoxysulfuron. After the calculation of the log-logistic analysis of herbicide dose-response relationships (Seefeldt et al. 1995), the 50 % lethal dose ( $LD_{50}$ ) values for resistant *L. micrantha* varied from 105 g a.i.  $ha^{-1}$  for PSE (approximately 141 times higher than for the susceptible biotype) to 719 g for BSM (282 times higher) and 804 g for imazosulfuron (81 times higher) (Itoh et al. 1999).

The same method was conducted using *L. sessiliflora* which had survived SU-based herbicide treatments in a paddy field of Sennan Village, Akita Prefecture in 1997. The resistant biotype of *L. sessiliflora* also exhibited a high level of resistance to BSM, and the biotype showed cross resistance to PSE, imazosulfuron and ethoxysulfuron. The  $LD_{50}$  values for resistant *L. sessiliflora* varied from 110 g a.i.  $ha^{-1}$  for PSE (approximately 334 times higher than the susceptible biotype) to 1316 g for BSM (896 times higher) and 1245 g for imazosulfuron (655 times higher) (Wang et al. 2000).



It was concluded that the resistant biotypes of these Scrophulariaceae weeds are about 100 to 1000 times more to the four kinds of SU herbicides than the susceptible ones (Itoh et al. 1999).

The difference in LD<sub>50</sub> values of the SU-resistant biotype in *S. juncooides* sampled from Iwamizawa City, Nakafurano Town and Kuriyama Town in Hokkaido Prefecture, were 40 to 80 (PSE), 75 to 90 (Imazosulfuron) and 55 to 140 (BSM) times higher, respectively, than the susceptible biotype sampled from the experimental paddy field in Hokkaido Central Agricultural Experiment Station (Kohara et al. 1999).

In the farmer's fields, SU herbicides are applied at rates that result in mortality of over 99% of susceptible individuals in Japan. Initial gene frequencies, selection pressure and inheritance manner seed bank in soil are important factors for resistant gene flow (Jasieniuk 1996; Diggle and Neve 2001).

#### 4. Breeding system and inheritance of the resistance

To know the gene dispersal process *Lindernia* by insects, the species composition and activity of insects visiting the flowers were studied at the fallow rice fields in Kawanishi town and Nangai village Northern Japan. Among seven species, five bees and two hover-flies were collected from the flowers. *Lasiogloum scitulum* (Halictidae, Hymenoptera) and *Sphaerophoria macrogaster* (Syrphidae, Diptera) were major visitors in a number of individuals. Observation of flower-visiting behavior proved that *L. scitulum* is the most important pollinator of *Lindernia* flowers. Comparison between two *Lindernia* flowers showed that *L. micrantha* is a cross-pollinated type and *L. dubia* var. *major* is self-pollinated type (Matsumura et al. 1999).

A SU-resistant biotype of *L. micrantha* from Kawanishi Town and a susceptible biotype from Omagari were used. One day before flower opening, all anthers were emasculated and the flowers bagged. In the morning of the flowering day, the tips of the stigma were introduced with pollen grains from different flowers. The F<sub>1</sub> seeds from crosses of S×S, S×R, R×S and R×R, were collected in July 1997. These seeds were stocked in the refrigerator, and seeded in 200 cm<sup>2</sup> pots filled with paddy sterile soil in early Feb. 1998, in a green house. After counting the seedlings on Feb. 20, these were treated with 21 g a.i. ha<sup>-1</sup> of PSE. Plants that survived were counted in April. Remaining plants of the S×R progenies were grown and crossed with susceptible pollens (back cross), and the seeds produced were collected late June, and were seeded October. Backcrossed seedlings were counted on Nov. 14, and were treated with 75 g a.i. ha<sup>-1</sup> of BSM. Surviving plants were counted 41 days after (Dec. 25). Chi-square test for a single gene was calculated.

Flowers of SU-resistant plants (R) were sequentially crossed with susceptible plants (S). Some bags had sterile seeds. Over 50 seeds per capsule were used in the experiment. All seedlings from the F<sub>1</sub> progenies resulting from S×S died after treatment with PSE, but some progenies from crosses S×R, R×S and R×R survived. This suggests that dominant nucleic genes control the inheritance manner of the SU resistant *L. micrantha*. The number of survivors varied significantly because: (1) seedlings were very small, (2) surface soil in pots was exfoliated, (3) algae developed, and (4) *Daphnia* spp. and small aquatic insects were present.

After PSE treatment, the remaining S×R progenies were cultivated, and the pollen grains were crossed with susceptible ovules. The results provided good evidence that a single dominant gene controls the SU resistance in *L. micrantha* (Itoh et al. 1999).

Wang et al. (1995) reported that the flower morphology and pollination biology of *Monochoria* genus. *M. vaginalis* is a self-pollinated type. The flower of *M. korsakowii* has somatic enantiostyly, i.e., each plant bears two morphs of flowers, left- and right-banded flowers, with the style deflecting to the left and right, respectively. The enantiostyly is accompanied by stamen dimorphism, i.e., each flower has five small and one large stamen. The large anther and the stigma are symmetrical with



respect to the median plane of the flower. The major pollinators were *Apis cerana japonica*, *Xylocopa circumvolans* and *Bombus* spp. The large anther of a flower morph touches the bee's abdomen in the same position as the style of the other morph, thereby apparently facilitating intermorph pollination. However, since the enantiostyly is somatic, this seems to lead to cross-pollination only when flowers of only one morph are open by chance, on an individual. The flower of the species was found to be self-compatible and capable of setting seed even in the absence of pollinators. It is the small stamen that contributes to autogamy.

Since the capsules of *M. korsakowii* and *M. vaginalis* contain hundreds of seeds, we detected heterozygotes with two isozyme markers, *Adh-1* and *Pgi-2* loci, and measured outcrossing rates for single fruits in each of the target genotypes. Outcrossing rates were measured on an experimental population of *Adh-1*<sup>1</sup>/*Adh-1*<sup>1</sup> homozygotes, in which target plants of the *Adh-1*<sup>0</sup>/*Adh-1*<sup>0</sup> homozygote were planted at three different positions.

Outcrossing rates for *M. korsakowii* ranged from 37% to 80% with pollinators and were 0% without pollinators. The variation in the outcrossing rates was significantly affected by the species of pollinators but was not affected by the position of target in the experimental population. The outcrossing rate for *M. vaginalis* was zero because it had been self-pollinated before the flowers opened, or it was cleistogamous (Wang et al. 1998, 2003).

Crossing test of SU- resistant and susceptible biotypes on *M. korsakowii* was conducted. All seedlings from the F<sub>1</sub> progenies resulting from S×S died after being treated with BSM, but all progenies from crosses S×R, R×S and R×R survived. This suggests that dominant nucleic genes control the inheritance manner of the SU resistant *M. korsakowii*. Both parents of the species used in the experiment were homozygous. Gene flow in an experimental population of SU-resistant *M. korsakowii* was also conducted in Omagari. Bee, *Apis* is one of main pollinators of *M. korsakowii*. A number of insects visited the flowers, and the outcross ratio was 10-65 % (Wang et al. 1998, 2003).

Inheritance of resistance to sulfonylurea (SU) herbicides was studied in *S. juncoides* var. *ohwianus* T. Koyama collected from Miyagi Prefecture Japan. At first, we found an F<sub>1</sub> progeny from susceptible ovule sprinkled to resistant pollens. The seed developed from susceptible plant after shaking and drop resistant pollens in the middle of Feb. 2001. The analysis of self of the F<sub>1</sub> indicated that the best fit for Chi Square analysis of the bensulfuron-methyl (a SU herbicide, at 75 g and 25 g a.i. ha<sup>-1</sup> on algae medium) treated F<sub>2</sub> generations was a 3:1 (R: S) ratio. F<sub>3</sub> seedlings of twenty plants of F<sub>2</sub> generation were tested of the segregation ration. Fifteen populations were segregated resistant and susceptible biotypes. Five populations were all resistant biotype (no segregation). It is indicating that the resistance was dominant and was controlled by a single, nuclear gene (Itoh et al. 2002).

## 5. Rapid Diagnostic Tests

The basis for the diagnosis is the comparison of acetoin accumulation in plant tissue treated with either a ketol-acid reductoisomerase (KARI) inhibitor alone, or a KARI inhibitor and the desired ALS inhibitor (Gerwick et al. 1993). In the presence of a KARI inhibitor, the branched chain amino acid pathway is inhibited after acetolactate synthesis, and acetolactate accumulation depends on ALS activity in the plant tissue. In the presence of an ALS inhibitor in addition to the KARI inhibitor, the acetolactate accumulation resulting from ALS activity is inhibited in the susceptible biotype, but not in the resistant biotype. The accumulated acetolactate can be converted to acetoin, which is then quantified colorimetrically. The distinct color reveals whether acetoin does (red or pink: resistant biotype) or does not (yellow or brown: susceptible biotype) accumulate.

For diagnosis, it is important to compare the results in the presence and absence of an ALS inhibitor. If little or no acetoin accumulates without an ALS inhibitor, then the test is not valid. If this control



without an ALS inhibitor is not included, a resistant biotype could be misdiagnosed as a susceptible biotype. Therefore, in our experiments we first established the conditions for stable acetoin accumulation without an ALS inhibitor.

For the *L. dubia* var. *dubia*, the acetoin accumulation increased with incubation time under illumination. It increased linearly for 25 h and an obvious red color was seen at this point in both biotypes. Gerwick et al. (1993) reported that illumination during the incubation did not significantly affect the diagnosis with velvetleaf. However, in the *L. dubia* var. *major* very little acetoin accumulated in the dark during the 25 h incubation period. This light requirement was also observed in *L. dubia* var. *dubia* and *L. micrantha*. High acetoin accumulation was observed in the light, while very low accumulation was observed in the dark in these three species, regardless of biotype. Therefore, light is crucial to acetoin accumulation in both biotypes of *Lindernia* spp., suggesting that carbon flux through branched-chain amino acid biosynthesis is light dependent in these species (Uchino et al. 1999).

To diagnose resistant weeds, we used thifensulfuron-methyl as an ALS-inhibiting sulfonylurea herbicide because BSM, the major sulfonylurea herbicide used in rice cultivation in Japan, has low solubility in water (120 ppm at pH 7.0, 25°C), and is not sold as a wettable powder formulation in Japan.

The experiment was performed with 0, 0.75, 7.5, and 75 ng a.i. ml<sup>-1</sup> thifensulfuron-methyl. In the resistant biotype, the accumulated levels were high enough to produce the red color under all these conditions, although a decrease was observed with 75 ng a.i. ml<sup>-1</sup>. In the susceptible biotype, the levels of accumulation were clearly lower than in the resistant biotype with 7.5 and 75 ng a.i. ml<sup>-1</sup> thifensulfuron-methyl. A yellow color was observed under these conditions. Hence, in the *L. dubia* the appropriate concentrations of thifensulfuron-methyl for resistance determination were 7.5 or 75 ng a.i. ml<sup>-1</sup>. These concentrations were also appropriate for the other *Lindernia* species. The resistant biotypes produced a red color, while the susceptible biotypes produced brown or yellow at concentrations of 7.5 or 75 ng a.i. ml<sup>-1</sup>.

To use this diagnostic method, other assay parameters must be considered in addition to the above. For the diagnosis in velvetleaf, Gerwick et al. (1993) recommended using the youngest apical leaf because acetoin accumulation was lower in older or lower leaves. However, the level in older leaves was still sufficient for diagnosis. In *Lindernia* spp., the younger apical leaves from young plants must be used because older leaves have very low levels of acetoin accumulation, which are insufficient for the diagnosis. Similarly, young leaves from older plants did not accumulate acetoin level sufficient for diagnosis. Kuk et al. (2003) developed a diagnostic method used *Monochoria vaginalis*.

Lovell et al. (1996) demonstrated that the in vivo ALS assay could effectively determine the degree of resistance or cross-resistance. Our data was able to show the degree of resistance (Uchino et al. 1999), even though the leaves were treated in a petri dish. The concentration that inhibited 50% acetoin accumulation was about 20 to 40 times more in the resistant biotype than in the susceptible biotype of *L. dubia* var. *major*. The in vivo ALS assay using our method could also provide information about the degree of resistance of sulfonylurea-resistant weeds.

The requirement for light suggests that light plays an important role in acetolactate accumulation in *Lindernia* spp. Light may be important for ALS activity, the activation of ALS, or production of a substrate for ALS. Light may also influence the metabolism of acetolactate. There has been no report on the effect of light on carbon flux through branched chain amino acid biosynthesis, at least to our knowledge (Uchino et al. 2000). Further in-depth experiments are needed to elucidate the role of light in the diagnosis. At present, quicker and easier method (ITO kit) is developed and being tested.



More easy diagnose using a novel method based on young plants rooting responses was developed by Hashimoto et al. 2001 and Hamamura et al. 2003.

## 6. Aspect of Molecular Biology

To investigate the process of resistance evolution to ALS-inhibitors by *L. micrantha*, molecular markers were used. The results indicated that the populations of the resistant biotypes contained less variation than those of the susceptible biotypes. In some cases, the populations of resistant biotypes were nested in the populations of susceptible biotypes. We concluded that the multiple founding events of resistant biotypes occurred in *L. micrantha* population in Japan (Shibaike et al. 1999).

Uchino and Watanabe presented a report of DNA sequences in Domain A of the acetolactate synthase (ALS) gene of *Lindernia* spp. They identified that all susceptible biotypes of a 196 base in Domain A gene were proline codons, and the resistant biotypes had other amino acids (Uchino and Watanabe 1999). Shibuya et al. (1999) also identified that nucleotide sequences and inferred amino acid sequences of ALS clones isolated from resistant and susceptible *S. juncooides*. Shimizu et al. (2002) showed the molecular characterization of ALS in resistant weeds and crops.

Now we can see all DNA sequence data in the following Heap's home page.

<http://www.weedscience.org/in.asp>

## ALS Mutations from Herbicide-Resistant Weeds

This new section of the website focuses on the amino acid substitutions that confer resistance to ALS inhibitor herbicides. We aim to expand this section to include data on mutations that confer resistance to other herbicide modes of action. The current section is based on a recent paper: "Tranel, P. J., and Wright, T. R. (2002). Resistance of weeds to ALS-inhibiting herbicides: What have we learned? *Weed Science*, 50: 700-712."

## 7. How to Control the Resistant Species

The biotypes were observed to infest paddy fields and unplanted or partly unplanted rice fields for mass production of rice in Japan. In these fields, we observed that applied SU based herbicides continuously used for 3 to 7 years failed to control these weeds. A combination of a soil application herbicide (400 g a.i. ha<sup>-1</sup> for pretilachlor) and a foliar- and soil- applied herbicide (240 g a.i. ha<sup>-1</sup> for MCPB+450g a.i. ha<sup>-1</sup> for simetryn + 3.0 kg a.i. ha<sup>-1</sup> for thiobencarb), was effective for SU resistant annual paddy weeds (Itoh et al. 1997; Itoh et al. 1999). Pentoxasone is also effective for SU-resistant annual broad leaves such as *Lindernia* spp. (Watanabe et al. 1998).

Because of the infestation of resistant biotypes of *S. juncooides* in paddy fields, there is a need to choose some one-shot-treatment herbicides including bromobutide and SUs (Fukumoto et al. 1999), or an one-shot herbicide like pyrazolate, pretilachlor/ SU (Sugiura et al. 1999) or herbicides with different mode of action, pretilachlor/ benfuresate/ pyrazolate/ dimepiperate (mixed with 4 compounds) or pyributicarb/ bromobutide/ benzofenap (mixed with 3 compounds) (Kohara et al. 1999).

Asian rice cultivation is generally once or twice a year; and most of case is monoculture. Weed problems under dry seeding culture are serious. If some transgenic herbicide-resistant rice will be developed, weed problems of genetically manipulated crops will occur (Gressel 2002). I agree with him. Single compound SUs are used for rice fields in South East Asian countries. Also, a high risk of occurrence of resistant biotypes is present in these countries.



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## Allelopathic research and development-A world view on breeding of allelopathic rice

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**Abstract:** An improvement in allelopathic potential in crop varieties will have a great impact on both low-input and high-input management systems. Allelopathy alone is not likely to totally replace other weed control practices due to its effectiveness and influence of many other factors. However, marginally reduced use of herbicide over time will be a significant economical benefit to farmers and will also reduce the ecological impact on the environment. At present, no commercial cultivar carrying allelopathic properties is available, but there is the possibility of breeding new allelopathic crops by regulating their capacity to produce allelochemicals. In association with the development of breeding new allelopathic crop cultivars, much progress has been made in screening methods, identification of specific allelochemicals and related genes that can be applicable in breeding programs. Particularly, the insertion of two specific genes such as CA4H for *p*-coumaric acid and OsDTS2 for momilactone into one cultivated rice variety by biotech will give us a new era for the development of allelopathic crops. Of course, in order for this technology to be practically used in rice production systems, the potential problems such as auto toxicity, metabolic imbalance, residual effect and development of a tolerant population upon repeated culture of such cultivars should be thoroughly solved before these cultivars are released. Nevertheless, development of the new allelopathic crop cultivars through either a classical breeding method or biotech can be an attractive alternative to meet the demand of consumers and environmentalists.

**Key words:** Allelopathy, allelopathic crops, allelochemicals, breeding, CA4H, OsDTS2

### INTRODUCTION

Allelopathy is defined as the direct influence from a chemical released from one plant on the development and growth of another. Allelochemicals are secreted to the rhizosphere and suppress the growth of neighboring plants (Bais et al. 2004), while phytoalexins are produced in response to microbial infections and exhibit antimicrobial activity (VanEtten et al. 2000). Allelochemicals, if present in crop varieties, may reduce the need for weed management, particularly herbicide use. Although, allelopathy alone may not be a perfect weed management technology, it may be a supplementary measure to weed management.

An improvement in crop cultivars is the only area that has not been exploited to any great extent as weed management strategy (Khush 1996). The possibility of incorporating allelopathic traits into improved rice cultivars, which would reduce the need for applying herbicides to the crop is worth exploring (Khush 1996). Of course, thus far, no commercial cultivars carrying allelopathic properties have been developed (Duke et al. 2001).

Recently, the allelopathic potential of rice has received a great deal of attention since Dilday et al. (1991) identified rice cultivars exhibiting allelopathic potential against duckweed [*Heteranthera limosa* (Sw.) Willd]. In addition, allelopathic potential has been also reported from numerous crops like barley (Lovett and Houlst 1995), cucumber (Putnam and Duke 1974), oats (Fay and Duke 1977), rice (Dilday et al. 1998), sorghum (Nimbal et al. 1996), sunflower (Leather 1983), tobacco (Patrick et al. 1963) and wheat (Wu et al. 1999). As indicated above, allelopathy works have been done in a broad range of plant species.



Although a breeding approach alone cannot overcome weed problems, an increase in the allelopathic potential of rice varieties will likely have a great impact on crop cultivation regardless of input levels. Moreover, allelopathy-based technology is also more easily transferable to farmers in low-input management systems than those in high-input management systems, which dependent heavily on herbicide.

There are a number of review papers on this subject: one is on strategies to enhance weed control by engineered allelochemical production (Duke et al. 2001); the other two reviews are by Olofsdotter (2001a, 2001b) on rice allelopathy and the other by Kim and Shin (2003) who introduced the importance of breeding allelopathic rice varieties. Thus this paper will briefly introduce some important aspects of breeding allelopathic rice crops.

## RICE ALLELOCHEMICALS

**Allelochemical synthetic pathways.** All phenylpropanoids are derived from cinnamic acid which is formed from phenylalanine by the catalytic action of phenylalanine ammonia-lyase (PAL), the branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism. Many phenolic compounds have not only a physiologically functional ability, but also plant allelopathic potential.

Isoprenoid compounds are produced from C<sub>5</sub> isoprenoid units and the classification of different families of isoprenoids is based on the number of C<sub>5</sub> isoprenoid units present in the skeleton of compounds (Gershenzon and Croteau 1993). In particular, diterpenoids are known to play an important role in the self-defense mechanism of rice as well as alelopathic potential. Terpenoids play diverse functional roles in plants as hormones (gibberellins, abscisic acid), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastophates), mediators of polysaccharide assembly (polyprenyl phosphates), and structural components of membranes (phytosterols). In addition to universal physiological, metabolic and structural functions, many specific terpenoid compounds (commonly in the C<sub>10</sub>, C<sub>15</sub>, and C<sub>20</sub> families) serve in communication and defense. It has been determined that induced specific terpenoids are correlated with plant-plant, plant-insect, and plant-pathogen interaction.

Environmental stresses have been well documented for inducing defence chemicals in plants. For example, enhanced UV-B light induces the accumulation of phenylpropanoids and flavonoids in different plant species, such as bean, parsley, potato, tomato, maize, rye, barley and rice (Hahlbrock and Scheel 1989; Ballare et al. 1995; Tevini et al. 1991; Liu et al. 1995; Kim et al. 2000a).

**Putative rice allelochemicals.** A number of studies indicate that common putative allelochemicals found in rice were phenolic compounds (Rice 1987; Chou et al. 1991; Inderjit 1996; Mattice et al. 1998; Blum 1998). Kim et al. (2000b) identified several compounds by GC/MS analysis from Kouketsumochi, a potential allelopathic rice, such as sterols, benzaldehydes, benzene derivatives, long chain fatty acid esters, aldehydes, ketones and amines from fractions with biological activity. Several compounds from Taichung Native 1, an allelopathic rice, have been identified by the bioassay-guided isolation method (Rimando et al. 2001). They were azelaic acid, *p*-coumaric acid, 1*H*-indole-carboxaldehyde, 1*H*-indole-3-carboxylic acid, 1*H*-indole-5-carboxylic acid and 1,2-benzenedicarboxylic acid bis (2-ethylhexyl) ester. Among the allelopathic substances identified, *p*-coumaric acid, a known allelochemical, inhibited the germination of lettuce (*Lactuca sativa* L.) seedlings at 1 mM, but was active against barnyardgrass only at concentrations higher than 3 mM (Rimando et al. 2001). A similar trend in results was reported by Kim et al. (2001b) in that *p*-coumaric acid was present in allelopathic rice such as Kouketsumochi, Tang Gan and Taichung Native 1. In addition, the content of this compound was markedly increased by UV irradiation.



These results strongly suggest that *p*-coumaric acid may be a compound regulated by environmental stresses and play a role in rice allelopathy. On the contrary, there were different reports that concentration of a single phenolic acid and combinations of all phenolic acids determined in rice ecosystems do not reach phytotoxic levels (Tanaka et al. 1990; Olofsdotter et al. 2001). Thus far, it is assumed that a single allelochemical responsible for rice allelopathy has not been determined. On the contrary, more than one chemical is likely responsible for rice allelopathy. Thus, identification of rice allelochemicals is of utmost important for use as markers in gene identification and regulation.

## IMPORTANCE OF ALLELOPATHY IN BREEDING NEW CROP

A number of rice cultivars or accessions with allelopathic potential have been determined in different places (Dilday et al. 1991; Fujii 1992; Kim and Shin 1998, Olofsdotter et al. 1999). However, no commercial allelopathic rice cultivar has been developed yet. For this, we have to utilize such allelopathic potential in breeding programs. In order to implement this technology, the anticipated problems such as autotoxicity, residual effects of allelopathic cultivars and tolerance of weed population upon repeated cultivation of allelopathic cultivars in the same fields should be thoroughly studied before allelopathic cultivars are released to farmers.

All the recommended herbicides are known to be very safe not only for humans and cattle, but also for the environment when they are properly used. Sulfonylurea type herbicides have been intensively used in far-east Asia since 1990, due to high efficacy against a broad spectrum of paddy weeds even at extremely low dose (20-50 g ai ha<sup>-1</sup>). However, intensive and repeated application of this type of herbicide has resulted in several negative effects such as (1) evolving resistant weeds (Valverde et al. 2000), (2) residual effects on the following crops and (3) the disappearance of some susceptible weeds such as *Brasenia schreberi* and *Sagittaria aginashi*, which affects weed biodiversity (Itoh 2000).

All these factors might provide enough reasons to attract public concern and worry regarding the negative effects of herbicides that may originate from intensive herbicide application in the environment. Thus, an alternative or a supplementary method to the heavy dependence on herbicide is needed. Such an alternative might be the use of allelopathy.

**Allelopathic traits.** What are allelopathic traits? Morphological characteristics such as early seedling emergence, seedling vigor, fast growth rates that produce a dense canopy, greater plant height, greater root volume and longer growth duration are known to increase the ability of rice cultivars to compete with weeds (Minotti and Sweet 1981, Bekowitz 1988). Plant height is often described as one of the most important factors in the total competitive ability of a crop and accounts for a similar percentage of total competitive ability (Gaudet and Keddy 1988; Garrity et al. 1992). However, it is not certain that all of these characteristics are also related to allelopathy. If there is no specific characteristic, the most visible plant characteristics such as plant height, root length and dry weight of testing plants can be used as measurable parameters to evaluate allelopathic potential. To breed new allelopathic crops, the first requirement is to identify allelopathic accessions or cultivars. An effective screening method that can test large amounts of accessions in a limited-space in an easy, cheap, reproducible and fast manner must be established.

**Screening methods.** To screen rice allelopathic potential, several methods such as the stairstep method (Bonner 1950; Liu and Lovett 1993), hydroponic culture test (Einhelig 1985), relay-seeding technique (Navarez and Olofsdotter 1996), agar medium test (Fujii 1992; Wu et al. 1999), cluster analysis using HPLC (Mattice et al. 2001), water extract method (Kim et al. 1999; Ebana et al. 2001) and 24-well plate bioassay (Rimando et al. 1998) have been suggested. Each of the methods mentioned can be used as a valuable tool to evaluate allelopathic potential. However, it has also



been observed that the screened accessions or cultivars in one method may not be always active in other methods employed. In terms of screening, the direct field test might be the best method to evaluate allelopathic potential, but it has many difficulties in dealing with a large number of germplasm. Thus, what is actually needed is a reliable and universal bioassay method that generates a similar trend under different conditions. For this purpose, the continued development of reliable screening systems is needed.

**Variation in allelopathic potential.** Dilday et al. (1998) identified 412 accessions with allelopathic potential against duckweed [*Heteranthera limosa* (Sw.) Willd] among 12,000 accessions that originated from 31 different countries. These accessions were genetically very diverse, indicating that allelopathic potential is widely present in rice germplasm. A number of studies have been conducted to evaluate the allelopathic potential from rice germplasm. It was reported that a large variation in allelopathic potential among rice cultivars exists in rice germplasm. Further, it is assumed that allelopathic potential might be polygenically controlled because it shows continuous variation in the germplasm. Moreover, allelopathic potential is often attributed to several inhibitors that act in an additive or synergistic way rather than in an isolated way (Courtois and Olofsdotter 1998). Tropical *japonica* rice varieties have shown a greater allelopathic potential against weeds, especially *Echinochloa* spp. than other rice (Jensen et al. 2001).

**Quantitative trait loci analysis.** There is very limited knowledge on allelopathy genetics. Moreover, no intentional breeding effort has been made to genetically improve the allelopathic potential of crops, mainly due to our poor knowledge of this phenomenon. In this regard, a recent work conducted by Jensen et al. (2001) seemed to be very valuable; quantitative trait loci (QTL) mapping using 142 DNA markers were located in 142 recombinant inbred lines derived from a cross between cultivar IAC 165 (*japonica* upland variety), which has strong allelopathic potential and cultivar CO 39 (*indica* irrigated variety), which has weak allelopathic potential. Three main loci, each accounting for about 10% of the upregulation of allelochemical production were localized in rice chromosomes 2 and 3. The two QTL traits on chromosome 3 were closely linked, so they could easily be manipulated. Although QTLs for allelopathic effect against barnyardgrass were identified, it is not presently known what kinds of gene are responsible for the allelopathic effect or production of allelochemicals correlated with plant-plant, plant-insect, and plant-pathogen interactions responsible for rice allelopathy has not been determined.

## STRATEGIES FOR BREEDING ALLELOPATHIC CROP

Two approaches to create more allelopathic crops are: (1) the classical breeding method; and (2) genetic engineering. From the breeding perspective, the distinction between allelopathy and competition is somewhat difficult in a farmer's field because interference is the phenomenon that really counts (Courtois and Olofsdotter 1998). In this regard, the best way might be to breed allelopathic cultivars with high competitiveness. If breeding of allelopathic cultivars is achieved, it can truly be beneficial to farmers in rice producing countries.

**Classical breeding method.** If a high number of QTLs with little effect are involved, a traditional breeding method can be a reasonable alternative (Courtois and Olofsdotter 1998). The principle of traditional breeding for the genetic studies is simple--two parents with contrasting behavior are crossed and recombinant inbred lines (RILs) are derived through the single-seed descent (SSD) method.

This procedure consists of advancing the  $F_2$  without selection for two or three generations (sometime four to five generations) in such a way that each  $F_4$  or  $F_5$  seed traces back to a different  $F_2$  plant. Only one seed is retained from each plant in each generation. This will repeat for two or more generations and afterwards, plants with desirable characteristics can be selected. Once a



reasonable degree of fixation is obtained, the allelopathic potential of the RILs can be evaluated. Then the seed is increased and tested under field conditions for use as allelopathic cultivars.

Table 1. Inhibitory effect of potent allelopathic lines on the shoot and root growth of barnyardgrass.

Cultivar	Extract		% inhibition of barnyardgrass growth					
	Plant part	Conc. (%)	Shoot			Root		
			1.0	2.5	5.0	1.0	2.5	5.0
Dongjinbyeo	Leaf		6.3	12.1	23.6	6.0	13.8	40.8
	Root		16.2	17.2	20.0	19.2	24.1	46.8
K21	Leaf		31.2	63.9	64.4	27.6	52.2	76.6
	Root		14.4	22.7	26.2	24.9	36.5	54.3
Kouketsumochi	Leaf		16.8	44.2	64.3	18.1	40.6	96.5
	Root		-7.8	12.9	46.6	-8.1	13.7	11.8

A cross between Donginbyeo (non-allelopathic cultivar, but a high yielding variety with good quality) and Kouketsumochi (an allelopathic cultivar, more similar to a wild type) was made and advanced by the SSD breeding method. We identified the F<sub>5</sub> generation of this cross which had a desirable agronomic traits and which exhibited allelopathic potential when determined by the water extract method. This was repeated in F<sub>5</sub> and F<sub>6</sub> generations under field conditions. We selected one line (K21) which has allelopathic potential with similar agronomic characteristics to female parent, Donjinbyeo.

Table 1 showed allelopathic effects of their leaf and root extract on the growth of barnyardgrass. A newly bred K21 line showed higher inhibitory effect than the female parent, but lower than the male parent which has allelopathic potential. Total phenolic compounds of K21 were in between its parents, showing a similar trend like inhibitory effect.

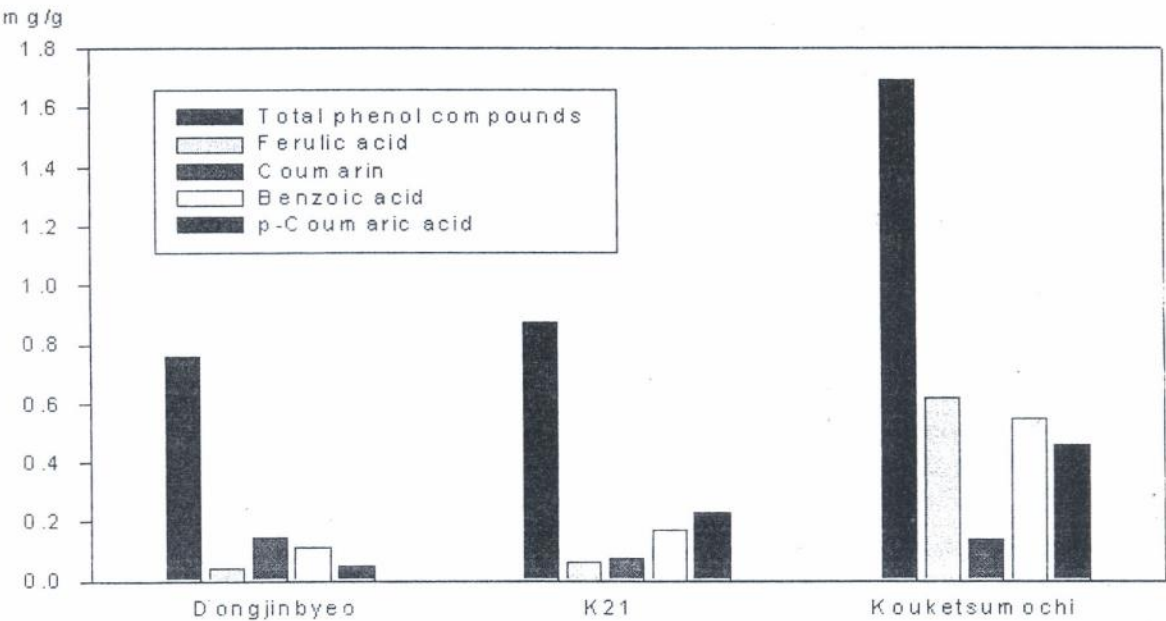


Figure 1. Total phenolic compounds of a newly bred rice line, K21 with its parents.



The SSD method is simpler than the pedigree method. The SSD method has several advantages including (1) a quick increase of the additive variance among families, (2) the need for only small breeding space and saving of work, and (3) suitability for low heritability traits where visual selection is not effective (Moreno-Conzalez and Cubero (1993). Another method is to cross two genotypes with contrasting behavior through the backcrossing method to produce near-isogenic lines (NILs) carrying different genes. The allelopathic potential of the NILs can be determined when a reasonable degree of fixation is obtained.

Although biotechnology and genetic engineering are widely employed as a future method for breeding new crop varieties, research on traditional plant breeding and breeding methodology will play a significant role in future breeding programs. Further refinement of these methods and better knowledge of classical knowledge are prerequisite for the rational use of new tools such as molecular markers.

**Molecular approach.** Two methods to create desirable allelopathic crops have been suggested: (1) regulation of gene expression related to allelochemical biosynthesis; and (2) insertion of genes to produce allelochemicals that are not found in the crop (Duke et al. 2001). Much of the presently available information on engineering allelochemicals comes to us through efforts to overproduce valuable secondary metabolites in plants (Canel 1999). Relating to the molecular approach for weed control, there is a good reference recently published by Gressel (2002).

Most secondary metabolites being used as allelochemicals are very complex and a multi-gene system might have to be developed and transformed into the specific crop to produce allelochemicals (Gressel 2002). This seems to still be hypothetical, but new areas open up to us. Various plant species suppress other species by production of allelochemicals, which are not toxic to the originating plant but toxic to other vegetation. It is presently known that molecular approaches in breeding allelopathic cultivars are potentially much more complicated than developing a herbicide resistant crop or producing a crop with resistance to insects or pathogens.

**Regulation of gene expression.** In order to regulate gene expression, the first requirement is to identify the target allelochemical(s), to determine enzymes and the genes encoding them, and thus a specific promoter can be inserted to crop plants to enhance allelochemical production.

In our recent work, *p*-coumaric acid was the highest content among intermediates of the phenylpropanoid pathway in several allelopathic rice cultivars used. Cinnamic acid 4-hydroxylase (CA4H) is the enzyme catalyzing cinnamic acid to *p*-coumaric acid, which is a key reaction in the biosynthesis of a large number of phenolic compounds in higher plants. The activity of CA4H was measured to elucidate how the activity is influenced by UV irradiation in rice leaves. CA4H activity in Kouketsumochi was induced by UV and its peak activity was observed at 24 h after UV irradiation for 20 min, however AUS 196 showed no response. There was a differential response to UV or other environmental stresses among rice cultivars. This further indicates that some metabolites with allelopathic potential might be newly synthesized or highly elevated in rice plants by UV irradiation (Kim et al. 2000a). Due to increased content of *p*-coumaric acid in rice plant irradiated by UV, it is assumed that the CA4H gene plays a role in the elevation of the allelopathic function in rice plants.

In order to investigate a specific promoter that confers responsiveness to UV light, the constructs of various CASC (*Capsicum annum* sesquiterpene cyclase) promoters were fused into GUS gene. The levels of GUS activity for transgenic plants with pBI121-KF1 and pBI121-KF6 were significantly elevated by UV-irradiation and had approximately a 2- to 3-fold increase over the untreated-transgenic plants. In contrast, GUS expression in the transgenic plants with pBI121-CaMV 35S was not changed by UV, and in the other constructs, had only a very small increase (Shin et al. 2000).



The regulation of genes associated with allelopathy can be achieved by developing a specific promoter responsive to plant-weed competition or environmental stresses (Shin et al. 2001). The specific promoters, CASC-KF1 and KF6, were fused to CA4H gene. The gene constructs were introduced into the binary plant expression vector pIG121-HMR with reverse primer harboring *Bam*HI site and forward primer harboring *Hind*III site. At present, we are investigating the regulatory effects on the gene coding for allelochemical producing enzymes.

**Insertion of gene to produce allelochemicals.** Rice produces momilactone diterpenoids as both phytoalexins and allelochemicals. Wilderman et al. (2004) identified the enzyme, a *syn*-copalyl diphosphate specific 9 $\beta$ -pimara-7,15-diene synthase (OsDTS2) catalyzing *syn*-CPP to be converted to polycyclic hydrocarbon intermediate *syn*-pimara-7,15-diene which is the precursor of momilactone A & B. This approach employs altering existing biochemical pathways by insertion of two genes such as CA4H and OsDTS2 into one recommended rice variety to produce *p*-coumaric acid and momilactone at the same time. It seems to be one of the promising molecular approaches that we are undertaking now. In this regard, there was a good review paper done by Duke *et al.* (2001) and a reference book, "molecular biology of weed control" recently published by Gressel (2002).

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## The World's landscapes of weedy rice – An overview

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**Abstracts.** Weedy rice (WR) is a scourge worldwide comprising many accessions of several species, viz. *Oryza sativa*, *O. spontanea*, *O. barthii*, *O. longistimata*, *O. granulata*, *O. officinalis*, *O. rufipogon*, *O. nivara*, *O. ridleyi*, *O. meyeriana*, and *O. minuta*. The wild species of *O. barthii* and *O. longistimata*, the weedy biotypes of the cultivated *O. glaberrima*, are among the worst weeds of rice in West Africa and Sahel. *Oryza officinalis*, *O. rufipogon*, *O. nivara*, and weedy biotypes of *O. sativa* infest severely the rice fields of Vietnam, Malaysia, and other South-East Asian countries. In Korea, the long- and short-grain weedy types of *O. sativa* predominate the weedy rice populations. The red rice (RR) is the most common weedy biotype of the cultivated rice (*Oryza sativa* var. *sylvatica*) in all temperate rice-growing areas worldwide. The WR and RR alike display a wide range of morphological, anatomical and physiological traits among their accessions. These WR biotypes are almost indistinguishable from their commercial counterparts at the seedling stage. The gross morphological differences of WR biotypes *vis-à-vis* the commercial varieties at the tillering and post-tillering stages with more numerous, longer and slender tillers, leaves are more often hispid on both surfaces, taller plant stature, the easy-shattering or deciduousness traits of seeds, pigmentation of several plant parts, principally the pericarp, and the presence of awns of variable lengths, in many instances, make the former more distinguishable *vis-à-vis* the commercial varieties. The spread of WR parallels that of RR, and this became significant principally after the shift of rice culture from transplanting to direct seeding in many European and Asian countries in the last two decades. Sowing of rice seeds contaminated with WR or RR, the season-mediated left over of volunteer crops, WR- or RR-contaminated tillage implements and harvesters and their sharing among farmers, and the failure of rice farming communities to recognise the potential severity of infestation of WR or RR at the onset, are some attributes responsible for the continued spread of the scourge in the rice ecosystems worldwide. WR and RR affects rice yield due to their shorter maturation period whereby early shattering of seeds and plant establishment, these WR or RR pre-empt resources and space, and at the competitive advantage *vis-à-vis* the commercial rice crops. WR- and RR-contaminated rice harvest reduces grain quality, and incurs extramilling costs. This milling operation results in broken grains and grade reduction. Close similarity of WR or RR with the commercial rice allows no herbicide selectivity to control the weed. The main control strategies include preventive measures by sowing or planting of clean seeds, agro-technical control through cultural and mechanical means, augmented with pre- and post-emergence herbicidal treatments of WR or RR in crop pre-planting or pre-seeding via stale seed bed technique, or post-planting and in-crop situation by cutting the panicle, systematic roughing, crop rotation, and planting of herbicide-resistant rice cultivars. The last option while offering an excellent control option for RR/WR accentuates the risk of gene flow. These strategies need to apply in a holistic manner within the framework of Integrated Weed Management Systems for both short- and long-term results.

**Key words:** Weedy rice, red rice, wild rice, commercial rice, herbicide-resistant rice, *Oryza sativa*, *O. spontanea*, *O. nivara*, ecology, management.



## INTRODUCTION

“If there is no man, there will be no woman.  
If there is no weed science there will be no agriculture.  
If there is no agriculture, there will be no mankind”  
(Baki B.B. 2005)

Weedy rice (WR) aggregates including red rice (RR) and their wild relatives represent some of the noxious and millennial weed species (*sensu* Gressel 2000) in rice ecosystems worldwide (Chin *et al.* 2000; Vaughan *et al.* 2002; Baki 2005; Mislamah 2005). Weedy rices impact on the rice industry world-wide. Impact studies of WR or RR on the rice industry registered measurable loss in terms of yields and quality of harvest by farmers (Fisher & Ramirez 1993; Chin *et al.* 2000; Baki 2004). Weedy rice infestation reduced growth and yields of commercial rice and is undesirable to rice farmers, to the milling industry, and to consumers alike. Millers complain that WR/RR reduce total and head rice recovery, and consequential grade reduction, besides inflating the processing cost when WR/RR are separated (Dunand 1988; Menzes *et al.* 1997) thereby fetching lower prices at the mills (Azmi, M. *pers. comms.*). These reductions are augmented with parallel increase in the costs of crop management and care. Diara *et al.* (1985), and Eleftherohorinos *et al.* (2002) recorded cultivar-mediated variations in yield reductions due to RR interference, ranging from 8 – 34%, and 46 -58%, respectively. Baki (2004) estimated that a 5% field infestation of weedy rice in Malaysia led to an economic impact in yield loss of *ca.* 64,880 tons of rice valued at MYR 137,876,375/year or US\$35,999,053/year.

Additionally, weedy rice infestations incurs further costs to farmers. Farmers need to practise thorough land preparation, water management, and herbicide-based weed management to ensure total control of weedy rices and other weeds prior to seeding. Measures of proper agronomic practices and proper crop care will inevitably lead to more hours spent in the fields. For some farmers these valuable hours should be spent elsewhere to generate better income or better-paid jobs. In the same vein, inculcation of the zero-tolerance concept and practice of weed infestation among farmers is difficult and expensive, especially among aging rice farmers in the country. This failure will aggravate weedy rice problems for many years to come.

This review summarizes advancements made in weed ecology and management of weedy rice in the rice ecosystems for the past decades. Special emphasis on weedy rice ecology include listings on noxious weed species, direct, and the rationale, general principles and concepts, and approaches of integrated weed management in weedy rice, namely, indirect, preventive, and substitutive agro-technical and cultural methods of control of weedy rice were made. The paper ends with a note on future challenges faced by the rice industry in managing weedy rice worldwide.

## WEEDY RICE ECOLOGY

“We sow rice seeds but weeds grow and establish” (Malay proverb)

**Weedy Rice Aggregates.** The weedy rice aggregates comprise many accessions of several species, viz. *Oryza sativa*, *O. spontanea*, *O. barthii*, *O. longistimata*, *O. granulata*, *O. officinalis*, *O. rufipogon* syn. *O. fatua*, *O. fatua* var. *longe-aristata*, *O. nivara*, *O. ridleyi*, *O. meyeriana*, *O. perennis*, or *O. punctata* and *O. minuta* (Table 1, Fig. 1). One weedy rice grouping, from Japan, Brazil, USA, and the upper Yangtze valley of China, comprised primarily of “indica-like” crop mimics with low levels of seed dormancy and seed shattering, may have ancestors from the cultivated varieties (Tang & Morishima 1996). A second group, the true indigenes of tropical Asia, are of indica type, self-propagating individuals, displaying high levels of dormancy and seed shattering, may have been the resultant populations following gene flow between the abundant wild



rice in the region and its cultivated relatives. The third group, originated from the Korean Peninsula and lower Yangtze valley of China, consisted of “japonica-like” self-propagating individuals.

The wild species of *O. barthii* and *O. longistimata*, the weedy biotypes of the cultivated *O. glaberrima*, are among the worst weeds of rice in West Africa and Sahel. *Oryza officinalis*, *O. rufipogon*, *O. nivara*, and weedy biotypes of *O. sativa* infest severely the rice fields of Vietnam, Malaysia, and other South-East Asian countries (Watanabe *et al.* 1996, 2000; Chin *et al.* 2000). In Korea, Choi (2000) recorded closely clustered japonica- and indica-cultivars of the short- and long-grain-type, respectively, among weedy rice accessions, through polymorphism of isozymes or RFLPs and RAPDs. No less than 74% of the 111 weedy rice accessions were the short-grain type. Overall, short-grain Korean weedy rices displayed 65-81% genetic similarity with the japonica Gihobyeo, while the long-grain type showed 49-57% genetic similarity with IR 26 and 5-11% with Gihobyeo. The red rice (RR) is the most common weedy biotype of the cultivated rice (*Oryza sativa* var. *sylvatica*) in many temperate rice-growing areas in Europe and elsewhere (MED-Rice 2005). In the Americas, RR is a common weed in most irrigated rice-growing areas, spreading primarily as a contaminant in rice seeds (Noldin 2000). The RR accessions or biotypes were highly polymorphic (Craigmiles 1978; Lago 1982) but display strong crop mimicry with the cultivated counterparts suggesting natural hybridization with rice (Noldin *et al.* 1999). Traditionally, all the RR found in commercial rice in USA has been classified as *Oryza sativa* ssp. *indica* based on phenotypic traits and high rate of fertility in crosses between red rice and cultivated rice (Diara *et al.* 1985; Langevin *et al.* 1990; Kwon *et al.* 1992). Such contention is debatable. It has been argued that red rice is actually *O. barthii*, *O. longistaminata*, *O. rufipogon*, *O. perennis*, or *O. punctata* (Parker & Dean 1976; Craigmiles 1978; Kwon *et al.* 1991; Oka 1991). Recent studies by Vaughan *et al.* (2001) and Gealy *et al.* (2002) demonstrated that the red rice populations in USA were actually a collection of *Oryza sativa* ssp. *indica*-like red rice, *Oryza sativa* ssp. *japonica*-like red rice, *O. nivara*, and *O. rufipogon*, based on DNA markers, and these are in general agreement with classification based on morphological traits. Ferrero (2001) in Italy, employed microsatellites to show that Mediterranean RR was closely related to japonica rice, where as the Brazilian RR was most related to indica rice. In Uruguay, Federici *et al.* (2001), using amplified fragment-linked polymorphisms, showed that straw hull awnless and blackhull awned RR clustered into genetically distinct groups, and a third group clustered with the cultivated rice. In many world rice-production areas domestic rice cultivation and the range of sexually compatible relatives can overlap (Gealy *et al.* 2003).

Table 1. Weedy and wild rice aggregates in selected rice-growing areas of the world (Adapted from Baki 2005).

Countries	Weedy Aggregates and Wild Rice Species/References
Australia	<i>Oryza sativa</i> , <i>O. australiensis</i> , <i>O. meriodinalis</i> , <i>O. officinalis</i> (Oka 1991).
Bangladesh	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. officinalis</i> (Karim, R.U., <i>pers.comms.</i> )
Belize	<i>Oryza alta</i> (Vaughan 1989).
Brazil	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> , <i>O. glumaepatula</i> , <i>O. alta</i> (Noldin <i>et al.</i> 1999; Noldin 2000; Valverde 2004).
Cambodia	<i>Oryza officinalis</i> , <i>O. ridleyi</i> (FAO 2005).
China	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. officinalis</i> (Oka 1991) <i>O. sativa</i> f. <i>spontanea</i> , <i>O. meyeriana</i> (Zhang 2000)
Columbia	<i>Oryza sativa</i> , <i>O. glumaepatula</i> , <i>O. alta</i> , <i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> (Rafael & Olga 2004; Valverde 2004).
Costa Rica	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> , <i>O. glumaepatula</i> (Valverde 2004).
Guyana	<i>Oryza alta</i> , <i>O. glumaepatula</i> (Vaughan 1989)
Greece	<i>Oryza sativa</i> var. <i>sylvatica</i> , <i>O. sativa</i> (Eleftherohorinos <i>et al.</i> 2002).
India	<i>O. officinalis</i> <i>O. sativa</i> f. <i>spontanea</i> , <i>O. rufipogon</i> , <i>O. nivara</i> , <i>O. granulata</i> , <i>O. minuta</i> (Vaughan 1989)



Indonesia	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. officinalis</i> , <i>O. ridleyi</i> Soerjani <i>et al.</i> 1987; Soekisman, T., <i>pers. comms.</i> ).
Italy	<i>Oryza sativa</i> var. <i>sylvatica</i> (Sparacino <i>et al.</i> 2000; MED-Rice 2005)
Japan	<i>O. nivara</i> , <i>O. rufipogon</i> (FAO 2005). <i>O. sativa</i> f. <i>spontanea</i> (Sato 2000)
Korea	<i>Oryza sativa</i> ssp. <i>indica</i> , <i>O. sativa</i> ssp. <i>japonica</i> , <i>O. sativa</i> ssp. <i>japonica</i> (long-grain type); <i>O. sativa</i> ssp. <i>japonica</i> (short-grain type); <i>O. sativa</i> ssp. <i>japonica</i> (Local Korean <i>japonica</i> ) (Choi 2000).
Kenya	<i>O. eichingeri</i> (Vaughan 1989, Oka 1991).
Laos	<i>Oryza officinalis</i> , <i>O. ridleyi</i> , <i>O. nivara</i> (Appa Rao <i>et al.</i> 2000) <i>O. rufipogon</i> , <i>O. granulata</i> , <i>O. sativa</i> f. <i>spontanea</i> , <i>O. minuta</i> (Oka 1991).
Malaysia	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. officinalis</i> , <i>O. ridleyi</i> , <i>O. meyeriana</i> , <i>O. minuta</i> (Abdullah, M.Z. ( <i>pers. comms.</i> )).
Mexico	<i>O. glumaepatula</i> (Vaughan 1989).
Myanmar	<i>Oryza officinalis</i> , <i>O. ridleyi</i> , <i>O. barthii</i> , <i>O. granulata</i> , <i>O. meyeriana</i> , <i>O. rufipogon</i> (Vaughan 1989).
Nepal	<i>Oryza officinalis</i> , <i>O. sativa</i> f. <i>spontanea</i> , <i>O. rufipogon</i> , <i>O. nivara</i> , <i>O. granulata</i> , <i>Hygroryza aristata</i> (Gupta & Upadhyay 2000).
Nicaragua	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> (Valverde 2004).
Pakistan	<i>Oryza sativa</i> , <i>O. rufipogon</i> (Oka 1991; FAO 2005)
Panama	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> ; <i>O. glumaepatula</i> (Valverde 2004).
Papua New Guinea	<i>O. officinalis</i> , <i>O. ridleyi</i> , <i>O. australiensis</i> , <i>O. meriodinalis</i> (Vaughan 1989).
Paraguay	<i>O. alta</i> (Vaughan 1989; Oka 1991).
Philippines	<i>O. rufipogon</i> , <i>O. officinalis</i> (Obien, S. <i>pers. comms.</i> )
Portugal	<i>O. sativa</i> var. <i>sylvatica</i> (MED-Rice 2005).
Spain	<i>O. sativa</i> var. <i>sylvatica</i> (MED-Rice 2005).
Sri Lanka	<i>O. eichingeri</i> , <i>O. sativa</i> f. <i>spontanea</i> (Vaughan 1989; Marambe & Amarasinghe 2000)
Surinam	<i>O. sativa</i> (Watanabe 2000).
Taiwan	<i>O. sativa</i> f. <i>spontanea</i> (FAO 2005).
Tanzania	<i>O. eichingeri</i> , <i>O. brachyantha</i> (FAO 2005).
Thailand	<i>Oryza officinalis</i> , <i>O. ridleyi</i> , <i>O. officinalis</i> , <i>O. sativa</i> f. <i>spontanea</i> , <i>O. nivara</i> , <i>O. ridleyi</i> , <i>O. granulata</i> (Vongsaroj 2000).
Uganda	<i>Oryza eichingeri</i> <i>O. brachyantha</i> (FAO 2005).
Uruguay	<i>Oryza sativa</i> (Deambrosi & Saldain 2000). <i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> (Valverde 2004).
Venezuela	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. latifolia</i> , <i>O. glumaepatula</i> (Valverde 2004).
Vietnam	<i>Oryza sativa</i> , <i>O. rufipogon</i> , <i>O. nivara</i> , <i>O. officinalis</i> (Bui 2000, Chin <i>et al.</i> 2003).
Western & Tropical Africa	<i>Oryza sativa</i> , <i>O. breviligulata</i> <i>O. perennis</i> complex, <i>O. longistamina</i> , <i>O. eichingeri</i>
(Gambia, Sierra Leone, Mali, Senegal)	<i>O. brachyantha</i> (Morishima & Oka 1991; FAO 2005).
USA	<i>Oryza sativa</i> ssp. <i>indica</i> , <i>O. sativa</i> ssp. <i>japonica</i> , <i>O. nivara</i> ; <i>O. rufipogon</i> (Diara <i>et al.</i> 1985; Langevin <i>et al.</i> 1990; Kwon <i>et al.</i> 1992; Baldwin <i>et al.</i> 2000; Vaughan <i>et al.</i> 2001; Gealy <i>et al.</i> 2000, 2002).



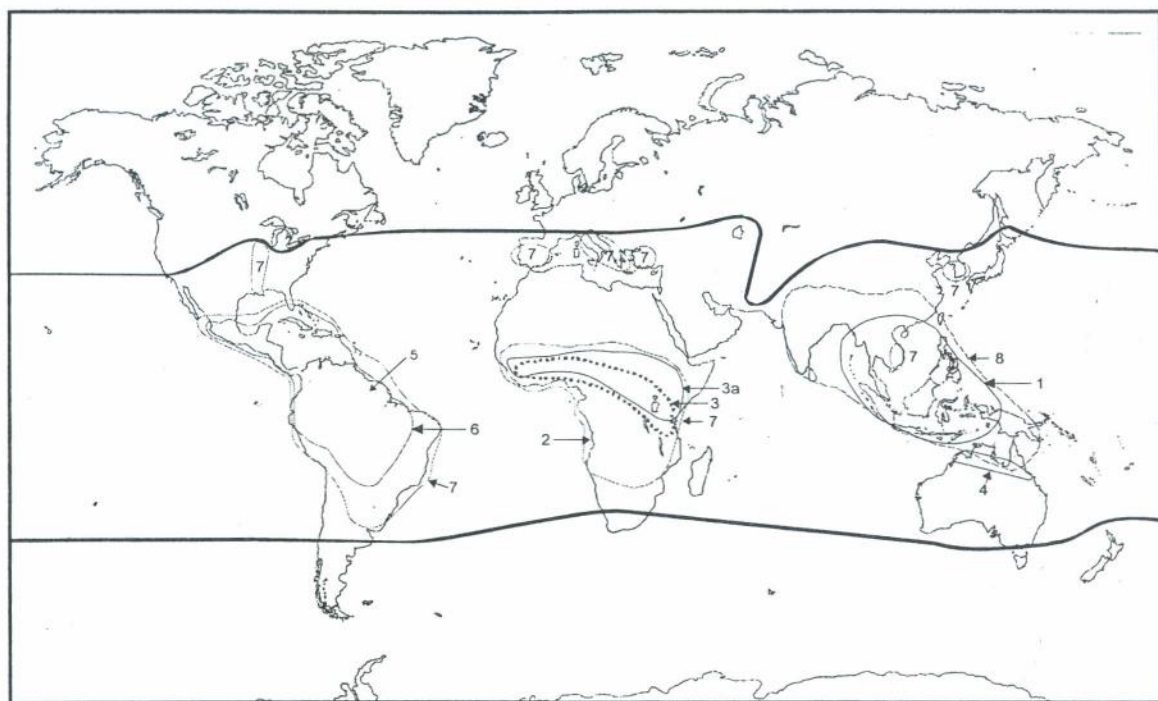


Fig. 1. The world's distribution of wild and weedy rices 1. Weedy rice (*Oryza sativa*), 2. *O. brachyantha*, 3. *O. eichingeri*, 3a. *O. barthii*, 4. *O. australensis*, 5. *O. alta*, 6. *O. glumaepedulla*, 7. Red rice (*Oryza sativa*), 8. *O. rufipogon*, *O. officinalis*, *O. ridleyi*. The lines across the globe represent the northern and southern latitudinal extremes where rice can be grown (After Baki 2005).

The origin of weedy rices remains a frontier issue. Sato (2000) argued that some autogamous strains as intermediates between wild and domesticated forms often prevailing as weeds in or within the fringes of rice fields, particularly in direct-seeded rice habitats. The strains have become differentiated from natural hybridization between cultivars (Arashi 1974) or between the perennial wild and domesticated types (Morishima *et al.* 1984). In the latter case, introgression between genes occurs commonly from the domesticated to the wild type, but such occurrence is rare in the opposite direction because of low fertility of the pollen grains and high outcrossing rate in the perennial type. The recurrent gene flow of this direction may lead to the production of a weedy type having an indica-type nuclear genome and a japonica-type cytoplasmic genome (Sato 2000). Oka (1991) argued that the prevalence of weedy rices as sympatric with wild rices, the other occurs in localities where no wild rice is found, perhaps explain the contrasting views as to the role of weedy rices in crop, namely, one assumes them as the intermediate progenitor of crops, the other considers them to have evolved side by side with the domesticated crops from common progenitor (Harlan 1969). Abdullah *et al.* (1996) and Vongsaroj (2000) argued that the origin of weedy rice was through the segregation of the deciduous "off-types" from extensively planted cultivars during the periods of volunteer seeding. Their populations contain various domesticated cultivar genes, and serve as a gene reservoir (Harlan 1969). Intrapopulation genetic variations in RR were probably the result of natural cross pollination of RR and rice (Lago 1982; Langevin *et al.* 1990). Following intraspecific crossing between RR or WR and rice, innumerable ecotypes with enhanced compatibility with the local habitat, displaying morphological characteristics resembling rice cultivars (Langevin *et al.* 1990, Abdullah, M.Z., *pers. comms.*).

Both WR and RR alike display considerable crop mimicry, and a wide range of morphological, anatomical and physiological traits among their accessions. These WR/RR biotypes are almost indistinguishable from their commercial counterparts at the seedling stage. The gross morpho-ecological and physiological traits and evolutionary characteristics of WR/RR biotypes *vis-à-vis* the



commercial varieties at the tillering and post-tillering stages with more numerous, longer and slender tillers, leaves are more often hispid on both surfaces, taller plant stature, the easy-shattering or deciduousness traits of grains, pigmentation of several plant parts, principally the pericarp, and the presence of awns of variable lengths, in many instances, making WR/RR more distinguishable *vis-à-vis* the commercial varieties. Besides, WR and RR also display variable seed dormancy and longevity, a wide window of adaptability to wet-seeded fields, and relative tolerance to some rice herbicides (Watanabe *et al.* 1996).

## MANAGEMENT OF WEEDY RICE

*“The greatest challenge facing weed scientists worldwide remains in convincing growers and those advising them (extension agents) the benefits of using a weed management decision aid justify the effort”* (Wilkerson *et al.* 2002).

**Integrated Weed Management of Weedy Rice.** The story of agriculture is indeed the story of weed interference (Dekker 1997). In the context of rice ecosystems, the classical concept and practice of weed management is managing weed interference to minimize the effects of weed competition on the rice crops. Today the modern neoclassical, functional and economic approaches of rice weed management go beyond arresting the damage of weed competition on the rice crop. Such approaches are knowledge-based, requiring knowledge-intensive management skills and inputs of crop-weed ecology, weed community dynamics, economic thresholds, production costs (including risk and ethical analysis, and costs to the environment following successive applications of herbicides), innovative ecologically based management practices, competitive rice cultivars, and transgenic rice engineered for herbicide resistance and improved herbicide application methods.

No single weed management component or control method can effectively control WR/RR in rice (Azmi *et al.* 2000; Noldin 2000; Valverde 2004). Farmers normally employ a battery of indirect and direct control methods to achieve satisfactory results. These include, principally, the agro-technical and preventive methods comprising land preparation and tillage, water management and manual weeding; crop manipulation through seeding rates, planting density allelopathy and a choice of competitive cultivars; and chemical weed control (Dunand 1988; Noldin 2000). In certain cases control measures are particularly suitable to a certain rice culture while others transcend rice cultures. Invariably, the most effective methods are those which provide favourable stand establishment and growth for rice but are simultaneously unfavourable to weeds; and probably most important, net profit obtained must be of higher equivalents *vis-a-vis* the cost of control inputs.

**Indirect Methods of Control: Preventive, Substitutive, Agro-technical and Cultural.** The indirect control measures of RR/WR are shown in Table 2. Each of these methods has its own merits and demerits, and when used within the context of integrated weed management systems, should augment each other in reducing RR/WR populations.

Preventing the introduction of invasive weeds is the most effective method for their management and is an essential component of a noxious weed management strategy. However, this is difficult to enforce. The major elements of a prevention programme are to stop the introduction of noxious weed seeds or vegetative propagules, reduce the susceptibility of the ecosystem to invasive weed establishment, develop effective education and extension materials and activities, and establish a programme for early detection and monitoring. Encroachment by weeds happens through establishment of small populations in close proximity to a larger infestation (Sheley *et al.* 1999). To prevent this kind of encroachment, effective containment of neighbouring weed infestations through herbicide sprays on the borders of infested areas should be made. Strict quarantine enforcement preventing free movement of animals, vehicles and farm machines from infested lands should also be carried out. This is a difficult and an expensive routine to carry out. In the case of weedy rices, only certified high quality seed-free rice seeds should be planted by farmers (Baki *et al.* 2000; Vongsaroj 2000; Azmi *et al.* 2004). This is an achievable target with close monitoring and political



will of the policy makers in the countries concern. In countries where certified rice seeds are not easily available or inadequate in supply, or where farmers rely on their own collection of seeds for planting, weedy rice contaminants are a risk to content with. High-quality or high-vigour seeds can result in faster and better seedling establishment. Noldin (2000) reported that planting rice field free of RR with rice seeds with two RR seeds  $\text{kg}^{-1}$  may result in a soil seed bank of 100 kg of RR seeds  $\text{ha}^{-1}$  after just three seasons! In Uruguay, RR is not allowed in any class of commercial seed.

**Table 2.** Components of indirect control methods of weedy- or red rice.

Control components	Merits/Demerits	Reference
Prevention/Eradication	Quarantine measures, difficult & expensive to enforce use of WR/RR-free certified seeds effective in long-term control, require effective quarantine and extension services. Panicle cutting of WR/RR before maturity; expensive to carry out in large farms.	Baki <i>et al.</i> 2000; Vongsaroj 2000; Noldin 2000; Chin 2001.
Tillage/Stale seedbed	2-3 rounds of tillage, augmented by blanket or spot sprays with low doses of glyphosate. Field leveling is necessary. Tillage implements should be free from WR/RR contaminants, difficult to enforce in systems where farmers hire tillage/harvesting machines	Watanabe <i>et al.</i> 1996. Azmi <i>et al.</i> 2000.
Water management	Fields inundated 5 -10 cm, 5 days after wet-seeding or 14 days after dry seeding. Inundation of rice fields suppresses WR/RR emergence and establishment.	Azmi <i>et al.</i> 2000. Noldin 2000
Seeding technique/rate	Pre-germinated wet seeding gives better seedling establishment than dry-seeding. 80 -100 kg/ha <i>vis-a-vis</i> the optimum 60 kg/ha seeding rate as insurance against establishment uncertainties.	Baki <i>et al.</i> 2000.
Crop rotation	Rice-soybean rotation grown at alternate years for 4 years controlled RR effectively. Brings about changes to the overall crop-weed ecology. Allows rotation of herbicides; prevent subsequent build-up of weed resistance. Farmers plant high-priced market-driven produce, rather than rice for better income.	Smith, Jr. 1989; Azmi <i>et al.</i> 2000; Valverde 2004.
Choice of cultivars and/or planting date	Competitive short maturation cultivars can outcompete WR/RR, e.g. IR 64. Use of transgenic or herbicide-resistance rice cultivars. Unacceptable to farmers/policy makers in some countries for environmental concern and food safety.	Azmi, M. ( <i>pers. comms.</i> )
GIS-monitoring of	Useful to monitor spatio-temporal spread of WR/RR. Less effective with WR accessions closely mimicking cultivated rice. Expensive and not accessible to farmers in many developing rice-growing countries.	Park 2000.

Eradication is an expensive undertaking and is often a stepchild in the field of introduced species management (Simberloff 2002, 2003). Rather, maintenance management is usually seen as the appropriate response – controlling an invader at a density sufficiently low that we can tolerate it. Simberloff (2003), in his treatise on the economics of eradication, lamented that although the success of permanent eradication of an invader from a site is alluring, society cannot undertake to eradicate every pestiferous invader, given the costs of successful eradication may entail. Eradication becomes the best management option when the benefits derived are clearly overwhelming (Arrow

*et al.* 1996). Given the rate of spread and infestation, eradication campaigns to eradicate large populations of weedy rice from any rice-growing area is an illusive battle with little chance of success.

Cultural practice of WR/RR include the use of stale bed techniques, water-seeded rice with pre-germinated seeds, crop rotation, and management practices to reduce RR/WR seed bank. WR/RR seed longevity increases when the seed is buried deep in the soil (Nordin 1995; Watanabe *et al.* 1996; Azmi *et al.* 2000; Vongsaroj 2000). Following harvest, rice fields infested with RR/WR should be left fallow, leaving the RR/WR seeds near or on the soil surface, allowing them to germinate or losing viability faster than when buried deep in the soil.

The size of seed bank plays an important role in determining the severity of infestation by weeds, including WR/RR. Proper tillage must be undertaken to reduce this seed reservoir. Land preparation, especially puddling and harrowing, provides a weed-free environment at planting and often aids in good crop establishment while minimizing weed growth and proliferation in the established crop. Azmi *et al.* (2000) advocated sequential tillage operations within the framework of integrated weed management to reduce WR populations in direct-seeded fields (Fig. 2). The initial tillage should be shallow enough to encourage sizeable germination of WR seeds while subsequent tillage must be thorough enough to ensure that all volunteer seedlings are annihilated.

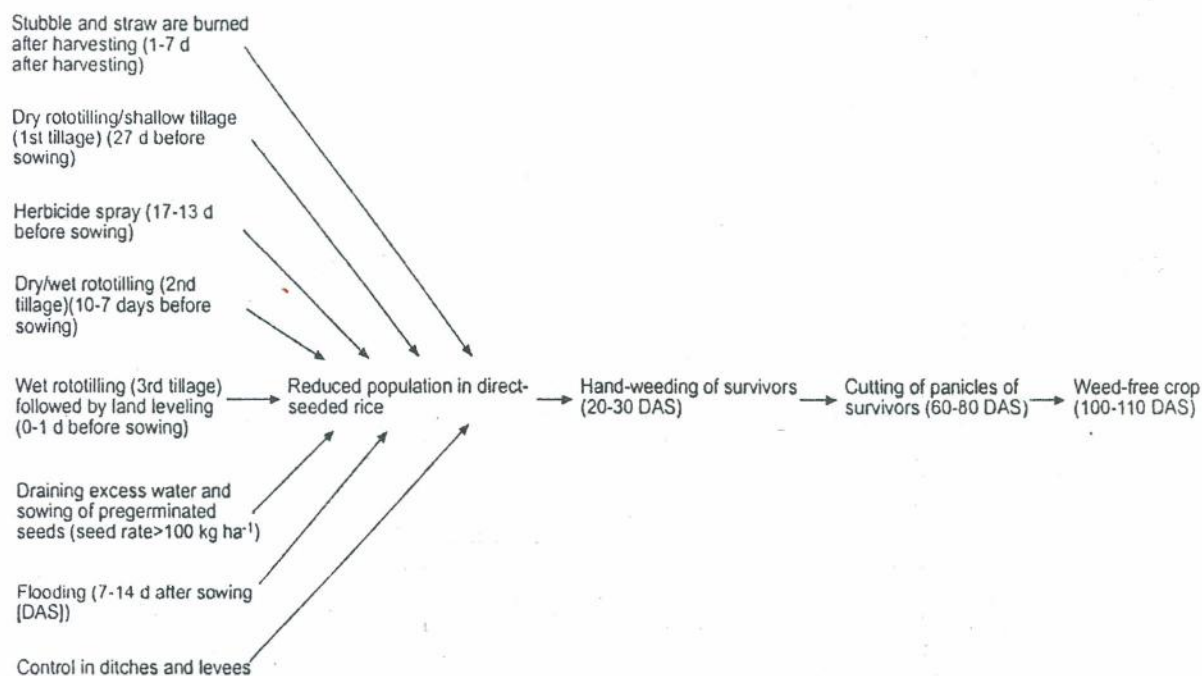


Fig. 2. Integrated approach to control weedy rice in direct-seeded fields in Malaysia (after Azmi *et al.* 2000).

Three rounds of tillage at 10-d intervals are effective in reducing WR populations. To ensure total kill, preplanting or pre-seeding sprays with non-selective herbicides such as glyphosate or glufosinate ammonium are occasionally needed. Minimum or zero tillage (MZT) systems are used in many areas with severe WR/RR problems. Seed drilling or water-seeding follows 15 - 20 days after land preparation and spraying the fields with non-selective herbicides such as glyphosate or glufosinate ammonium (Nordin 2000; Azmi *et al.* 2003). Appropriate water management is singularly important for controlling weeds irrespective of rice cultures. Inundation of rice fields suppresses weed emergence and establishment, including RR/WR. Rice fields should be flooded soon after rice emergence, preferably at 5 -10 cm until booting stage, otherwise RR control is



ineffective. In water-seeded rice, pre-germinated seeds are broadcasted in the water in leveled fields soon after seedbed preparation. This system warrants permanent levees, and offer one of the best alternatives for WR/RR control in Brazil and elsewhere. Field drainage is monitored so as not to expose soil to air and increases oxygen concentration in the soil, thereby stimulating RR/WR germination.

An important component in the indirect cultural and preventive weed control methods in rice is the use of suppressive and competitive rice cultivars against WR/RR. A short-maturing variety, IR 64, outcompetes WR, where early harvesting of IR 64 at the time when WR is still at its flowering stage, will indirectly help reduce the WR seed bank. (Azmi *et al.* (2000). Puckridge *et al.* (1988) recommended the planting of cultivars with distinguishing colour traits (e.g. Khao Niew Dam), a cultivar with purplish stems and leaves to differentiate crop from weedy forms, and so aid in manual weeding. However this tactic will only be successful in the long term, and if hybridization with wild rice is prevented, such the choice of planting date.

**Developing Herbicide-Tolerant Rice Cultivars.** Commercial development is under way for three HR rice varieties: Clearfield® (imidazolinone resistant), Roundup Ready (glyphosate resistant), and LibertyLink® (glufosinate resistant). The respective varieties will be resistant to imidazolinone, glycine, and phosphinic acid chemical families (Schmidt 1997). The transgenes for glyphosate resistance are biological derived from a plant or bacterial source and encode for an alternative enzyme with an active site that is not inhibited by the herbicide (OECD 1999a). The transgene for glufosinate resistance was derived from a soil bacterium (Michiels & Johnson 2001). According to Croughan (1994), an ethyl methane sulfonate-induced mutation of the acetolactate synthase (ALS) gene is the basis for herbicide resistance in the imidazolinone rice varieties, conferring resistance to imidazolinones and certain sulfonylurea herbicides.

The imidazolinone-resistant rice varieties Clearfield®, viz. CL 121, CL 141, were planted in southern USA on a wide scale for the first time in 2002, while the varieties CL 161, and Clearfield XL8 are now in the field since 2003. The imazethapyr herbicide Newpath® has been approved for use on these varieties by the EPA of USA. Both imidazolinone- and glufosinate-resistant rice varieties are in the advanced stages of development in Brazil. Further developments in Arkansas, USA indicated that imazethapyr and glufosinate could be used for weed control in a chemically mutated Clearfield® rice (Baldwin *et al.* 2000; Dillon *et al.* 2000), although the development status for glyphosate in Roundup Ready rice is uncertain and tortuous.

Riches & Valverde (2002) and Madsen *et al.* (2002), among others, argued that prior to the introduction of HR crops including HR/HT rice, their short- and long-term risks should be thoroughly assessed. Generally, the cultivation of HR/HT rice would lead to parallel increase of volunteer rice crop problems. Sankula *et al.* (1998) in their genetic analysis of glufosinate resistance in crosses between Gulfmont rice transformed rice with the bialaphos resistance (BAR) gene and RR and BAR-transformed Kohihikari rice and RR reported glufosinate resistance in all F<sub>1</sub> hybrids, with either of the rice types as a maternal parent. Braverman (1997) observed no competitive advantage of resistance in the glufosinate resistance rice, although the competitive ability of glufosinate-resistant red rice hybrids was unknown. He argued that the ontogeny of RR plants escaping herbicide treatment may be delayed *vis-à-vis* glufosinate-resistant rice, and flowering may not be synchronized, thus preventing hybridization. Such argument is flawed as WR/RR plants may emerge out in frequent time-mediated flushes, and hybridization, however, low in frequency, may still prevail. In fact, and based on long-term HR/HT rice cultivation in Latin America, relying on glufosinate-resistant rice for weed control, Madsen *et al.* (2002), using a ten-year run, predicted that resistance to glufosinate would occur within 3 -8 years of monoculture. Increasing hybridization from 1 to 5% decreased the time for resistance to occur by 1 to 3 year. Further, the risk assessment process of HR/HT rice revealed that several weed species have already evolved resistance to



herbicides used in conventional rice crops. It is likely that that this development be enhanced by lack of crop rotation and the persistent use of herbicides having the same mode of action, imposing a high selection pressure on weed populations, and especially, if the trait is transferred to compatible weedy crop relatives.

Herbicide-resistant crops may serve as effective alternatives to selectively control problem weeds (Braverman 1997; Sankula *et al.* 1997a,b; Baldwin *et al.* 2000; Dillon *et al.* 2000; Howard *et al.* 2000). The use of herbicide-tolerant crops has been demonstrated to be cost efficient, and it allows the farmer more flexibility in the timing of herbicide application to control weeds not controlled by conventional means or products. For example, the insertion of the bialaphos resistance gene allows the use of non-selective herbicide glufosinate for the selective control of red rice and other problem weeds in rice cropping systems (Braverman & Linscombe 1994). In the case of HR/HT rice, improved control of weed flora associated with the crop, namely the conspecific RR or other weedy rice species (Gealy & Dilday 1997; Olofsdotter *et al.* 2000). Other perceived benefits derived from improved crop production with HR/HT rice and substitution of some of the currently used herbicides with others that are less detrimental to the environment (Burnside 1992; Olofsdotter *et al.* 2000). There are enough evidences of that the introduction of these herbicide-tolerant (HT) or herbicide-resistant (HR) rices accentuate the risks of gene flow into weedy relative, the RR (Gealy *et al.* 2003; Shivrain *et al.* 2004) or the potential development of herbicide-resistant or ferality in RR (Gealy & Estorninos, Jr. 2004). Such risks are monitored through SSR marker and phenotypic analyses of segregating populations to identify, quantify, and track the RR hybrids in farmers' and research fields. Rice and WR/RR are primarily self-pollinated, but can cross-pollinate one another, providing avenue for the transfer of genetic traits such as herbicide resistance from rice or weedy rice. Once hybrids are formed, they may introgress into a population within a few generations. Gealy *et al.* (2003) believed that intense, continuous use of HR/HT rice variety would provide strong selection pressure in favour of such hybrid families. However, and based on relatively low outcrossing rates between these sympatric populations of RR, IMI Rice, or non-HR rice are always <0.5%, Gealy & Estorninos, Jr. (2004) argued that the establishment of feral populations of hybrid, weedy rice in non-rice or non-agricultural areas remain low, at least in the short-term. Similar contentions were forwarded by Shivrain *et al.* (2004) with Clearfield® rices CL 121 and CL 161, although the presence of herbicide-resistance trait among RR populations was detected.

**Direct Methods of Control.** Physiological similarities between RR/WR limit chemical control options (Jordan & Sanders 1999). However, Vietnamese farmers applying oxadiazon at 250 g a.i. ha<sup>-1</sup> or oxadiargyl at 72 -100 g a.i. ha<sup>-1</sup> to control WR, obtained significantly higher rice yields than the control (Chin *et al.* 2000). Pyon *et al.* (2000) registered good control of WR in Korea also with oxadiazon applied at 240 g a.i. ha<sup>-1</sup> at six DAS. Slight injury was observed under drained field conditions. In Malaysia and Japan, Azmi *et al.* (2000) and Sadohara *et al.* (2000) reported good control of WR with molinate at 4.5 kg a.i. ha<sup>-1</sup> and thiobencarb at 300 g a.i. ha<sup>-1</sup>, respectively. Graminicides such as clethodim, fluazifop-P, quizalofop-P and sethoxydim were efficacious for control and seed head suppression of RR especially at booting stages than when applied at early growth stage (Guy & Helms 1995; Askew *et al.* 2000) although the efficacy was dependent on application timing and environmental conditions during treatment. With the absence of herbicides for RR control in commercial rice, rice farmers are encouraged to rotate rice fields with soybean, and take advantage of herbicides available to control graminaceous weed species (Khodayari *et al.* 1987). Crop rotation, very often, for reasons of selectivity, may also mean rotation of herbicides. Such rotation of crops not only will bring about changes to the overall crop-weed ecology, but also prevent the continuous use of same herbicide(s) and the subsequent build-up of weed resistance (De Datta & Baltazar 1994; Valverde *et al.* 2000). Some of the chemical control attempts of RR/WR are shown in Table 3.



**Table 3.** Chemical control of red (RR) and weedy rice (WR).

Herbicides	Rates/Comments/Reference
AC 263,222	EPOST <sup>a</sup> , 30 g a.e. ha <sup>-1</sup> suppressed seedhead of RR by 95%. Caused 30% injury to soybean; reduced yield 200kg ha <sup>-1</sup> (Askew <i>et al.</i> 1998a)
Acetolachlor	PRE <sup>b</sup> application at 1.5 kg a.i. ha <sup>-1</sup> controls 92% RR (Eleftherohorinos & Dhima 2002)
Alachlor	PPI <sup>c</sup> or PRE, 4.5 kg a.i. ha <sup>-1</sup> controls 83-95% RR for 2 of 3 years; PRE application at 2.4 kg a.i. ha <sup>-1</sup> controls 84 % RR (Noldin <i>et al.</i> 1998; Eleftherohorinos & Dhima 2002).
Cicloxidim	0.40 kg ha <sup>-1</sup> provided excellent control of RR (Sparacino <i>et al.</i> 2000)
Clethodim	EPOST, 110 kg ha <sup>-1</sup> followed by 0.70 - 110 kg ha <sup>-1</sup> controlled 54 - 83% RR ; LPOST <sup>d</sup> , 110 kg ha <sup>-1</sup> controlled RR, suppressing seedheads at least 95%. 0.70 - 110 kg ha <sup>-1</sup> controlled 54 - 83% RR at 2 - 4 leaf stage, or 51 - 83% at 2-3tiller stage, 2 - 6 WAT <sup>e</sup> or 50% at booting stage, 2 WAT in soyabean (Askew <i>et al.</i> 1998a; 2000)
Dimethenamid	PRE application at 1.4 kg a.i. ha <sup>-1</sup> controls 92% RR (Eleftherohorinos & Dhima 2002).
Fluazifop-P	0.210 kg ha <sup>-1</sup> controlled 53 - 58 % RR at 2 - 4 leaf stage, or 55 -66% at 2-3 tiller stage, 2 - 6 WAT or 57% at booting stage, 2 WAT. Reduced RR seedhead by 50% in soyabean (Askew <i>et al.</i> 2000).
Glufosinate	<p>POST, 0.6 kg a.i. ha<sup>-1</sup> controlled 96% of RR. Reduced plant height, RR. Reduced plant height, panicle maturity, and 100-seed weight by 16, 31, and 24%, respectively, when mixed with 3.4 kg a.i. ha<sup>-1</sup> propanil, or 0.6 kg a.i. ha<sup>-1</sup> acifluorfen. Less toxic to BAR-transformed rice when applied with 0.4 kg a.i. ha<sup>-1</sup> of triclopyr or 0.6 kg a.i. ha<sup>-1</sup> of acifluorfen. POST, 1.1 - 2.2 kg a.i. ha<sup>-1</sup> injured BAR-transformed Koshihikari rice from 0-53%. No injury on transgenic Gulfmont rice &amp; 6 out 9 lines Koshihikari rice. Good control of RR but efficacy reduced with 25-50% flooding of RR plant height. POST, 2.2 kg a.i. ha<sup>-1</sup> injured 23-26% Gulfmont rice when applied at 1 to 2-leaf stage, 13-19% injury when applied at 3 to 4-leaf stage. POST, 1.1 kg a.i. ha<sup>-1</sup> controlled 91% of RR at 3 to 4-leaf stage, 74% at panicle initiation or 77% at booting stage. Injury to RR was 2 -11 times more than BAR-transformed Gulfmont rice (Sankula <i>et al.</i> 1997 a,b).</p> <p>POST, 1.0 kg ha<sup>-1</sup> controls 81% RR seedlings (Eleftherohorinos &amp; Dhima 2002).</p> <p>POST, 0.8 - 1.7 kg ae ha<sup>-1</sup> controls 88 - 91% RR at 2-leaf to 2 to 3 tiller-stage, 2 WAT Reduced seedheads of RR by 56-87% and 97% with POST applications at 2 to 4-leaf stage and 2 to 3 tiller-stage (Askew <i>et al.</i> 1998a).</p> <p>POST, 1.8 kg ha<sup>-1</sup> controls 89% RR seedlings (Eleftherohorinos &amp; Dhima 2002). POST, 0.36 - 1.8 kg ha<sup>-1</sup> reduced seed viability of strawhull &amp; blackhull RR by 36-74% (Deambrosi &amp; Saldain 2000).</p>
Imazethapyr	> 70 g ha <sup>-1</sup> gave 90% control of RR (Klingman <i>et al.</i> 1992)

Sequential POST applications at 0.07 kg ha<sup>-1</sup> control RR of Clearfield, Liberty Link, and Round Ready rice. 5 – 50% crop injury prevailed (Baldwin *et al.* 2000; Dillon *et al.* 2000).

Better RR control when applied POST<sup>+</sup> *vis-à-vis* PPI (Wei *et al.* 2001)

Singular PPI (70 -110 g ha<sup>-1</sup>) applications control RR better ( 85 – 98%) than PRE applications (75 – 95%) application in imidazolinone-tolerant rice var. 93AA3510 (Steele *et al.* 2002).

Sequential PPI followed by POST Application gave 92 – 98% RR control. Visual injury to rice. 94% RR control applied at PPI or PRE at 0.070 to 0.10 kg ha<sup>-1</sup> followed by EPOST application. Sequential POST applications led to 95% RR control. Early-season imidazolinone-tolerant rice

var. CL 131 injury of 0 -34% with POST applications resulting in some rice yield reductions (Ottis *et al.* 2003).

90 -96% RR control at 21 DAT applied to soil either before seedling flood (surface SURF applied) followed by POST application at the respective rate of 0.053 kg ha<sup>-1</sup> respectively, at 28 DAT (Pellerin *et al.* 2003).

Imazaquin POST, 140 - 280 g a.i. ha<sup>-1</sup> controls 82 – 96% RR at 14 and 28 DAT, respectively. Low injury to IMI Rice (Webster & Masson 2001).

Imazapyr POST, 32 - 64 g a.i. ha<sup>-1</sup> controls 82 -89%RR at 14 and 28 DAT, respectively. Injury to IMI<sup>f</sup> Rice (Webster & Masson 2001).

Maleic POST, 2.16 kg a.e. ha<sup>-1</sup> lowered seed viability by 31% for strawhull RR, 15% for blackhull RR hydrazide (Deambrosi & Saldain 2000).

Metolachlor PPI at 3.4 kg a.i. ha<sup>-1</sup> control RR late season 90-92% (Noldin *et al.* 1998). PRE application at 2.5 kg a.i. ha<sup>-1</sup> controls >85% RR (Eleftherohorinos & Dhima 2002).

Molinate POST application at 4.5 kg a.i. ha<sup>-1</sup> controls WR (Azmi *et al.* 2000)

Nicosulfuron POST, 35 g a.i. ha<sup>-1</sup> controls 73 and 95% RR at 14 and 28 DAT, respectively. Low injury to IMI Rice 93AS3510 (Webster & Masson 2001).

Oxadiargyl POST, 72 -100 g a.i. ha<sup>-1</sup> controls WR (Chin *et al.* 2000)

Oxadiazon POST, 240 - 250 g a.i. ha<sup>-1</sup> controls WR (Chin *et al.* 2000; Pyon *et al.* 2000)

Paraquat POST, 0.8 kg ha<sup>-1</sup> controls 92% of RR seedlings (Eleftherohorinos & Dhima 2002)

Quizalofop-ethyl 0.10 kg ha<sup>-1</sup> provided excellent control of RR (Sparacino *et al.* 2000). 0.070 kg ha<sup>-1</sup> controlled 65-86 RR at 2 – 4 leaf stage, 2 – 6 WAT or 86 – 90% at 2-3 tiller stage. Reduced RR seedhead by 50% in soyabean (Askew *et al.* 2000). POST, 0.1 kg ha<sup>-1</sup> controls 100% RR seedlings (Eleftherohorinos & Dhima 2002).

Quizalofop-P POST, 0.07 kg ha<sup>-1</sup> controls 83-95% RR, at least for 2-3 years (Noldin *et al.* 1998)

Rimsulfuron POST, 14 -28 g ha<sup>-1</sup> control 63 – 98% RR at 14 and 28 DAT, respectively. Injury to IMI Rice (Webster & Masson 2001).

Ropaquizafop 0.078 kg ha<sup>-1</sup> provided excellent control off RR (Sparacino *et al.* 2000).



SAN 582H    PPI or PRE at 2.2 – 3.4 kg a.i. ha<sup>-1</sup> controlled 83 – 95% RR (Noldin *et al.* 1998)

Sethoxydim    0.420 kg ha<sup>-1</sup> controlled 60 – 84 %    RR at 2 – 4 leaf stage, or 77 - 84% at 2-3 tiller stage, 2 – 6 WAT or 39% at booting stage, 2 WAT in soyabean. Reduced RR seedhead by 50% in soyabean (Askew *et al.* 2000).

Thiobencarb 300 g a.i. ha<sup>-1</sup> controls WR (Sadohara & Watanabe 2000).

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<sup>a</sup> Early post-emergence application; <sup>b</sup> Pre-emergence application; <sup>c</sup> Pre-plant incorporated; <sup>d</sup> Late post-emergence application; <sup>e</sup> Weeks after transplanting; <sup>f</sup> Imidazolinone-resistant rice.

## FUTURE CHALLENGES

The new millennium witnesses food security and food scarcity (FSFS) as major issues haunting the world populace at large, notably in developing countries. These countries depend to a large extent on adequate supply of cereals, notably rice, as a staple food for their populations. With demand for rice outclassing supply coupled with increasing world's population spell uncertainty in the world rice market. The apparent discrepancy in the rice production capacity and requirement between countries aggravated the problems supply and demand in world's rice market. While rice farmers in USA and

Australia produce more rice exceeding the domestic demand by both countries, countries like Uruguay, Brazil, and Malaysia for examples, require importation from abroad to meet the domestic demand of their populace. Recently, two of the world's biggest supplier of rice, Thailand and Vietnam, plagued by drought and floods, announced shortfalls in supply, hence the export quantity of the commodity.

A perennial issue facing rice farmers worldwide is pest outbreak with weeds taking the centre stage. The advent of recalcitrant, hard-to-control millennial weeds like WR and RR, coupled with increasing incidences of herbicide-resistant weed species in rice ecosystems worldwide are challenges facing the rice industry at large thereby requiring farmers to invest higher input costs to control these weeds. The ability to institute control measures against WR/RR with minimal input costs yet inflicting no yield loss to commercial rice warrants the commitment of farmers, extension agents and other players in the rice industry. WR/RR infestations inflict yield loss to a variable degree. The ability of extension agents to inculcate awareness among rice farmers that WR/RR infestations inflict yield loss to a variable degree, and that integrated control measures against the scourge must be carried out early enough, are other key challenges facing the rice industry. This is especially important to small-scale peasantry rice farming communities, where state of the art control inputs and credit facilities are not always at their disposal. Farmers must be advised that intensive rice monoculture breeds resistance in other weed species. Where possible crop rotation should be practised so as to break the RR/WR infestation cycles allowing the change of cultural and agronomic practices, and the change in the spectrum of herbicide use as well. The use of certified WR/RR-free rice seeds, the sharing or use of WR/RR-clean tillage and harvesting implements, and cutting of immature WR/RR panicles are other ways to prevent the spread in fields devoid of the scourge.

The planting of HR/HT rice cultivars as an alternative measure to arrest WR/RR infestation is a controversial issue not only among rice farmers, but also among weed science fraternities, and policy makers alike. While proponents of HR/HT or transgenic rice cultivars advocate strongly for their adoption as an effective way to control RR/WR infestation though selective use of herbicides which are claimed to be environmentally benign, strong reservations prevailed against such adoption due to the unknown long-term risks of gene introgression from HR/HT rice to their weedy counterparts. In fact such reservations against the adoption of HR/HT rice are not without foundation. Gene introgression do occur between HR/HT rice and RR/WR or between transgenic



rice and RR (Sankula *et al.* 1997a, b, 1998) or between HR/HT rice and RR/WR (Madsen *et al.* 2002; Gealy *et al.* 2003; Gealy & Estorninos, Jr. 2004; Shivrain *et al.* 2004).

With innumerable accessions RR/WR of the deciduous “off-types” segregants from extensively planted cultivars during the periods of volunteer seeding (Abdullah *et al.* 1996; Vongsaroj 2000; Choi 2000; Vaughan *et al.* 2001; Valverde 2004), problems of WR/RR in rice fields are here to stay. Our ability to manage these WR/RR populations below the economic threshold levels season after season require the sharing of knowledge and experience for the common good of humanity. The establishment of global databases and RR/WR management network is one way that the entire world rice community can share and benefit from each other. The MED-Rice is one of such network. Such networking facilities are not common in rice-growing areas of the developing countries for a variety of reasons. The digital divide between the rice-growing areas of USA, Europe and Japan, and those in the developing world is one reason for the “apparent lack of dialogue and cooperation” to solve RR/WR problems worldwide. The weed science fraternities, especially those working in RR/WR management need to bridge this divide.

Another teething problem is the farm subsidies in USA, Europe, or Japan or lack of them in many developing countries. While rice farmers in USA, Europe, or Japan enjoy considerable farm subsidies in terms of price-support system, and their produce are competitive in the world rice market, many rice farmers elsewhere can only dream of such subsidies or price-support system. In this way another of divide – “market divide” prevails, making farmers in the developing world of Africa, Asia, and Latin America at a disadvantage *vis-à-vis* their counterparts in USA, Europe or Japan. Bridging such market divide within the context of the World Trade Organization will go a long way to help peasantry rice farmers in the developing countries to get a fair price in the world rice market. This divide becomes more warranting due to the inadequate or lack of farm credits made available to many rice farmers in the developing world. I believe strongly that if back-breaking manual weeding is still instituted in weed control against RR/WR or any weed species for that matter in this so called post-modern era of the new millennium, then something is wrong with our attitude towards humanity as a whole. Either the world community is not sensitive to the needs and suffering of the human race, or there are real gaps in our extension activities failing to outreach the rice farmers to help modernise their weed management effort and boost their rice yields. Sadly, many rice-growing countries in Africa, Latin America and Asia are not able to provide price-support for rice produce or farm subsidies to rice farmers. I can say with pride that rice farmers in Malaysia enjoy not only price-support system and subsidies in many forms from the government but also good extension and technical-support services from government-run agencies.

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Rice (*Oryza sativa*) is the most important food crop worldwide but as yet, little transgenic rice is commercially planted. Among factors that have prevented the commercial release of transgenic rice, including herbicide resistant (HR) varieties, gene flow to compatible relatives and concern about consumer and market response in importing countries are most important. In this presentation we consider general aspects of gene flow between plants with novel traits and their relatives and the specifics about rice. We also elaborate on possible ways to prevent and mitigate gene flow.

## Gene flow and introgression

Crops and weeds come from wild plants but domesticated crops have been subjected for millennia to selection for certain traits such as self-fertility, non-shattering seeds, lack of dormancy and particular plant architecture to the point of becoming highly dependent on humans for establishment and propagation (see Warwick and Stewart 2005 for an excellent review). Thus it is not surprising that many crops, especially those that exist as a crop-weed-wild complex such as rice, remain exchanging genes when they are sympatric under conducive conditions (i.e. overlapping flowering time, presence of pollinators if required). Warwick and Stewart (2005) list the most important world food crops and their related, sexually compatible and economically important weeds. Five crops (rice, oilseed rape, sugarcane, oat, and sorghum) among the 25 most cultivated food crops are known to have introgressing weedy relatives among the 180 weeds that are estimated to cause 90% of the weed damage worldwide. The ecological effects of transgenic crops and of the escape of transgenes to wild and weedy populations have been the subject of several reviews and books (Dale et al. 2002, Ellstrand 2003, Gressel 2002, Keeler et al. 1996, Pilson and Prendeville 2004, Stewart 2004, Wolfenbarger and Phifer 2000), including some in relation to rice (Gealy 2005, Lu et al. 2003, Olofsdotter et al. 2000).

Vaughan et al. (2005a) describe the taxonomic status of *Oryza* species, some of which are weeds in rice fields at particular locations. There are 23 species and nine identified genomes in the genus *Oryza*. The *O. sativa* complex contains seven species, all diploid and sharing the AA genome. Hybrids between species in this complex can be produced without the need for embryo rescue, some occurring in nature. The most widely documented weedy rice species is *O. sativa* itself. Wild rice species, particularly *O. rufipogon*, the immediate ancestor of cultivated Asian rice (*O. sativa*), is also weedy especially in Asia (Holm et al. 1997, Song et al. 2003, Vaughn et al. 2005a). *O. rufipogon*-like populations that invade rice fields are generally forms with introgressed genes from cultivated rice that associate intimately with the crop. These weedy forms have been documented in the Americas (Lentini and Espinoza 2005, Peña-Deyán and Ortiz 2001, Sánchez-Olguín 2001, Vaughan and Morishima 2003, Vaughan and Tomooka 1999, Vaughan et al. 2001, B. Valverde, E. Skov and S. Andersen, unpublished) and in Australia (Vaughan and Morishima 2003). Weedy rice is also increasing in importance in Europe (Vidotto and Ferrero 2005). In parts of West Africa, where *O. glaberrima* is the cultivated rice species, its ancestor *O. barthii* has become a major weedy-rice form particularly in direct-seeded rice (Davies 1984, Johnson 1997). *O. longistaminata* is also weedy in rice fields in Africa (Johnson et al. 2004), and it hybridizes with *O. sativa* (Vaughan and Morishima 2003) and the diploid form of *O. punctata* (Johnson et al. 2000, Vaughan and Morishima 2003). *O. officinalis* (CC genome) is a weed in rice in the Philippines (Vaughan et



al. 2005a). In Central America, the native tetraploid (CCDD) species, *O. latifolia* is weedy in rice fields (Valverde 2005). *O. glumaepatula* (CC genome), also native to this area, has the potential to become weedy in rice fields (Lentini and Espinoza 2005, Morishima 2001).

Genes, including those for novel traits such as transgenes, can spread by pollen and seed dispersal to other crop populations and to compatible weeds and wild relatives. Proximity and some flowering overlap are key for pollen flow. Successful cross-pollination requires that the donor plants produce viable pollen that survives long enough to interact with the stigmas of the receptive plant. Still, there are natural barriers that minimize cross-pollination in rice, yet gene flow does occur in the field. Rice florets open once for a short period limiting the time for fertilization. Pollen viability is also limited to a few minutes once exposed to the external environment. The stigma is more tolerant to environmental stress, and remains viable for a few days, allowing it to be fertilized by foreign pollen (Gealy et al. 2003).

Using pollen traps, Song et al. (2004a) determined horizontal and vertical pollen dispersal patterns of a cultivated rice variety. Dispersal decreased with increasing distances from pollen sources and was influenced by wind direction and speed. At a wind speed of  $2.5 \text{ m s}^{-1}$  rice pollen traveled 38 m. More pollen grains were detected at the height of 1.0–1.5 m than at 2.0 m, indicating that rice pollen mainly disperses at relatively low heights.

Herbicide resistance (HR) genes are easy to measure markers of gene flow. HR genes incorporated into rice varieties can also escape through crop volunteers arising from seed lost before or during crop harvest. The amount of crop seed lost is about 35 and 40–75  $\text{kg ha}^{-1}$  for stripper headers and for conventional combines, respectively, enough to plant a third of a hectare. Volunteer HR plants in rice monoculture that emerge together with “rotational” conventional varieties would have an additional opportunity to outcross with the conventional variety and the weedy rice populations associated with it. Transgenic seeds could also potentially become locally established as persisting feral (partially de-domesticated) transgenic populations, or can be transported long distances by farm equipment or trucks. A thorough discussion of this issue in several crops, including rice is available (Gressel 2005). Feral transgenic populations may also serve as sources or bridges for transgene flow to wild relatives (Claessen et al 2005b).

### **Introgression of transgenes from transgenic rice to other rice varieties and landraces**

Rice is predominately self-pollinated and cleistogamous but cross-pollination does occur albeit at low rates, usually less than 1% (Table 1). Recent field studies with glufosinate-resistant rice conducted in the US confirmed limited gene flow between transgenic and conventional rice varieties. In a study in Louisiana, using a central plot of 90% transgenic rice and 10% conventional rice surrounded by conventional rice, outcrossing was 0.08% when the two rice varieties were inter-seeded. Hybrids were detected only between adjacent plants and none at distances of up to 21 m from the pollen source. In California, maximum outcrossing was 0.4% with no hybrids detected beyond 1.8 m from the pollen source (Table 1). Although crop-to-crop gene flow is perceived as a minor ecological problem, it is of agronomic importance. Cross-pollination of conventional varieties being grown for certified seed may result in the seed being disposed as grain. In seed certification there are strict tolerances for weedy rice seeds and, though more relaxed, for varietal contamination. In California, for example, certified rice seed must contain no weedy rice at all but a maximum of nine seeds of other crop seed or other varieties  $\text{kg}^{-1}$  is allowed (i.e. a maximum contamination level of about 0.02%). Furthermore, under California Rice Certification Act of 2000 transgenic rice seed or conventional seed commingled with transgenic rice seed will fall into a classification of seed having “characteristics of commercial impact,” meaning traits that may adversely affect the marketability of rice. The presence of transgenic rice in organic rice may result in denial of organic certification and decreased value.



Table 1. Estimated outcrossing rates among cultivated, weedy and wild rice (*Oryza* spp) from selected gene flow studies.

Country	Estimated outcrossing rate	Study details	References
<u>Crop to crop (both <i>O. sativa</i>)</u>			
USA	0.08% when two varieties were intersown. No hybrids found outside central plot	Central plot of 90% transgenic GFR <sup>1</sup> and 10% conventional rice surrounded by conventional rice	Studies conducted by S. Linscombe (Louisiana Sta. Univ.) and S. Pinson (USDA ARS, Beaumont, Texas). D. Mitten (Bayer Crop Science, Woodland, California). 2005. Pers. Comm.
USA	Maximum rate: 0.4%	Central core of GFR donor surrounded by conventional variety. No hybrids found beyond 1.8 m	D. Cheetham thesis <sup>2</sup> . A. J. Fischer (Univ. California Davis) and D. Mitten. 2005. Pers. Comm.
China	56% to 67% to male sterile lines and 2% to 4% to restorer lines	Side by side sowing of GFR rice and male sterile or restorer lines	Jia 2002
China	< 1% from hybrid rice to landrace	Mixed planting of landrace and hybrid rice	Rong et al. 2004
Spain and Italy	0.01% to 0.1% depending on distance	Circular field designs with core of GFR rice surrounded by conventional variety	Messeguer et al. 2001, 2004
<u>Crop to weedy</u>			
Brazil	0.14% to 0.26% depending on weedy type	1-m <sup>2</sup> plots of GFR rice with weedy rice plots (two biotypes) of identical area on each side	Noldin et al. 2002
USA	1% to 52% depending on crop variety	Common garden of cultivated varieties and weedy rices	Langevin et al. 1990
Korea	0.01% to 0.05%	Mixture of GFR and weedy rice	Chen et al. 2004
Spain	0.04%	Circular field designs with core of GFR rice	Messeguer et al. 2004
Costa Rica	0.2% to 3.8% using marker variety and 0.1% to 0.4% with GFR rice	Varying densities of marker variety with colored stem base or GFR variety and weedy rices	Lentini and Espinoza 2005
Colombia	0.03% to 0.3%	GFR rice intermingled with 20% weedy rice	Lentini and Espinoza 2005
USA	From less than 0.01% to 0.14%	Row planting and progenies of plants surviving field application of IMI herbicides, and circular designs	Burgos et al. 2005, Estorninos et al. 2003a, 2003b



Two more circumstances where movement of transgenes from crop to crop is important are when they introgress into landraces, especially in the centers of origin and diversity, and in relation to hybrid seed production. A real controversy occurred when it was reported that landraces of maize had introgressed transgenes in southern Mexico (Quist and Chapela 2001), something that was perceived as tampering with traditional genetic resources and biodiversity. This report was later considered to be erroneous due to systematic errors in measurement (Editorial Note 2002), and a more recent study found no transgenes in local landraces in that area (Ortiz-Garcia et al. 2005). An estimated 29% of the rice area in Asia is still planted to landraces. In some countries, especially those in which rice is cultivated under rainfed conditions, farmers still grow traditional varieties on a high proportion of the planted land (Hossain et al. 2003). Farmers may abandon planting their landraces to take advantage of new opportunities in the changing rice cropping environment and to realize higher yields (Bellon 2004) providing an opportunity to these landraces to go feral and become weedy. Volunteer landrace plants may go undetected because the farmer does not see them as weeds and also via the soil seed bank if rice production is abandoned (Valverde 2005). Under these conditions, if the landrace had introgressed a HR gene the result would be a HR weedy rice. Additionally, rice left in abandoned paddies can go feral in a few generations, exacerbating weedy rice problems in nearby operating paddies by gene flow (Marambe 2005).

A major concern with hybrid rice is the transfer of transgenes to male sterile lines used in hybrid rice production (Jia and Peng 2002). Male sterile lines are selected for stretched stigma to improve hybrid seed production and thus have a higher outcrossing rate than regular cultivars, restorer lines, and hybrids themselves. Movement of the *bar* gene from transgenic rice to *indica* or *japonica* male sterile lines growing side by side in a field trial was 56% to 67%, respectively, compared to 4% and less than 2% to the same maintainer lines, respectively (Jia 2002). However, the *bar* gene has been used as a research tool in the production of hybrid rice seed (Lin et al. 2000). If HR hybrid rice were to be planted commercially then another situation can arise. Some farmers in China intercrop traditional varieties with high-yielding hybrid rice aiming to reduce rice blast disease and improve yields of the traditional variety (Zhu et al. 2000) so there is concern about gene exchange that could result in degeneration of the traditional varieties. However, very limited gene flow between intercropped hybrid rice and a traditional variety was experimentally detected (Rong et al. 2004). Although gene flow from hybrid rice to the traditional variety was five times higher than the opposite, total gene flow was less than 1%. If selection pressure for the transgenic trait is strong (e.g. the herbicide is used), the transgene will become rapidly established in the population. Non-transgenic imidazolinone (IMI)-resistant (Clearfield®) hybrid rice is commercially planted in the U.S. but to our knowledge all gene flow studies conducted so far with IMI rice have involved varieties, not hybrids. The rate of pollen-mediated transgene flow from a glufosinate-resistant *japonica* rice to a conventional *japonica* variety was 0.1% and 0.01% for plants grown at 1- and 5-m distance from the transgenic source (Messeguer et al. 2001). Similarly, in a follow-up experiment gene flow averaged 0.09% (Messeguer et al. 2004). In both cases, pollination frequency was influenced by prevailing winds.

### **Gene flow between herbicide-resistant rice and weedy rice**

The main purpose of introducing HR rice is to control weedy rice, which being con-generic or con-specific with the crop cannot be effectively controlled with conventional herbicides without delayed planting, with concomitant potential yield loss due to a shorter season. Thus the introgression of resistance genes into weedy rice defeats the reason of this technology. Although cultivated rice, and probably weedy rice as well, is mostly autogamous, old breeding literature (e.g. Beachell et al. 1938) as well as recent publications using transgenic rice varieties and field observations, all indicate that outcrossing and introgression do occur.



Work by Langevin et al. (1990) has been widely cited in recent literature dealing with gene flow in rice. In their study, field-collected seed of weedy rice growing in association with six rice cultivars was planted in a common garden. Emerging hybrid plants were confirmed by morphological comparison and electrophoretic patterns; hybrids occurred with all cultivars. Those plants confirmed as hybrids showed heterosis, growing taller and more robust than any of the parents, except those arising from the Nortai cultivar. Hybridization was between 1% and 52%, the latter with the late-flowering cultivar Nortai, which coincides in flowering with weedy rice. It was postulated that hybrids with Nortai may actually represent previously-formed hybrids (since the seed was collected from plots where both the crop and the weed had interacted for three cropping season) that had already backcrossed.

Most recent studies, as reviewed by Gealy (2005), conclude that hybridization between cultivated and weedy rice occurs at lower levels than those reported by Langevin et al. (2000), typically at about 1% or less. Determination of gene flow is usually carried out by either planting a core of the pollen source (the variety with the trait of interest) and determining the presence of hybrids at varying distances and positions from the core. In some cases, weedy rice is also planted in mixture with the HR variety. Hybrids are identified by their resistance to the herbicide of interest, but to corroborate their hybrid nature another means should also be used. Microsatellites and other genetic markers have been used successfully to distinguish between cultivated and weedy rice and hybrids between them (Burgos et al. 2005, Gealy et al. 2002, Lentini and Espinoza 2005, Zhang et al. 2003). Because of the restrictions for the release of the transgenic varieties, most of these experiments are located in fields where rice is not normally planted, away from other commercial rice crops and using a single weedy rice biotype collected in a different field. To make conditions more conducive for hybridization, planting dates are adjusted to synchronize flowering, or biotypes similar in life-cycle duration to the variety are selected. But in actual rice fields, as it is common in Latin America, many morphologically-distinct biotypes grow in association with the crop and have been doing so in many cases for long periods. The result is the presence of highly variable weedy rice populations with individuals resembling the varieties within which they grow, not only phenotypically but also genetically (Federici et al. 2001, Lentini and Espinoza 2005, Skov et al. unpublished, Valverde 2005). Sometimes the mimicry is such that farmers describe the weedy types as “varietal” weedy rices as they are extremely difficult to tell apart except by seed shattering, when it is too late (Valverde 2005). Under these conditions, where varying levels of introgression had already occurred, one would expect that weedy-rice hybridization with varieties with novel traits would occur more rapidly and at a higher frequency. The presence of putative hybrids between *indica*-type weedy rice (105 days to maturity) and IMI-rice (tropical *japonica*) in Costa Rica just after two succeeding seasons, even though the variety introduced is very precocious (about 90 days to harvest), suggests that without proper containment and management, resistant weedy rice populations will rapidly increase provided that a seed bank accumulates in the soil. Langevin et al. (1990) have already indicated that the presence of a broad range of flowering times in weedy rice ensures that hybrids could be produced with both early- and late-season rice varieties.

Recent studies in several countries on pollen mediated gene flow using glufosinate resistant rice as the donor confirm outcrossing rates of less than 1% (Chen et al. 2004, Messeguer et al. 2004, Noldin et al. 2002) although slightly higher rates have been detected. In field studies in Costa Rica, the marker commercial variety (Setesa 9) that has anthocyanin-colored culms and a locally-transformed, glufosinate-resistant *indica* variety were allowed to hybridize with weedy rice. Hybridization between Setesa 9 and the two weedy rice biotypes included in the study was less than 3.8% and slightly varied with biotype and weedy rice density. Hybrids were confirmed phenotypically and by microsatellite analysis. Hybridization between the transgenic rice variety and six weedy rice biotypes ranged between 0.1% and 0.4% and was more common with weedy rice biotypes having *indica* characteristics than with *rufipogon*-like biotypes. Similar results were also obtained in Colombia (Lentini and Espinoza. 2005).



Glufosinate resistant transgenic rice was cleared by US regulatory authorities in 1999 (USDA-APHIS 1999) but is not yet commercially available. Commercial release has been postponed on several occasions and it is now anticipated that it will be marketed in 2007 (Mitten, D., 2005, Bayer Crop Science, California, USA, personal communication). Although recent studies demonstrate economic and environmental benefits of glufosinate-resistant rice in California (Bond et al. 2004), resistant varieties have not been commercially released and many rice farmers are concerned about the impact of such varieties in the export market, especially that of Japan. The picture about trade concerns related to transgenic rice could change dramatically if transgenic rice is allowed in Asia, particularly in China and India. Transgenic varieties with resistance to diseases, insect pests and herbicides, including glufosinate and glyphosate, and with other traits (e.g. drought and salt tolerance) are being developed and tested in China (Jia 2002, 2004, Jia and Peng 2002). Approval of transgenic rice in China will probably be speeded up after substantial benefits in terms of pesticide use reduction and diminished health effects were demonstrated among farmers planting transgenic insect resistant rice varieties in pre-commercial production field trials (Huang et al. 2005). There are already reports of illegal planting of unapproved insect resistant transgenic rice varieties in China (Zi 2005). The National Academy of Agricultural Sciences of India has stated that no scientifically valid environmental or ecological impact issues were identified with respect to releasing transgenic rice varieties in centers of origin and diversity (Chopra et al. 2005).

Experimental results for gene flow between non-transgenic IMI rice and weedy rice are similar to those obtained with glufosinate-resistant rice, with outcrossing rates below 1% (Burgos et al. 2005, Estorninos et al. 2003a, 2003b). For example, outcrossing at a low frequency occurred up to 6.5 m away from the IMI-rice pollen source (maximum distance allowed by experimental design) regardless of wind direction in a field trial using a circular design (Burgos et al. 2005). The most interesting, revealing and relevant information about hybridization between HR rice and weedy rice, however, comes from the commercial production of IMI rice or from experiments resembling commercial situations. A few IMI rice varieties and hybrids have been commercialized in the US and Latin America. Putative weedy rice hybrids were found in a field in Costa Rica that had been planted sequentially with IMI-rice (i.e., two cropping seasons, one year) and then rotated to a conventional variety following recommendations of the IMI stewardship program. The conventional crop became severely infested with weedy rice (the soil seed bank was not depleted with the IMI-rice crops) and at harvest time, after a meticulous survey, the senior author found a few plants that appeared to be hybrids between IMI varieties and weedy rice. As in the USA, weedy rice in tropical America is predominantly *indica* types. In Costa Rica all important rice varieties are also *indica*. Under Costa Rican conditions, IMI-rice (cv CFX-18) matures early and as a *japonica* rice, it has smooth leaves. This has been used as a diagnostic feature to differentiate CFX-18 from both other varieties and weedy rice that have rough (pubescent) leaves. The alleged hybrids were tall; weedy-rice type plants with smooth leaves, some with red pericarp others with white pericarp. Progenies of these plants were resistant to high doses of imazapyr, similar to IMI-rice. After the conventional rice was harvested, the farmer decided to plant, once again, an IMI-rice crop. Another survey at harvest time allowed the senior author collect a larger sample of surviving plants and seed. The progenies of these plants are under scrutiny and most of them are resistant to imazapyr. Genetic characterization in progress is expected to verify the hybrid nature of the plants. Interestingly, some of the putative hybrid progenies grow quite tall (up to 2 m in the greenhouse), have smooth leaves (a recessive trait), and mature late but produce few seed (most seeds in the panicle are empty).  $F_1$  individuals resulting from hybridization of dissimilar parents are often sterile and relatively few populations persist unless when they backcross with their parents or if they are able to spread vegetatively (Raybould and Grey 1993). High infertility of hybrids between glufosinate-resistant *japonica* rice and *indica* weedy rice was also observed in hybrids between IMI and weedy rice (Burgos et al. 2005, Gealy et al. 2002, Zhang et al. 2003). Using a preliminary model, it was predicted that under rainfed rice cropping conditions in Costa Rica, herbicide resistant weedy rice would become a problem (using a threshold of 30% resistance based on biomass) after



three to four cropping seasons when limited control was exercised between crops (Madsen et al 2002). The recently gained field experience seems not only to confirm the predictions but indicates that resistant populations could build up more rapidly because of the absence of some of the practices assumed in the model (e.g. some tillage operations, a fallow period, pre-plant volunteer and weedy rice control with glyphosate). The model is now being refined.

When putative hybrid plants are found in the field, morphological characteristics do not allow determining if they are first generation hybrids or later progenies coming from selfing or backcrosses to initial parents. Simultaneously flowering IMI and weedy rice outcrossed in the field and seed was allowed to shatter. In the population emerging after shattering, all eight possible combinations of pale and dark green, rough and smooth, and droopy and erect leaves were found among plants surviving imidazolinone herbicides, although two thirds had pale, rough leaves typical of weedy rice (Shivrain et al. 2003). Segregation for these characteristics was also notorious in plants derived from the predominant survivor type that had pale, rough, droopy leaves. Alleged hybrids between IMI-rice cultivars and weedy rice from several locations in Arkansas (USA) were separated into first generation crosses or products of varying degrees of selfing using 17 microsatellite markers (Gealy et al. 2005). Segregation for pubescent and glabrous leaves also has also been observed in plants derived from hybrids between transgenic *japonica*-type glufosinate-resistant rice varieties and weedy rice (Oard et al. 2000). Both glufosinate resistance and pubescent leaf are controlled and independently inherited as single dominant genes (Zhang et al. 2003, Sankula et al. 1998).

Table 1 (contd.) Estimated outcrossing rates among cultivated, weedy and wild rice (*Oryza* spp) from selected gene flow studies.

Country	Estimated outcrossing rate	Study details	References
<u>Weedy to crop</u>			
Brazil	0.02% to 0.22% depending on weedy type	1-m <sup>2</sup> plots of GFR rice with weedy rice plots (two biotypes) of identical area on each side	Noldin et al. 2002
USA	0.10% to 0.23% depending on crop variety	Row planting of IMI and weedy rice	Estorninos et al. 2003b
<u>Crop to wild</u>			
China	1.2% to 2.2%	Alternating rows of GFR rice and <i>O. rufipogon</i>	Chen et al. 2004
China	1.5% to 3.0% depending on design and distance from donor. Hybrids found at maximum distance of 43 m.	Four designs including two circular arrangements, an alternating row planting and a unidirectional setup. Donor was a conventional rice variety and recipient was <i>O. rufipogon</i>	Song et al. 2003

<sup>1</sup> GFR: glufosinate resistant; IMI: imidazolinone resistant.

<sup>2</sup> Cheetham, D. 2004. Outcrossing study between transgenic herbicide-resistant rice and non-transgenic rice in California. Thesis. University of California-Davis, 91 pp.

Better adapted IMI rice varieties are being introduced in Latin America based on well known, widely planted conventional varieties. Thus a new generation of *indica* (not *japonica*) varieties will soon dominate the IMI rice market in this area. Weedy rice populations have been associated with these kinds of varieties for decades and have exchanged genes accordingly. Thus we may expect



greater rates of gene flow and introgression with the new varieties that are more closely related to prevalent weedy rices (Valverde 2005).

Gene flow can also occur from the weedy type to the cultivated rice and often the rates of outcrossing are greater in this direction because typically weedy rice plants are taller (Gealy 2005). Outcrossing rates of 0.22% and 0.02% were reported in Brazil when strawhull and blackhull weedy rice were pollen donors and glufosinate-resistant rice was the receptor (Noldin et al. 2002). Gene flow from weedy rice to HR rice is important because the genetic dominance of many weedy traits may convert cultivated HR-rice to a HR-weedy rice hybrid (Messeguer et al. 2001). Plant stature probably affects outcrossing. Field outcrossing between a short stature, purple-marker rice line and weedy rice (tall and with green leaves) was 0.76% whereas its reciprocal outcrossing was nil (Zhang et al. 2003). In a field study in Arkansas outcrossing was also higher when tall weedy rice was the pollen donor and rice (shorter plant) was the pollen acceptor (Estorninos et al. 2003b, 2004).

### Gene flow between herbicide-resistant rice and wild rice

The closest wild species to the rice crop is *O. rufipogon*, its immediate progenitor. Therefore both species are highly compatible and naturally hybridize (Majumder et al. 1997, Song et al. 2001, Suh et al. 1997). There is still some controversy about the taxonomy of *O. rufipogon*. Vaughan et al. (2005a) suggest considering the annual form that is referred to in the literature as *O. nivara* as *O. rufipogon sensu lato*, and the perennial form as *O. rufipogon sensu stricto*. Although overlapping in flowering between wild rice and rice is less common than between rice and weedy rice, it does occur in areas where both are sympatric (Snow et al. 2004, Song et al. 2001). Indeed, hybridization between the two species is one of the likely origins of weedy rice. Natural hybrids between cultivated rice and *O. rufipogon* or *O. nivara* backcross in either direction to produce the morphological, weedy intergrades that invade rice fields (Buu 2000). Most *O. rufipogon* found adjacent to rice fields in tropical Asia is actually this type of weedy rice, making it difficult to find truly wild populations without introgression from cultivated rice in these areas (Lu et al. 2002, Tang and Morishima 1996, Morishima 2001). Some weedy rice biotypes may have originated from gene flow between *indica* and *japonica* rice and wild types (Suh et al. 1997). In some cases, differences in daily flowering time between *O. rufipogon* and *O. sativa* provide opportunities for the cultivated rice to pollinate *O. rufipogon* earlier than conspecific pollen when the two species grow nearby. A proportion of the resulting hybrids produce fertile pollen and a transgene in a partially fertile hybrid could be passed on to its selfed and backcrossed progenies (Song et al. 2001).

The maximum gene flow under optimized conditions from commercial rice to perennial *O. rufipogon* varied between less than 1.5% to 3.0% depending on experimental design and distance from the source. Wind influenced the distribution of the crop-to-wild gene flow; more hybridization events occurred in *O. rufipogon* plants growing down wind during the flowering period. The frequency of hybridization decreased with increasing of distance from the pollen sources; hybrids were found at a maximum distance of 43 m. These rates are higher than those obtained in other studies owing to conducive conditions and probably to the use of a crop-introgressed form of *O. rufipogon* (Song et al. 2003). Lower gene flow frequencies (1.2-2.2%) were obtained from glufosinate-resistant rice to perennial wild *O. rufipogon* under field conditions in China (Chen et al. 2004).

Intermediate weedy-rice resulting from gene flow between traditional varieties and wild *O. rufipogon* in India proliferated as a result of ecological changes and human intervention almost bringing the founder *O. rufipogon* population to extinction (Majumder et al. 1997). A similar case of extinction of a wild *O. rufipogon* population was recently reported in the central plain of Thailand (Akimoto et al. 1999). No transgenes were involved in these cases.



Information regarding gene flow from rice to other wild species under natural or agronomic conditions is scarce. Recent studies conducted in Costa Rica corroborated outcrossing between cultivated rice and native *O. glumaepatula* (also AA genome) in natural environments (Lentini and Espinoza 2005). Since cultivated rice is sometimes planted in close proximity to *O. glumaepatula* populations and because of rice management practices and altered weather patterns can make flowering of both species coincide, it is likely that they would outcross. Natural crosses appear to be primarily sterile but interspecific hybrids backcrossing spontaneously to rice may have a comparative fitness advantage (Lentini and Espinoza 2005). It is more likely that there will be sporadic spontaneous gene introgression from rice to non-AA genome *Oryza* spp. that are polyploid than to diploid species, by analogy to wheat, where more is known (Vaughan et al. 2005b, Levy and Feldman 2002). This is because polyploid species are less affected by interspecific hybridization and have less problems in meiosis than diploids due to the genomic redundancies. This is evident in wheat where such sporadic but stable introgressions have occurred into wild polyploid *Aegilops* species (Weissmann et al. 2005). *Aegilops* spp. include the progenitors of wheat.

Song et al (2002) tried to force hybridization of two glufosinate-resistant *japonica* cultivars with *O. officinalis* (CC genome) by anther emasculation. Although pollen from the transgenic plants germinated on the stigma of the wild rice and its pollen tube penetrated the stigma, actual fertilization failed and seed was not set. Thus incompatibility between the two species should overrule any possibility of gene flow (Liu et al. 2004, Song et al. 2002).

### **Persistence of hybrids between rice and weedy and wild rice**

The fate of a transgene that is neutral or costly to the wild plant will be determined in the wild population by genetic drift, but those that increase survival or fecundity will naturally increase in frequency if the genes have a selective advantage (Pilson and Prendeville 2004). HR genes are neutral in the absence of selection by the herbicide, so it is not expected that resistant weedy or wild rice will be a more severe problem where the herbicides are not used. A different situation arises with transgenes that confer adaptive advantages such as those for insect and disease resistance and tolerance to environmental stress (e. g. salinity, drought, heavy metals). Insect herbivores and fungal and bacterial pathogens that are common pests on cultivated rice also infest both weedy rice and *O. rufipogon*, some of them causing extensive damage to weedy and wild plants (Snow et al. 2004). Resistance to these pathogens and insects may alter the population dynamics of the weedy and wild rices. There is a report of *indica*-rice varieties co-transformed for hoja blanca virus and glufosinate resistances being taller and producing more tillers and grain than the untransformed varieties under field conditions (Saborío 2004), but the possible implications for fitness and persistence as feral populations have yet to be addressed.

Persistence of hybrids between wild and cultivated rice will also depend on the selective advantage or fitness of the hybrids in relation to both parents, particularly in terms of reproductive output. Hybrid vigor is not synonymous with competitive advantage and increased fitness. The relative productivity of interspecific F<sub>1</sub> hybrids between rice and perennial *O. rufipogon* to that of their parental species was compared under cultivation conditions (Song et al. 2004b). Hybrid plants resembled more closely *O. rufipogon* but were taller, produced more tillers and panicles (hybrid vigor) than their parents. Hybrids, however, had diminished seedling survival, pollen viability and seed production. The overall productivity of hybrids was similar to that of their wild parent, although they had less seed dormancy, and thus neutral crop (trans) genes such as those of herbicide resistance could persist in wild rice populations at low frequencies.

The productivity of eight F<sub>2</sub> populations produced from controlled crosses of glufosinate-resistant rice lines and four weedy rice biotypes were evaluated at two field locations in the USA. Populations were similar in plant vigor, seed production and seed dormancy to populations



produced from crosses between conventional (non-transgenic) rice and weedy rice (Oard et al. 2000). Fitness experiments are required to ascertain whether transgenes can survive in the population. The hybrids and their progeny must be able to compete with both crops and with weedy rice. True natural and controlled  $F_1$  hybrids between glufosinate resistant and weedy rice and between a purple-marker rice line and weedy rice exhibited hybrid vigor for maturity, plant height and flag leaf length, but had decreased fecundity. Some hybrids matured about 2 months after their parents and were highly infertile (Zhang et al. 2003). Natural outcrossing between these lines did not occur when the pollen donor was the cultivated variety. Plant maturity of  $F_2$  populations was greatly influenced by maternal effects, with less of heading plants when the pollen donor was the cultivated line. Similar studies were conducted in Brazil (Noldin et al. 2004) using  $F_2$  populations originating from artificial hybrids between transformed rice (glufosinate-resistant Bengal) and two weedy rice biotypes (strawhull and blackhull). More seed was obtained with cultivated rice as the pollen donor. Second-generation hybrids originated from cultivated and strawhull weedy rice had a longer life cycle than both original parents whereas those arising from cultivated and blackhull rice were intermediate between their original parents.  $F_2$  hybrids did not appear to have a competitive advantage over the weedy rice biotypes, but they were not studied under competitive conditions.  $F_2$  hybrids were shorter, more sterile, produced less tillers, similar or less number of leaves, and shattered less than their respective weedy rice parent.

Reduced fecundity of a hybrid, however, is no guarantee of a low competitive fitness, as increased seed survival may outweigh the decreased fecundity (Claessen et al 2005a). For crops such as rice that do not build up to high levels in the soil seed bank, a transgene that improves seed survival or the dormancy fraction in the soil seed bank is likely to increase its persistence. Thus it has been suggested that transgenic lines should be tested for seed survival. It also means that it is advisable to use cultivars with low seed survival as the basis for transgenic lines (Claessen et al 2005a, 2005b). Additionally, fertility limitations can be overcome if the hybrids are able to backcross to any of their parents. Some rice x weedy rice hybrids as well as their progeny and back crosses to the weedy parent ( $BC_1$ ) had greater fecundity and biomass than their weedy parent suggesting that crop genes that are selectively neutral or beneficial are likely to persist and even be boosted in progeny of later generation (Snow et al, 2004), but this cannot be verified without competition studies.

Seed dormancy in weedy rice is imposed by the tissues covering the hulls or the pericarps/testas or both (Gu et al. 2003, 2005). Weakly dormant or nondormant weedy rice strains sometimes share certain morphological characteristics with cultivars and probably were derived from hybridization with the crop (Gu et al. 2005). Backcross  $F_1$  populations ( $BC_1F_1$ ) were obtained from strongly-dormant weedy strains and a receptor non-dormant breeding line to study the relationship between dormancy and weediness. Weedy seed characteristics (shattering, presence of awn, dark hull-color, and colored pericarp) associated alone and in combination with dormancy in the  $BC_1F_1$  populations, stressing the importance of combined effects of dormancy and other weedy characteristics in the adaptation of weedy populations to agroecosystems. This also agrees with the notion that domestication has eliminated dormancy alleles at loci near the genes for shattering and other morphological weedy traits (Gu et al. 2005). No apparent effect on seed dormancy was observed in the progeny of populations of artificial hybrids between glufosinate-resistant rice and weedy rice in Brazil (Noldin et al. 2004).

### Dealing with transgene flow

There are two general approaches to dealing with gene flow: (1) '**contain**' the transgenes in the rice novel variety so that gene inflow, gene outflow or both are precluded depending on the mechanism; (2) '**mitigate**' gene flow effects if there are inevitable 'leaks' in the containment system, which should also prevent volunteer populations of the transgenic variety from establishing and/or reaching maturity so that they cannot evolve into problems. Most discussions so far have dealt with



'containing' gene flow from managed ecosystems to 'natural' ecosystems with less on 'mitigation' of the effects of gene flow after it has occurred (Ellstrand 2003, Gressel 1999, 2002, Jenzewski et al. 2003, Stewart 2004). Only recently has discussion begun dealing with gene flow within the agroecosystems, both on preventing and mitigating endo-feral (evolution within the biotype) and exo-feral (evolution of less domesticated forms by crossing with wild or weedy forms) dedomestication of species as volunteer weeds (Gressel and Al-Ahmad 2005). Containment and mitigation are discussed below in the general context of bi-directional containment as well as mitigation.

### **Containing gene flow by molecular genetic means**

Large isolation distances and other physical containment mechanisms are impractical for crops such as rice, except in special cases such as transgenic rice bearing pharmaceutical genes. Several molecular mechanisms have been suggested for containing gene flow (i.e., to prevent gene flow between rice and relatives), especially by pollen.

#### *Containment by targeting transgenes to a cytoplasmic genome*

The most widely discussed containment possibility is to integrate the transgene of choice in the plastid or mitochondrial genomes (Daniell 2002, Maliga 2002, 2004). There are good reasons to engineer transgenes into chloroplasts besides the presumed biosafety. Many genes of value come from bacteria with similar codon usage as chloroplasts. Such genes often need to be re-engineered to plant codon usage before inserting into the nuclear genome. The opportunity of gene outflow is limited due to the predominantly maternal inheritance of these genomes in many species, including rice, but far from all species. This is presently an arduous technology, which so far is limited to a few species. It would not preclude wild or weedy rice from pollinating the crop, and then acting as the recurrent pollen parent.

The claim of strict maternal inheritance of plastome-encoded traits (Maliga 2004, Daniell et al. 1998, Bock 2001) has not been substantiated. Tobacco (Avni and Edelman 1991) and other species (Darmency 1994) often have between a  $10^{-3}$ – $10^{-4}$  frequency of pollen transfer of plastid inherited traits. Pollen transmission of plastome traits can only be easily detected using both large samples and selectable genetic markers. A large-scale field experiment utilized a *Setaria italica* (foxtail millet) with chloroplast-inherited atrazine resistance (bearing a nuclear dominant leaf marker) crossed with five different male sterile herbicide susceptible lines. Chloroplast-inherited resistance was pollen transmitted at a frequency of  $3 \times 10^{-4}$  in >780,000 hybrid offspring (Wang et al. 2004). At this transmission frequency, the probability of transgene movement via plastomic gene flow is orders of magnitude greater than by spontaneous nuclear genome mutations. Thus, chloroplast transformation is probably unacceptable for preventing transgene outflow, unless stacked with additional mechanisms, and as noted above, will not at all impede gene inflow. Maliga (2004) discounts the relevance of the findings with tobacco and *Setaria* as being due to an origin of the plastids from interspecific (closely related) cytoplasmic substitution, where pollen transmission barriers can break down (Kiang et al. 1994). *Setaria viridis*, the wild progenitor of *Setaria italica* is biologically con-specific with it (Darmency 2005). There are two problems with this denigration of the relevance of pollen movement of plastome encoded genes: 1) it is just such interspecific movement that could be a problem between rice and related species; 2) Maliga (2004) ignores the discussion in Darmency (1994) of cases of intraspecific transmission of plastomic traits by pollen at about the same frequency, within the same species, as reported above between species.

#### *Male sterility coupled with transplastomic traits*

A novel additional combination that considerably lowers the risk of plastome gene outflow within a field (but not gene influx from related strains or species) can come from utilizing male sterility with transplastomic traits (Wang et al. 2004). Introducing plastome-inherited traits into varieties with



complete male sterility would vastly reduce the risk of transgene flow, except in the small isolated areas required for line maintenance. Such a double failsafe containment method might be considered sufficient where there are highly stringent requirements for preventing gene outflow to interbreeding species adjacent to rice. Plastome-encoded transgenes for non-selectable traits could be transformed into the chloroplasts together with a trait such as atrazine resistance as a selectable plastome marker. With such mechanisms to further reduce out-crossing risk, plastome transformation may possibly meet the initial expectations.

#### *Genetic use restriction technologies and recoverable block of function*

Other molecular approaches suggested for transgene containment include: seed sterility, utilizing the genetic use restriction technologies (GURT) ('terminator gene') (Oliver et al. 1998, Crouch 1998), and recoverable block of function (RBF) (Kuvshinov et al. 2001) to prevent transgene flow by producing nonviable crop seed or hybrids. Such proposed technologies control both the gene influx and efflux (except in fields to produce the seed for planting), but theoretically if the controlling element of the transgene is silenced, expression would occur, rendering a critical defect in principle and practice. The frequency of loss of such controlling elements is yet unclear, as there have been no large-scale field trials to test either method.

#### *Repressible seed lethal technologies*

An impractical technology has been proposed to use a "repressible seed-lethal system" (Schernthaler et al. 2003). The seed-lethal trait and its repressor must be simultaneously inserted at the same locus on homologous chromosomes in hybrids used for planting to prevent recombination (crossing over), a technology that is not yet workable in plants. The hemizygote transgenic seed lethal parent of the hybrid cannot reproduce by itself, as its seeds are not viable. If the hybrid could be made, half the progeny would not carry the seed lethal trait (or the trait of interest linked to it) and they would have to be culled, which would not be easy without a marker gene. A containment technology should leave no viable volunteers with the transgene, but this complex technology would kill only 25% of the progeny and 50% would be like the hybrid parents and 25% would contain just the repressor. Thus, the repressor can cross from the volunteers to related weeds, and so can the trait of choice linked with the lethal, and viable hybrid plants could form. The death of a quarter of the seeds in all future generations is inconsequential to plants that copiously produce seed, as long as the transgenic trait provides some selective advantage.

#### *Transient transgenics*

It is possible to insert useful traits encoding transgenes on RNA viruses or in endomycorrhizae that are expressed in the plant, but are not carried through meiosis into reproductive cells, and thus there will be no gene flow via seeds or pollen. Attempts had been made to use endophytes to carry useful genes into plants by pressure-infiltrating the endophytes into seeds (Fahey and Anders 1995, Tomasino et al. 1995). The advantage of the technology is that it was not variety specific, such that hybrids, varieties, and landraces can be used. The same or other infection procedures could be used to introduce useful traits by disarmed plant disease viruses as the vector. The possibility that such a procedure might work was borne out in many cases with dicots showing that they express virus encoded genes. It was possible to infect *Arabidopsis* with tobacco etch virus carrying the *bar* gene encoding glufosinate resistance; the gene was fully expressed in the plants (Whitman et al. 1999). Cucurbits artificially infected with an attenuated zucchini yellow mosaic potyvirus containing the same transgene were resistant in the field (Shiboleth et al. 2001). An NPTII carrying wheat streak mosaic virus was used to infect various grains, and the gene was expressed (immunologically) (Choi et al. 2001). Considerable technological obstacles of infection of the crop species will have to be worked out. While no gene flow from the plants is expected, endophytic bacteria are prone to horizontal gene transfer among themselves, which bacterial biosafety experts will have to consider. There are biosafety issues relating to the mode of disarming to be considered, and it must be demonstrated that there is no gene introgression from the virus to the plant chromosomes, as well as



no-extra-nuclear transmission of the virus through ovules or pollen in very large numbers of individuals. It is necessary to transfect the crop every generation, which would be no problem with hybrid rice, but would be with farmer-saved varietal material.

In summary, none of the above containment mechanisms is absolute, but the risk could be reduced by stacking a combination of containment mechanisms, compounding the infrequency of gene introgression. Still, even at very low frequencies of gene transfer, once gene transfer occurs, the new bearer of the transgene could disperse throughout the population if it has just a small fitness advantage.

### **Preventing establishment by transgenic mitigation**

If a transgene confers even a small fitness disadvantage, the less fit transgenic volunteers and their own or hybrid progeny should only be able to exist as a very small proportion of the population. Therefore, it should be possible to mitigate volunteer establishment and gene flow by lowering the fitness of transgene recipients below the fitness of competitors, so that the volunteer or hybrid offspring will reproduce with considerably less success than its non-transgenic competitors. A concept of "transgenic mitigation" (TM) was proposed (Gressel 1999), in which mitigator genes are linked or fused to the desired primary transgene. Thus, a transgene with a desired trait is directly linked to a transgene that decreases fitness in volunteers and hybrid progeny.

This TM approach is based on the premises that: 1) tandem constructs act as tightly linked genes, and their segregation from each other is exceedingly rare; 2) the gain of function dominant or semi-dominant TM traits chosen are neutral or favorable to the crop, but deleterious to volunteer progeny and hybrids due to a negative selection pressure; and 3) individuals bearing even mildly harmful TM traits will be kept at very low frequencies in volunteer/hybrid populations because strong competition with their own wild type or with other species should eliminate even marginally unfit individuals, and prevent them from persisting in the field population (Gressel 1999). Thus, it was predicted that the primary gene(s) being engineered into rice will not persist in future generations if it is flanked by TM gene(s), such as genes (for rice) encoding dwarfing, strong apical dominance to prevent tillering, uniform seed ripening, non-shattering, and/or anti-secondary dormancy. When they are in such a tandem construct, the overall effect would be deleterious to hybrids with weedy or wild rice. Indeed a TM gene such as anti-shattering should decrease re-seeding, and thus the number of initial volunteers. Dwarfing of taller rice would increase yield (harvest index). The same transgenes would not further dwarf modern dwarf varieties, but as they are functionally dominant transgenes, the appearance of tall off-types would be precluded. Rice typically has a small amount of shattering due to imperfect harvesting equipment, which may leave a few seeds behind. Because the TM genes will reduce the competitive ability of the rare hybrids with weedy or wild rice, they should not be able to compete and persist in easily measurable or biologically significant frequencies in agroecosystems (Gressel 1999, 2002).

Once TM genes are isolated, the actual cost of cloning them into TM constructs is minimal, compared to the total time and effort in producing a transgenic rice. The cost is even inconsequential in systems where biolistic co-transformation allows introducing genes into the same site such that the tandem construct is made by the plant.

#### *Demonstration of the utility of Transgenic Mitigation in tobacco and oilseed rape*

We used tobacco (*Nicotiana tabacum*) as a model plant to test the TM concept: a tandem construct was made containing an *ahas*<sup>R</sup> (acetoxy acid synthase) gene for herbicide resistance as the primary desirable gene of choice, and the dwarfing *Δgai* (gibberellic acid-insensitive) truncated gene as a mitigator (Al-Ahmad et al 2004, 2005a). Dwarfing would be disadvantageous to the rare weeds introgressing the TM construct, as they could no longer compete, but is desirable in many



crops, preventing lodging and producing less stem with more leaves. The dwarf and herbicide resistant TM transgenic hybrid tobacco plants (simulating a TM introgressed hybrid) were more reproductive than the wild type when cultivated alone (without herbicide). They formed many more flowers than the wild type when cultivated separately, which is indicative of a higher harvest index. Conversely, the TM transgenics were weak competitors and highly unfit when co-cultivated with the wild type in ecological simulation of competition. The inability to achieve flowering on the TM plants in the competitive situation resulted in zero reproductive fitness of the TM plants grown in an equal mixture with the wild type at typical field spacing of plants resulting from seed rain of volunteer weeds (Al-Ahmad et al. 2004).

From the data above it is clear that transgenic mitigation should be advantageous to a rice growing alone, while disadvantageous to a weed or wild rice hybrid with it living in the competitive environment of the paddy, or off site. If a rare pollen grain bearing tandem transgenic traits bypasses containment, it must compete with multitudes of wild type pollen to produce a hybrid. Its rare progeny must then compete with more fit wild type cohorts during self-thinning and establishment. Even a small degree of unfitness encoded in the TM construct would bring about the elimination of the vast majority of progeny in all future generations, as long as the primary gene provides no selective advantage that counterbalances the unfitness of the linked TM gene. Typically such crosses between a crop such as rice and its weedy or wild relatives are rare (as discussed in previous sections), yet even if there are many more hybrids than crop, the unfitness of the hybrids will cause the TM bearing individuals to disappear, as evidenced by using a replacement series with the tobacco (Al-Ahmad et al. 2005a).

We have inserted the same construct into oilseed rape and have tested the selfed progeny, as well as hybrids with the weed *Brassica campestris*=*B. rapa* (Al-Ahmad et al. 2005b, Al-Ahmad and Gressel 2005). When cultivated separately, the dwarf transgenic oilseed rape grew at almost the same rate as the transgenic (Fig. 1A), but produced almost twice as much seed as the non-transgenic isoline (Fig. 1C). When the TM transgenic oilseed rape plants were co-cultivated in competition with the wild type, they were unable to grow normally (Fig. 1B), and hardly set seed (Fig. 1C) because they were so unfit to reproduce.

The rare hybrid offspring from escaped pollen bearing transgenic mitigator genes would not pose a dire threat, especially to wild species outside fields, as the amount of pollen reaching the pristine wild environment would only be at a minuscule fraction of the pollen from the wild type. This depends on the distance, source size, and on fertility barriers. Large scale cultivation creates large pollen sources, and in theory a wild population having its niche on “the edge of agriculture” with coincident pollen shed could be swamped. There has been pollen flow, but no swamping with native DNA of wheat sporadically appearing in a ruderal *Aegilops* sp. (Weissmann et al. 2005). Presently, there are no well documented cases where fertility barriers do not prevent more than the formation of a few infertile hybrids near the borders, as well as the rare introgressions, as have been happening for time immemorial. Any unfit hybrids and their rare backcross offspring containing transgenes linked to TM genes should still be eliminated. Further large-scale field studies will be needed with rice/weed pairs to continue to evaluate the positive implications of risk mitigation.



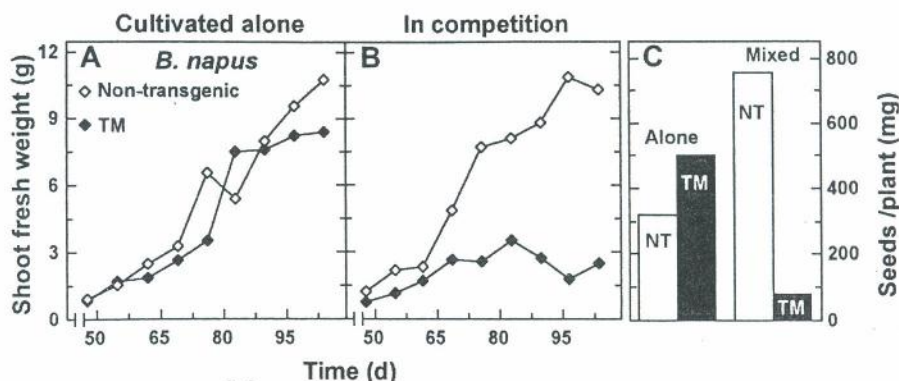


Fig. 1. Suppression of (B) growth and (C) seed yield of TM (transgenic mitigator) bearing oilseed rape plants carrying a dwarfing gene in tandem with a herbicide resistance gene (closed symbols and bars) when in competition with non-transgenic plants (open symbols and bars), and (A) near-normal growth of the transgenics and (C) much higher seed yield of the transgenics when cultivated separately without herbicide at 3 cm spacing in a biocontainment screenhouse. (Adapted from Al-Ahmad et al. 2005b).

Thus, systems exist that can theoretically preclude rice transgenes from becoming established in wild or weedy relatives, whether by containing gene flow or by preventing the establishment of hybrids by mitigation. There is evidence that some of these systems are efficient in other crops, and there is no reason they could not be used in rice, where a risk of transgene flow is perceived. The actual magnitude of risk can be estimated using decision trees, which help preclude biases, such as the one developed by Gressel and Rotteveel (2000). Risks should not preclude developing transgenic rice – they should stimulate the imagination to devise and then test systems to deal with the potential problems.

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## Procedures for weed risk assessment

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**Abstract:** Due to the current intensive free trade of goods, some exotic plants may be introduced to new countries/territories and spread to other areas, affecting agricultural production, plant diversity and other systems. It is for this reason that weed risk assessment systems are becoming more and necessary tools for the prevention and effective control of introduced weeds.

This present paper briefly explains the new FAO procedures on weed risk assessment, which was developed in close collaboration with New Zealand and Australian experts in this field. The procedures provide guidelines for countries wishing to strengthen quarantine protocols and to make efficient use of their limited resources for prioritizing weed control. Obviously all these procedures may be modified in the future based on the experience gained by the specialists on this matter, including the evaluation of new aspects of the behaviour of plants thought to have the potential to become weeds once introduced into a new country/territory.

Two types of weed risk assessment are used; pre-entry assessment is used for species not yet introduced, where preventive quarantine measures play a major role, while post-entry assessment is used to classify those plants already established in new territories. To this end, a generic scoring system will be necessary to rank the national importance of established weeds and prioritize control measures to prevent their further spread. In both cases it is essential to have good technical information about the plants' behaviour in other countries, their eco-biology and control. In most cases Internet access makes this process easier and faster.

**Keywords:** Risk assessment, invasiveness, plant quarantine

### INTRODUCTION

Since the discovery of the Americas, the introduction of many new plants from the western hemisphere into Eurasia, Africa and Oceania, and vice versa has increased tremendously. Plants previously unknown in countries on both sides of the globe became either excellent crops or problems for biodiversity and agriculture.

Due to this exchange, plants such as perennial grass *Sorghum halepense* (L.) Pers. were introduced as potential pasture into the USA. Similarly *Cynodon dactylon* (L.) Pers., although it found some use as pasture, also became a serious weed in orchards in various countries of the region (Labrada, 2003). With the use of chlorotriazine herbicides, annual grass *Rottboellia cochinchinensis* (Lour.) Hayton, originally from South East Asia, became a problem in maize and sugarcane in Central America and the Caribbean (Labrada, 1994).

In Africa and Asia, *Chromolaena odorata* (L.) R.M. King & H. Robinson, (formerly *Eupatorium loratum* L.) was introduced as potential beneficial cover in various perennial plantations. *C. odorata* is a harmful perennial plant of humid tropical regions which usually creates dense thickets in infected perennial plantations, annual crop areas, sandy banks along shores of the rivers or in lowland areas. This plant, which originated in South and Central America, was introduced into Asia in the mid-19th century. In 1937, it was also introduced accidentally into Nigeria from Sri Lanka (then Ceylon). Since then, the plant has become particularly harmful in humid zones of tropical Africa. Here, dense stands of *C. odorata* are found in plantations of rubber, cocoa, coffee, oil palm and orchards (FAO,



1995). Some years later the plant is now recognized as a serious weed in Asia as are several others plants like *Mimosa pigra* L. (Lonsdale & Forno, 1994).

In recent years, the annual *Parthenium hysterophorus* L. has been introduced into Australia (Navie *et al.*, 1996), India (Bhan *et al.*, 1997) and Ethiopia (Cock, 2001). In all cases, it is assumed that the seeds of the plant were introduced in shipments of wheat from the USA.

Another plant causing serious problems, but introduced as a panacea in arid zones of East Africa and Near East countries, is the leguminous *Prosopis juliflora*. Its high capacity for proliferation enables this plant to spread easily to vast areas of fertile land (FAO, 2004).

Various scientists and collectors of ornamentals introduced and spread the floating plant *Eichhornia crassipes*, which is at present the world's most troublesome weed in tropical and sub-tropical areas. Most of these plants are examples of invasive species, which are a severe threat to biodiversity, second only to habitat loss (Wilcove *et al.*, 1998).

More exotic introduced plants are becoming invasive, i.e. naturalized plants that produce reproductive offspring, often in large numbers, at considerable distances from parent plants, and thus have the potential to spread widely (Richardson *et al.*, 2000). The likelihood of spread of such plants expresses the invasiveness of the species. Some of these invasive plants may become transformers, which should be understood as a subset of invasive plants that change the character, condition, form or nature of ecosystems over a substantial area relative to the extent of those ecosystems (Richardson *et al.*, 2000).

The situation of new introductions of exotic plants is aggravated by the current active free trade, compelling countries to implement preventive control measures as well as risk assessments of plants with the potential to adapt and become established in new habitats.

Here prevention becomes crucial, but to really develop a related comprehensive programme it is imperative to conduct risk assessment. Weed risk assessment (WRA) is the use of standard technical criteria to determine the relative weed threats posed by plant species (Virtue and Panetta, 2002). Given the future threat of new weeds and the magnitude of the current weed problem, there is a need for efficient pre-entry screening procedures to prevent the introduction of new weeds as well as post-entry procedures to effectively control those weeds already established in the new habitat.

### PRE-ENTRY WEED RISK ASSESSMENT

According to Williams (2003), any weed risk assessment is normally based on pest risk analysis (PRA) prepared and accepted by the IPPC, which consists of three stages:

1. **initiating the process for analyzing risk**, i.e the identification of a pathway, usually an imported commodity, that may allow the introduction and/or spread of quarantine pests; and the identification of a pest that may qualify as a quarantine pest.
2. **assessing pest risk**, considers all aspects of each pest and in particular actual information about its geographical distribution, biology and economic importance. Expert judgement is then used to assess the establishment, spread and economic importance potential in the PRA area.
3. **managing pest risk**, which involves developing, evaluating, comparing and selecting options for reducing the risk.



Initiating the PRA is only meaningful in relation to a defined "PRA area" considered to be at risk. This is usually a country, but can also be a territory within a country, or an area covering all or parts of several countries, e.g. a geographical region.

Australia and New Zealand are countries with effective quarantine protocols for preventing the entry and spread of weeds, which have been developed on the basis of existing PRA standards. In Australia, the procedure for pre-entry - the so-called "Pheloung system" - consists of 49 questions covering a plant's domestication, climate preferences, weed history, undesirable traits, growth form, reproduction, dispersal and persistence attributes (Pheloung, 1995, Pheloung, 2001). Questions are mostly answered as yes/no/don't know with a +1 score for a weedy attribute and a -1 score for a non-weedy attribute. A minimum number of questions must be answered to generate a score.

To carry out such an assessment it is really important to have as much information as possible about the spread of the plant likely to enter into the new territory/country and its eco-biology.

Based on the above experiences, FAO panel of experts decided to draw up simple procedures for weed risk assessment (FAO, 2005) modelled on the above-mentioned systems (fig. 1). Naturally they are based on the approved guidelines for pest risk analysis of quarantine pests and include information on primary pathways of entry of plants for horticultural or forestry purposes; through weed seeds contaminating agricultural products for direct consumption by people or livestock; or, the least frequent, seeds on shoes or adhering to the clothes of passengers. National plant protection authorities and associated scientific personnel should analyse the most important pathways for possible entry of such plants. The new procedures contain far less questions than the Australian one. The scoring system adopted (table 1) gives the opportunity to identify a plant that may pose a threat of becoming a weed in a new territory.

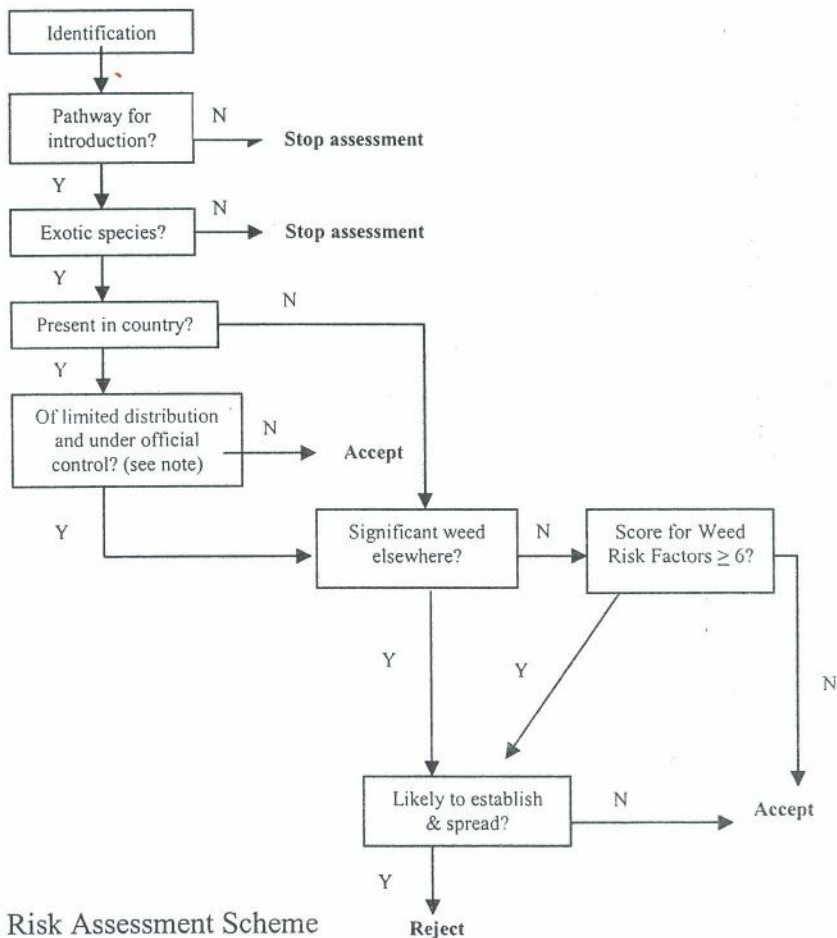


Fig. 1 Weed Risk Assessment Scheme



Table 1. Scoring for Weed Risk Factors (critical score = 6)

Aquatic plant	Y=3
Congeneric weed	Y=2
Propagules likely to be dispersed intentionally or unintentionally by human activity?	Y=2
Produces spines, thorns or burrs?	Y=1
Parasitic?	Y=1
Unpalatable or toxic to grazing animals?	Y=1
Host for recognized pests and pathogens?	Y=1
Causes allergies or otherwise toxic to humans?	Y=1
Climbing or smothering growth habit?	Y=1
Produces viable seed?	Y=1
Seed persists for > 1 year?	Y=1
Reproduction by vegetative propagation?	Y=1
Tolerates or benefits from mutilation, cultivation or fire?	Y=1

NB Where the status of a risk factor is unknown; it should be scored as a 'yes'.

### POST-BORDER WEED RISK ASSESSMENT

We may not always be able to prevent the introduction of plants, and this compels plant protection services to devise a management procedure to contain the spread of a new exotic plant thought likely to become a weed. Therefore, once the plant is introduced into a new territory it is imperative to discover whether it will spread in order to assess its potential as an invasive, and its impacts as a weed. Obviously, it is also important to know how to prevent such impacts and which control methods can be implemented.

Again, as in the case of the pre-entry assessment, the system should be simple and easy to use. To this end, in Australia a National Weed Strategy system was developed in 1997-98 (Virtue *et al.*, 2001), which included a generic scoring system to rank the national importance of established weeds. Four main criteria were used in the final version of the system: (i) invasiveness, (ii) impacts, (iii) potential for spread and (iv) socio-economic and environmental values. A literature review was conducted for each introduced species with separate scores for invasiveness, impacts and distribution. The first score would provide a substitute measurement to rate of spread.

In Australia, the above-mentioned work was conducted by panels of experts for each species or group of species. Information gathered was the basis for the new Weed Strategy system.

FAO, in close collaboration with Australian experts on post-border weed risk assessment, is also preparing a new procedure for countries to develop a system similar to that already available in Australia and New Zealand.

### REQUIREMENTS FOR FUTURE DEVELOPMENTS

Weed experts from most developing countries need to be trained in these new methodologies and will require access to literature and compendia on weeds and also to Internet web sites giving updated information on weeds. There is no doubt that donor support for these activities will also be necessary.

The new procedures for weed risk assessment will have to be modified in the future based on the current work of national weed specialists. In some cases, the systems may include far more



questions than the initial procedures but this will be dictated by practice and experiences of the specialists.

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# Effect of global warming on weed invasion world wide

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**Abstract:** Global warming due to the increase of “Green house gases” would have a considerable impact on weed invasion worldwide. Rise in CO<sub>2</sub> concentration, changes in precipitation patterns, maximum and minimum temperatures and sea-level rise that are regarded as the consequences of global warming would alter the nature of vegetation and agriculture. Elevated CO<sub>2</sub> concentration has been shown to favor invasive traits of weeds such as rapid germination of smaller seeds, ability to tolerate high and low temperatures and resistance to control measures. Accelerated evolution in some weed species in response to changing atmospheric carbon dioxide concentration has also been suggested. These observations indicate the possibility of range extension of aggressive weeds. Further, alteration in the precipitation and evaporation pattern coupled with sea level rise and frequent inundation or drought leaves open niches for better adapted alien species to invade the ecological habitats. Literatures revealing the adaptability of invasive weeds to changing climate linked to global warming and invasions of habitats by invasive alien weeds due to climate change are reviewed.

**Key words:** Adaptability, climate change, invasiveness, weed shift.

## INTRODUCTION

The World Meteorological Day, celebrated on 23<sup>rd</sup> March 2005, chose the theme “Weather, climate, water and sustainable development” that converges on the significant impact of climate on human progress all along. The climatic change due to global warming in the last century has been greater than at any other time during the last millennium. The concentration of carbon dioxide is 33 per cent higher than it was before the industrial revolution. The sea level has been rising at the rate of 2 mm a year since the beginning of the 20<sup>th</sup> century. Droughts and floods have become more common (The Hindu 2005). The year 1990 was the hottest in the last century with all other five of the warmest years in the century falling within the last 22 years. Scientists agree that the planet’s temperature has risen 0.5 degree Celsius since 1900 and will continue to increase at an increasing rate. Unabated use of fossil fuel and forest destruction are chiefly responsible for this global warming. This sort of a climate change either directly or indirectly induces changes in land use. Land use changes involve conversion from one type of land cover to another along with changes in management practices. These include fertilization, type of land preparation and changes in availability of surface water and river flow. Because of change in the land use pattern, the terrestrial biosphere of the 21<sup>st</sup> century would probably be further impoverished in species richness. The biosphere will be generally weedy (Walker and Steffen 1997).

## INFLUENCE OF CLIMATE CHANGE ON THE INVASIVITY OF WEEDS

Climate change is likely to cause a spread of tropical and subtropical species into temperate areas and to increase the activity of species currently limited by thermal accumulation at higher latitudes. Because of globalization of economies and subsequent movement of people and materials, invasion by alien species is going to be the second important factor causing loss of biodiversity next to land use pattern. Changes in atmospheric composition and climate are regarded as long term factors influencing weed invasion, increasing in relative importance over time.

Elevated carbon dioxide and competitive ability of weeds



Several biological traits of weeds show positive response to elevated carbon dioxide concentration in the atmosphere and global warming. Plants with  $C_4$  photosynthetic mechanism theoretically gets saturated for  $CO_2$  use even below the current concentration of  $CO_2$  in the atmosphere of  $370 \mu\text{mol/mol}^{-1}$ , whereas plants with  $C_3$  mechanism of photosynthesis are favored by increases in  $CO_2$  content (Tissue et al. 1995). Though the competition between  $C_4$  plants and  $C_4$  weeds has not been studied so far as a function of carbon dioxide concentration, it is observed that elevated carbon dioxide stimulates  $C_4$  weeds to a larger extent than  $C_4$  crop species (Ziska and Bunce 1997). It is also observed that relative increase in biomass at elevated carbon dioxide is larger for fast growing than for slow growing  $C_3$  species (Bunce 1997). Few studies compared the competitive ability of  $C_3$  weeds and  $C_3$  crops as influenced by different carbon dioxide concentrations. They revealed that elevated carbon dioxide levels have most frequently favored weed species like *Chenopodium album* as against soybean, *Taraxacum officinale* as against alfalfa and *Plantago lanceolata* as against several grasses (Bunce 2001). The reason attributed is inherent genetics in crops for yield at high stand density, reducing elongation response to competition in crop species, offering advantage to weeds. Elevated carbon dioxide has also been found to accelerate seed germination in species with smaller seeds (Ziska and Bunce 1993) and by virtue of possessing smaller seeds, weeds surpass annual crops under higher carbon dioxide levels. Further, elevated carbon dioxide levels increase the ability of plants to tolerate both high and low temperatures, enabling the weeds to disperse and extend their geographical range as allowed by the environmental change. The mechanisms involved are, at warm temperatures, increased temperature optima for photosynthesis of  $C_3$  species with increase in carbon dioxide concentration and lower stomatal conductance mitigating the dehydrating effects of high temperature stress (Long 1991). Tolerance to low temperature by higher carbon dioxide concentration, in chilling sensitive as well as other plants is attributed to lower stomatal conductance and mitigation of chilling (Boese et al. 1997). Reduced stomatal conductance with increasing carbon dioxide is often observed to impart drought tolerance as atmospheric carbon dioxide level increases (Drake et al. 1997). Reduced stomatal conductance with increasing carbon dioxide levels would ensure reduced exposure to atmospheric pollutants (Polle and Pell 1999). The weedy attribute such as persistence and resistance to control measure has also been observed to be strengthened by increasing carbon dioxide levels. More rapid growth under such circumstances of elevated carbon dioxide levels, narrowing the window of opportunity for control and increased potential of underground propagules could be responsible for such a phenomenon. Glyphosate has been shown to be less effective in *Chenopodium album*, *Elytriga repens* and *Cirsium arvense* grown at elevated carbon dioxide level (Ziska et al. 1999). Experiments with four annual weed species *Abutilon theophrasti*, *Chenopodium album*, *Datura stramonium* and *Xanthium strumarium* at three different levels of carbon dioxide, viz. 280, 370 and  $460 \mu\text{mol/mol}^{-1}$ , indicated that these weeds are adapted to  $370 \mu\text{mol/mol}^{-1}$ , approximately the current level of carbon dioxide rather than pre-industrial concentration (Bunce 2002). Accordingly, rapid evolution in some weed species in response to changing atmospheric carbon dioxide concentration has also been suggested.

#### Increasing global temperature and weed shift

Indirect temperature effects will be more significant than direct effects. Thermal acclimation reduces the direct impact of increased air temperature on plant growth than that is often expected. However, developmental acceleration and stimulation of litter decomposition could be expected. Indirect temperature effects are mainly associated with warming of permafrost in the high latitudes that may lead to thermokarst expansion, substantial changes in species composition and increased nutrient availability. Though shift in biomes as intact entities could not be speculated, differential response is possible in terms of competitive abilities of species, migration rates and recovery from disturbance. This could pave way for the new combinations of species. Accordingly, invasion by alien species in to the natural ecosystem would be increasingly troublesome. Such an invasion by alien weed species is likely to be exacerbated by trends in land use and increased disturbance through globalization of trade (Walker and Steffen 1997).



Invasive species are extremely adaptable to a changing climate as shown by their large latitudinal ranges. They also possess rapid dispersal characteristics enabling them to shift ranges quickly in response to climate change (Dukes and Mooney 1999). A major weed that has been invading irrigated upland agro eco-systems in several tropical Asian countries is *Trianthema portulacastrum*. This weed is reported to have originated from tropical Africa and has invaded several continents, viz., Australia, Africa and Asia (Rawson and Bath 1984; Yaduraju et al. 1980). A survey conducted in different irrigated upland crops of Veeranum Ayacut in Tamilnadu, India indicated that *T. portulacastrum* predominates as the dominant species in all the three crops viz., sugarcane, sunflower and gingelly with Important Value Index percentages of 28.73, 26.83 and 25.99, respectively. This weed tops the list of 15 weed species recorded in all these crops in different locations (Kathiresan 2004). One of the most important characters responsible for its invasiveness is thermal induction of seed germination with soil temperature around 35° C favoring synchronized and mass germination of seeds, covering the soil as a green carpet. In a field study conducted at Annamalai University, it was observed that increasing soil temperature with the summer months of June and July triggered the mass germination of seeds of this weed suppressing the native species (Table 1). The seeds of this weed undergo dormancy during winter and thermo-induction to break the dormancy requires soil temperatures above 35° C (Sundari and Kathiresan 2001).

Table 1. Thermo induction of seed germination in *Trianthema portulacastrum*.

Month	Weed emergence 15 days after land preparation at the beginning of the month (m <sup>-2</sup> )	Monthly average max. temperature(°C)
January	8.5	28.5
February	11.6	30.4
March	16.7	33.1
April	23.4	34.2
May	32.9	36.9
June	85.6	37.3
July	102.4	36.2
August	126.8	35.0
September	20.5	33.9
October	-	31.2
November	-	28.7
December	-	28.1

Another important concern regarding global warming is that increasing tolerance of temperature extremes triggers potential pole ward range extension of some particularly aggressive weeds whose agronomic impact is limited at present by low temperatures. Bunce and Ziska (2000) indicated that weeds have larger impact on maize and soybean crops in the southern than in the Northern United States.

### Rainfall pattern and weed invasion

Global warming directly reflects on rising sea levels due to melting of ice caps and natural expansion of seawater as it becomes warmer. Consequently, areas adjoining the coast and wetlands could be frequently flooded and the distribution pattern of monsoon rains gets altered with more intense downpours, storms and hurricanes. The meteorological data available at the Annamalai University, in tail end of Cauvery river delta region of Tamilnadu state, India, shows that the average annual rainfall during the period of 1991 to 2000 has increased by 129 mm compared to that during 1981 to 1990. The record also reveals that the annual evaporation has reduced by 255 mm from the period between 1981 to 1990 & 1991 to 2000. Further, frequent wet years (years with excess average annual rainfall by more than ten per cent) are also seen in between 1991 to 2000



(Table 2). Phyto-sociological survey of floristic composition of weeds in this region reveals the recent invasion of these rice fields by alien invasive weeds *Leptochloa chinensis* and *Marsilea quadrifolia* (Table 3). These two weed species dominated over the native weeds such as *Echinochloa sp.* and others by virtue of their amphibious adaptation to alternating flooded and residual soil moisture conditions prevalent during recent years in this region (Yaduraju and Kathiresan 2003; Kathiresan 2004).

Table 2. Rainfall and evaporation pattern in the Cauvery river delta region of India.

Year	Average annual rainfall (mm)	Annual evaporation (mm)	Moisture deficit / excess percentage
1981	1192.0	2701.0	-14.86
1982	926.8	2482.0	-33.8
1983	1148.6	2336.0	-18.0
1984	1479.6	1861.5	5.69
1985	1985.2	1971.0	41.8
1986	1257.5	1971.0	-10.18
1987	1438.7	2080.5	2.76
1988	1132.6	2007.5	-19.10
1989	1448.3	2153.5	3.45
1990	1543.0	1971.0	10.21
Average	1355.2	2153.5	
1991	1196.4	1934.5	-14.54
1992	1257.3	2007.5	-10.19
1993	2024.1	1934.5	44.58
1994	1349.8	1971.0	-3.59
1995	1124.8	1861.5	-19.66
1996	1701.5	1825.0	21.54
1997	1636.0	1861.5	16.85
1998	1560.1	1861.5	11.44
1999	1434.0	1861.5	2.43
2000	1555.0	1861.5	11.07
Average	1483.9	1898.0	
2001	1343.2	1788.5	-4.06
2002	1281.0	2007.5	-8.50
2003	938.7	1825.0	-32.95

#### Wetlands as landscape sink for weed invasion

Wetlands are more prone to weed invasions in part, because they serve as landscape sinks that accumulate materials resulting from both terrestrial and wetland disturbances (excess water, debris, nutrients, sediments, salts and other contaminants). Invasive weeds in wetlands differ from many upland invaders in respect of invasive traits such as water aided seed dispersal, vegetative plant bits floating in water serving perennation, abundant aerenchyma to tide over submerged conditions and rapid nutrient uptake. Opportunities that make wetland more prone to weed invasions are that the riparian habitats are subjected to flood pulses and inflows from surface water. Habitats fed by surface water are low in species richness and the inhabitants are low in quality with co-efficient of conservatism scoring less than five (Kercher and Zedler 2004). Studies in Wisconsin showed that



Table 3. Floristic composition of weeds in rice fields irrigated by channels in Cauvery river delta (IVI %), India.

Weed species	1992			2002		
	Channel I	Channel II	Channel III	Channel I	Channel II	Channel III
<i>Echinochloa colonum</i>	25.56	28.48	27.52	5.45	4.42	5.34
<i>Leptochloa chinensis</i>	22.74	24.81	23.64	28.81	29.79	29.50
<i>Cyperus rotundus</i>	17.23	22.28	17.01	9.13	7.61	3.60
<i>Echinochloa crusgalli</i>	8.33	6.00	5.80	3.44	2.96	3.00
<i>Sphenoclea zeylanica</i>	2.02	0.68	1.68	4.60	4.47	5.08
<i>Marsilea quadrifolia</i>	1.46	0.63	0.46	19.10	20.98	21.97

wetlands with a history of hydrological disturbance had more widespread invasions (Zedler and Kercher 2004). Despite the emphasis on increased influxes as causing disturbance, it has also been observed that some invasive weeds are abundant where the regions have reduced flood flows. Both increased and decreased runoff will alter wetland water regimes and the floristic composition could be invaded by the floating weeds like *Eichhornia crassipes* under inundation and *Ipomoea aquatica* under semi or complete dry situations of watersheds in Southern India. Accidentally introduced in England in 1872, *Spartina alternifolia*, hybridized with *S. maritima* to descend to *S. anglica* after chromosome doubling. Fertile seeds, vegetative spread, salt tolerance and allopolyploid's vigor contributed for its invasive behavior. *S. alternifolia* has become highly invasive in three states of USA namely Washington, Oregon and California (Ayres et al. 1999). Other such invasive species in wetland eco-systems are *Phalaris aurindinaceae* in North America dominating more than 80 per cent cover in 40,000 ha (Bernthal and Willis 2004) in Wisconsin alone, *Typha orientalis* in Australia favored by urban run off reducing soil salinity (Zedler et al. 1990), *Juncus articulatus* in Australia (Smith and Brock 1995), *Polygonum cuspidatum* a native of Eastern Asia in Central and Northern Europe (Weber 2000) and *Impatiens glandulifera* from Himalaya in UK and Europe (Beerling and Perrins 1993; Tickner et al. 2001). The hypotheses that substantiate invasiveness of wetland species are (i) release from their natural enemies (ii) broader tolerance to environmental extremes (iii) efficient use of resources through extended growth duration and advantageous architecture (iv) hybrid vigor for invaders with different parents and (v) allelopathy to suppress native species (Zedler and Kercher 2004).

### PREDICTING WEED INVASION IN RESPONSE TO GLOBAL WARMING

The interdisciplinary research required to predict how migration might constrain the response of the plant kingdom to climate shifts is at a preliminary stage. The evidence consists of loose links. Some of the literature pertains to fossil record of plant migration following ancient climatic upheavals. Others relate contemporary invasions of exotic species where as some of them attempt at predicting and modeling invasions by virtue of correlating dispersal mechanisms and contemporary landscape patterns. These three lines of research offer disparate and even contradictory insights (Pitelka 1997). The high migration rates and the ability of some tree species to jump large water bodies as evidenced by accumulation of their pollen in lake sediments during the early Holocene epoch, reveal an underlying potential for relatively rapid response to climate change. Fossil records reflect that plants have moved fast enough to track climate change, as fast as rapid warming predicted for the next few decades. During the last glacial period, there were repeated episodes of warming in which the mean annual global temperature rose by 5 degrees Celsius or more with in a few decades (Nicholls et al. 1996). However, such a rapid migration is also dependant on other factors such as local topography and landscape patterning. Research on contemporary invasions also offers leads to support rapid plant migration in response to climate change. The typical example is the entry of cheat grass *Bromus tectorum*, native of Eurasia in the inter mountain West of North America in 1880s as contaminant of Agricultural seeds, invading its current range of about 200000 km<sup>-1</sup> within



the last decade of 40 year invasion process (Marck 1986). Two phases of invasion have been observed with *B. tectorum* i) a distinct quiescent phase during which the range shift is only to lesser magnitude and ii) active phase during which the explosive expansion of the alien species is triggered (Forcella and Harvey 1981). The duration of the lag phase may vary from decades to century depending on the species and such duration is too short to be inferred from paleorecords, but they are too long for the species to persist withstanding rapid climate changes as predicted by some scenario.

Alien weed or plant species that gain entry in to a new geographic range either aided by human intentions or accidentally might pose a threat and deserves monitoring as a quarantine pest in any country, if it is to possess any of the invasive traits comprising biological attributes, ecological traits, damaging potential and geographic range extension capability. The best examples are Cogan grass in USA, water hyacinth and carrot grass in many continents. Mesquite (*Prosopis juliflora*), known for its drought tolerance, introduced into India for greening the deserts in 1914, has incurred the ability to withstand water stagnation and has invaded many watershed environs of Southern India. This region with comparatively lesser perennial water sources and where the rainwater harvesting and pond or lake irrigation has been the traditional source of irrigation and domestic water use suffered appreciable water loss with in the last two decades because of invasion by the weed. Intermittent flooding and drought in the catchments zones have favored invasion by this weed. Range extension and invasion of plant community by both an exotic invader and a native species though differs in several aspects, have one attribute in common. In both cases, plants invade new territory through efficient reproduction and dispersal. The long-haul mechanisms of dispersal including dispersal by wind and vertebrates deserve special attention in predicting large-scale migration of invasive species. For some, these natural agents are likely to be superseded by human activities. Water hyacinth (*Eichhornia crassipes*) native of Brazil was first traced in history as to have been gifted as a compliment to visitors of cotton Expo in New Orleans state of USA in late 1870s. At present, the weed has invaded all continents except temperate Europe. However, dispersal is only the first phase ensuring entry of an invader but to pass through successive phases of establishment, expansion and explosion, the species need to successfully germinate, grow and reproduce. The possibility of a new species establishing itself is resisted by the existing vegetation and favored by the type and frequency of disturbances in that particular habitat. In general, the disturbances of established vegetation by fire, flood and grazing mammals render it more vulnerable for invasion. This indicates that climate changes in the future that suppress native vegetation would increase the probability for open niches in the ecosystem favoring invasion by alien weeds. An alien species getting naturalized involves escaping from cultivation and establishing self-sustaining populations in the wild. This critical step before the species becoming invasive was studied for different introduced species in Australia and New Zealand by Williams et al. 2004. Results indicated the difference in naturalization rates for the same species in these two countries. This emphasizes the important role of climate and climate matching in predicting invasive species.

Though weed invasion has been influenced by the cultural practices at the field scale, in the larger context, regional weed invasions have been mainly influenced by climatic factors. Global warming with the causal and consequent effect of elevated carbon dioxide levels, increased temperature regimes and changes in precipitation pattern might influence the invasive potential of different weed species in various parts of the globe under differing situations. Accordingly, any programme for prediction and prevention of weed invasion should consider the climatic factor as one of the vital component.



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Bozgne

# **History of Weed Science**



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**Abstracts:** Malaysian initiatives in weed science research for the past 85 years since early 1900 focused on descriptive taxonomy, ecology and biology of weeds, and weed management aspects with emphasis on manual, mechanical, biological, and chemical control. More than 2,000 papers in weed science, encompassing a wide range of areas and disciplines, were published in journals, book chapters, occasional periodicals, theses, newsletters, monographs and proceedings between 1918 and 2003. These publications divided into eight categories, viz., (i) Weed Biology/Ecology; (ii) Environmental Fate/Transport; (iii) Herbicide Metabolism/Mode of Action/Physiology/Biochemistry/Phytochemistry; (iv) Application Technology; (v) Herbicide Activity/Selectivity/Efficacy; (vi) Herbicide Resistance; (vii) Economics and Extension; and (viii) Biocontrol. Research focus in the last three decades include weed systematics, weed-crop competition studies between crops and weed species while weed community studies focused on spatio-temporal population patterns, modular dynamics and structural demography, succession and species shift, allometric response, and self-thinning in weed species of economic importance. Research on efficacy of herbicides with/without adjuvants and surfactants, application safety, herbicides bioactivity sorption, attenuation, persistence, mobility and degradation were also given emphasis in the last two decades. Due to increased incidences of herbicide-resistant weeds, namely, goosegrass to propaquizafop and clodinafop, *Fimbristylis miliacae* and *Limncharis flava* to 2,4-D, *Limncharis flava* to bensulfuron-methyl, and *C. crepidiodes* and *S. nigrum* to paraquat, research focus on these weeds took shape. Research on ecology and physiology of herbicides, include metabolism, uptake and translocation of  $^{14}\text{C}$ -paraquat in resistant and susceptible biotypes of *Crassocephalum crepidiodes*, or  $^{14}\text{C}$ -glyphosate in goosegrass (*Eleusine indica*) were conducted. Work on the biological control of weeds using insects or bioherbicides was also conducted. Of these successful suppression of *C. curassavica* by *Schematiza cordiae* syn. *Metrogaleruca onscura* and *Eurytoma attiva*, and *S. molesta* by *Cyrtobagous salviniae* has been recorded. Sheep labour has been used successfully to control plantation weeds such as *A. intrusa*, *A. coromandeliana*, *Pennisetum* spp., *Ischaemum* spp., and *Digitaria* spp., but not *C. odorata*, *Clidemia hirta* or *M. malabathricum*. Recent interest includes weed utilization and biotechnology as animal feed supplements, fertilizers and composts, or as medicinal plants, or even as nutrigenics. For example, the biochemical basis of *Phyllanthus* spp. used against liver, kidney, and urinary bladder ailments forms new generations of research activities, especially among phyto-chemists in public universities, and research institutions. I envisage that weed science research in Malaysia in the next decades will emphasize on biotechnology to produce transgenic crops, resistant to commonly used herbicides, alongside the environmental-friendly and computer-aided weed management strategies with less inputs of herbicides yet sustainable for crop production and resource management.

**Key words:** Weed science research priorities, weed ecology, weed management.

## INTRODUCTION

Malaysian agriculture is very much plantation-based with great economic emphasis on cash crops, namely oil palm, rubber, and cocoa, although sizeable acreages of the arable lands are planted with food crops like rice, pepper, fruit orchards, and vegetables. Weed management likewise has been principally, very much herbicide-based, with herbicides comprising no less than 76% of the Malaysian pesticide market or RM276 millions annually for the past two decades (Anon 2003, Baki



2004). In rice, fruits and vegetables, a combination of manual, chemical and cultural weeding is practiced.

The increasing dependence on herbicides for weed management in the country is a common cause for environmental concern. With this continued usage unabated, one can easily visualize the extent of measurable economic impact of these invasives on Malaysian agriculture. These include many unwarranted environmental and social problems, increased incidences of herbicide resistance, loss of beneficial organisms and almost total disappearance of fresh water fish in the rice granaries (Baki 2004). Further, the largely monoculture pursuits in Malaysian agriculture have led to high incidences of difficult-to-control invasive weed species. The highly invasive millennial weeds (*sensu* Gressel 2000), namely, *I. cylindrica*, *P. polystachion*, *R. cochinenis*, *Mikania micrantha*, *A. gangetica*, *E. indica*, and *I. rogosum* are common sights in many young oil palm, rubber, cocoa, and sugar cane plantations, exposed areas along road sides, railway tracks, and other areas within the fringes of plantations. The continued prevalence of these millennial weed species and the increasing incidences of herbicide-resistance weed species in Malaysia (Heap 2004) are a common cause of concern for weed scientists, agrochemical companies, policy makers, extension workers, and farmers alike.

The recent re-emphasis and renewed interest in agriculture of the Malaysian government, especially for food production in the 9<sup>th</sup> Malaysia Plan, principally to off-set the unhealthy trend of steady increase in food imports to the tune of RM15 billion in 2003 (Anon 2004) augurs well in promoting agricultural development in the country, and will add yet another dimension in weed management strategies.

This paper discusses the research initiatives on weed science in Malaysia since the early 1900. Future directions in weed science research on in the country are also discussed. The paper ends with some notes on future trends and challenges in weed management in Malaysian agriculture.

## WEED SCIENCE RESEARCH PRIORITIES

### Research Priority Areas

The Malaysian initiatives in weed science research for the past 85 years since early 1900 focused on descriptive taxonomy, ecology and biology of weeds, and weed management aspects with emphasis on manual, mechanical, biological, and chemical control. More than 2000 papers in weed science, encompassing a wide range of areas and disciplines, were published between 1918 and 2003. Over the years, research priority areas included Weed Biology and Ecology, Environmental Fate and Transport, Herbicide Metabolism, Mode of Action, Physiology, Biochemistry, and Phytochemistry Application Technology, and Herbicide Activity, Selectivity, and Efficacy; Herbicide Resistance, Economics and extension, and Biocontrol. However, research emphasis has evolved initially from descriptive biology and ecology of weeds to herbicide efficacy.

Table 1 and Figures 1 to 8 show the partition analysis on the number of papers and their percentages out of the total publications accorded for each broad category of research areas over the last 85 years. Overall, the bulk of the papers and theses written was on the Weed Ecology and Biology, forming no less than 40%, or 863 articles of the total papers and theses in weed science in Malaysia. Papers and theses on Herbicide Activity/Selectivity/Efficacy come next comprising 37.89%, or 783 articles of the total papers and theses written. The next hierarchical order based on the number of papers and theses written was Environmental Fate/Transport (169) > Biocontrol (102) > Herbicide Resistance (95) > Herbicide Metabolism/Mode of Action/Physiology/ Biochemistry/Phytochemistry (77) > Application Technology (56) > Economics/Extension (30). There was a steady increase in the number of papers written on Weed Biology/Ecology over the years up to the 1970s. However, the



post 1970s period saw a measurable decline in papers and theses written on this area. The period between 1990 and 2003 witnessed a sharp decline in the percentage of papers and theses written on Weed Biology/Ecology. Arguably, the increasing awareness and interest on the possible impact of continuous use of herbicides on the environment has led to more publications on Environmental Fate/Transport of Herbicides and Herbicide Resistance in the post 1970s period. From the nominal publication of a paper by Riepma (1962) on the breakdown of amitrole in soil to about 187 papers and theses by several authors ranging from the breakdown of paraquat, glyphosate, glufosinate ammonium and sulfonylureas, to reports on the incidences and mechanisms of resistance in weed species such as *Monochoria vaginalis*, *Lindernia spp.*, *Eleusine indica*, and *Paspalum pliculatum* in the 1990's and the new millenium. Interest on Herbicide Metabolism/Mode of Action/Physiology, and Economics of weed control and extension took a back seat in the overall profile of research priority among researchers and students in weed science in Malaysia, forming slightly just about 5% of the total number of papers and theses written in 85 years. Research activities on Application Technology of herbicides and Biocontrol of weeds were given less emphasis by Malaysian researchers. Together they comprised slightly more than 7% of the total number of papers and theses written on weed science in more than eight decades. It is anticipated that in the coming decades of the new millennium, papers dealing with biotechnology will dominate the publication landscapes of weed science. With the increasing incidence of herbicide resistance in Malaysia, and the "spilling over" of herbicide resistant crops (HRCs) and transgenics, either through international trade and or deliberate imports, research emphasis on management of herbicide-resistant weeds, and herbicide resistant crops will likely to take the centre stage in the coming decades. This is aggravated by the legislative silence in the part of the government on the core issue of management of herbicide-resistant weeds, and herbicide resistant crops in the country. Further, it is also anticipated that herbicide development would continue to evolve with safer, more environmentally-benign and friendly, low use rate, and short residual herbicides. While there is a growing need for the development of alternative control measures, parallel increase in funding opportunities in Weed Biology/Ecology, IWM, and Biocontrol does not prevail at the Malaysian agricultural stage.

#### Research Emphasis Before the Second World War, in the 1950's and 1960's

The formative years between 1918 and the Second World War saw that research work undertaken was mostly on biological, ecological, taxonomy and botanical description of the weeds with only eight published papers on record. As early as 1938, experiments were conducted to assess the competitive effect of lalang (*Imperata arundinacea* syn. *I. cylindrica*) on the growth of young rubber (*Hevea brasiliensis*) trees. Likewise, the effects of *I. arundinacea* on the growth of coconut (*Cocos nucifera*) were also studied. Occasionally, interest on the exploitation of weeds as organic fertilizers, vegetables, and medicinal plants were also recorded. The 1950s and 1960s saw a ten-fold increase in the number of published papers and occasional reports, with the emphasis on Weed Biology/Ecology, Herbicide Efficacy/Selectivity/Activity and general weed control. In 1953, chemical weed control using sodium arsenite, sodium chloride, TCA, DNOC, calcium cyanamide, and copper sulphate were introduced as in rubber estates for lalang (*I. arundinacea*) control in Malaya. Papers dealing with the ecology and establishment of legume covers such as *Centrosema pubescens*, *Calopogonium mucunoides*, *Pueraria phaseoloides*, *Flemingia congesta*, *Stylosanthes gracilis*, *S. sundaica*, and *Desmodium ovalifolium* in rubber estates appeared in 1954 and 1955. By 1959, new and more potent and effective herbicides such as 2,4-D amine and other isomers, 2,4,5-T, and dalapon were then available in the market for general weed control in the estates, silviculture, and paddy. Paraquat and simazine were first introduced in 1962, with the former herbicide taking a lion market share in sales and research activities, notably among the planters. Intriguingly, studies on the degradation of amitrole in tropical soils were initiated in 1962. Comparative studies on weed control efficacy in tropical legumes with several herbicides, namely amitrole, paraquat, dimethyl arsenic acid, disodium methylarsonate, TCA, 2,4-D, 2,4,5-T, 2,4,5-TP,



were undertaken. The mechanical Holt Weed Breaker of Models Mark V1 and Mark V111B to chop upright weeds weed control on holt was introduced in 1957.

The post-independent years witnessed an upsurge in herbicide-based weed science research in the country. New players such as atrazine, diuron, MSMA, picloram, trifluralin and simazine, along side paraquat, dimethyl arsenic acid disodium methylarsonate, TCA, 2,4-D, and paraquat were experimented with, especially in estates. By 1975, research emphasis was on the use of paraquat, alachlor, MSMA, 2,4-D, diuron, and glyphosate in both crop and non-crop areas of rubber, pineapple, groundnut and oil palm. Research experimented with the use of CP50144, atrazine, ametryne, simazine, BAS 201311, and glyphosate for weed control in soybean.

#### Research Emphasis in the 1970's and Beyond

The 1970's witnessed further increase in research activities principally on Weed Biology/Ecology and Weed Control/Herbicide Efficacy with more than 90% of the total publications recorded. Concerns on environmental impact on the increasing use of herbicides for weed control in the country began to surface in the 1970s. This was exemplified by initial work on the breakdown and residual toxicity of sodium chlorate and alachlor in 1971. Further concern on the excessive use of herbicides in the 'no-till' systems, replacing the mechanical ploughing, the former aggravating in soil erosion, took shape as well among researchers. Successful biological control on the weed *Cordia curassavica* by *Schematiza cordiae* under coconut was recorded in Kuala Selangor in 1976 - 1977 (Ung et al. 1979).

Research focus in the 1980s was still on weed ecology and biology, and herbicide-based weed management in plantation crops, rice, vegetables, and fruits with emphasis on chemical weed control, herbicide physiology and ecology, and the establishment and weed management in legume cover crops. Concerns over herbicide toxicity to crops, especially incidences of parthenocarpy, and legume covers, also received wide coverage. As in the preceding years, research emphasis on the efficacy of herbicides against weeds, their phytotoxicity on crops, and application techniques dominated the papers published. The efficacy of herbicide combinations as mixtures, or sequentially applied *vis-à-vis* singular application against weeds with minimum crop injury was quite prominent among herbicide-based research effort in 1977-1981. The basis of herbicide selectivity in crops and weeds received attention by researchers. This included physiological studies on paraquat, diquat, and dalapon, and their effects on photosynthesis and ion leakage, and electron transport in plants and fungi. Studies on herbicide application techniques in plantation crops continued in the late 1970's and early 1980's. This includes controlled droplet application (CDA) of herbicides that had advantages in cost savings, application time and ease of usage in hilly or difficult terrain. Commercial evaluation of ULV/VLV/LV/CDA spray systems were conducted to assess their suitability and risks of contaminations to the workers. In rubber and oil palm plantations, sheep and goat labour were used to control weeds, while providing extra income.

The 1990s and the first three years of the new millenium witnessed continued interest in weed science research in Malaysia, with 1166 publications, or 56.4% of the total papers, theses, monographs and reports on the subject. Again studies on Herbicide Selectivity/Efficacy and general weed control, and Weed Biology/Ecology dominated the research activity landscapes. Research inroads were made on the fate, attenuation, and persistence of herbicides in the environment, and herbicide resistance. A significant upsurge of interest on the economics of weed control and extension was evident with the generation of 28 papers and reports during the period.

Chemical weed control in rice, rubber, oil palm, vegetables, fruit orchards, golf courses and turfs were a dominant part of research activities in the 1990s and beyond. These were augmented with parallel studies on herbicide application technology, an integral part of herbicide-based weed



management. Research on formulation and application techniques (e.g. Samurai CDA, F120 Knapsack sprayer and Turbo CDA), carrier volume, controlled-released formulation (e.g. thibencarb and diuron), and rainfastness of herbicides. So were research on efficacy of herbicides with/without adjuvants and surfactants, application safety, herbicides bioactivity sorption, attenuation, persistence, mobility and degradation. Direct-seeded rice culture and continuous use of phenoxy herbicides then have resulted in species shift in weed community structure favouring the more competitive graminaceous species such as *Echinochoa crus-galli* complex, *E.colona*, *Leptochloa chinensis*, *Ischaemum rugosum* and *Paspalum distichum*, and of late, weedy rice in Malaysia. Research on herbicide resistance found increased incidences of herbicide-resistant weeds in Malaysia such as cross- and multiple-resistance (e.g., goosegrass on propaquizafop and clodinafop), *Fimbristylis miliacea* and *Limnocharis flava* to 2,4-D, *Limnocharis flava* to bensulfuron-methyl, and *C. crepidiodes* and *S. nigrum* to paraquat, to name a few. Research on ecology and physiology of herbicides, include metabolism, uptake and translocation of <sup>14</sup>C-paraquat in resistant and susceptible biotypes of *Crassocephalum crepidiodes*, or <sup>14</sup>C-glyphosate in goosegrass (*Eleusine indica*) were conducted. Tran et al. (1999) recorded for the first time the incidences of goosegrass resistance biotypes to glyphosate in Johore in Malaysia. Dill et al. (2000) illustrated the mechanism and the basis of glyphosate resistance in goosegrass biotypes prevailing in Malaysia.

Table 1. Weed science publications abstracted through Eighty-Five Years of the Malaysian Initiatives in Weed Science Research (after Baki 2005)

Category	Pre-1970		1970's		1980's		1990's & Beyond		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Weed Biology/Ecology	39	46.43	127	56.19	365	48.55	332	28.46	863	41.77
Environmental Fate/Transport	1	1.19	8	3.54	66	9.56	94	8.13	169	8.18
Herbicide Metabolism/Mode of Action/Physiology	1	1.19	-	-	29	4.20	47	4.06	77	3.73
Biochemistry/Phytochemistry										
Application Technology	1	1.19	3	1.33	21	3.04	31	2.65	56	2.71
Herbicide Activity/Selectivity/Efficacy	41	48.81	80	35.40	202	29.26	460	39.45	783	37.89
Herbicide Resistance	-	-	-	-	2	0.02	93	7.98	95	4.60
Economics/Extension	-	-	1	0.04	1	0.01	28	2.40	30	1.45
Biocontrol	-	-	7	3.09	14	2.03	81	6.94	102	4.93
Total	84	4.05	226	10.92	690	33.39	1166	56.44	2066	100

Work on biological control of weeds using insects on *Mikania micrantha*, *Mimosa pigra*, *Chromolaena odorata* and *Echhornia crassipes*, or using on bioherbicides such as *Dactylaria higginsii* for purple nutsedge did prevailed. Interest on allelopathy and allelochemicals, and economics of control also took shape. Baki (2001) reviewed documented evidences on biological control initiatives in Malaysia since the 1950's. Of these successful suppression of *C. curassavica* by *Schematiza cordiae* syn. *Metrogaleruca onscura* and *Eurytoma attiva*, and *S. molesta* by *Cyrtobagous salviniae* has been recorded. Sheep labour has been used successfully to control plantation weeds such as *A. intrusa*, *A. coromandeliana* Pennnisetum spp., *Ischaemum* spp., and *Digitaria* spp. but not *C. odorata*, *Clidemia hirta* or *M. malabathricum*. In paddy fields, ducks and chickens as potential bio-control agents have been evaluated.



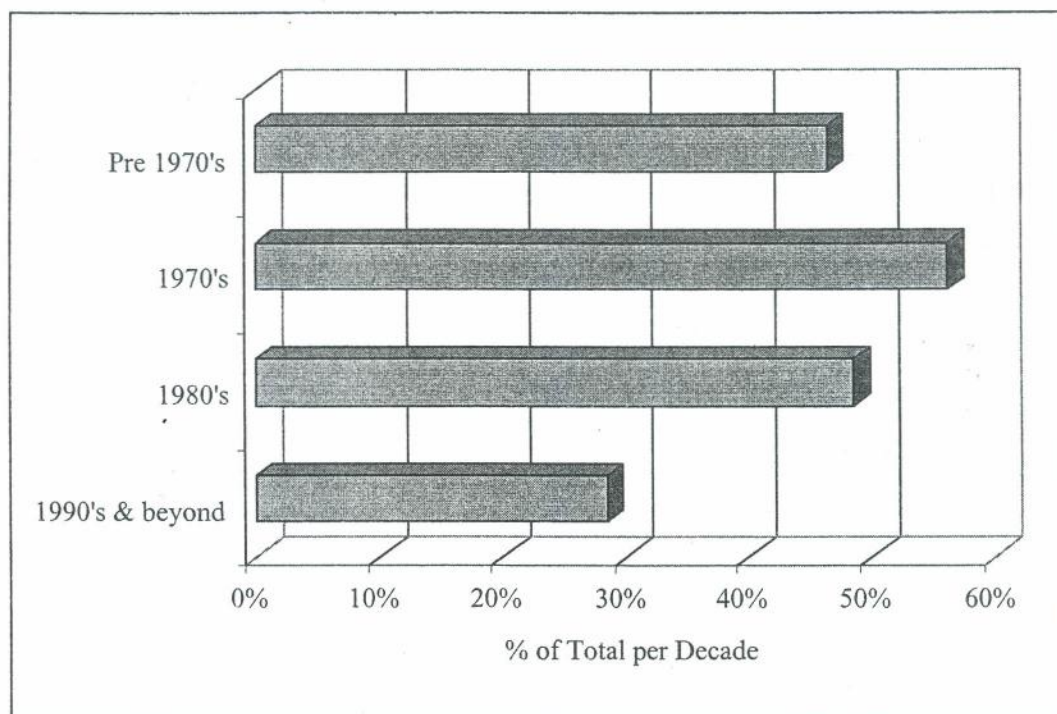


Figure 1. Publications in weed research category of Biology/Ecology for each respective decade as expressed as a percentage of the total per decade.

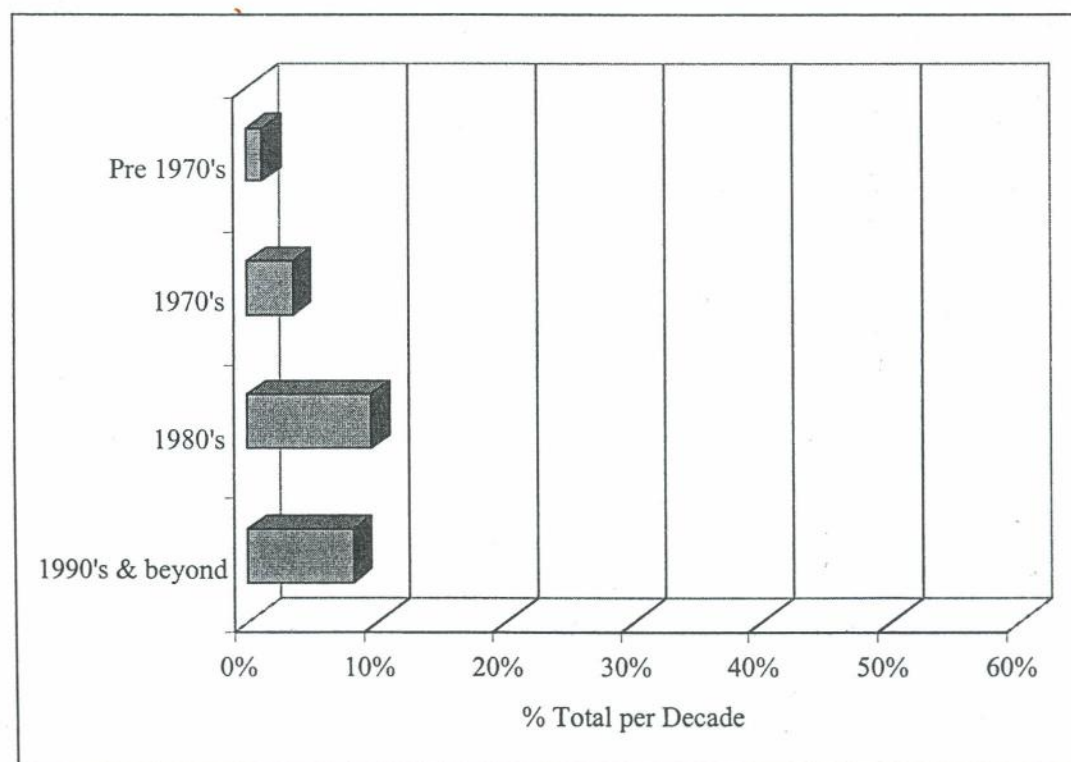


Figure 2. Publications in weed research category of Environmental Fate/Transport for each respective decade as expressed as a percentage of the total per decade.



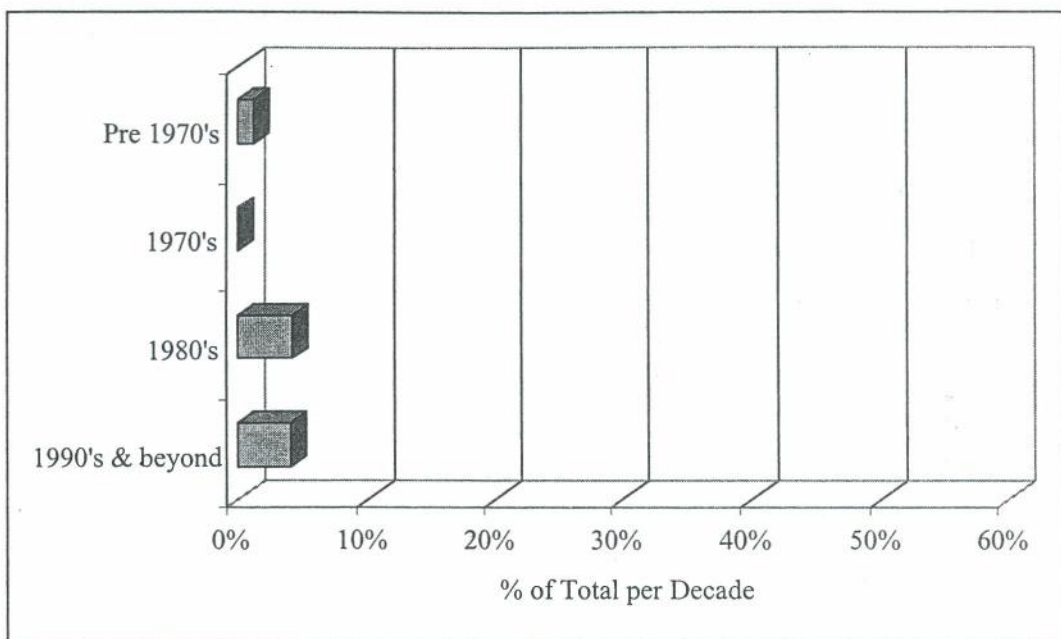


Figure 3. Publications in weed research category of Herbicide Metabolism/Mode of Action/Physiology/Biochemistry/Phytochemistry for each respective decade as expressed as a percentage of the total per decade.

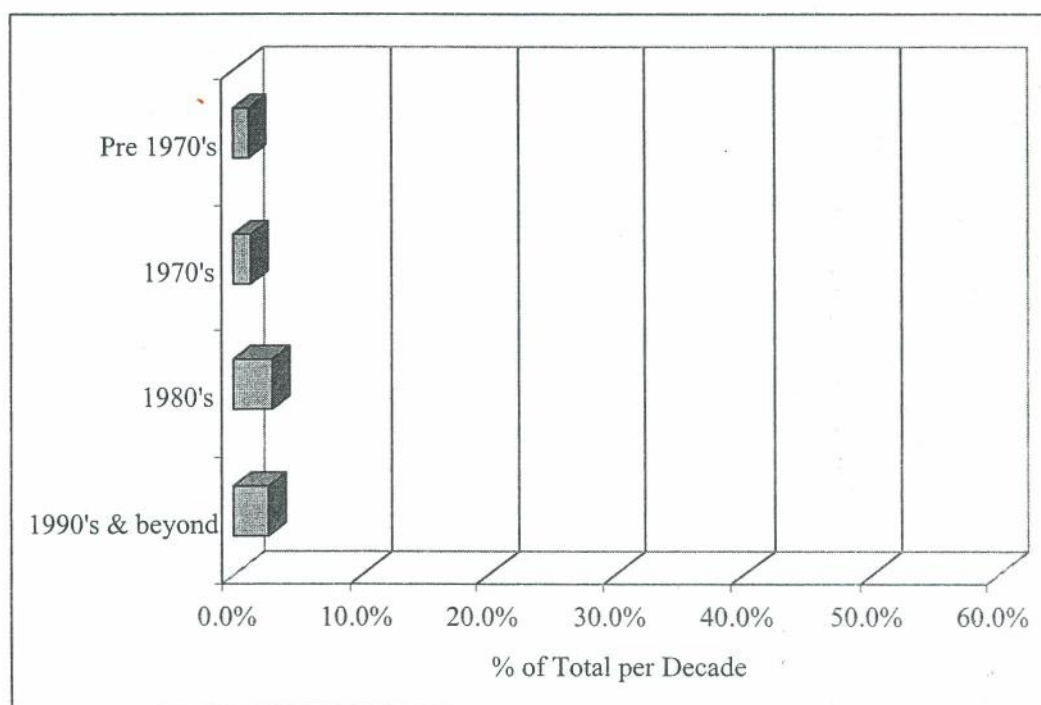


Figure 4. Publications in weed research category of Application Technology for each respective decade as expressed as a percentage of the total per decade.



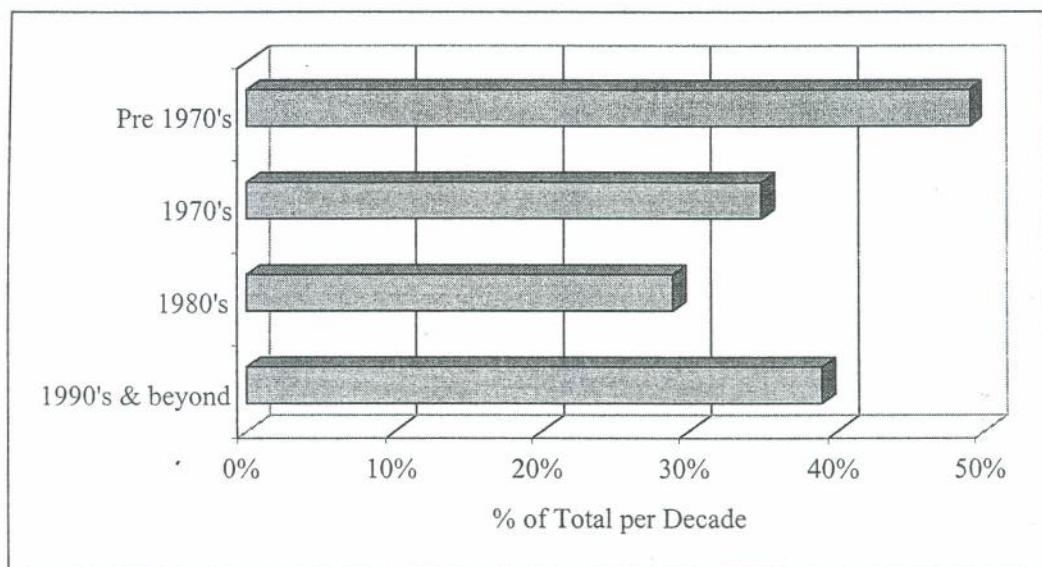


Figure 5. Publications in weed research category of Herbicide Activity/Selectivity/Efficacy for each respective decade as expressed as a percentage of the total per decade.

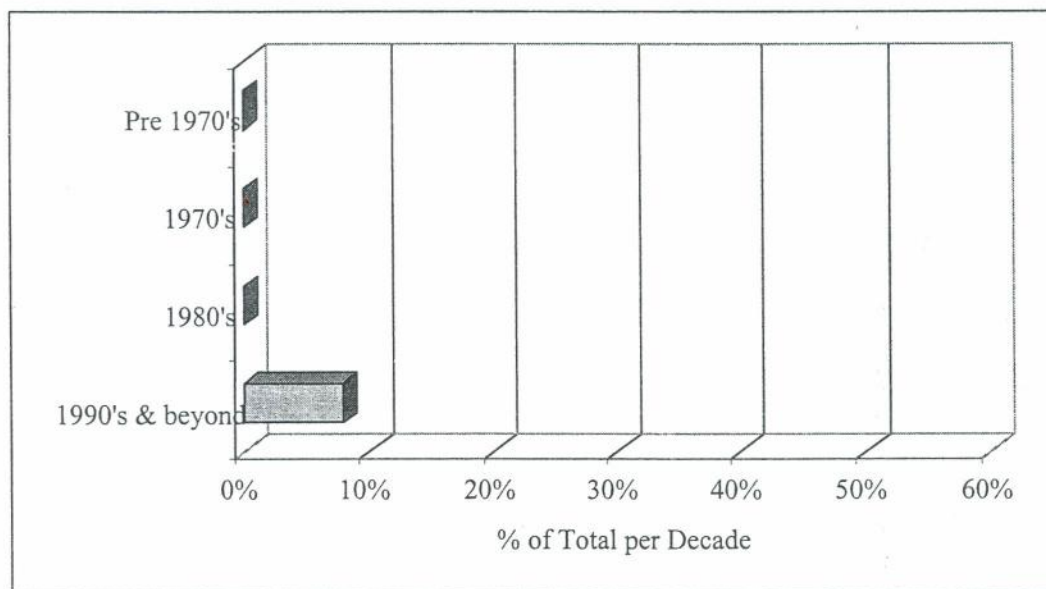


Figure 6. Publications in weed research category of Herbicide Resistance for each respective decade as expressed as a percentage of the total per decade.

A noticeable advancement of research in weed science includes research activities on weed utilization and biotechnology in the first half of 1980's and beyond, especially on the medicinal and nutraceutical properties and values of weeds. For example, the biochemical basis of *Phyllanthus* spp. used against liver, kidney, and urinary bladder ailments forms new generations of research activities, especially among phyto-chemists in public universities, and research institutions. Ultimately, Malaysian initiatives on weed science are likely to focus on biotechnology research to produce transgenic crops, resistant to commonly used herbicides, alongside the environmental-friendly weed management strategies with less inputs of herbicides yet sustainable for crop production and resource management.



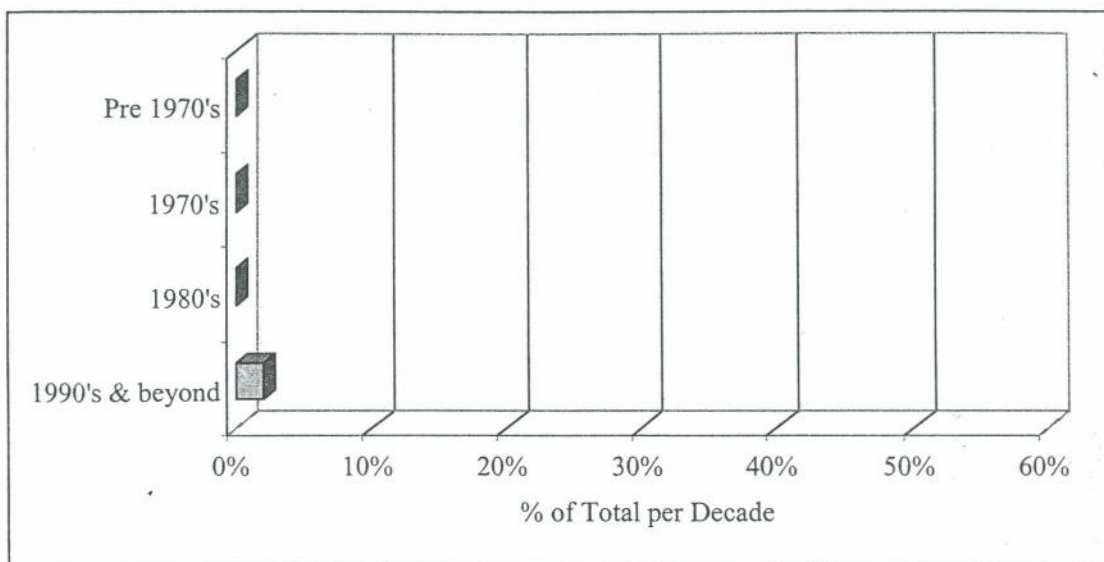


Figure 7. Publications in weed research category of Economics/Extension for each respective decade as expressed as a percentage of the total per decade.

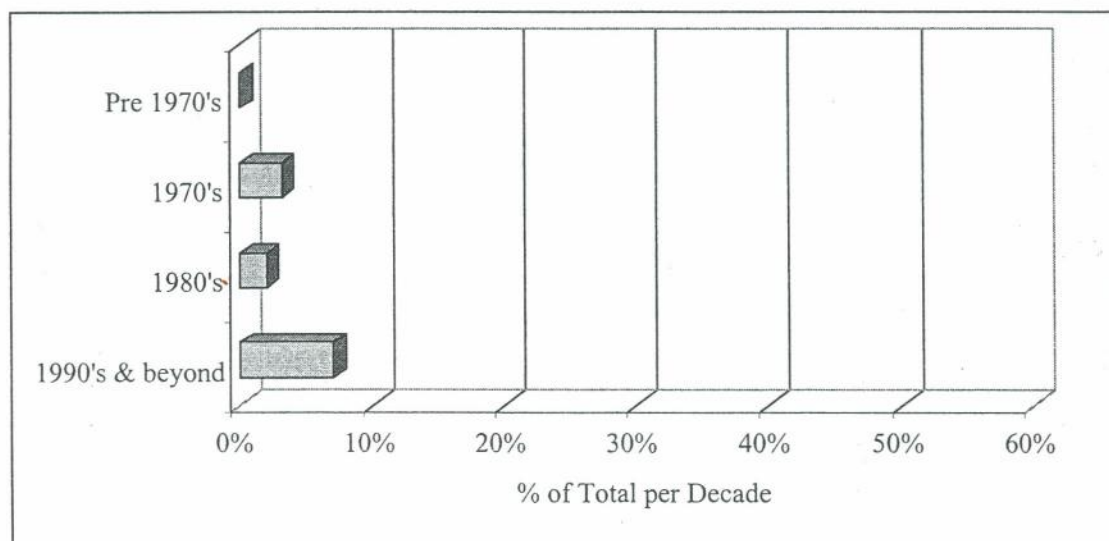


Figure 8. Publications in weed research category of Biocontrol for each respective decade as expressed as a percentage of the total per decade.

### FUTURE TRENDS AND CHALLENGES

Food scarcity and food security (FSFS) are synonymous with 21<sup>st</sup> century agriculture. Our monumental import food bills in excess of RM15 billion annually are synonymous with the lingering concern for FSFS to feed the growing populace, despite the burgeoning economy. While Malaysia made enviable progress in plantation-based agriculture dominated by rubber, oil palm and cocoa, there is a definite need for intensification and sustainability in agriculture, especially in food production. Research emphasis in the past has always been herbicide-based science principally when food security was the domain of public concern. It is only proper that future directions in weed science research in Malaysia need a reframing to include the broad areas of (i) Knowledge-Based and Systems Approach-Based Decision; (ii) Weed Biology and Ecology; (iii) Weed Control and Management; (iv) Herbicide Resistance; (v) Issues Related to Transgenic Plants; (vi) Environmental Issues, and (vii) Potential Benefits of Plant Species Generally Classified as Weeds



The increasing problems of environmental pollution due to agrichemicals, increasing incidences of herbicide-resistance, and noxious millennial weeds, water shortage, and technology divide between the plantation-based sector and the small farmers are some of the current issues facing Malaysian agriculture in general, and weed management practices in particular. The total absence of WRA within the framework of quarantine protocols and infrastructures may lead to increased risk of further introduction and subsequent invasion and spread by plant invasives (weeds!) into the country in the future. The strong dependence on herbicide-based control measures, notably in estates, will lead to a parallel increase in herbicide-resistant weed species. There are evidences of increased incidences of endemics becoming invasive – this is worrisome as native species can become naturalized and become weedy and invasive quite quickly, especially in disturbed habitats, as most Malaysian agro-ecosystems are.

Future initiatives in weed science in Malaysia hinge on the ability to provide multi-dimensional approach in weed control technologies. Such a system will work toward a socially permissible, environmentally sound, economically feasible, productive and sustainable agricultural system. I believe that the initiatives on agriculture and weed science today and tomorrow should focus on the (i) expansion of the science and application of plant genomics to provide the basic knowledge and technology required to increase food production and resource utility; (ii) development of efficient sustainable systems for the production of food and fiber, and preservation of the natural resource base; and (iii) development of mechanisms that enhance producer profitability while minimizing financial risks and ensuring food safety and security.

With the continued use of herbicides and adoption of HRCs come the inevitable ecological risks to the environment. These will require stringent ecological risk assessment of herbicide resistant crops and screening for increased incidence of herbicide-resistant weeds to be in place. While ecological risk assessments are encouraged by the FAO for member states, regulatory implementation is a difficult task. There is a definite link between FSFS with the development of weed science research and application in this country. The major issues and challenges facing weed science in the era of biotechnology include (i) relationships with industry, (ii) funding opportunities, (iii) graduate education, (iv) visibility and status of weed science as an academic discipline, (v) networking with other academic disciplines, and (vi) creative outreach programmes. The old definition of weeds as plants whose uses have not been discovered will serve us well in the era of biotechnology. Weeds are now used as a source of specialty genes for the production of transgenic crops or sources and bioproduction of nutraceuticals, or drugs (Duke et al. 2002) representing some concrete success of biotechnology. It is heartening to note that The Scientific Committee on Problems of the Environment (SCOPE) in collaboration with the United Nations Environment Programme (UNEP), the International Union for the Conservation of Nature (IUCN), and Commonwealth Agricultural Bureau International (CABI) is embarking on a new programme on invasive species, this time with the explicit objective of providing new tools for understanding and dealing with invasive species (Mooney 1999). This venture is under the umbrella of the Global Invasive Species Program (GISP) where the scientific community along with policy makers, legal experts and people from industry and government are engaged in serious deliberations under 11 elements on building a comprehensive approach needed for dealing with invasive species. Four of these elements deal with synthesizing our current knowledge on invasives, and these include (i) the ecology of invasive species, (ii) the current species and new methods for assessing their changing distributions and abundance, (iii) how society views and evaluates invasive species, and (iv) how global change will impact the success of invaders. It is my hope that the Malaysian scientific community plays its role in GISP.

Despite almost a century of research initiatives in weed science, weed problems remain a central issue in Malaysian agricultural landscapes. Research emphasis at present as in the past has always been herbicide-based science principally when food security was the domain of public concern.



There is a serious need for collaborative research within public institutions, and between them and private institutions in this country. Such collaborations will help to break institutional barriers and optimisation of manpower and facilities, while setting up research priorities at the same time. The real challenge is to set research directions in weed science in the country so as to generate knowledge-based and systems approach-based decision, at least in principal economic crops. Strategising the development of effective weed management entails full understanding of the fundamental relationships of weeds and crops in agricultural systems. Benefits arising from adoption of new technologies such as herbicide-resistant crops (HRC) by farmers, mechanistic research must be conducted in weed ecology, genetics, and physiology to increase understanding of basic regulatory processes in weed-crop interactions, weed population dynamics under various management practices, and other aspects of weed invasion, adaptation and persistence. An ill-conceived idea on weed biology and ecology may lead to ineffective weed control measures and cultivation practices. Further, research on weed ecology and biology must address social and economic aspects of weed control, namely, practices that select for herbicide-resistant weed populations, declining agricultural profitability, and the belief that herbicide use poses environmental and health risks. For example with the adoption of direct-seeded rice culture, has resulted in the emergence and heavy infestations of weedy rice. With regard to selection pressure, it is recommended that research be conducted in the following areas of investigation: (i) the ecological relationships between weeds and cropping systems to understand and predict shifts in weed species response to selection pressure while comparing existing and new, innovative crop production systems; (ii) short- and long-term changes in species dynamics of weed communities within the contexts of successional theory; and (iii) dynamics of weed populations that shift in response to selection by control practices, including herbicides. In order to develop descriptive and predictive models of population behaviour, such research should be conducted in the context of population and metapopulation theories. Unfortunately, there has been little support for this type of research, as indicated by the disconnection between ecological and weed science literature. Weed scientists need to demonstrate the relevance of this research, *vis-à-vis* societal goals.

Recommendations of future research include (i) investigation on the dynamics of weed seed banks in relation to above ground flora, and response to different management systems; (ii) investigation on ecological and physiological mechanisms of weed-crop and weed-weed competition and weed adaptation to environmental factors, based on extensive descriptive literature on weed-crop competition; (iii) investigation on changes in competitive abilities of weeds and crops in relation to biotic and abiotic factors in order to develop new models and to parameterize existing models of crop-weed competition; and (iv) Conduct long-term field-scale studies to determine the effect of utilizing weed thresholds in management.

Several action plans are necessary to deal with invasive weeds, viz., (i) strict quarantine measures should be enforced to prevent introduction, sales, and importation of invasives at every port of entry. Methods to efficiently recover and identify taxa moving as contaminants in trade goods and posing a significant threat based must be developed; (ii) coordinated mitigation and control programmes within the contexts of IWM should be developed. Experiments on herbicide efficacy focussing on herbicide application techniques maximising efficacy on target weeds but with minimum effects on no-target species should be undertaken; (iii) increased emphasis on basic research on the biology, ecology, physiology and epidemiology of invasive weeds should be made; (iv) continuous and effective campaigns to educate and increase awareness among the public on the danger of invasive weeds to the environment and economy should be carried out. Capacity building by increasing the number of weed scientists to carry out research on invasive weeds is required; and (v) assessment methods on economic losses by invasive weeds to agricultural land management operations.



Within the context of weed control and management, in depth studies of practical importance include (i) herbicide efficacy enhancement, (ii) alternative weed management methods, (iii) weed management systems. Further, issues associated with herbicide resistance management, the use of HRCs or/and transgenic crops, and the environmental health following heavy reliance on herbicide-based weed management require strong will among the policy makers, scientists alike to strategise action plans to minimise any unhealthy trends in weed management practices while ensuring food security for the populace.

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# **Weed Biology and Ecology**



## Assessing the risks of weed seeds on horticultural imports

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**Abstract:** New weed species are spreading quickly around the world and no country is immune. With growing tourism and trade in New Zealand, the potential for accidental introductions is high, but not all these are able to survive or establish here. This project assessed the risk posed to New Zealand agriculture and the environment by weed species whose seeds were found on imported fresh produce. Regular inspections of fruit imported from the Philippines and Thailand yielded seeds of 12 species which are all serious weeds in tropical and subtropical areas and could potentially survive in some parts of New Zealand. For each species information on biology, ecology, distribution and weed status was collected and used in two Weed Risk Assessment (WRA) systems. The weed seeds were also grown in a containment facility to evaluate their potential to establish, grow and reproduce under local conditions. The species considered most likely to be serious weeds at least in some parts of New Zealand included the fast growing shrub, *Chromolaena odorata*; weedy perennial grasses *Brachiaria mutica*, *Paspalum conjugatum* and *Saccharum spontaneum*; the annual grass *Rhynchelytrum roseum* and the free seeding annual grass *Ageratum conyzoides*. The other species, including *Borreria alata*, *Bothriochloa ewartiana*, *Dactyloctenium redulans* and *Digitaria brownii* do not appear to present a serious threat to New Zealand agriculture or environment. These could probably establish here but are unlikely to be competitive with other species, and would probably only survive in open, disturbed habitats in warm northern areas. Based on the WRA scores and on the establishment and reproduction in a controlled environment, Maximum Pest Levels (the number of seeds per sample acceptable for imports) were prepared for quarantine and border protection purposes.

**Key words:** Biosecurity, fresh produce, import contamination, quarantine, weed risk assessment, weed seeds.

## INTRODUCTION

New Zealand has been geographically isolated for a very long time resulting in unique plant species and floral communities that are found nowhere else in the world. It also lacks many of the common pest and weed species found in other countries. While this is a great advantage, it also means that it is very difficult to predict what will happen to new species once they have entered the New Zealand environment. On the other hand, New Zealand has one of the highest percentages of introduced plants in the world (Williamson 1996) and all its agricultural crops are introduced species. The problem is to balance the risks and benefits of exotic plant species.

The huge increase in trade and international travel resulting from globalization brings with it the possibility of introducing a range of organisms, including plant species. Current records of the Ministry of Agriculture and Forestry (MAF) show that approximately 400,000 containers are unloaded each year in New Zealand. Of these around 39% are contaminated. Fourteen per cent of cargo is inspected because of risk factors such as the point of origin, and another 10% is randomly chosen for inspection. Thus, there is no doubt that contaminated containers are entering New Zealand even if interception rates were perfect (Goldson et al. 2002). A further study of the external surfaces of shipping containers also showed considerable potential for entry by unwanted organisms (Gadgill et al. 2000). In addition there are about 3.8 million passenger arrivals each year. Estimates suggest that about 50 unwanted species enter New Zealand every year (Goldson et al. 2002). However, not all of these are able to establish, or their presence may not be noted for sometime. In



both New Zealand and Australia most weeds are invasive, and most threats of new weed species come from outside the region (Williamson 2001).

Seeds of exotic weed species attached to imported fresh produce bring the risk of introducing new weeds to New Zealand. If they become established, these weed species have the potential to become serious problems in this country. For the present study 12 such species were investigated for the risk they posed to New Zealand, if they were accidentally introduced into the country. All these species are warm-zone weeds whose seeds are most frequently intercepted on fresh fruit imported from countries like the Philippines and Thailand. These weeds are all largely restricted to the tropics or sub-tropics, however, many sub-tropical weed species already flourish in New Zealand and climate change is likely to allow these and other species to spread and become more damaging. The overall objective of this work was to provide information to the MAF Biosecurity Authority to help set maximum pest levels (MPLs), i.e. the number of seeds per sample that would be acceptable for fresh imports.

## MATERIALS AND METHODS

### Weed risk assessment

For each of the 12 species (Table 1), information on ecology, distribution and weed status was collected. This information was used in two weed risk assessment (WRA) systems. The first WRA, devised by Pheloung (1996) and revised for New Zealand by Pheloung et al. (1999), is based on 49 questions about the main attributes and impact of a weed. Its aim is to test species not yet present in the country for their likely impact if they were introduced. It is probably the best effort yet available as an objective, credible and publicly accepted risk assessment system to predict the invasive potential of the thousands of likely new entries. It also differentiates between plants that may become either agricultural or environmental weeds. Final scores range from minus 14, for a benign species, to plus 29, the maximum weediness score. A score of less than 0 means the species can be accepted into the country, 1 to 6 means that further evaluation is necessary, while 7 or more indicates the species should not be allowed entry.

The second WRA, devised by Esler et al. (1993), for assessing biological success and weediness for species already present in New Zealand was used to compare the potential risk of these weeds. This model is an objective and transparent method of assessing the relative importance of pest plants. It results in two separate scores, one for biological success, and one for weediness. Biological success ratings are associated with the ability of the species to establish and spread and range from 0 to 21. Weed status ratings refer to the nuisance value of the species, and range from 0 – 24.

### Germination and growth

Weed seeds intercepted on imported fresh fruit were collected at port of entry and sent to the New Zealand National Seed Laboratory. For germination test, the seeds were placed on top of blotters moistened with water. Blotters were placed on a cotton wick over water at 20°C night (16hrs) and 30°C day (8hrs). Dormant/slow seeds of *Digitaria brownii* had the seed coats (glumes) removed 7 days before the final germination count. Seeds of several species were chipped to speed up their germination.

As soon as a seed germinated, it was transferred to a quarantine greenhouse. The minimum temperature in the greenhouse ranged from 6°C to 8°C and the maximum was between 22°C and 23°C. These were winter temperatures in the greenhouse and are below average summer temperatures for most of the North Island. No supplementary heating or lighting was provided. Trays were drenched with the fungicide etridiazole three times while seedlings were establishing to control fungal infections.



As the plants in the greenhouse reached maturity, their seeds were collected and forwarded to the New Zealand National Seed Laboratory to test their viability and germination using the procedure described above. Dormant/slow seeds of some species were chipped to speed up their germination.

## RESULTS

### Weed risk assessments

The 12 species included in this study and their brief details are given below. Results of the two weed risk assessments are presented in Table 1. For comparison, assessment scores are also included for four weeds already present in New Zealand which have shown excellent ability to establish and spread.

*Ageratum conyzoides*: A free-seeding annual weed of crops and waste areas, widespread in the tropics and sub-tropics. Can produce seed within two months of emergence and populations regenerate very quickly from seed. Can apparently grow as a summer annual in Britain.

*Bothriochloa ewartiana*: An upright perennial grass growing to 90 cm tall. It is found in dry regions of Australia and known as desert bluegrass. Occurs on river banks, drainage floors, valley slopes or stony hills.

*Borreria (or Spermacoce) alata*: A low growing annual herbaceous shrub that has spread noticeably over the last 20 years to become serious crop weed in Caribbean, West Africa and South Pacific.

*Brachiaria (or Urochloa) mutica*: A coarse, trailing perennial grass, widely introduced into tropical areas as a pasture grass. Mainly a weed of wet areas, although adaptable to varying moisture conditions. It is somewhat shade-tolerant but very susceptible to frost.

*Chromolaena odorata*: Is common in many tropical regions and recently discovered in Queensland but being eradicated. It is an erect or sprawling fast-growing shrub, forming dense tangled thickets 1.5 – 5 m high or higher when supported on adjacent plants or other objects. It is very competitive with pastures and plantation crops; also a fire hazard and threat to natural areas.

*Cleome rutidosperma/viscosa*: A blue-flowered annual herb usually growing up to 0.5 m high. *Cleome viscosa* is an invasive on Nuie, and its presence in central Australia is causing some concern there.

*Coccinia grandis*: A vine which is proving very invasive on Hawaii and also found in other Pacific islands. It can be a very aggressive weed of natural areas and sometimes smothers forest.

*Dactyloctenium radulans*: A soft, sprawling, tufted annual grass up to 30 cm in height, occasionally toxic (nitrate). It is found in semi arid and saline regions of Australia where it is known as button grass. It can become dominant under heavy grazing, and occurs as a weed of gardens, cultivation and roadsides.

*Digitaria brownii*: This tussock-forming perennial grass is found in central Australia where it is considered a valuable pasture perennial, with relatively soft foliage that is palatable to stock.

*Paspalum conjugatum*: Widespread in the tropics and subtropics, where it is an aggressive and persistent perennial stoloniferous grass that rapidly invades wet habitats, often forming a dense ground cover. It has led to the extinction of some native forests.

*Rhynchelytrum roseum*: A common annual grass weed and colonizer of bare ground in tropical and some temperate areas but is not considered particularly competitive. In Australia it is a widespread roadside and railway embankment weed.



*Saccharum spontaneum*: A perennial rhizomatous grass which is widely distributed in Asia and some parts of the Pacific. It has wind-borne seed, and is regarded as a serious weed in some areas. Sometimes used in sugar cane breeding programmes, it tends to be restricted to tropical areas, although it does grow in northern India.

According to both WRA systems, the worst potential weed among the 12 species investigated is *Chromolaena odorata* with high scores for maturation rate, seeding, dispersal and establishment, competitive ability, health impairment and resistance to management, etc. Its scores are similar to those for three of the four of our existing weeds in Table 1 that are among the worst pasture weeds. *Paspalum conjugatum* is not far behind it on weed scores. The other two perennial grasses, *Brachiaria mutica* and *Saccharum spontaneum* come next and again are found mainly in tropical areas. *Ageratum conyzoides*, sometimes a serious annual weed of crops, has a moderate score for weediness. The two annuals *Cleome rutidosperma* and *Rhynchelytrum repens* have lower weediness scores, mostly ranking as weeds of open ground rather than as serious weeds of crops. *Digitaria brownii* seems to have few weedy characters.

#### Germination and growth

Most of the intercepted seed of weed species evaluated in this study germinated rapidly and within the first 7 – 10 days. In the case of *Bothriochloa ewartiana* germination was slow and spread over 3 weeks. For this species and also in the case of *Brachiaria mutica*, *Cleome rutidosperma* and *Rhynchelytrum roseum*, many seeds needed chipping to initiate the germination process. Although some seedlings died, often due to disease, particularly in the case of *Cleome rutidosperma* and *Borreria alata*, most grew and established well in the greenhouse. The only two intercepted seeds of *Saccharum spontaneum* failed to germinate and *Borreria alata* plants failed to reach maturity.

Table 1: Weed risk assessment scores for the 12 intercepted and 4 local weed species and recommended acceptance levels for importation.

Species	Pheloung et al. (1999) NZ weed score	Esler et al. (1993)		Acceptance level* (seed/600 fruit sample)
		Biological success rating	Weed status assessment	
<i>Chromolaena odorata</i>	23	16	21	8
<i>Paspalum conjugatum</i>	22	19	15	8
<i>Brachiaria mutica</i>	21	14	12	8
<i>Saccharum spontaneum</i>	17	15	10	15
<i>Rhynchelytrum roseum</i>	14	11	4	15
<i>Cleome rutidosperma</i>	12	11	6	15
<i>Ageratum conyzoides</i>	12	16	9	15
<i>Borreria alata</i>	12	14	11	30
<i>Dactyloctenium radulans</i>	12	14	10	15
<i>Coccinia grandis</i>	8	12	13	30
<i>Bothriochloa ewartiana</i>	6	11	8	30
<i>Digitaria brownii</i>	4	11	4	100
<i>Rubus fruticosus</i> **	29	17	15	-
<i>Sorghum halepense</i> **	25	21	18	-
<i>Senecio jacobaea</i> **	22	15	14	-
<i>Ulex europaeus</i> **	13	15	19	-

\* Based on the two WRA scores and their establishment and reproduction in a controlled environment.

\*\* These species are already present in NZ. Their WRA scores are included here for comparison.



Viable seeds were obtained from eight of the 10 test species which reached maturity. The only species that did not flower was *Chromolaena odorata*. *Coccinia grandis* grew rampant but no seed was set, possibly because of the dioecious nature of this species. Seed was collected from the remaining species and their viability was tested. Results showed they all produced viable seed with germination rates ranging from 55–100%.

The MAF Biosecurity Authority (Plants) has phytosanitary guidelines (Standard 152.02) for importation and clearance of fresh fruit and vegetables into New Zealand for human consumption. Using this Standard and the results of two weed risk assessments plus the germination, growth, reproduction and viability studies, we developed the Acceptance Levels (MPLs) for seeds of the individual weed species on imported produce (Table 1). These will be used at New Zealand ports for quarantine and border protection purposes.

## DISCUSSION

Most of the weed species evaluated in this study demonstrated their ability to germinate, grow and set viable seed under the right conditions. Although the test plants were grown in a secure greenhouse, the actual growing conditions could be replicated in most northern areas of New Zealand. Thus all of these weeds could probably find a niche somewhere in New Zealand. In many cases that niche would be small, as many of these weeds are widespread mostly in the tropics and do not stray far into temperate areas. Some of them – notably *Brachiaria mutica*, *Chromolaena odorata*, *Paspalum conjugatum* and *Saccharum spontaneum* have the potential to be very serious weeds of both agriculture and the environment as they are in other countries. However, this would initially be only in the northern areas and for some of them, only in sheltered areas of Northland. *Brachiaria mutica*, for example, is very susceptible to frost. *Chromolaena grandis* is proving a serious weed of native environments in Pacific Islands and could become a problem in similar areas here.

Species like *Cleome rutidosperma* and *Rhynchelytrum roseum* could probably establish here but are unlikely to be competitive with other species and would probably only survive in open and disturbed habitats in warm northern areas. *Ageratum conyzoides* could become established as a crop weed and a plant of waste places in northern parts of New Zealand as demonstrated by its weediness potential overseas (Holm et al. 1977). *Bothriochloa ewartiana* and *Dactyloctenium radulans* both occur in central Australia and could probably establish in northern New Zealand, but are unlikely to present a threat to either agriculture or the environment. *Digitaria brownii* seems to have little in the way of weedy characteristics and is unlikely to become established here.

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**Abstract:** *Ludwigia peruviana* (Primrose Willow; Family: Onagraceae), a native of South America with a wide geographical distribution in tropical climates, has become a significant aquatic weed in eastern Australia, mainly in the Sydney basin. A second species- *Ludwigia longifolia* (Long-leaf Willow Primrose), first recorded in 1991 as an escapee from nurseries, is also recognised as a significant weed of aquatic habitats in the same region. Until recently, these species have been considered relatively minor weeds or naturalised plants, mainly in the tropics. However, their potential to become major threats to aquatic habitats over a wide geographical area in the tropics or sub-tropics needs to be recognised so that infestations detected early can be managed.

Since 1971, when *L. peruviana* was first recorded in Botany Wetlands in Sydney, it has spread widely from the initial infestation. The magnitude of the infestations in wet habitats, such as wetlands, creek and drains, and the rate at which the infestations have spread from a Sydney locus indicate how a recently introduced plant (a new 'immigrant') could rapidly become an 'invader'. Although *L. longifolia* is yet to invade so wide a territory, the tenaciousness of its established populations and resistance to control efforts have been noted in several locations.

Issues related to the successful invasion of aquatic habitats by *L. peruviana* and *L. longifolia* are discussed. Case studies are presented, which demonstrate the success, as well as limitations of implementing integrated weed management to locally contain the two species. Whilst recognising that control of biotic invasions becomes most effective when it employs a long-term, ecosystem-wide strategy rather than a tactical approach focusing on individual invaders, it is suggested that in the case of relatively recent invaders such as *L. peruviana* and *L. longifolia*, an individualist approach appears necessary, if the considerable additional threat posed by these weeds are to be averted. Containment and/or local eradication strategies should include early detection, early intervention to control individuals reaching maturity and prevention of spread via stormwater runoff, wind and other dispersal mechanisms.

**Key words:** Aquatic weeds, *Ludwigia longifolia*, *Ludwigia peruviana*, primrose willow.

## INTRODUCTION

Biotic invasions occur when organisms are transported to new, often distant ranges, where their descendants proliferate, spread and persist, establishing successful breeding populations (Mack *et al.* 2000). Such invasions are neither novel nor exclusively human-driven. Nevertheless, the frequency and number of species invading across continents have grown enormously, especially in the last 200 years. This phenomenon is probably a consequence of expanding inter-continental transport of goods and people, with humans serving as both accidental and deliberate dispersal agents.

The establishment of Primrose Willow (*Ludwigia peruviana* (L.) Hara) in Sydney is a classic case of a recent biotic invasion of Australia. The history of its introduction in Sydney, first as a botanical specimen, and the nature and rate of spread in the region are similar to an invasion, rather than a gentle immigration (an introduction), naturalisation (assimilation) and intermingling with existing vegetation communities. A second species- Long-leaf Willow Primrose (*Ludwigia longifolia* (DC.)



Hara) was first recorded in NSW, Australia in 1991 as an escapee from nurseries. This species is also threatening to become a significant aquatic weed in eastern Australia. Both *L. peruviana* and *L. longifolia* have not been recorded as major weeds in the world, but are considered as relatively minor weeds and naturalised plants in tropical and sub-tropical countries. However, the threat posed by both species to sensitive aquatic habitats in these areas is much greater than has been recognised.

In this paper, some issues related to the successful invasion of aquatic habitats by *L. peruviana* and *L. longifolia* in eastern Australia are reviewed. Experiences in the control of infestations are also discussed, arguing the case for a possible eradication strategy.

## THE INVADERS

### *Ludwigia peruviana*

*Ludwigia peruviana*, first named by Linnaeus as *Jussiaea peruviana* L, is known by other synonyms, including *Jussiaea grandiflora* L. It is a semi-aquatic, cold-deciduous shrub, which can grow up to 3-4 m height; its flowers are bright yellow, and the plant is a profuse seed setter. A native of the New World, *L. peruviana* occurs widely in South-eastern United States, nearly throughout tropical and sub-tropical South America. It has not been regarded as a weed in these areas.

According to Raven (1963), introduced in the Old World, the species became naturalised in Asia, South India, Sri Lanka (Ceylon), Singapore, Northern Sumatra and Java. Referring to specimens collected from these countries dating back to mid-1850s Raven (1963) suggested that the spread of genus *Ludwigia* to Australia and Pacific islands might have been relatively recent. The species is regarded as a minor weed in Asia, Indonesia and North America (Ramamoorthy and Zardini 1987).

Specimens in herbaria, such as in Sri Lanka's National Herbarium, are from moist habitats, from 0-1450 m altitude. In Australia, infestations south of Sydney, in Sutherlandshire, latitude  $\approx 30^{\circ}$  South, are the southern-most limit of its current spread. The pantropical occurrence, across several continents, and tolerance of conditions from sea level to  $> 1450$  m altitude indicates the wide ecological amplitude of the species.

Humans introduced *L. peruviana* to Australia; it was cultivated at the Botanic Gardens in Sydney in 1907 (Jacobs et al. 1994), possibly after introduction to Australia from South East Asia by botanists. Humans probably moved the species out of the Gardens as well, or humans may have acted as accidental carriers of propagules.

The central locus of first establishment of *L. peruviana* appears to be the Botany Wetlands in Sydney (Jacobs et al. 1993), less than 10 km south of the Botanic Gardens (Fig. 1). This extensive nutrient-rich pond system is historically significant in Australia because they once served as a drinking water supply to early Sydney settlers. First recorded in 1971 as naturalised in Botany Wetlands, *L. peruviana* infestations expanded in the next 20 years and by 1991, dense, mono-specific stands in the ponds covered approximately 30-31% of the Wetlands (Jacobs et al 1993; Chandrasena & Sim 1998) (Table 1).

Infestations in some ponds were so extensive that they covered almost 70% of the area, causing large scale changes to water flow and vegetation (Fig. 2). The potential of *L. peruviana* to spread throughout tropical and temperate Australian waterways and damage other wetlands was recognised with these vast infestations (Jacobs et al. 1993).



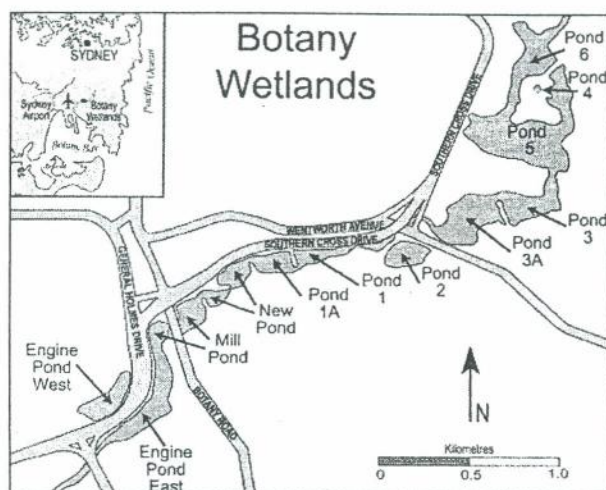


Figure 1.

Botany Wetlands pond system (59 ha) in Sydney's eastern suburbs, where large infestations of *L. peruviana* were first found. By 1990s, 30-31% of pond system was covered by entrenched, dense infestations and floating islands. Note major arterial roads impacting on the Wetlands.

Table 1. Extent of *L. peruviana* infestations in Botany Wetlands (1991).

POND Area (ha)	<i>L. peruviana</i> (ha)	Other aquatic weeds & invasive trees (ha)	Open water (ha)
Ponds 6, 5, 4, 3, 3A, 2 (34.1)	6.6	4.5	23.0
Ponds 1, 1A, New Pond (10.0)	7.9	0.7	1.4
Mill Pond & Engine Ponds (15.0)	3.7	7.8	3.5
Total Area (59.1)	18.2 (31%)	13.0 (20%)	28.0 (49%)

### *Ludwigia longifolia*

*Ludwigia longifolia* ( $\approx$  *Jussiaea longifolia* DC.) is also a New World species, whose native range stretches from Brazil to Argentina. Its habitats are also tropical and sub-tropical swamps. The species may look like *L. peruviana*, but its leaves are much narrower, and often have a reddish tinge.

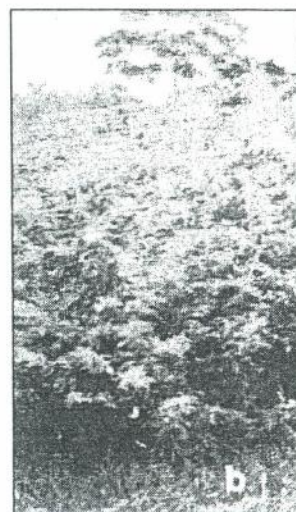
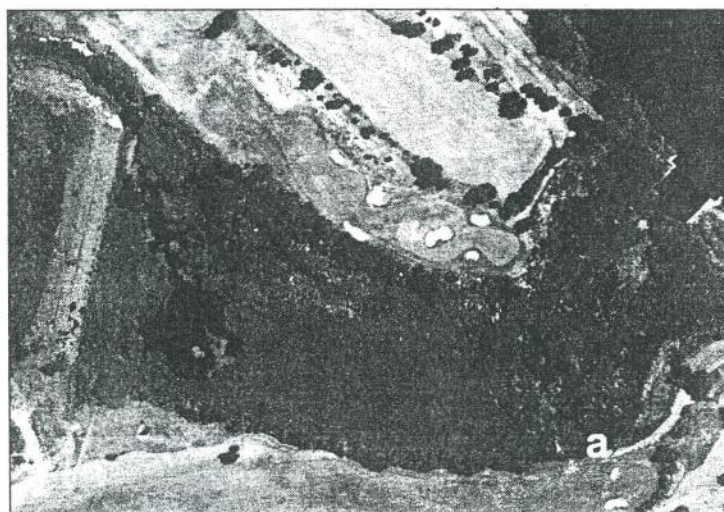


Figure 2. *Ludwigia peruviana* in Pond 3, Botany Wetlands (1991) (a) aerial photograph showing >70% of area was covered (b) 2-3 m tall dense shrubs.



Small *L. longifolia* infestations were first found in New South Wales, Australia, in areas between Sydney and Port Stephens near Newcastle (Gorham 2003). Newcastle is a port city approximately 150 km north of Sydney on Australia's eastern shoreline. Several large infestations were found in the Mambo Wetlands located in Salamander Bay on the southern foreshore of Port Stephens (Fig. 3) (McCall 2004).

In the early 1990s, a few plants of *L. longifolia* were found in the upstream sections (Pond 6) of Botany Wetlands. In 2004, a new infestation was found almost on the same location where the initial patches were found (Fig. 4).

### FROM IMMIGRANT TO INVADER

Mack et al. (2000) suggested that the transformation from an immigrant to an invader entails a long lag phase, followed by a phase of rapid exponential increase, which continues until the species reaches the limits of its new range, and its population growth slackens. The spread and establishment of *L. peruviana* in Sydney fits this model.

It is possible that *L. peruviana* infestations in Botany Wetlands, which existed for nearly 20 years, may have been the source of infestation of the weed for further spread in the Sydney basin. By late-1980s, infestations were found in Heathcote, about 40 km south of Sydney, and in Gosford, about 80 km north of Sydney (Jacobs et al. 1993).

The pattern of *L. peruviana* spread in the northwest region of Sydney, along several creek systems, indicates that a now defunct nursery may have been a second source (Peter Gorham, NSW Dept of Primary Industries, pers. comm.). Creeks in the northwest drain to the Hawkesbury River- a major river in the Sydney basin. Established populations are regularly encountered on its flood zones and banks.

The species is now widespread in the Sydney basin over a large area and is regarded as a common weed of aquatic habitats. Spread has occurred largely along stormwater drainage creeks, ditches, riverbanks and other wet habitats, where silt accretion occurs.

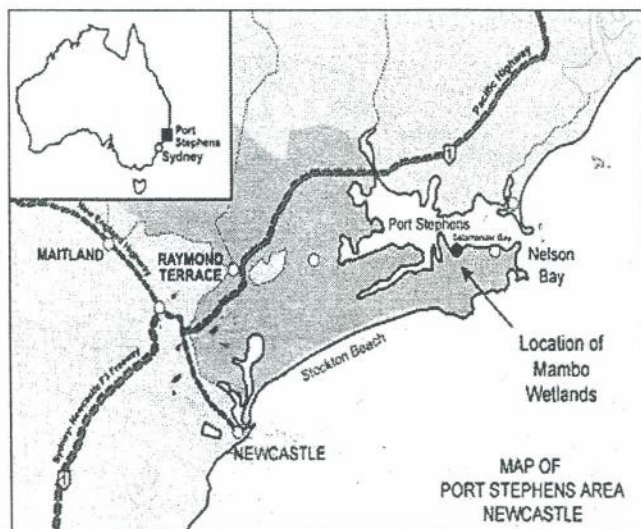


Figure 3. Location of the culturally significant Mambo Wetlands (175 ha), Salamander Bay, Port Stephens, where the largest infestations of *L. longifolia* were first found. Note closeness to the port city Newcastle, north of Sydney. Other infestations found thus far are small and sporadic in occurrence.

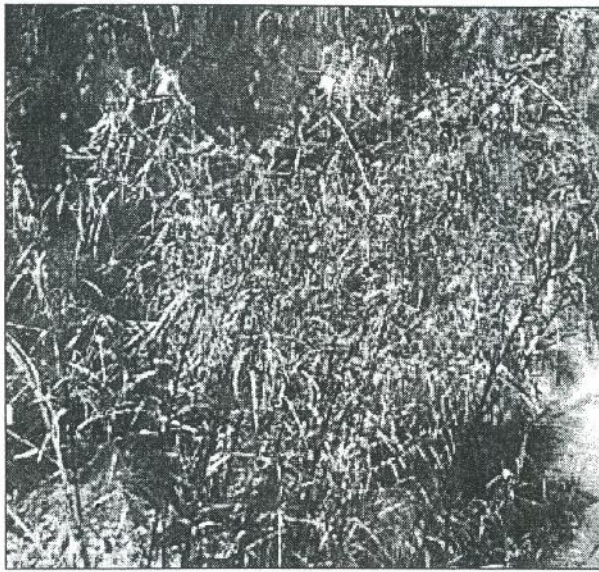


Figure 4. *Ludwigia longifolia* in Pond 6 Botany Wetlands (2004). Note reddish tinge in leaves and on young stems.

Ramamoorthy and Zardini (1987) noted the ability of *L. longifolia* also to form floating islands during its late succession stages.

All municipalities in the Sydney basin have now declared *L. peruviana* a noxious plant, to be controlled within their local government areas. The establishment of extremely successful populations in the Sydney basin, over a short space of 30 years, indicates the invasive potential of the immigrant turned 'invader' to Australia (Fig. 5).

The known extent of *L. longifolia* spread is currently limited to several wetlands and moist locations along the eastern shoreline of NSW between Sydney and Newcastle.

#### WHY *L. peruviana* AND *L. longifolia* ARE PROBLEMS?

The main potential deleterious effect of both species is their ability to supplant native species in wetlands and in riparian zones of waterways, which could result in permanent changes to flora, fauna and ecological diversity in such ecosystems. Dense stands of *L. peruviana* intercepted 93% of incident light (Jacobs et al. 1993), which led to dramatic losses of smaller native freshwater wetland plants and a reduction in bird populations.

In the interconnected Botany Ponds, dense *L. peruviana* stands choked water flow between ponds and increased sedimentation.

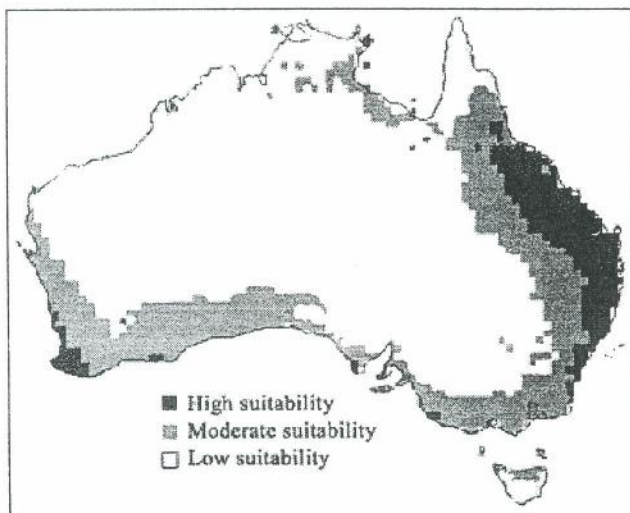


Figure 5. The CLIMATE computer-generated distribution map for *Ludwigia peruviana* (Source: Dept of Natural Resources, Queensland 2003).

Note the vast area of sub-tropical and tropical Australia potentially available to be exploited by the weed.



Reduced water flow increased the risk of flooding of adjacent properties. The addition of vast amounts of organic material over decades led to deoxygenation of ponds and wide ranging ecological damage. Recurrent toxic blue-green algal blooms were common in the ponds, which indicated nutrient enrichment and a breakdown of natural food webs. These adverse changes threatened cultural, social, aesthetic and economic values of the Botany Wetlands (Chandrasena and Sim 1998). Negative social impacts included reduced opportunity for recreational use by the public.

McCall (2004) noted that the near pristine state of the Mambo Wetlands was under threat by *L. longifolia* as it increased the risk of flooding and sedimentation of the Wetlands, and the reduced the Wetlands' recreational values.

It could also be argued that there are other environmental impacts, which could arise due to applying weed control technologies, such as herbicides or mechanical removal. Economic factors affected include costs of such controls, plus increased maintenance costs of stormwater drainage systems, to prevent re-establishment of the weeds.

## KNOWLEDGE OF BIOLOGY AND ECOLOGY

Understanding relevant aspects of the invader's biology and ecology is critical to effectively manage the invasions. Such understanding, particularly the invader's strengths and weaknesses, allows strategies to be developed targeting the weaknesses.

### Seed production

Seed production is the main reproductive strategy of both species ("r-strategists"). Mature *L. peruviana* stands in Botany Wetlands produced  $\approx 450,000$  seeds  $\text{m}^{-2}$  (Jacobs et al. 1994). In addition, there were  $\approx 65,000$  seeds  $\text{m}^{-2}$  in the soil seed bank and  $\approx 300,000$  seeds  $\text{m}^{-2}$  in old fruits, which remained on stems over winter.

Young plants flowered within two years. Within a year, there are two periods of flowering (spring and late summer) in Sydney. Seed viability was extremely high, in the range of 80-99% in the first year, declining significantly within 2 years (Jacobs et al. 1993). The small seeds germinate readily in mud throughout spring and summer.

There was some evidence of dormancy, possibly due to the hard-seed component of the seed bank, but this appears to break down after about one year (Jacobs et al. 1993).

The seeds of *L. peruviana* are hydrophobic, which make them germinate while afloat or underwater (Jacobs et al. 1993). But the seedlings eventually float to the surface for establishment along shorelines, and also allow *L. peruviana* to form floating islands.

McCall (2004) reported that a *L. longifolia* plant, one year old, produced about 5 capsules, equivalent to 35,000 seeds. A more mature plant can average 35 capsules  $\text{stem}^{-1}$  and with 6-10 stems  $\text{plant}^{-1}$ , this equates to  $\approx 2.45$  million seeds  $\text{plant}^{-1}$ . Annual seed production of heavily infested sites (10 plants  $\text{m}^{-2}$ ) can reach 25 Million seeds  $\text{m}^{-2}$ . The seed germination rate of *L. longifolia* over 45 days in this study was 94%. The young plants grew at a growth rate of 125 mm  $\text{month}^{-1}$ . The sediments had a pH range of 3.6-5.8 and very high levels of aluminium and ferrous.

Seed bank depletion would occur due to germination of those which germinate, and decay over time of those that are not germinating. Seeds survive by being buried in mud and shallow buried seeds are probably lost by intermittent exposure. Based on flushes of new seedlings, which appear on exposed mud at Botany Ponds, where previous stands occurred, it is evident that *L. peruviana* seeds have moderate long-term longevity.

The primary dispersal agents of seeds are water, wind and birds. Machinery, vehicles, footwear and clothes, which are contaminated with mud or seeds, also cause spread.



## Vegetative Reproduction

A major strength of both species is their ability for vegetative propagation, mainly by stem layering. Dislodged branches and stem pieces can take root after dispersal by flood or by machinery during removal and develop new plants. The capacity for vegetative reproduction greatly exacerbates the threat posed, particularly in environments where established small to medium infestations occur.

## CONTROL OPTIONS

From the trials conducted at Botany Wetlands (McCorkelle et al. 1995) and subsequent on-ground weed management works (Chandrasena et al. 2002), a significant amount of information is available on options to control *L. peruviana* infestations. Given that both species are well adapted to exploit the ecological niches left open in environments altered by man, the overall focus of integrating the weed management options should be to create conditions unfavourable to weed establishment and growth, while maintaining suitable conditions for other beneficial vegetation.

The integrated weed management (IWM) strategy implemented at Botany Wetlands combined the following: (a) water level management, (b) herbicides (Biactive® Glyphosate, 2,4-D Amine), (c) mechanical weed clearing, (d) controlled burning, (e) early detection and control of new infestations, and (f) large-scale revegetation. Over 6-7 years (1996-2002), the program reduced the once dominant infestations to negligible levels, with concomitant increases in native vegetation cover (Chandrasena et al 2002).

### Preventative Control

Preventative control is strategic weed management. However, to be successful, prevention has to apply to all levels of scale from the whole of Australia down to small areas, creek lines, roadside culverts, private properties, water bodies or paddocks.

To manage invaders such as the *Ludwigia* species, early detection and control of isolated plants is critical. Early interventions have to be applied aggressively to a wider area, region or catchment, and are by far the most effective means of controlling and arresting the invasions of the weed species.

### Herbicides

Experiences at Botany Wetlands and other areas in the Sydney basin indicate that both *L. peruviana* and *L. longifolia* can be relatively easily controlled by the non-selective glyphosate and highly selective 2,4-D amine.

However, treatment efficacy can be sub-optimal, because of poor access to infestations and difficulties in applying herbicides in locations such as wetlands. Therefore, repeat treatments, after some regrowth had occurred, are often required to control mature stands. Biodegradable adjuvants increase the efficacy of treatments (data not presented), and these would ensure that the overall amounts of chemicals needed are reduced.

Lower herbicide rates are often preferred in wetlands to reduce potential damage to native vegetation. In such situations, split applications with lower rates can be used. Control of seedling flushes can also be achieved with much reduced rates.

### Controlled Burning



*Ludwigia* stands, killed by herbicides, can be removed by burning. This method of weed-clearing has been successful in Botany Wetlands (Chandrasena et al. 2002).

#### Mechanical and manual clearing

Mechanical clearing of *L. peruviana* stands is possible, but this option is only suitable after killing the plants with herbicides. Machinery is also limited by access to sites and potential environmental impacts they may have on adjacent native vegetation.

Seedlings of both species are easily removed by hand pulling, but mature plants are difficult to remove because of extensive root systems embedded in the mud.

#### Biological Control

Biological control possibilities for the two *Ludwigia* species have not yet been explored. If they were found, the use of any natural enemies of the weeds (parasites, predators and pathogens) would be by far the cheapest method of long-term control.

#### Revegetation

Reclaiming *Ludwigia*-infested habitats will succeed only if competitive, perennial native macrophytes, including sedges, rushes and grasses, would displace the invaders. Hence, revegetation needs to be an integral component of fighting the invasions.

To ecologically complement wetland plant communities, preference should be for natural regeneration over active replanting. Once the original *L. peruviana* infestations were controlled in Botany Wetlands (Chandrasena et al. 2002), the growth of competitive macrophytes drastically reduced recolonisation by the weed.

Supplementing natural regeneration by purposeful planting of a range of wetland species may also be necessary, to maximise species diversity to achieve resilience to future disturbances, resist further weed invasion and enhance ecological values. Local seed sources or propagules should be used in revegetation to retain genetic resources.

### A CASE FOR AN ERADICATION STRATEGY

Eradication of a weed species requires control aimed at destruction of every propagule capable of growing up to a breeding individual, from an area or region, thus preventing re-establishment of a larger population. In contrast, containment is control, which targets prevention of spread, possibly with reductions of the size of populations.

Eradication of a widespread invader has rarely been achieved in any country. However, local eradications of weed infestations are commonly achieved. In general, successful eradication depends more on sustained weed control over a period of time, backed by diligent monitoring, than on the efficacy of any specific control method.

The decision to use eradication as a management strategy is a complex one. It involves assessing the following: (a) long-term impact of the weed species on native ecosystems; (b) the value placed by the public on those vulnerable ecosystems; (c) ease of achieving eradication; (d) costs and benefits of containment control; and possibly (e) the potential environmental disruptions caused by eradication treatments.

In the case of relatively recent invaders in the Sydney basin, like *L. peruviana* and *L. longifolia*, a 'weed-led' individualist eradication approach appears necessary, if these species are not to become

permanent major weed problems in fragile environments. The main rationale for eradication is that: (a) the existing knowledge on the biology and ecology is sufficient to formulate reasonable management strategies; (b) the species are still rather limited in distribution; (c) the area occupied by the infestations is small in most cases; (d) control methods are relatively well known; and (e) relatively quick and sustained action on small infestations has achieved local eradications.

An effective eradication plan requires legislative backing, which exists in New South Wales with *L. peruviana* classed as a W2 category noxious weed, under the *Noxious Weeds Act*. It also requires a commitment from those involved to stop the invaders.

Invasive species thrive in new habitats replacing other species, because the newcomer is better suited to exploit the environment. In most cases this occurs not because the newcomer is necessarily 'environmentally fit', but because the existing environment has undergone changes due to disturbances caused by humans or by natural causes. The examples of the *Ludwigia* species in Sydney represent the human-disturbance scenario.

Accumulation of nutrient-enriched sediments in urban drainage systems, traceable to human activities, created conditions conducive to the establishment and expansion of the *Ludwigia* species. Absence of natural enemies may have been the main cause, which allowed the new invaders to be successful in their new habitats. Their innate capacity to tolerate a wide range of ecological conditions, combined with 'r-strategist' life cycles (enormous reproductive potential involving seed production, rapid growth and vegetative reproduction) allowed them to maintain breeding populations and spread.

In my view, *L. peruviana* and *L. longifolia* represent unwarranted new weed invasions, which pose considerable threats to Australia's fragile natural ecosystems. The most significant and long-lasting adverse effect of the invaders is the alteration of the integrity of wetland ecosystems, through modification of biological inter-relationships.

As well documented in Botany Wetlands, and probably seen to some extent at Mambo Wetlands, the two *Ludwigia* species altered the floral composition of the wetlands, community structure and stability and along with the above, trophic relationships, natural cycling and productivity. The aggregate effect would be loss of bio- diversity and our inability to protect natural ecosystems and native vegetation communities.

There is a case for weed managers in Australia to adopt eradication as a strategy, rather than containment, to deal with both *Ludwigia* species in the Sydney basin, based on the previously discussed rationale. Where there are still small infestations, there should be quick action focused on eradication; where there are relatively large infestations, sustained action is needed, complemented by monitoring.

The tools required for a successful eradication campaign- integration of biological information into management and control options, are well established. The need is really for a change of mindset, early detection of new infestations, and a commitment of resources to cause local eradication through a systematic, co-ordinated approach.



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## Emergence and growth strategies of some common lowland rice (*Oryza sativa* L.) weeds to submergence

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**Abstract:** Screen house studies at the International Rice Research Institute were conducted to examine the influence of flooding times (0-7 days after seeding, DAS) and water depth (0-120 mm) on germination, growth and survival of common lowland weeds. Species consisted of the grasses *Echinochloa crus-galli* (L.) Beauv, *Ischaemum rugosum* Salisb., and *Leptochloa chinensis* (L.) Nees, sedges *Cyperus difformis* L. and *Cyperus iria* L., broadleaves *Sphenochlea zeylanica* Gaertn. and *Ludwigia octovalvis* Jacq. *S. zeylanica* and *C. iria* which germinated well under water. However, seedling number of *E. crus-galli*, *I. rugosum*, *L. chinensis*, *C. difformis*, and *L. octovalvis* decreased with early flooding. Growth of *I. rugosum*, *L. chinensis*, and *E. crus-galli* decreased with increasing water depth. In contrast, the biomass of *S. zeylanica*, *L. octovalvis*, and *C. difformis*, though depressed by flooding, was not affected by increasing water depth. Weed species employ different strategies for their survival under flooding. The root-shoot ratios of *C. difformis*, *L. octovalvis* and *S. zeylanica* were largely unaffected by flooding, while the ratio in the grasses decreased as plants invested biomass in stem growth for height extension. *C. difformis* can photosynthesize under water and produced similar biomass whether they are growing below or above the water surface. A different growth strategy was shown by *S. zeylanica*, which partitioned a high proportion of biomass to roots. The results demonstrate that weeds have different abilities and strategies to survive flooding stress and exploiting these differences could lead to improved weed management practices.

**Key words:** Growth strategy, root and shoot biomass, root-shoot ratio, submergence

### INTRODUCTION

Flooding the soil in rice (*Oryza sativa* L.) fields reduces weed emergence and suppresses weed growth by degrees depending upon water depth, the weed species, and timing and duration of flooding. (Civico and Moody 1979; Mukhopadhyay 1983; Diop and Moody 1984; Baghat et al. 1996, Kent and Johnson 2002). Many weeds cannot germinate in submerged soil and this serves as an effective means of weed control (Moody 1978). For example, the germination of *Ludwigia hyssopifolia*, *E. crus-galli* and *C. iria* was drastically reduced by flooding while *Monochoria vaginalis* was virtually unaffected (Prusty et al. 1999; Pons 1982). Other studies have also shown that submergence reduced weed density and dry matter production (Subbulakshmi et al. 2002). In submerged soil, the available oxygen near the upper layer of soil where most weed seeds are found may be insufficient for germination. Further, prolonged submergence of terrestrial plants may result in their impaired growth and death. Plants, however, can adjust to flooded environments. Some plants can adapt to flooding by adventitious root formation, aerenchyma development and shoot elongation (Voesenek and Van Der Veen 1994). The following studies were conducted to determine the emergence, growth strategies, and survival of some common lowland rice weeds in submerged condition.



## MATERIALS AND METHODS

*General procedures.* Fifty seeds of the test weed species were uniformly sown on plastic trays (95 mm x 95 mm x 55 mm) filled with fine sterilized upland soil and thinly covered. The soil was moistened with fine mist spray. The trays were then transferred onto a "step ladder" placed inside the growth cubicle for the flooding treatment. We used the "step-ladder" design to achieve desired water depths depending on the position of the trays. A growth cubicle is a wooden tray (136 cm long, 100 cm wide, 33 cm tall) lined with polyethylene sheets positioned over a concrete slab. A water system with inflow/outflow valves provided a continuous supply of fresh water for each growth cubicle.

*Initial studies.* The weed species used were *C. iria*, *S. zeylanica*, and *L. octovalvis*. The treatments were time of flooding (0, 1 and 2 DAS) and water regimes (aerobic, 0 mm or saturated, 10, 20, 30, 40, 50, 60, 70, 80, 100, and 120 mm water depths). Data presented are means of six samples per water regime.

*Extended flooding times studies.* The weed species used were *C. difformis*, *L. chinensis*, *E. crus-galli*, and *I. rugosum*. Flooding times were 1, 3, 5, and 7 DAS while the water regimes were 0, 10, 20, 30, 40, 50, and 80 mm water depths. A split-plot design with two replications was used with time of flooding as the main plots and water regime as the sub-plots. ANOVA was used to analyze all collected data. Only root and shoot biomass at 3 and 7 DAS flooding times are presented.

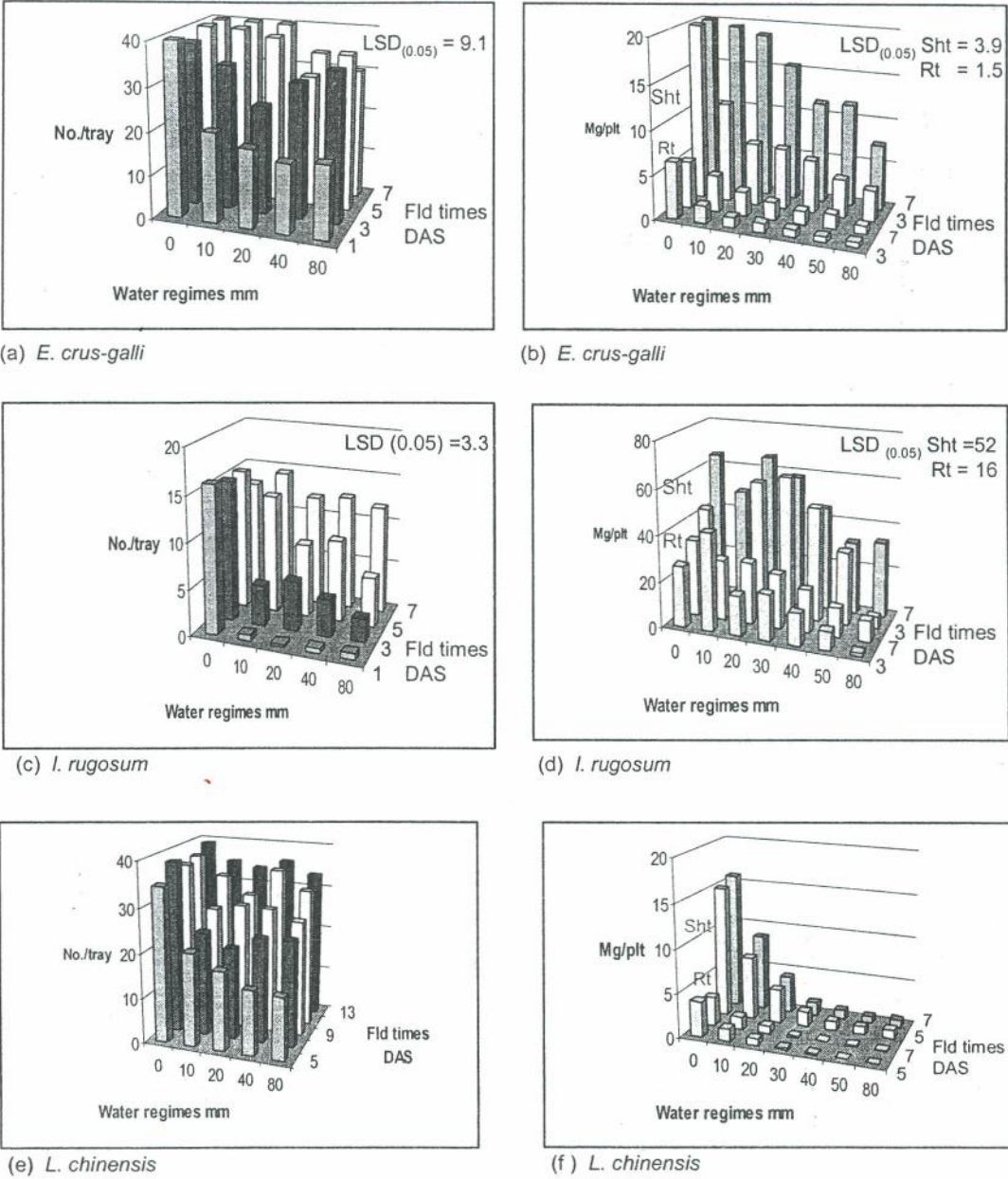
*Data gathered.* Plant count from each tray was recorded starting at first emergence. At harvest (14 DAS for *E. crus-galli*, 21 DAS for *I. rugosum*, *L. chinensis*, *C. difformis* and *C. iria*, and 40 DAS for *S. zeylanica*), plant number, shoot height and root length were measured. To determine the biomass, the plants were separated into roots and shoots, dried at 70 °C for three days, and weighed separately in an analytical balance (Mettler-Toledo AG204). The number of plants that emerged through the water surface was also recorded.

## RESULTS

### Emergence and growth

*Grasses.* Flooding times and depth had significant ( $P < 0.05$ ) effects on plant numbers and biomass of *E. crus-galli* and *I. rugosum*. *E. crus-galli* flooded 1 DAS (Fig. 1a) and *I. rugosum* flooded 1 and 3 DAS (Fig. 1c) had least plant number at all depths compared to 0 mm water level. Flooding at 7 DAS had no effect on plant number. Seedling biomass decreased with increased flooding depth and earlier flooding. Root and shoot biomass of *E. crus-galli* (Fig. 1b) and *I. rugosum* (Fig. 1d) decreased with water depths when flooded at 3 or 7 DAS. *L. chinensis* plant numbers were depressed by flooding at 5, 7, and 9 DAS (Fig. 1e) and growth was greatly reduced at all depths greater than 10 mm (Fig. 1f) regardless of flooding time.

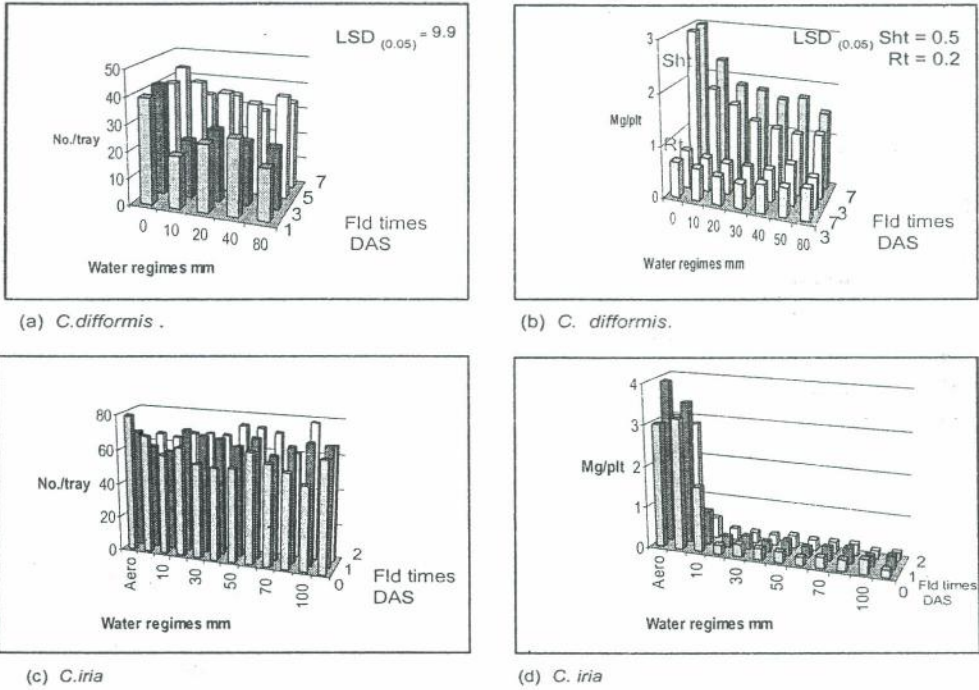
Figure 1. Plant number (a, c and d) and biomass (b, d and f) at harvest. Sht = shoot; Rt = root; Fld times = flooding times.



*Sedges.* Flooding times and water depths had significant ( $P<0.05$ ) effects on *C. difformis* with reduced plant numbers with early flooding (Fig. 2a). Flooded at 3 and 7 DAS, shoot biomass of *C. difformis* decreased from 0 mm to 10 mm water depths but greater depths had no greater effect, and there was no effect on roots (Fig. 2b). *C. iria* germinated well regardless of water regimes (Fig. 2c). Compared to aerobic and 0 mm, flooding had substantial impact on *C. iria* total biomass but there was no increased effect beyond 20 mm (Fig. 2d).

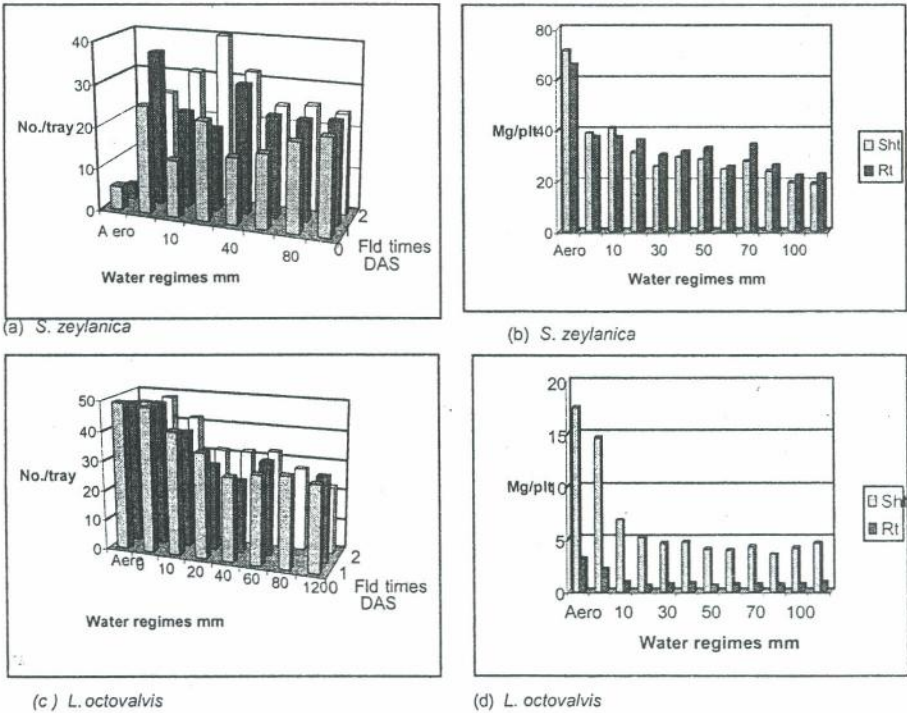


Figure 2. Plant number (a and c) and biomass (b and d) at harvest. Sht = shoot; Rt = root, Fld times = flooding times.



*Broadleaves.* *S. zeylanica* had higher rates of germination under flooding than when aerobic (Fig. 3a) but growth was greatest under aerobic conditions (Fig. 3b). There was no decrease in root and shoot biomass with increasing water depth. *S. zeylanica* was the only weed species with similar root and shoot biomass values and with roots longer than shoots. There was a slight decrease in plant numbers of *L. octovalvis* beyond 10 mm water depth (Fig. 3c). *L. octovalvis* attained greater root and shoot biomass under aerobic and saturated conditions but there was no further effect beyond 10 mm water depth (Fig. 3d).

Figure 3. Plant number (a and c) and biomass (b and d) emergence at harvest. Sht = shoot; Rt = root; Fld times = flooding times.



## Root-shoot ratio

The root-shoot ratio of *E. crus-galli*, *I. rugosum* and *L. chinensis*, in general, decreased while that of *C. difformis*, *S. zeylanica* and *L. octovalvis* tended to be either stable or increase with water depths when flooded 3 or 7 DAS (Table 1).

Table 1. Effect of submergence and water depths on root-shoot ratio of some weeds.

Root-shoot ratio – flooded 3 DAS						
Water depths (mm)	<i>E. crus-galli</i>	<i>I. rugosum</i>	<i>L. chinensis</i>	<i>C. difformis</i>	<i>S. zeylanica</i>	<i>L. octovalvis</i>
0	0.27	0.61	0.27	0.22	1.06	0.11
10	0.18	0.34	0.19	0.27	1.03	0.11
20	0.17	0.30	0.20	0.34	1.01	0.11
30	0.15	0.32	0.12	0.35	1.05	0.11
40	0.14	0.28	0.12	0.39	1.10	0.11
50	0.15	0.25	0.09	0.45	1.15	0.12
80	0.16	0.24	0.07	0.41	1.31	0.14
Root-shoot ratio – flooded 7 DAS						
0	0.23	0.52	0.19	0.23	0.96	0.15
10	0.21	0.53	0.21	0.34	0.92	0.13
20	0.15	0.41	0.18	0.34	1.16	0.11
30	0.13	0.40	0.19	0.36	1.18	0.13
40	0.13	0.41	0.16	0.44	1.09	0.16
50	0.14	0.40	0.16	0.47	1.16	0.15
80	0.12	0.27	0.14	0.50	1.10	0.18
LSD (0.05)	0.06	0.34	0.04	0.06	0.17	0.02

## DISCUSSION

The results show differences in the response of different weed species to submergence and the influence of time of flooding and water depth. *S. zeylanica* and *C. iria* germinated well under flooded conditions while others were suppressed by early flooding. The growth of *I. rugosum* and *E. crus-galli* was reduced by flooding, with earlier and deeper flooding having the greatest effect. In contrast, increasing depth beyond 10 mm appeared to have little additional effect on the growth of *S. zeylanica* and *C. difformis*.

Earlier studies have shown that *C. difformis* (McIntyre 1985) and some broadleaves like *Monochoria vaginalis* and *Ludwigia hyssopifolia* (Sathiasagar et al. 1992) are more tolerant of submerged environment than others. Further, whether *C. difformis* was submerged or growing above water surface a similar biomass was obtained demonstrating that it can utilize the low light intensity to photosynthesize under submergence as a mechanism for survival. Namuco et al. (2003) reported that *C. difformis* continued photosynthesis while submerged though stem extension was delayed whereas *E. crus-galli* relied on conversion of seed reserves and grew toward the water surface. A different growth strategy was shown in this study by *S. zeylanica* which partitioned a high proportion of biomass to roots. *C. difformis* showed an increasing root-shoot ratio with flooding depth. In contrast, other species in this study partitioned most biomass to the stem. In particular, the three grasses showed a marked drop in root-shoot ratio in response to flooding. This characteristic in *E. crus-galli* was also reported by Namuco et al. (2003).



The results clearly demonstrate varied tolerance and response of weed species to flooding timing and depth. Information on such differences could provide the basis for improved weed management systems that utilize differential tolerance to flooding. The control of many of the species tested in this study could be greatly improved by early flooding to a depth of 10 mm. Even where the weed seeds germinate the plants may fail to develop with early flooding as in the case of *C. iria*, *E. crus-galli* and *L. chinensis*. In this example, however, full exploitation of differences in flood tolerance between rice and weeds would also require a varied water management regime so as to avoid a shift towards flood tolerant aquatic species.

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**Abstract:** Watergrasses (*Echinochloa phyllopogon* and *E. oryzoides*) are typically the most serious weeds in California rice fields. Our research over the past decade has been directed towards assessing if watergrass impacts on rice could be reduced by manipulating interspecific competition. A new rice model has predicted that rice grain yield should be proportional to the number of fruiting rice panicles, which in turn responds to total tiller density, the duration and timing of coexistence, and to a lesser extent, the competitive differential between watergrass and rice. Experiments have confirmed that grain production is stable across a range of rice densities because of compensatory survival of grain-bearing tillers. Some of these experiments also show that grain loss and panicle size reduction do occur under extreme circumstances, but do not seem to be a major component of yield loss. The agreement between model predictions and experimental data suggests that control of watergrass by enhanced competition by rice is unlikely to provide satisfactory weed control without large and unlikely changes in competitiveness, not only in California, but worldwide.

**Key words:** Watergrass competition, compensatory tillering, rice grain yield, tiller density

## INTRODUCTION

California rice farmers are under increasing pressure to reduce their dependence on herbicides for weed control. Rapidly increasing herbicide resistance is one reason (Fischer *et al.* 2000). The cost and limits on new alternative herbicides represents a substantial cost to the farmer in an uncertain market. Pressure is increasing to reduce water use and to limit chemical residues, frequently represented as potential regulatory mandates or as part of a campaign to increase agricultural sustainability. As a result, there is growing interest in maximizing competitive suppression of weeds. Farmers, however, have limited options to manipulate competition. A traditional alternative by routine crop rotation (Liebman and Dyck 1993) is difficult in California because of the heavy, poorly-drained soils. Increasing planting densities is not generally effective because competitive suppression by rice on watergrass is weak. Nevertheless, this is an area of active research at the present time. But how substantial is the promise of effective weed control by manipulated competition?

## MATERIALS AND METHODS

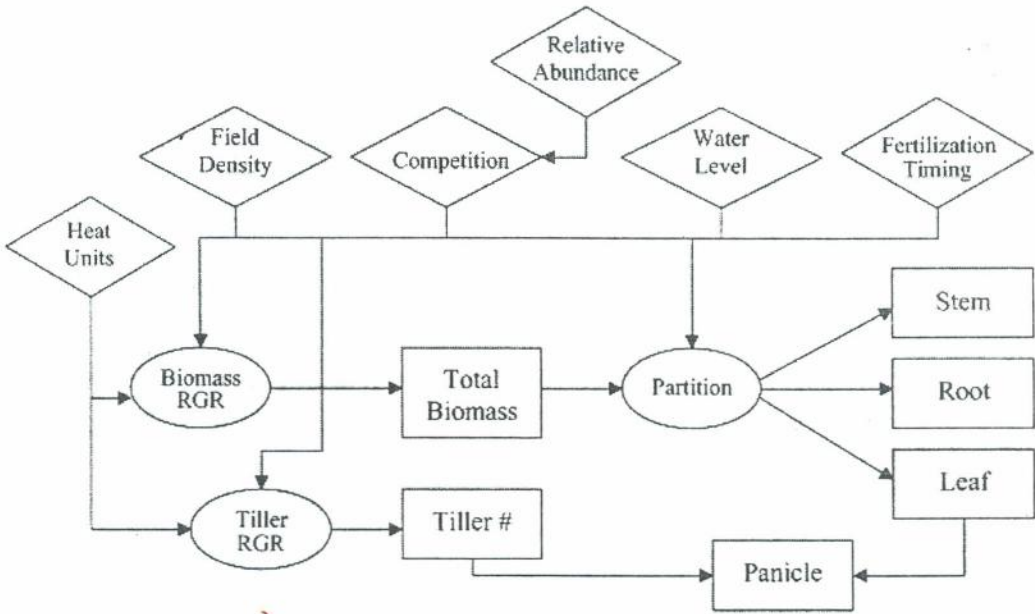
The VAMP model explicitly represents competition in flooded systems of rice culture. VAMP evaluates the effect of stand density, phenological development, and competitive interactions. The results can then be scaled up to match field densities. A flowchart of the VAMP model is shown in Figure 1.

A series of experiments have been conducted to both support and validate VAMP. All pot experiments have been set up outdoors in large flooded basins in 25 cm pots. Pregerminated seed were sown into rice soil (Stockton Clay Adobe: fine, montmorillonite, thermic, Typic Pelloxerts.). In one experimental series, we manipulated initial density ratios. In a second series, we removed the weeds at set times in the experiment, and in an experiment currently in progress, we also reduced rice density in control pots at the same time weeds were removed. Other experiments were done



manipulating contact between shoots, roots, or both (Cahill 1999). Still other experiments used flood depth and fertilizer timing as experimental variables. All of these experiments were designed to support the development of VAMP and ultimately to test it.

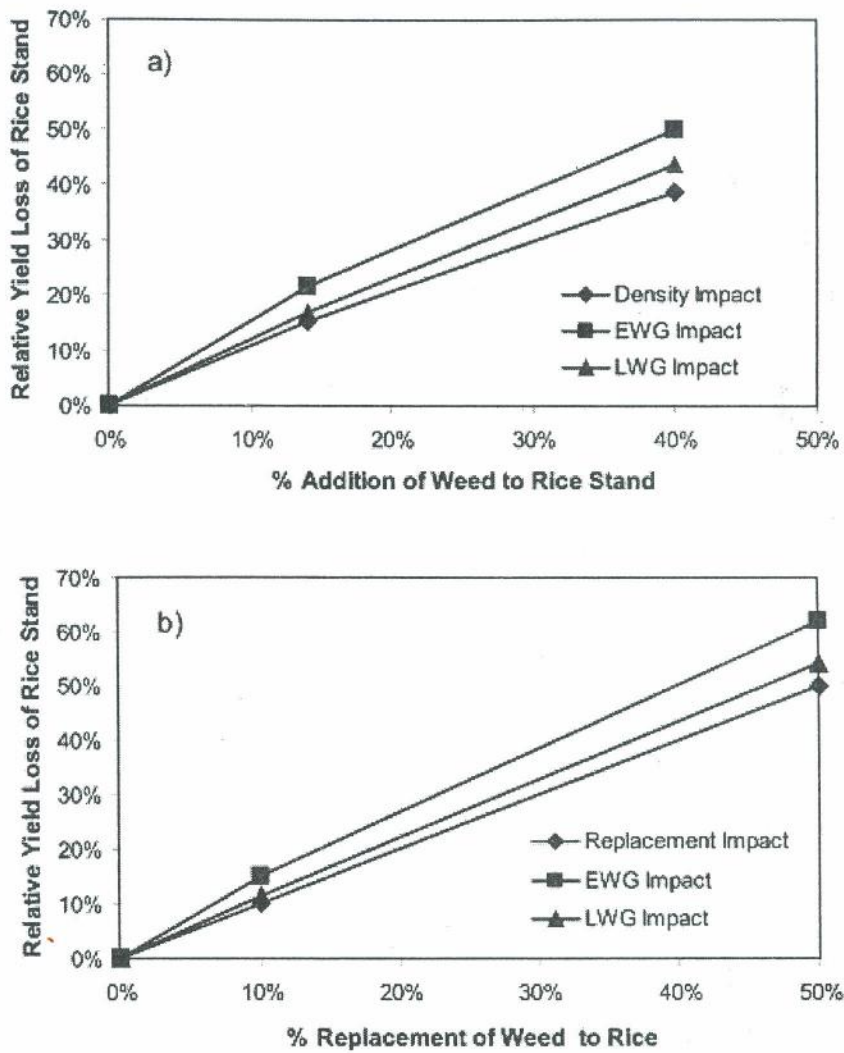
A number of field experiments have also been conducted. The fertilizer timing and timed weed removal treatments have been conducted in field trials on local farms. These were conducted with small (2x2 m) plots replicated across fields. In addition, experiments with dry-down were conducted on an organic farm to assess the relative effects on rice and weed control.



**Figure 1.** Schematic flowchart of VAMP, showing steps in the calculation of stand development.

**RESULTS AND DISCUSSION**

*Predictions generated by VAMP.* Multiple simulation experiments with VAMP have repeatedly shown a small set of characteristic patterns of behavior. The most important pattern is that final grain yield is determined predominately by the number of rice panicles in the final stand rather than by variation in head size, seed number per panicle, or individual seed weight. It should be noted that this was an assumption of model structure whose independent validity remains to be demonstrated. In turn, panicle number is predominately determined by total tiller density, counting both rice and watergrass (Fig. 2 a, b). This prediction is important because it implies that interspecific competition is expressed more through relative tiller density than through competitive effects of one individual on another.



**Figure 2.** VAMP predictions of the effect of density on relative yield loss compared to direct competitive impact of one individual on another. EWG=early watergrass; LWG=late watergrass. Panel a represents an additive experiment and Panel b represents a replacement series.

Simulation of water depth effects and fertilization delay was also done using VAMP. Water depth has been hypothesized to slow the growth of watergrass more than rice, reducing the competitiveness of watergrass (Williams *et al.* 1996). Fertilization delay is expected to operate in a similar fashion, by reducing the growth advantage of watergrass relative to rice (Cahill 1999, Liebman and Davis 2000). Simulation results show that water depth is not a useful way to manage competition, as the effects on rice at least equal the effects on watergrass (Fig. 3). Fertilization delay is somewhat more effective, but is dependent on weed proportion and the specific competitive interaction. Early watergrass (*E. oryzoides*) is little affected by fertilization delay, maintaining its competitive advantage over rice, but least so when total density is high. Late watergrass (*E. phyllopogon*) is more affected by a range of densities, even becoming the less competitive species it is more abundant. On balance, fertilization delay is only effective as a stand management tool when watergrass infestation of the rice stand is so high that subsequent yields of rice are already too low to be acceptable.



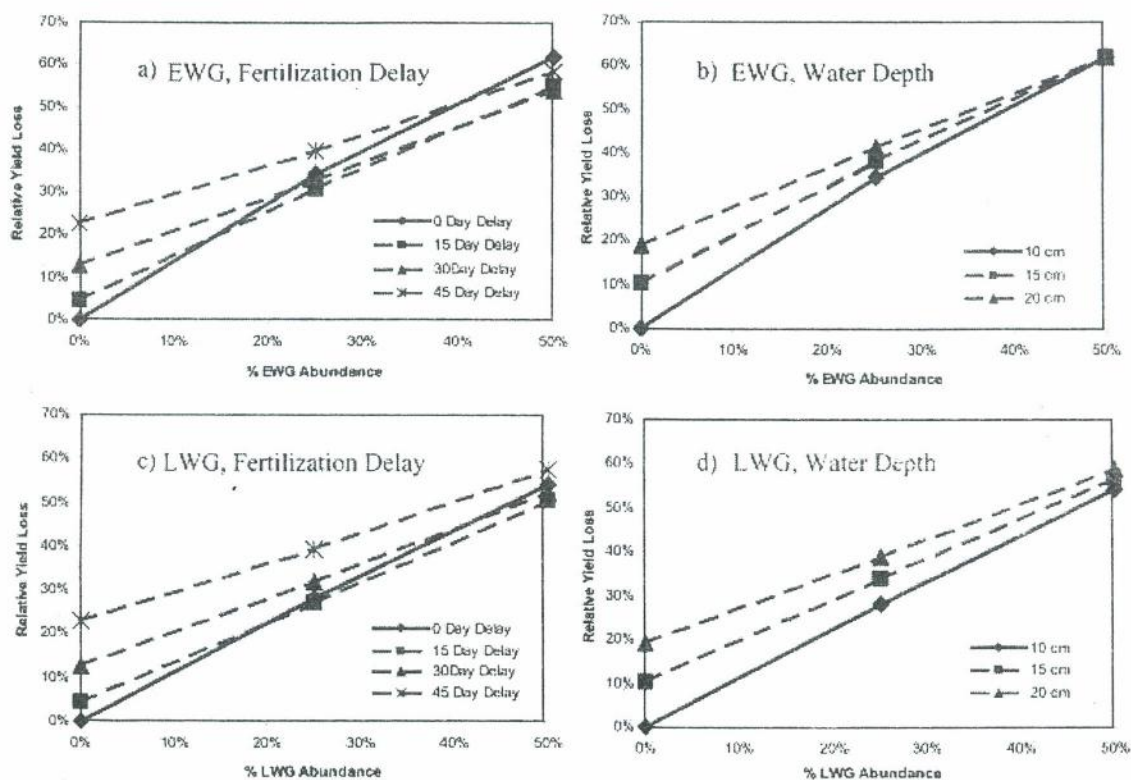


Figure 3. Response of relative rice yield loss to competition from EWG or LWG under water or fertilization management.

*Validation experiments.* Several experiments yield results showing that the tight linear relationship between yield and panicle number is realistic over a wide range of realistic field densities and mixture ratios (Fig. 4). When panicle counts are high, there is some evidence of reduction in seed size as the relationship shows some curvilinearity. When panicle counts are very low the curvilinearity is even less evident. In either case, this effect is small relative to the effect of panicle number. Field experiments demonstrate that this effect is field-realistic, although there are unexplained effects in weed-free fields and further complexities arising from drydown (see Table 1).

#### Grain mass and rice panicle number

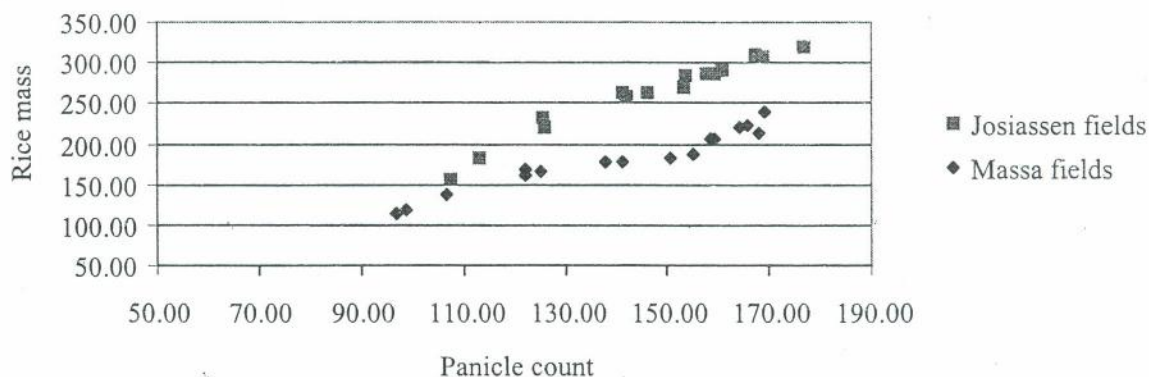


Figure 4. Rice grain mass as a function of panicle numbers in an organic field (Massa fields) and a conventional one (Josiassen fields).

Table 1. Tukey tests of significant differences in the analysis of variance of rice grain weight and panicle numbers under different weed: rice fractions and the presence or absence of a dry-down imposed during stand development. Drydown only occurred in the Massa fields. Different letters denote significant differences at the 0.05 level.

Response Variable	Grain mass	Panicle number
<i>Massa fields</i>		
	no weeds <sup>a</sup>	none <sup>a</sup>
	low weeds (1-5%) <sup>a</sup>	low <sup>a</sup>
	moderate (6-10%) <sup>b</sup>	moderate <sup>b</sup>
	high (>10%) <sup>c</sup>	high <sup>b</sup>
<i>Josiassen fields</i>		
	no weeds <sup>ab</sup>	none <sup>ab</sup>
	low weeds (1-2%) <sup>a</sup>	low <sup>a</sup>
	moderate (5-10%) <sup>b</sup>	moderate <sup>b</sup>
	high (>20%) <sup>c</sup>	high <sup>c</sup>

Recent experiments manipulating the timing of weed removal demonstrates the impact of duration of contact between species (Figure 5). The earlier the watergrass is removed from the rice stand, the less the final reduction in panicle number and the lower the loss in grain yield. Conversely, rice yield is less sensitive to late removal of weeds, as grain loss becomes asymptotic to 50% late in stand life.

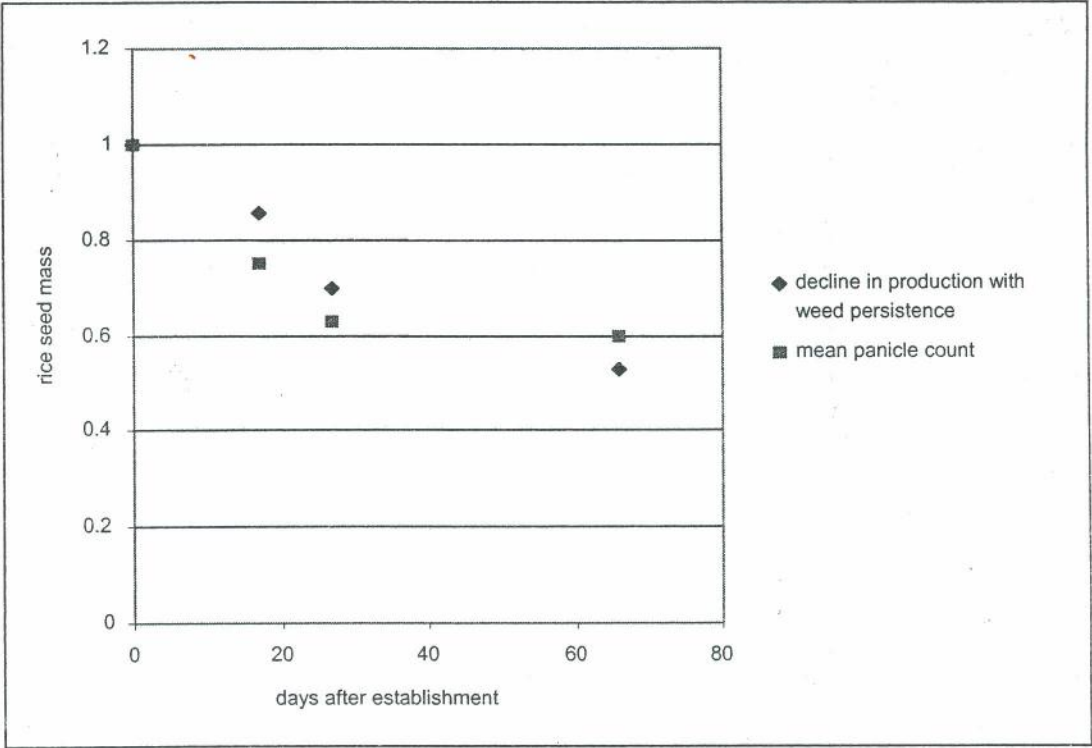


Figure 5. Decline in rice grain mass with timing of weed removal, normalized to grain mass in weed-free stands. Note the close association with panicle number.



*Implications.* The correspondence between model predictions and experimental data is not perfect, but thus far is encouraging. If further evidence validating these predictions is obtained, we could then concentrate on the dynamics of tiller formation, survival, and maturation as the most important key to weed management.

Regarding the larger question of the potential role of enhanced competition in weed control in rice, the picture is less encouraging. *Echinochloa* spp. are well-known as rice weeds because they grow so well over a range of rice cultural conditions. Our results to date have not revealed a feasible path towards some combination of enhanced rice competitiveness and/or reduced watergrass competitiveness, despite some earlier expectations (Gibson *et al.* 2003). Although this cannot be a firm conclusion because we have not explored all alternatives, it at least suggests a less optimistic future for competitive management of weeds. In turn, this emphasizes that herbicides may play an important, but much more selective role, in future weed management as a component in integrated weed management.

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**Abstract:** One hundred sixty seven species of exotic weeds belonging to 28 families were identified and recorded through a re-survey of over 25 provinces after referring to related literatures up to 2003. Among those originating from all five continents in the world, the exotic weeds introduced from America accounted for 57.48%. Sixty eight percent of the exotic weeds were introduced as useful plants for ornamental, medicinal, forage, vegetable, environmental utilization, while the others were introduced accidentally accompanying transportation, imports of agricultural products or invaded China by natural spread. Analysis of introduction time indicated that invasion of the exotic weeds had been occurring since ancient times, and invasion incident augmented from ancient time comprised 18.56%, reaching 56.29% to the present as the international exchanges increase. The most dangerous invasion weeds of China were *Alternanthera philoxeroides*, *Eupatorium adenophorum*, *Ambrosia artemisiifolia*, *Eichhornia crassipes*, *Eupatorium odoratum*, *Conyza canadensis*, *Erigeron annuus*, *Conyza bonariensis*, *Spartina alterniflora*, and *Ageratum conyzoides*; and potentially dangerous ones *Sorghum halepense*, *Mikania micrinata*, *Solidago canadensis*, *Lolium temulentum*, and *Ambrosia trifida*. The distribution of the exotic weeds in China was governed by the integration of the inherent ecological adaptability of the weeds, their spread capability and the human spread activities.

**Key words:** Exotic weed, invasion, China, origin region, introduction way, introduction time.

## INTRODUCTION

The numbers of the exotic weeds of China have been documented since 1998. Fifty nine species, 108 species and 134 species of exotic weeds were recorded and accounted respectively by Ding and Wang (1998), Qiang and Cao (2000) and Li and Xie (2002). Obviously former reports on the list of exotic weeds in China were not completed. Thus, a re-survey of all over 25 provinces on species and occurrences of exotic weeds was conducted in northeastern, eastern, northern, southern and southwestern China during 2001-2002. The paper aims to describe and analyze the species, source and invasion of the exotic weeds of China.

Exotic weed is a plant that originated from a foreign country or exotic region, whose population can naturally persist its population through reproduction and growth wildly under man-made habitats. An exotic weed is different from exotic crops in that the latter needs artificial assistance to reproduce or grow. According to this definition, 167 species of exotic plants belonging to 28 families were identified and recorded as exotic weeds in China till 2002 (Cheng and Cao 2002; Cheng et al., 2001; Ding, 2000; Ding et al. 2003; Li 1998; Li et al. 2002; Liu et al. 2002; Peng 2000; Ng and Corlett 2002; Wang and Li 2002). The list of the exotic weeds is showed in Table 1 (Qiang and Cao 2000; <http://weed.njau.edu.cn/exowort/exoweeds.htm>). The families with the most species were *Compositae* (46), *Leguminosae* (27), *Gramineae* (26), *Amaranthaceae* (12) and *Onagraceae* (11). The other 23 families had less than 10 species.



Table 1. A list of exotic weeds in China.

ID	NAME	INV	D	ORIGIN	ID	NAME	INV	D	ORIGIN
<i>Amaranthaceae</i>					<i>Gramineae</i>				
1	<i>Alternanthera philoxeroides</i>	N	4	SA	84	<i>Aegilops squarrosa</i>	G	2	WAS
2	<i>Alternanthera pungens</i>	G	1	SA	85	<i>Axonopus compressus</i>	G	2	TA
3	<i>Amaranthus albus</i>	G	1	NA	86	<i>Brachiaria eruciformis</i>	G	1	SAS
4	<i>Amaranthus blitoides</i>	G	1	NA	87	<i>Brachiaria mutica</i>	G	3	AF,SAS
5	<i>Amaranthus caudatus</i>	C	2	SA	88	<i>Bromus unioloides</i>	G	1	SA
6	<i>Amaranthus paniculatus</i>	C	1	TA	89	<i>Buchloe dactyloides</i>	G	2	NA
7	<i>Amaranthus polygonoides</i>	C	3	EU, WAS	90	<i>Ehrharta erecta</i>	G	1	AF
8	<i>Amaranthus retroflexus</i>	rN	3	TA	91	<i>Hordeum jubatum</i>	G	2	NA
9	<i>Amaranthus spinosus</i>	C	3	TA	92	<i>Lolium multiflorum</i>	G	2	EU
10	<i>Amaranthus tricolor</i>	C	2	IN	93	<i>Lolium perenne</i>	C	3	EU
11	<i>Amaranthus viridis</i>	C	3	TA	94	<i>Lolium persicum</i>	G	1	EU
12	<i>Gomphrena celosioides</i>	G	2	TA	95	<i>Lolium temulentum</i>	C	2	EU
<i>Boraginaceae</i>					96	<i>Lolium temulentum</i> var . <i>arvense</i>	G	2	EU
13	<i>Heliotropium europaeum</i>	G	2	EU	97	<i>Lolium temulentum</i> var. <i>longiaristatum</i>	rN	3	EU
<i>Cabombaceae</i>					98	<i>Paspalum dilatatum</i>	G	3	SA
14	<i>Cabomba caroliniana</i>	G	2	NA	99	<i>Paspalum fimbriatum</i>	G	1	SA
<i>Cactaceae</i>					100	<i>Pennisetum setosum</i>	G	1	AF
15	<i>Opuntia dillenii</i>	G	2	NA	101	<i>Phalaris minor</i>	G	2	SAS
16	<i>Opuntia vulgaris</i>	C	3	SA	102	<i>Phalaris paradoxa</i>	G	1	SAS
<i>(Opuntia monacantha)</i>					103	<i>Phleum pratense</i>	G	2	EU,WAS
<i>Cannabinaceae</i>					104	<i>Poa compressa</i>	G	1	EU, WAS
17	<i>Cannabis sativa</i>	C	2	AS	105	<i>Setaria palmifolia</i>	G	2	SAS
<i>Caryophyllaceae</i>					106	<i>Sorghum halepense</i>	rN	2	ME
18	<i>Agrostemma githago</i>	G	1	EU	107	<i>Sorghum sudanenses</i>	G	2	ME
19	<i>Corrigiola littoralis</i>	G	1	NA	108	<i>Spartina alterniflora</i>	rN	3	NA
20	<i>Stellaria apetala</i>	C	3	EU	109	<i>Spartina anglica</i>	G	2	EU
21	<i>Vaccaria segetalis</i>	C	3	EU	<i>Leguminosae</i>				
<i>Chenopodiaceae</i>					110	<i>Cassia hirsuta</i>	G	1	SA
22	<i>Chenopodium ambrosioides</i>	C	3	TA	111	<i>Cassia mimosoides</i>	G	2	SA
<i>Compositae</i>					112	<i>Cassia occidentalis</i>	G	2	SA
23	<i>Acanthospermum</i> <i>anthemifolia</i>	G	3	SA	113	<i>Cassia tora</i>	G	4	SA
24	<i>Ageratum conyzoides</i>	rN	4	TA	114	<i>Crotalaria incana</i>	G	1	SA
25	<i>Ageratum houstonianum</i>	G	2	TA	115	<i>Crotalaria juncea</i>	G	1	IN
26	<i>Ambrosia artemisiifolia</i>	rN	4	NA	116	<i>Crotalaria lanceolata</i>	G	1	SA,AF
27	<i>Ambrosia trifida</i>	rN	3	NA	117	<i>Crotalaria micans</i>	G	1	SA
28	<i>Anthemis arvensis</i>	G	2	EU	118	<i>Crotalaria ochroleuca</i>	G	1	AF
29	<i>Aster subulatus</i>	C	2	NA	119	<i>Crotalaria zanzibarica</i>	G	1	SA,AF
30	<i>Bidens frondosa</i>	G	1	NA	120	<i>Desmodium tortuosum</i>	C	1	TA
31	<i>Chrysanthemum carinatum</i>	G	1	ME	121	<i>Medicago hispida</i>	C	3	IN
32	<i>Chrysanthemum coronarium</i>	G	1	ME	122	<i>Medicago sativa</i>	C	2	WAS
33	<i>Cichorium intybus</i>	G	1	EU	123	<i>Melilotus albus</i>	C	3	EU,WAS
34	<i>Conyza bonariensis</i>	rN	4	SA					

35	<i>Conyza Canadensis</i>	rN	4	NA	124	<i>Melilotus indicus</i>	C	1	IN
36	<i>Coreopsis grandiflora</i>	G	1	NA	125	<i>Mimosa invisa</i> var. <i>invisa</i>	G	1	SA
37	<i>Coreopsis lanceolata</i>	G	2	NA	126	<i>Mimosa pudica</i>	G	3	SA
38	<i>Coreopsis tinctoria</i>	G	1	NA	127	<i>Mimosa sepriaria</i>	G	1	SA
39	<i>Cosmos bipinnatus</i>	G	2	TA	128	<i>Neptunia plena</i>	G	1	SA
40	<i>Cosmos sulphureus</i>	G	2	TA	129	<i>Sesbania cannabina</i>	C	1	SAS,AF
41	<i>Erigeron annuus</i>	rN	4	NA	130	<i>Trifolium fragiferum</i>	C	2	EU,WAS
42	<i>Erigeron philadelphicus</i>	C	2	NA	131	<i>Trifolium hybridum</i>	G	2	EU
43	<i>Eupatorium adenophorum.</i>	rN	4	TA	132	<i>Trifolium incarnatum</i>	C	2	EU
44	<i>Eupatorium odoratum</i> ( <i>Chromolaena odorata</i> )	rN	4	TA	133	<i>Trifolium pratense</i>	G	3	EU
45	<i>Galinsoga ciliata</i>	C	4	TA	134	<i>Trifolium repens</i>	C	3	EU
46	<i>Galinsoga parviflora</i>	C	4	TA	135	<i>Ulex europaeus</i>	G	2	EU
47	<i>Halianthus tuberosus</i>	G	2	NA	136	<i>Vicia sativa</i>	rN	4	EU,WAS
48	<i>Helenium autumnale</i>	G	1	NA	<i>Malvaceae</i>				
49	<i>Leucanthemum vulgare</i>	G	1	EU	137	<i>Abutilon crispum</i>	G	1	TA
50	<i>Mikania micrantha</i>	rN	3	TA	138	<i>Hibiscus trionum</i>	C	2	EU,AF
51	<i>Parthenium hysterophorus</i>	G	1	SA	139	<i>Malvastrum</i> <i>coromandelianum</i>	G	1	TA
52	<i>Polymnia uvedalia</i>	G	1	NA	<i>Nyctaginaceae</i>				
53	<i>Pseudelephantopus spicatus</i>	G	1	SA,AF	140	<i>Mirabilis jalapa</i>	G	1	SA
54	<i>Pyrethrum parthenifolium</i>	G	1	WAS	<i>Onagraceae</i>				
55	<i>Senecio vulgaris</i>	G	2	EU	141	<i>Clarkia pulchella</i>	C	3	NA
56	<i>Silybum marianum</i>	G	1	EU,WAS	142	<i>Oenothera biennis</i>	C	3	NA
57	<i>Solidago canadensis</i>	rN	1	NA	143	<i>Oenothera drummondii</i>	C	1	NA
58	<i>Soliva anthemifolia</i>	G	3	AU	144	<i>Oenothera glazioviana</i> ( <i>O. erythrosepala</i> )	C	3	NA
59	<i>Sonchus asper</i>	C	3	EU	145	<i>Oenothera laciniata</i>	C	1	NA
60	<i>Sonchus oleraceus</i>	C	3	EU	146	<i>Oenothera oakesiana</i>	C	1	NA
61	<i>Synedrella nodiflora</i>	G	2	SA	147	<i>Oenothera parviflora</i>	C	1	NA
62	<i>Tagetes erecta.</i>	G	2	TA	148	<i>Oenothera rosae</i>	C	1	NA
63	<i>Tagetes patula</i>	G	2	TA	149	<i>Oenothera stricta</i> ( <i>O. odorata</i> )	C	1	SA
64	<i>Tithonia diversifolia</i>	G	3	TA	150	<i>Oenothera tetraptera</i>	C	1	TA
65	<i>Tridax procumbens</i>	G	1	TA	151	<i>Oenothera villosa</i>	C	1	NA
66	<i>Wedelia trilobata</i>	C	2	TA	<i>Oxalidaceae</i>				
67	<i>Xanthium spinosum</i>	G	2	TA	152	<i>Oxalis corymbosa</i>	C	3	TA
68	<i>Zinnia peruviana</i>	G	1	SA	<i>Phytolaccaceae</i>				
<i>Convolvulaceae</i>					153	<i>Phytolacca americana</i>	G	1	NA
69	<i>Ipomoea cairica</i>	G	2	SA	<i>Plantaginaceae</i>				
70	<i>Ipomoea triloba</i>	C	2	SA	154	<i>Plantago lanceolata</i>	G	2	EU
71	<i>Pharbitis nil</i>	C	2	SA	155	<i>Plantago virginica</i>	G	1	NA
72	<i>Pharbitis purpurea</i>	C	2	SA	<i>Pontederiaceae</i>				
<i>Cruciferae</i>					156	<i>Eichhornia crassipes</i>	rN	4	SA
73	<i>Brassica kaber</i>	G	1	EU, WAS	<i>Portulacaceae</i>				
74	<i>Chorispora tenella</i>	C	3	EU,WAS	157	<i>Talinum paniculatum</i>	G	1	SA
75	<i>Coronopus didymus</i>	C	2	EU	<i>Ranunculaceae</i>				
76	<i>Lepidium campestre</i>	G	1	EU,WAS	158	<i>Ranunculus arvensis</i>	G	1	EU,WAS
77	<i>Lepidium densiflorum</i>	G	1	NA	<i>Resedaceae</i>				



78	<i>Lepidium virginicum</i>	C	2	NA	159	<i>Reseda lutea</i>	G	1	EU, WAS, AF
<i>Curcubitaceae</i>					<i>Scrophulariaceae</i>				
79	<i>Coccinia grandis</i> ( <i>C. cordifolia</i> )	C	3	NA	160	<i>Scoparia dulcis</i>	G	2	TA
80	<i>Cucumis bisexualis</i>	G	2	AF	161	<i>Veronica arvensis</i>	C	2	EU
81	<i>Cucumis melo</i> var. <i>agrestis</i>	G	2	AF	162	<i>Veronica hederifolia</i>	G	1	EU
<i>Euphorbiaceae</i>					163	<i>Veronica persica</i>	rN	4	EU, WAS
83	<i>Ricinus communis</i>	C	2	AF	<i>Solanaceae</i>				
					164	<i>Physalis pubescens</i>	G	1	TA
Note: SA: South America; TA: Tropical America; NA: North America; WAS: Western Asia; IN: India and South Asia; SAS: South East Asia; AF: Africa; EU: Europe; ME: Mediterranean; AU: Australia					<i>Umbelliferae</i>				
					165	<i>Apium leptophyllum</i>	G	2	EU
					166	<i>Coriandrum sativum</i>	G	1	ME
					<i>Verbenaceae</i>				
					167	<i>Lantana camara</i>	G	3	SA

NOTE: INV: 1) Invasive capacity; D: dominance; N: most invasive to main part of China; rN: most invasive to limited regions. C: common but little invasive; G: limited

## INTRODUCTION TIME

Analysis of introduction time indicated that invasion of the exotic weeds had been occurring since ancient times, and 18.56% of invasion incidents augmented from ancient time, 25.15% modern time, and 56.29% at present as the international exchanges increase.

## INTRODUCTION PATHWAYS

### (1) Intentional introduction

Of the 167 species, 67.67% were intentionally introduced as useful plants. According to utilization of plants, introduction pathways can be classified into the following.

a) Forages: 35 species were introduced as forage, e.g., *Alternanthera philoxeroides*, *Trifolium* spp., *Malvastrum coromandelianum*, *Phleum pratense*, *Axonopus compressus*, *Bromus unioloides*, *Aegilops squarrosa*, *Paspalum dilatatum*, *Paspalum fimbriatum*, *Pennisetum setosum*, *Setaria palmifolia*, *Sorghum sudanenses*, *Lolium persicum*, *Lolium temulentum* var. *arvense*, *Eichhornia crassipes*, *Crotalaria* sp., *Desmodium tortuosum*, *Medicago hispida*, *Medicago sativa*, *Melilotus albus*, *Melilotus indicus*, *Sesbania cannabina*, *Trifolium* sp., *Vicia sativa*.

b) Ornamentals: 44 species were introduced as ornamentals, e.g., *Gomphrena celosioides*, *Ageratum houstonianum*, *Coreopsis lanceolata*, *Coreopsis tinctoria*, *Coreopsis grandiflora*, *Cosmos bipinnata*, *Cosmos sulphurens*, *Helenium autumnale*, *Leucanthemum vulgare*, *Tagetes erecta*, *Tagetes patula*, *Solidago canadensis*, *Zinnia peruviana*, *Pharbitis nil*, *Pharbitis purpurea*, *Lantana camara*, *Mirabilis jalapa*, *Mimosa*, *Oenothera* sp. and *Oxalis corymbosa*.

c) Fiber plant: *Cannabis sativa*, *Crotalaria juncea*, *Sesbania cannabina*.

d) Herb medicine: *Cassia* sp., *Talinum paniculatum*, *Phytolacca americana*, and *Datura metel*.

e) Vegetables: *Amaranthus* sp., *Chrysanthemum coronarium*, *Coriandrum sativum*, *Cichorium intybus*, and *Helianthus tuberosus*.

f) Turfgrass: *Axonopus compressus*, *Brachiaria mutica*, *Poa compressa* and *Lolium multiflorum*.

g) Environmental plants: *Spartina alterniflora* , *Spartina anglica*, and *Brachiaria eruciformis*.

The data above show that the introduction of alien plants as forages or ornamentals had the highest proportion of weediness incidents. Thus, an introduction may change into exotic weeds. Humans normally prefer alien plants that are easily grown as forages and ornamentals, which readily escape into wild habitats as exotic weeds.

(2) Unintentional introduction

a) Hitch-hikers

Unintentional introduction of exotic weeds occurs as hitch-hikers on clothing and vehicles, in containers, packaging materials, shipping waste, and ship's ballast, and as contaminants in shipments of agricultural seeds and other biological materials during transition of travelers and transported goods. *Sorghum halepense* was unintentionally introduced with imported soybean and corn in the 1980s because its seeds have often been found in farm products since then.

b) Natural spread

Exotic weeds invaded China through natural spread by wind, bird, and water. Crofton weed naturally spread into southern Yunnan through wind from Myanmar in the 1940s. So did *Eupatorium odoratum* through similar ways almost at the same time.

ORIGIN REGIONS

The exotic weeds originated from all five continents (<http://www.hear.org/gcw/index.html>). Exotic weeds from the American continent, accounted for 59.88%, while the least came from the Pacific continent. Thus, exotic plants from the American continent may have greater potential risks to become exotic weeds in China.

Table 2. Analysis of origin regions of exotic weeds in China.

Origin Areas	Abbreviation	Species	Percentage
American Continent (Total)		100	59.88
South America	SA	34	20.36
Tropical America	TA	30	17.96
North America	NA	36	21.56
Asia (Total)		27	16.17
Western Asia	WAS	17	10.18
India and South Asia	IN	4	2.4
South East Asia	SAS	6	3.59
Africa	AF	13	7.78
Europe and Mediterranean		46	27.55
	EU	41	24.55
	ME	5	2.99
Australia	AU	1	0.6



## 1) Harmfulness

Exotic weeds have invaded arable land, road transportation networks, forests and wetlands, thereby causing great economic and ecological losses in agriculture, forestry, biodiversity, ecosystem, transportation, and the landscape.

Some of the exotic weeds have become the most important arable and weeds. For example, *Alternanthera philoxeroides* invaded paddy, maize, cotton, soybean fields, orchards and lawns, amounting to 20 million ha. (Wang and Wang 1988; Wu and Qiang 2003). *Vicia sativa*, *Veronica persica*, *Vaccaria segetalis*, *Lolium temulentum*, and *Chorispora tenella* are main weeds of summer crop fields in Yangtze River valley and northward regions. *Conyza canadensis*, *Erigeron annuus*, *Conyza bonariensis*, *Ageratum conyzoides* and *Amaranthus retroflexus* are main weeds of the autumn crop fields in Yellow River valley and southward regions (Qiang 2002).

Water hyacinth (*Eichhornia crassipes*) has become one of the main aquatic plants in many ponds, lakes and rivers of tropic and sub-tropic regions in China. The rapid spread of this weed has resulted in great economic losses to fisheries, transportation and tourism, as well as declines in native aquatic plants and threats to local biodiversity (Ding et al. 1995).

Ragweed (*Ambrosia artemisiifolia*) has invaded main cities and neighboring areas and is spreading along highways and railways throughout China. *Ambrosia trifida* is distributed only in Tieling, Shenyang, Shijiazhuang and Beijing, but may extend through most of China (Wan et al. 1993).

*Erigeron annuus* and horseweed (*Conyza canadensis*) mainly occurs in disturbed areas such as suburban and rural waste land, roadsides, open hillsides throughout China, and has invaded orchards and other arable lands.

South American climber (*Mikania micrantha*) is overspreading in Hainan and south of Guangdong where it dominates large areas. It climbs trees, blocks sunshine and then kills the trees and other substratum plants so that it has almost destroyed the original biodiversity of invaded habitats (Zhuang 1999).

Crofton weed (*Eupatorium adenophorum*) is now widespread and covers an area of 350,000 km<sup>2</sup> in southwest China. It has invaded forests, hills and mountains and grasslands and decreased biodiversity dramatically. It is now spreading northward and eastward. Similar incidents have happens with *E. odoratum* (Qiang 1998).

*Sorghum halepense* has invaded five limited regions. However, it may become aggressive throughout most of China.

Tall goldenrod (*Solidago canadensis*) is particularly aggressive in disturbed areas, such as suburban wastelands, roadsides, banks, and in residential and industrial areas; and is spreading from such areas into surrounding orchards, arable lands, and vegetable fields in eastern China.

## 2). Most invasive exotic weeds in China

Based on invasive capacity, distribution range and harmfulness, the most dangerous invasion weeds of China are *Alternanthera philoxeroides*, *Eupatorium adenophorum*, *Ambrosia artemisiifolia*, *Eichhornia crassipes*, *Eupatorium odoratum*, *Conyza canadensis*, *Erigeron annuus*, *Conyza bonariensis*, *Spartina alterniflora*, *Ageratum conyzoides*, *Amaranthus retroflexus*, *Vicia sativa*, *Veronica persica* and potentially dangerous *Sorghum halepense*, *Mikania micrantha*, *Solidago Canadensis*, *Lolium temulentum*, and *Ambrosia trifida*.

## 3) Distribution of exotic weeds



The distribution of exotic weeds in China was governed by the integration of their inherent ecological adaptability, their spread capability and human spread activities (Guo 1995; Qiang and Cao 2001). Seventy eight exotic weeds originated from tropical regions of America, Africa and Asia, mostly distributed in tropical or subtropical areas southward to the Yangtze River. Only 18 exceptions extended to temperate areas of northern China. The over extension of *Alternanthera philoxeroides*, *Eichhornia crassipes*, *Pharbitis nil*, *Pharbitis purpurea* and *Oxalis corymbosa* resulted from the frequent introduction to those regions as forages or ornamentals while *Conyza bonariensis*, *Amaranthus retroflexus*, *Amaranthus viridis*, *Chenopodium ambrosioides*, and *Galinsoga parviflora* had strong spread capacity or became successful hitch-hikers. Thirty one species from subtropical areas mainly occurred in subtropical regions, too, of which 15 spread to temperate regions. Most of the 49 species that originated from temperate regions are vernal plants, which need low temperature to be vernalized. Thus, these are mainly distributed in the temperate regions northward to the Yangtze River.

Regulations for evaluating the potential risks of alien plants should be established and practiced for the introduction of alien plants for economic purposes. The related introduction should be restricted unless it meets the regulations. Effective control should focus mainly on the most invasive or potentially invasive weeds, which are still distributed in limited areas.

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# **Tuber sprouting and production of daughter plants and rhizomes under submerged conditions in yellow nutsedge (*Cyperus esculentus* L.)**

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**Abstract:** Yellow nutsedge (*Cyperus esculentus* L.) has infested paddy fields in south-western Japan since the 1990s. Sprout of tuber in water, regrowth from sprouted tubers, production of daughter plants and rhizomes were investigated under submerged conditions. More than 70% of tubers sprouted in the water with 5-7 mg L<sup>-1</sup> of dissolved oxygen (DO), while less than 25% of them could sprout under 1-2 mg L<sup>-1</sup> of DO. Sprouted tubers, whose shoots were removed at different size, were buried at different depth in puddled soil in 4 L pot under submergence on the end of April and May, 1993, with/without eleven days' drainage at one month after planted. Re-sprouting was observed during drainage period from tuber with drainage, while re-sprouting from tubers without drainage was observed after final drainage on the end of August, for tubers buried on the end of April. As for the tuber buried on the end of May, tubers buried at depth of 1-3cm could re-sprout during drainage period from tubers with drainage and after final drainage on the end of September from them without drainage, respectively. Re-sprouted plants produced tubers according to their sizes. A plant planted in a 4 L pot at the end of April produced 55 daughter plants from 6.5 m of rhizomes and 281 rhizomes producing tubers in 2.5 months after planting under submerged condition.

**Key words:** Daughter plant, rhizome, submerged condition, tuber sprouting, yellow nutsedge

## **INTRODUCTION**

Yellow nutsedge (*Cyperus esculentus* L.), one of the world's worst weeds (Holm et al. 1977; Mulligan and Junkins 1976), was found in a corn field in Tochigi Prefecture (Konnai et al. 1990), and has infested paddy fields in south-western Japan since 1990s (Morita and Nakayama 1992; Shibuya and Morita 2005). Clarifying its growth habit under irrigated paddy condition is necessary to establish control procedures to yellow nutsedge. Sprout of tuber in water, regrowth from sprouted tubers, production of daughter plants and rhizomes were investigated under submerged conditions.

## **MATERIALS AND METHODS**

Yellow nutsedge was collected in a paddy field at Hondo, Kumamoto Prefecture and propagated at the National Agricultural Research Center for Kyushu-Okinawa Region in 1992. Tubers were kept at 5°C after harvest.

Ratio of sprouting tuber was determined with tubers before sprouting and tubers whose shoot with 8 cm in length was removed after sprouting, in 500 ml of tap water adjusted to different level of dissolved oxygen (DO). DO was adjusted by using boiled water and aeration, and measured once a day during the experiment.

In order to clarify the re-growth of sprouted tubers buried into puddled soil under submerged condition, seedlings with tuber were buried into light heavy clay soil after being fertilized with 0.16 g of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O and puddle in 4 L pot under different conditions as below:



Planting date; a: 29<sup>th</sup> April, b: 28<sup>th</sup> May  
Degree of shoot removal; a: intact, b: upper half, c: all  
Burial depth; a: 1cm, b: 3cm, c: 5cm  
Inter drainage; a: drained for ten days, b: no drainage

Seedlings with shoot of 8.0 cm and 11.1 cm in length were used for planting on 29<sup>th</sup> April and 28<sup>th</sup> May, respectively. Re-sprouting of buried tuber was determined at 5 days after burial, end of 11 days' drainage at 35 days after planting as midseason drainage and at 5 days after final drainage at around 120 days after planting, respectively. Three tubers were used for each treatment.

A seedling of 11.1 cm in height was transplanted to puddled light heavy clay soil with 0.16 g of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O in 4 L pot on 29<sup>th</sup> April. Production of daughter plants and rhizomes under submerged condition was measured at 78 days after planning. Three pots were used for the experiment.

## RESULTS AND DISCUSSION

### Sprouting ratio under different dissolved oxygen (DO)

In the water with 5.5 mg to 7 mg L<sup>-1</sup> of dissolved oxygen (DO), 70% to 100% of tubers sprouted, while 0% to 25% of them could sprout below 2 mg L<sup>-1</sup> of DO for the unsprouting tubers (Table 1). Re-sprouting of tuber whose shoot was removed was observed in the water with 3.4 mg to 7 mg L<sup>-1</sup> of DO and the elongation of shoot was encouraged by higher DO (Table 2).

Table 1. Percentage of sprouted tubers in the water with different dissolved oxygen contents (DO) in *Cyperus esculentus*.

DO(mg L <sup>-1</sup> ) <sup>1)</sup>	Sprouted tubers at days after placement <sup>2)</sup>						
	1	2	3	4	5	6	7
1.0±0.7	0	5	5	5	5	5	5
2.2±0.9	0	15	25	25	25	25	25
2.9±0.7	0	10	10	20	20	40	40
5.5±0.3	20	40	100				
5.8±0.3	0	70	70	70	70	70	70
6.9±0.2	45	75	80	80	80	80	80

1) Average and SD measured at 9:00 AM through the experiment.

2) Percentage to 10 to 20 tubers used

Table 2. Number of re-sprout and shoot length of tubers in which shoot was removed in water with different dissolved oxygen contents (DO) in *Cyperus esculentus*.

DO (mg L <sup>-1</sup> ) <sup>1)</sup>	No. of re-sprouted tuber/ 10 tubers	Length of shoot (mm±SD)
2.8±0.7	0	0
3.4±0.3	3	3.7±1.4
5.4±0.3	1	1.8
6.7±0.3	4	13.4±9.8

1) Average and SD measured at 9:00 AM, for 7 days

2) 10 tubers were used

Re-sprouting of tubers buried into puddled soil after sprouted

Time of re-sprouting of individual tuber is shown in Figure 1. In the tubers buried at the end of April, re-sprouting was observed during the drainage period from tubers with drainage, while that from tubers without the drainage was observed after the final drainage on the end of August. For tubers buried at the end of May, those buried at the 1-3 cm deep could re-sprout during the drainage period when the drainage was practiced. When the drainage was not practiced, re-sprouting was observed after the final drainage at the end of September.

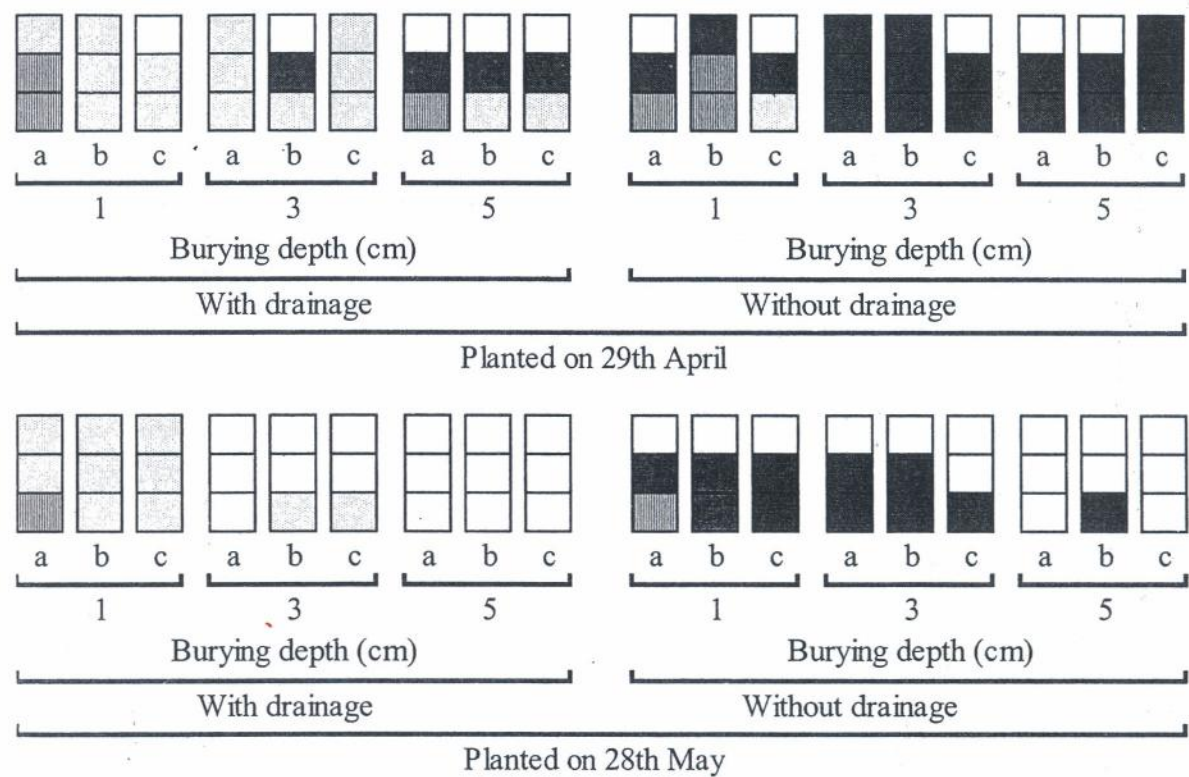


Fig. 1. Re-sprout of individual tuber under submerged condition buried into different depth of puddled soil at the end of April or May, with or without drainage in *Cyperus esculentus*.

- 1) intact (a) , upper half of shoot (b) and all shoot (c) was removed.
- 2) Time of re-sprout from individual buried tuber, as follows;

planting	drainage		final drainage
29/Apr.	04/June	14/June	25/Aug.
28/May	03/July	13/July	27/Sept.

□: extinction

Growth and formation of rhizome that could produce tuber in the plants re-sprouted during the drainage period were measured at the final drainage as shown in Table 3. These plants produced 40 to 50 daughter plants and 300 rhizomes approximately in both planting date, though percentage of rhizome with mature tuber was higher in re-sprouted plants buried on 28<sup>th</sup> May than those in April.



Table 3. Growth and rhizome formation under submerged condition in re-sprouted plant during drainage period in *Cyperus esculentus*.

Burial date and depth	No. of plant	No. of daughter plant	Top dry weight (g)	No. of rhizome for tuber	Details of tuber maturing (%)		
					mature	immature	unformed
29 April 1cm	6	44.0±16.6	17.7±9.3	279±170	23	26	52
29 April 3cm	6	49.5±28.7	22.9±9.3	311±113	20	22	58
28 May 1cm	8	39.3±14.6	12.6±5.3	297±124	44	32	24

1) Details are given in Figure 1.

2) Average and SD at the final drainage

On the plants planted on the end of April which re-sprouted after the final drainage, plant height was 3.6 cm to 20.4 cm at three weeks after the final drainage with 12 to 75 tubers when the aerial part was killed by frost. An exponential relationship,  $y=6.8149e^{0.1024x}$  was calculated between the plant height at three weeks after the final drainage (x cm) and the number of tuber as shown in Figure 2.

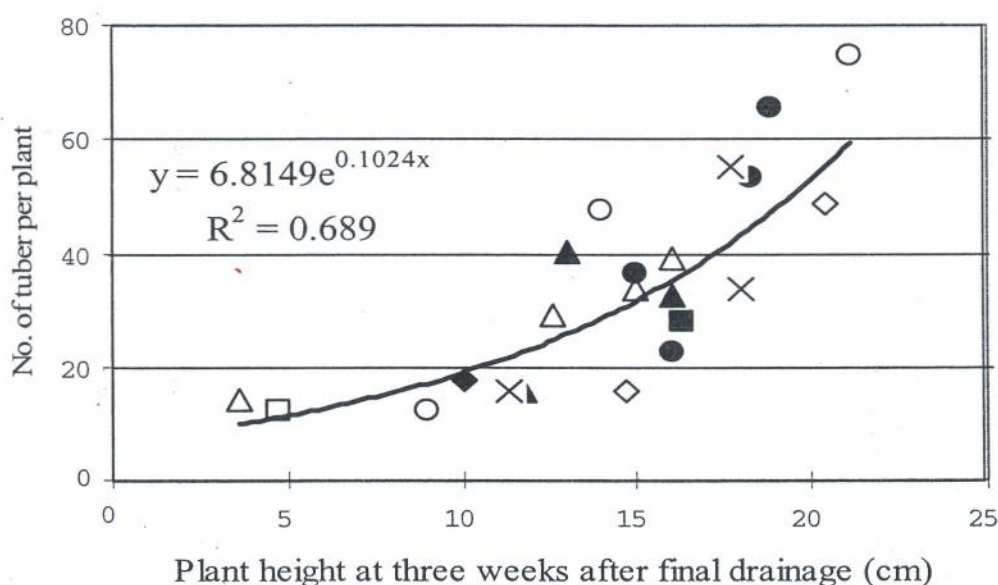


Fig. 2. Relationship between plant height at three weeks after final drainage on 25th August and number of tuber produced after aerial part was died in the plant re-sprouted after the final drainage in *Cyperus esculentus*.

Note: Tubers were buried on 29th April as below;

Buried depth (cm)	Degree of shoot removal		
	intact	half	all
1	□	■	◆
3	○	●	◇
5	△	▲	×

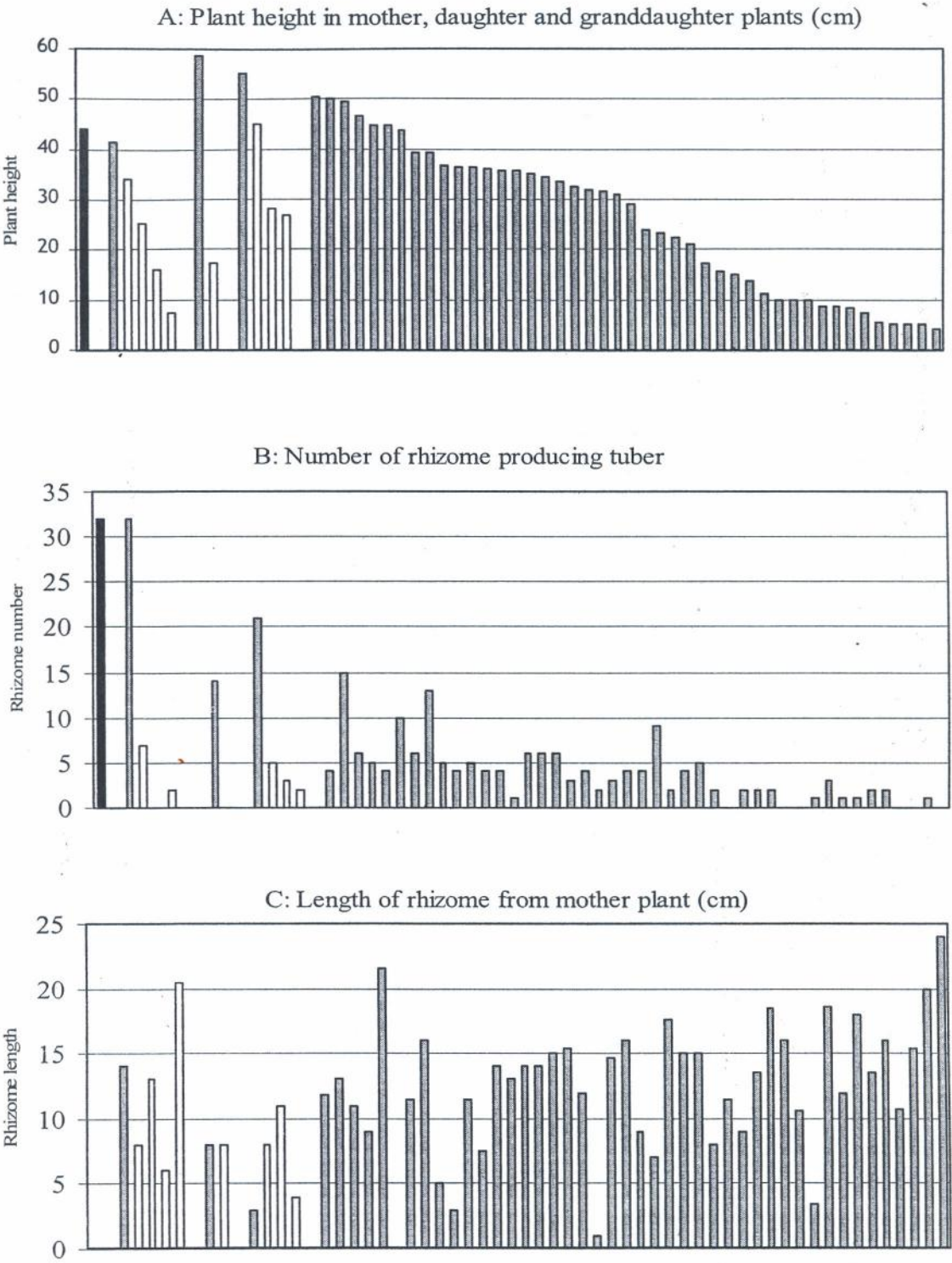


Fig. 3. Growth of *Cyperus esculentus* under submerged condition, planted in four litter pot on 29th, April, at 78 days after planted.

1) Bar shows plants as follows;  
■ : mother    ■ : daughter    □ : granddaughter



The result of analysis for one pot is shown in Figure 3, because the difference was not observed among three yellow nutsedge plants in the experiment. One plant of yellow nutsedge produced 47 daughter plants with 8 granddaughter plants, which were connected with 6.5 m of rhizomes in total and 281 rhizomes which could produce tubers at 2.5 months after planting under submerged condition.

Re-sprouting potential in yellow nutsedge has been reported under upland condition (Thullen and Keeley 1975). However, oxygen condition is important factor affecting sprouting and re-sprouting of tuber in yellow nutsedge growing in paddy fields where puddling and submergence were practiced for rice cultivation. That tuber sprouting was inhibited under about 2 mg of DO would contribute to clarify the performance of tuber in the paddy fields. Further study on relationship between tuber sprouting and oxygen condition in puddled soil is necessary.

Most tubers buried in puddled soil re-sprouted during the drainage period when drainage was practiced and after the final drainage when soil was submerged throughout the cropping season. Midseason drainage which is an important management practice in rice cropping, should be conducted avoiding excessive drainage because sprouting of buried tuber was observed when soil moisture ratio decreased to around 80% from submergence (Morita and Nakayama 1992).

Number of daughter plants and rhizomes in this study did not reach the result obtained under upland condition which should that one tuber produced 1900 plants and 6900 tubers to a depth of 23cm in a patch 21dm in diameter (Tumbleeson and Kommedahl 1961). Results in this study indicate that puddling and submergence suppress the growth of yellow nutsedge in paddy fields. Therefore, mechanism of above suppression should be investigated under the competition with rice plants to prevent further infestation of yellow nutsedge in paddy fields.

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**Abstract:** A study on the weed seed bank in rice fields at O Mon district, Can Tho province, Mekong Delta of Vietnam was carried out by the nursery testing method during 2001-2002 at Cuulong Delta Rice Research Institute. Research results show that the number of weed seeds along the river bank (zone I) is more than the far distant areas from the river (zone III). There were six species of grasses, two sedges and 16 broadleaved weeds observed. The dominant grasses were *Leptochloa chinensis* with high appearance frequency (52.64%) followed by *Oryza sativa* (13.06%) and *Echinochloa crus-galli* (11.25%). The two sedges were *Cyperus difformis* (55.42%) and *Fimbristylis miliacea* (53.33%). The three most important broadleaved weeds were *Euphorbia thymifolia* (44.44%), *Phyllanthus niruri* (30.69%) and *Lindernia crustacea* (27.78%). After 12 months of nursery testing, there were 5,191 plants m<sup>-2</sup> of grasses in zone I as compared to 2,889 plants m<sup>-2</sup> in zone III. The majority of grass seeds germinated and emerged within the first five months. The same trend was observed in the case of sedges with the corresponding data of 10,654 plants m<sup>-2</sup> and 6,663 plants m<sup>-2</sup>. Unlike the two monocotyledon groups, the population of broadleaved weeds was higher in zone III (13,842 plants m<sup>-2</sup>) as compared to that of zone I (10,740 plants m<sup>-2</sup>). These seeds also germinated and emerged later than grasses and sedges and the maximum number of weed seedlings fell into the third and fourth months. Generally, after 12 months, total number of weeds in zone I was 26,586 plants m<sup>-2</sup> and zone III with 23,396 plants m<sup>-2</sup>. The first five months contributed 93.09% and 95.13% of the total number of weeds observed.

**Key words:** Broadleaved weeds, grass, rice field, sedge, soil seed bank.

## INTRODUCTION

Mekong Delta is the most important rice growing area in Vietnam. The production in 2002 reached 17.5 million tons with the average yield of 4.63 tons ha<sup>-1</sup>. More than 95% of exported milled rice comes from this delta. However, rice yield in this area continues to be low as compared to that of Red River Delta and other advanced rice growing countries in the world. There are many factors affecting the growth and yield of rice in which weed competition plays an important role. Weeds cause the yield loss of 46% in direct seeded rice in the delta (Chin and Sadohara 1994). There are two major sources of weed pest in rice fields. The first one comes from the contaminated seeds. In 2004, only 30% of planted areas were sown by certified seeds approximately. The rest of areas was sown by contaminated seeds reserved from the farmers' stock product. The second factor is soil seed bank. The persistence of weed seeds in the soil is very important because the dormant seeds in the soil can germinate and emerge when the environment is favorable. This persistence is influenced by a number of factors, two of the most important being seed burial depth and soil type (Forcella 1992). 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 weed seeds in rice soil is important in determining the likelihood of weed infestation in rice fields. In this research the method of nursery testing has been used to determine the soil seed bank in rice fields of O Mon district, Can Tho province as a starting point for a larger study in the entire Mekong delta in the future.



## MATERIALS AND METHODS

Some simple materials and instruments were used for this research both in the fields as well as in the net house. Those are: metal soil cores (6.5 cm diameter) for soil sampling, plastic bags for containing soil samples, plastic trays (32 cm x 26cm x 7cm) for nursery. A small hole in the bottom of the tray was made to drain excess water after irrigation.

### Site selection

O Mon district has a total of 54,850 ha. It is located in the central areas of the Mekong Delta at 9° 55' to 10° 27' North latitude and 105°22' to 105° 43' East longitude. The entire land of this district is divided into three agro-ecological zones. Zone I is located along the bank of Bassac River. The elevation of zone I ranges from 1.0 to 1.5 meters above sea level (masl). Soil is alluvial with the pH nearly neutral, shallow submergence. Zone II has an elevation of 0.8 to 1.0 masl with lightly acid sulphate soil and medium submergence. The elevation of the zone III ranges from 0.4 to 0.8 masl with light to medium acid sulphate soil. In these areas, water submergence is a problem from middle of August to the end of December every year and the depth of water is deeper as compared to that of two previous zones. Three villages of the zone I namely Thoi Long, Thoi An and Phuoc Thoi were selected. The corresponding villages in zone III were Thoi Lai, Thoi Dong and Truong Xuan. In each village, from which ten fields were randomly selected. Five soil cores with a depth of 0-10 cm in each field were taken. These were mixed together and used for nursery testing in this research. The soil was placed into one tray and sprinkle irrigated to keep the soil moist all the time. Weed seedlings of each species were counted every month. After counting, the seedlings were buried deeply into the soil and beneath the layer of the soil was turned up to the surface for other seeds to germinate. The same work was done every month. A total of 60 trays were planted, 30 trays for each of zone. Every month each tray with a particular species emerged was counted. A total of 720 trays from the whole year was counted. The appearance frequency (A.F.) of each species was calculated by the formula:

A.F.(%) of species (a) = number of tray having species (a) divided by total number of trays (720) and multiplied by 100. The series number of weed population in zone I were compared to those in zone III by *t*-test.

## RESULTS AND DISCUSSION

### Weed Flora

After 12 months of nursery testing, 24 weed species were observed in the soil samples of the two zones in O Mon district. Those weeds are listed in the Table 1. Some of them are weeds of upland areas. This may be due to the dispersal of upland weed seeds from the upstream catchment of the Mekong River to the lower basin through water movement. The other factor may be the rotational cultivation. Those weeds can grow, and produce seeds in the pocket areas with high elevation along the bank of this big river when farmers grow upland crops such as maize, soybean, mung bean, sesame in rotation with rice during the dry season. There were six grass species of which the three most important ones include *Leptochloa chinensis*, *Oryza sativa* and *Echinochloa crus-galli*. *Oryza sativa* comprises of weedy and volunteer rice. The corresponding appearance frequencies of those weeds are 52.64%, 13.06% and 11.25%, respectively. The two sedge species were *Cyperus difformis* and *Fimbritylis miliacea*. Their appearance frequencies were 55.42% and 53.33% respectively. There are 16 species of broadleaved weeds. However, only three dominant species which include *Euphorbia thymifolia* (44.44%), *Phyllanthus niruri* (30.69%) and *Lindernia crustacea* (27.78%) were observed. The other weeds with the appearance frequency higher than one are *Hedyotis diffusa* (8.19%), *Ludwigia octovalvis* (4.17%), *Lindernia ciliata* (3.75%), *Lindernia*

*antipoda* (3.33%), *Nymphoides indica* (2.08%), *Vernonia cinerea* (2.08%) and *Monochoria vaginalis* (1.67%).

Table 1. Appearance frequency of weed species.

No.	Scientific name	Appearance frequency (%)
A) GRASSES		
1	<i>Leptochloa chinensis</i>	52.64
2	<i>Oryza sativa</i>	13.06
3	<i>Echinochloa crus-galli</i>	11.25
4	<i>Digitaria timorensis</i>	1.81
5	<i>Digitaria ciliaris</i>	1.11
6	<i>Eleusine indica</i>	0.56
B) SEDGES		
1	<i>Cyperus difformis</i>	55.42
2	<i>Fimbritylis miliacea</i>	53.33
C) BROADLEAVED WEEDS		
1	<i>Euphorbia thymifolia</i>	44.44
2	<i>Phyllanthus niruri</i>	30.69
3	<i>Lindernia crustacea</i>	27.78
4	<i>Hedyotis diffusa</i>	8.19
5	<i>Ludwigia octovalvis</i>	4.17
6	<i>Lindernia ciliata</i>	3.75
7	<i>Lindernia antipoda</i>	3.33
8	<i>Nymphoides indica</i>	2.08
9	<i>Vernonia cinerea</i>	2.08
10	<i>Monochoria vaginalis</i>	1.67
11	<i>Eclipta alba</i>	0.83
12	<i>Sphenoclea zeylanica</i>	0.83
13	<i>Ceratopteris pteridroides</i>	0.69
14	<i>Heliotropium indicum</i>	0.42
15	<i>Solanum americanum</i>	0.28
16	<i>Ipomoea aquatica</i>	0.14

### Grass Population at 0-10 cm Soil Depth at the Two Zones

The number of grasses observed every month is presented in Table 2. Total number of grasses in zone I was 5,191 plants m<sup>-2</sup> which was higher than those of zone III (2,889 plants m<sup>-2</sup>). Most of grasses emerge within the first five months. At the end of the first month, the number of grasses in zone I was 1,270 plants m<sup>-2</sup> which are higher than that of zone III (794 plants m<sup>-2</sup>). The same trend was observed in the second, third, fourth and fifth months. The corresponding data of zone I and zone III in those months were 1,021 plants m<sup>-2</sup> and 675 plants m<sup>-2</sup>, 1,494 plants m<sup>-2</sup> and 619 plants m<sup>-2</sup>, 557 plants m<sup>-2</sup> and 247 plants m<sup>-2</sup>, 649 plants m<sup>-2</sup> and 390 plants m<sup>-2</sup>, respectively. The grass population in the zone I was significantly higher than those in zone III in the first, second, third and fourth months. There was no significant difference in terms of grass population between the two zones from the fifth to twelfth month.



Table 2. Grass population (plants m<sup>-2</sup>) at one-month interval in two zones.

Month	Zone I	Zone III	T value
First	1,270	794	2.45 **
Second	1,021	675	2.18*
Third	1,494	619	2.98**
Fourth	557	247	2.37*
Fifth	649	390	1.73
Sixth	92	58	1.35
Seventh	64	34	1.09
Eight	26	6	1.86
Ninth	14	10	0.47
Tenth	4	56	-1.8
Eleventh	0	0	-
Twelfth	0	0	-
Total	5,191	2,889	

## Sedge Population at 0-10 cm Soil Depth at the Two Zones

Sedge weed population (no of plants m<sup>-2</sup>) observed monthly from soil samples of zone I and zone III are presented in Table 3. The total number of sedges in zone I are 10,654 plants m<sup>-2</sup>. This population was higher than that of zone III (6,663 plants m<sup>-2</sup>). The number of emerging and growing sedges were observed and counted every month. At the end of the first month, the number of sedges was 3,359 plants m<sup>-2</sup> in zone I. The corresponding data in zone III were 2,024 plants m<sup>-2</sup>. The difference between the two zones was significant. The number of sedges at the end of second month in zone I was higher than in zone III. The sedge population at this stage in zone I was 2,249 plants m<sup>-2</sup> whereas in zone III, it was 1,586 plants m<sup>-2</sup>. In the third month, 1,777 sedge plants were counted in zone I which was statistically higher than zone III (1,057 plants m<sup>-2</sup>). A special situation was observed at the end of fourth month. The difference between the two zones was significant at 1% level. In zone I, there were 1,236 sedge plants m<sup>-2</sup> compared to that of 583 plants m<sup>-2</sup> in zone III. At the end of fifth month, sedge population in zone I was 1,357 plants m<sup>-2</sup> which was higher than that of zone III (842 plants m<sup>-2</sup>). Majority of sedge seeds in the soil was germinated within five months under moist condition. In zone I, 93.65% and 91.43% in zone III of viable sedge seeds in rice soil germinated within 5 months.

Table 3. Sedge population (No of plants m<sup>-2</sup>) at one-month interval in two zones.

Month	Zone I	Zone II	T-value
First	3,359	2,024	2.22*
Second	2,249	1,586	1.72*
Third	1,777	1,057	2.08*
Fourth	1,236	583	4.06**
Fifth	1,357	842	2.16*
Sixth	107	133	-0.51
Seventh	358	366	-0.09
Eighth	107	28	2.32*
Ninth	52	20	1.80
Tenth	52	22	0.99
Eleventh	0	2	-1.00
Twelfth	0	0	-
Total	10,654	6,663	

## Broadleaf weed population at 0-10 cm Soil Depth at the Two Zones

Broadleaf weed populations in different zones observed every month are presented in Table 4. The trend in this group differs substantially as compared to grass and sedge groups. This may be due to the large variation among species in terms of dormancy and the response of these species to the environment. The total weed count during the 12 month duration in zone III (13,842 plants  $m^{-2}$ ) was higher than that of zone I (10,740 plants  $m^{-2}$ ). Unlike grasses and sedges, the time of maximum weed emergence was little late in broadleaved weeds. The highest weed population in both zones was observed in the third and fourth months. In the third month, the broadleaf weed population in zone III was 6,815 plants  $m^{-2}$  which was significantly higher than f zone I (5,221 plants  $m^{-2}$ ). The similar trend was observed in the fourth month. The corresponding data were 5,540 plants  $m^{-2}$  and 3,349 plants  $m^{-2}$ . In zone I, the total weed population in the third and fourth month was 8,570 plants  $m^{-2}$ , occupying 79.80 % of the total weeds emerged in 12 months. In the case of zone III, there were 12,355 plants  $m^{-2}$  in the third and fourth months. These two months contributed 89.26% of the total broadleaf weed population.

Table 4. Broadleaf weed population in 12 months (No of plants  $m^{-2}$ ) at one-month interval in two zones.

Month	Zone I	Zone III	T-value
First	105	314	-2.21*
Second	183	239	-0.61
Third	5,221	6,815	-2.00*
Fourth	3,349	5,540	-4.19**
Fifth	921	531	2.98**
Sixth	101	46	2.15*
Seventh	484	249	3.15**
Eighth	205	30	2.93**
Ninth	157	30	3.04**
Tenth	14	40	-0.96
Eleventh	0	8	-1.44
Twelfth	0	0	-
Total	10,740	13,842	

## Total weed population at 0-10 cm Soil Depth at the Two Zones

The data on total weed population in two zones are presented in Table 5. Although there is a difference in the emergence pattern of broadleaved weeds as compared to those of grasses and sedges, the total weed population represents the trends of soil weed seed bank in O Mon district. The total weed population in 12 months in zone I (25,586 plants  $m^{-2}$ ) tended to be higher than that of zone III (23,396 plants  $m^{-2}$ ). Except at fourth month, weed population in zone I was higher than that of zone III during the rest of the months. The first five months was very important for weed germination and emergence in both the zones. In zone I, 24,748 plants  $m^{-2}$  were counted within the first five months occupying 93.09% of the total weeds observed in 12 months. Similarly in zone III, 95.13% (22,257 plants  $m^{-2}$ ) of weed population was counted from the first to the fifth month. There were few weeds observed in the rest of seven months.



Table 5. Total weed population at 0-10 cm soil depth in two different zones.

Month	Zone I	Zone III	T-value
First	4,734	3,132	2.39*
Second	3,454	2,501	2.19*
Third	8,491	8,491	0.00
Fourth	5,142	6,370	-2.30*
Fifth	2,927	1,763	3.55**
Sixth	300	237	0.86
Seventh	907	649	2.09*
Eighth	338	64	3.70**
Ninth	223	60	3.54**
Tenth	70	119	-0.75
Eleventh	0	10	-1.72
Twelfth	0	0	-
Total	26,586	23,396	

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# Seed bank studies on *Parthenium hysterophorus* infested pastures: examining of different management approaches

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**Abstract:** *Parthenium* weed (*Parthenium hysterophorus* L.) has been in Australia for about 50 years, in which time it has spread from isolated infestations to established core populations in central Queensland. This annual herb is having a significant impact on pasture productivity and is causing serious health concerns in regional communities. The main management tool used is biological control, while the use of fire has been considered as another potential management tool. In an area where biological control is used, the soil seed bank of a heavily infested pasture was studied over a 6 year period (1995 to 2001). The studies were conducted shortly after the introduction and establishment of biological control agents. Initially the seed bank of parthenium weed was very large (34 682 seeds m<sup>-2</sup>). However, over time it decreased significantly (13 140 seed m<sup>-2</sup>). Though it is possible that other factors could also have had an impact on the seed bank, these factors alone are unlikely to explain the results obtained. Hence, this data suggests that biological control agents reduced the soil seed bank of parthenium weed over time. In the case of fire, the effect of heat and smoke was tested on the soil seed bank taken from a parthenium weed infested pasture. Parthenium weed seeds were stimulated to germinate by smoke, but to a much lesser degree than the seeds of other species. As a result of this dormancy-breaking effect of smoke, the portion of seedlings emerging represented by parthenium weed dropped from 87% to 79%. Hence, the findings suggest that smoke may promote the germination of pasture grasses and, hence, decrease the competitiveness of parthenium weed. However, fire will have other effects on the pasture community that were not measured in this study.

**Key words:** Biocontrol, fire management, parthenium weed, seed banks.

## INTRODUCTION

Parthenium weed (*Parthenium hysterophorus* L.) is a major pest plant in Queensland and has recently been declared one of the 20 weeds of national significance in Australia. This species has the ability to dramatically reduce the productivity of pastures, threaten the environmental integrity of natural ecosystems, cause serious allergic health problems, and become a significant contaminant of agricultural produce (Navie et al. 1998). Parthenium weed is also an important weed in many tropical and sub-tropical countries of the world, and it is especially damaging on the Indian sub-continent.

The main tool used for parthenium weed management is biological control, while the use of fire has been considered as another potential management tool. To date a number of biological control agents (including plant feeding insects and fungal pathogens) have been released in Australia. In certain areas these agents are having a significant effect on the weed populations, reducing both biomass and density. Certain agents are better adapted to certain areas and therefore the effect of the agents varies throughout the range of the weed.

Fire has also been considered as a possible management tool for parthenium weed. Fire not only destroys plant biomass but the heat and the smoke associated with it are also known to stimulate the germination of native grassland species (Orr et al. 1997). Hence, the use of fire has become an important pasture management tool in many areas. The role of high temperature in breaking seed



dormancy has also been known for some time (Fenner 1992) and more recently smoke has been shown to stimulate germination in many species (Keeley *et al.* 1985, De Lange and Boucher 1990). It has been suggested that native species from the naturally fire-prone regions of the country could be selectively stimulated over introduced weedy species originating from non fire-prone habitats (Adkins *et al.* 2000). However, very little examination of the effect of smoke on germination of pasture weed species has been carried out.

The purpose of the present study is two-fold, firstly to determine the size of the germinable seed bank of parthenium weed in grazed pastures in central Queensland over a number of years (1995 to 2001). It is hoped that this data will give some experimental evidence or indication on the effectiveness of biological control agents and lead to improved management practices in reducing the seed bank of this serious weed. Secondly, this study aims to determine the secondary effects of fire (those due to smoke and heat) on the soil seed bank of parthenium weed in an infested pasture and help determine if this management tool has potential for further study.

## METHODS AND MATERIALS

### Biological control study

The central Queensland site at Moolayember Creek was chosen for the seed bank study as it had been used as a beef pasture for several decades and parthenium weed had been a prominent weed for at least 15 years. Collections of soil samples from the site were undertaken from 1995 to 2001. Once back in the laboratory, the soil samples were spread thinly over sterilised soil in shallow trays that were randomly distributed on a bench in a glasshouse and kept watered to field capacity. Emerged seedlings were recorded and a Kruskal-Wallis one way ANOVA was used to compare the total seed densities, and the seed densities for common species, between sampling dates at this site.

### Heat and smoke study

The site chosen for this study was located in 'Albinia Downs' National Park. This site had been used as a beef pasture for several decades prior to the commencement of this research, but had recently been declared a national park and therefore grazing animals were removed. Parthenium weed had also been present at this site for many years. Collection of soil samples from the site was made in April 2000. Ten cylindrical soil cores were removed from each of 12 quadrats and pooled to make a single sample from each quadrat. Each of these pooled soil samples were then divided into six equal parts (by volume) and spread thinly over sterilised soil in shallow trays. Three of the six trays of soil from each sample were placed in a heating oven for one hour set to a temperature of 60°C (i.e., 'Heat treatment'). An apparatus was also set up with which to apply aerosol smoke to the soil in some of the trays. For each sample, a heat-treated tray and a tray that had not been heat-treated were treated with smoke for 10 or 60 minutes. Hence, there were six separate treatments used in this experiment. Once the smoke treatments were completed the trays were then randomly distributed on a bench in a glasshouse. The soil in these trays was kept watered to field capacity and observed regularly for newly emerging seedlings. To analyse any differences between the number of seed germinating from the seed banks of the different treatments, a non-parametric Friedman's test was carried out on the data.

## RESULTS AND DISCUSSION

### Biological control study

The germinable soil seed bank at the site ranged from 34 682 in 1995 to 13 140 seeds m<sup>-2</sup> in 2001 (Table 1). The seed bank was totally dominated by *P. hystrophorus* (65% in 1995, reducing to 45% in 2001) with grasses representing less than 3% of the total seed bank. A number of annual or short-lived perennial weeds were also very common in the seed bank. By the end of the study *C.*



*bonariensis* (26%), *Lepidium bonariense* L. (4.4%), *Sida* sp. (3.3%) and *Argemone ochroleuca* Sweet (1.4%) were the most abundant species.

Table 1. Temporal variation in the germinable soil seed bank of a pasture near Moolayember Creek, Queensland. Seed densities followed by the same letter in each row are not significantly different (SNK  $P < 0.05$ ).

Species	Seeds m <sup>-2</sup>					
	Mar 1995	Oct 1995	Mar 1996	Oct 1996	Apr 2000 <sup>#</sup>	Feb 2001 <sup>#</sup>
* <i>Argemone ochroleuca</i>	4388 c	60 a	1287 b	10 a	4446	184
* <i>Bidens pilosa</i>	0 a	248 b	362 b	117 b	25	15
<i>Casuarina cunninghamiana</i>	22	50	73	34	34	49
<i>Centaureum spicatum</i>	0	0	10	0	96	37
* <i>Ciclospermum leptophyllum</i>	6	4	78	0	165	101
* <i>Conyza bonariensis</i>	685 c	127 b	862 d	24 a	2341	3446
<i>Crassula sieberana</i>	647	21	279	0	84	0
<i>Einadia polygonoides</i>	122	71	117	0	1989	7
* <i>Euchiton sphaericus</i>	0	0	0	0	177	0
<i>Euphorbia</i> sp.	17	0	88	0	0	0
* <i>Gamochaeta subfalcata</i>	188 b	11 a	480 c	5 a	54	17
* <i>Lepidium bonariense</i>	536 b	230 b	357 b	0 a	1245	572
* <i>Oxalis corniculata</i>	613 c	39 b	558 c	0 a	680	91
* <i>Parthenium hysterophorus</i>	22597 ab	31662 b	33904 b	17579 a	6332	5433
<i>Sida</i> sp.	6	21	15	0	226	430
* <i>Solanum mauritianum</i>	497	258	113	108	555	324
* <i>Verbena</i> sp.	349	42	137	10	191	160
<i>Wahlenbergia</i> sp.	28	0	137	5	76	12
Minor species	42	61	172	5	169	127
Chenopodiaceae	88	96	54	39	27	39
Cyperaceae/Juncaceae	1304 b	425 a	1503 b	162 a	1360	769
Poaceae	641 a	1551 a	2345 b	1111 a	4087	354
Portulacaceae	0	39	10	10	28	46
Unidentified	1912	1282	1699	1381	334	925
Total	34682 b	36296 b	44639 b	20599 a	24728	13140

<sup>#</sup> not included in the statistical analysis due to variation in the sampling procedure on these dates. \* introduced species.

There were great changes in the seed bank at Moolayember Creek from the start of the study when compared to the end (Table 1). At the start of the study (1995/96), the total seed bank averaged 34 054 seeds m<sup>-2</sup>, and varied between 20 599 and 44 639 seeds m<sup>-2</sup>. However by the end of the study the seed bank became significantly smaller (13 140 seeds m<sup>-2</sup> February 2001). The seed bank of parthenium weed varied between 17579 and 33904 seeds m<sup>-2</sup> at the start of the study (average: 26436 seeds m<sup>-2</sup>) and was reasonably constant until October 1996, when a significant decrease was detected. By the end of the study the seed bank of parthenium weed had declined to be only 5433 seeds m<sup>-2</sup> (Table 1). This equates to less than a third of the lowest value recorded during the first two years of the study and its contribution to the entire seed bank had fallen to about 41%. The



grass seed bank started low, increased to 17% in 2000 but decreased greatly to about 3% in 2001. The seed banks of many annual weed species were also much lower than those recorded at the start of the study, with the notable exception of *Conyza bonariensis* (L.) Cronq. whose seed bank continued to increase (up to 26% of the entire seed bank in 2001). These annual weed species contributed a combined total of 43% in April 2000 and 33% in February 2001, much greater percentages than were seen at the start of the study (19% in March 1995, 7% in March 1996).

This study has confirmed a continuing decline in the seed bank of parthenium weed at one site in recent years. This reduction in the seed bank is most probably due to an increase in the activity of biological control agents at the site over the last five years. At the start of the study only *Epiblema strenuana* Walker was well established at the site, while outbreaks of *Zygogramma bicolorata* Pallister were becoming more numerous. The seed-feeding weevil *Smicronyx lutulentus* Dietz was first observed at this site in January 1996, and the arrival of this agent was suggested as a possible reason why the seed bank of parthenium weed dropped substantially in October 1996. It is likely that the increasing impact of these agents, along with favourable growing conditions for native grasses, has continued to decrease the dominance of this weed at the site during the last five years. The decrease of *P. hysterothorus* in the seed bank has also coincided with an increase in the seed bank of other annual weed species. These species may have moved in to fill that part of the niche in the ecology of this site previously occupied by parthenium weed. In particular, *C. bonariensis*, which is closely related to parthenium weed and quite similar in many respects, may be partially filling the gap in the vegetation that has been created at this site by the recent and continuing decline in importance of parthenium weed.

While the changes in the seed bank at this site during the time of the study are quite significant, a relatively large seed bank of the weed remains present in the soil. By simply observing changes in the vegetation at these sites during the last five years, one may wrongly come to the conclusion that this weed is no longer a threat. However, the numerical size and longevity of the seed bank of this species means that it will remain a concern for many years after it has declined in the extant vegetation. Even though the current situation with *P. hysterothorus* is much improved on that which was previously the case, and the biological control program has been relatively successful, a change in climatic conditions and a return to less sensible pasture management practices may see its return to some extent. Alternatively, such a scenario may allow the chance for another highly invasive introduced weed species to become established.

#### The heat and smoke study

The total germinable soil seed bank varied from 20 396 seeds  $m^{-2}$  emerging in the control treatment to 25 124 seeds  $m^{-2}$  in the intense smoke treatment (Table 2). Parthenium weed was by far the most abundant species present and accounted for 79 to 88% of the seed bank depending on the germination treatment. Grasses were also common in the seed bank (9-17% of the total), particularly *Cenchrus ciliaris* L., which represented the vast majority of the germinable grass seed bank. The number of parthenium weed seeds germinating in the 60 minute smoke treatment was significantly higher than those germinating in the heat treatment. However, the differences between other treatments were not statistically significant. The number of seeds from all other species germinating from the 60 minute smoke and 30 minute smoke + heat treatments were significantly higher than in the other treatments. Also, the total number of seed germinating from the soil was significantly lower in the control and heat treatments than in the other treatments.

In this study the vegetation where the soil samples were collected was dominated by a dense cover of buffel grass (*C. ciliaris*), but the germinable soil seed bank was dominated by parthenium weed (Table 2). On first inspection the germinable seed bank of parthenium weed seemed to be greater in the samples that underwent smoke treatments. However, there was a great degree of variation



between samples and the statistical analysis only detected one significant difference. This may be due to the heat treatment killing a small proportion of parthenium weed seeds and the smoke treatment stimulating a small proportion to germinate. While neither of these factors may have been significant in themselves, they were when so compared against each other.

Table 2. Germination from the soil seed bank of a pasture near 'Albinia Downs' National Park, Queensland after different smoke and heat treatments. Seed densities followed by the same letter in each row are not significantly different (SNK  $P < 0.05$ ).

Species	Treatment (seed m <sup>-2</sup> )					
	Control	Heat	Smoke (10 min)	Smoke (10 min) + Heat	Smoke (60 min)	Smoke (60 min) + Heat
<i>Parthenium hysterophorus</i>	17786ab	18359a	21685ab	19966ab	20713b	19526ab
<i>Cenchrus ciliaris</i>	1678	1811	1883	3500	3285	3920
<i>Brachiaria</i> sp.	133	72	102	51	154	184
Other Poaceae	215	327	266	266	225	215
<i>Argemone ochroleuca</i>	61	61	133	72	72	133
<i>Chenopodium cristatum</i>	31	10	20	41	41	0
<i>Conyza</i> sp.	0	0	20	10	10	0
<i>Cyperus</i> sp.	81	91	61	82	81	102
<i>Phyllanthus</i> sp.	10	10	10	20	0	0
<i>Portulaca</i> sp.	10	10	20	20	41	41
<i>Sida</i> sp.	41	61	72	61	92	41
Other Dicotyledons	112	82	81	70	80	162
Unknown	235	287	266	164	327	317
Total	20396a	21184a	24622b	24326b	25124b	24643b

The seed bank of all other species was stimulated by the smoke 60 treatment, and even more-so when in conjunction with heat. As this portion of the seed bank consisted mainly of grasses, greater germination is probably due to smoke and heat breaking seed dormancy in these species. It seems that parthenium weed seeds were stimulated by smoke to a much lesser degree than the seeds of other species and as a result, the portion of the germinable seed bank represented by parthenium weed dropped from 87% in the control treatment to 79% in the 60 minute smoke + heat treatment. Meanwhile, grass seedlings only accounted for 9% of the germinable seed bank in the control and as much as 17% in the 60 minute smoke + heat treatment. This means that there were nearly nine parthenium weed seedlings for every grass seedling in the control treatment trays and less than five parthenium weed seedlings for every grass seedling in the 60 minute smoke + heat treatment trays.

These findings suggest that smoke may promote the germination of pasture grasses and, hence, decrease the competitiveness of parthenium weed. However, there are many other aspects of fire that should be considered before it is promoted as a control measure. For instance, fire removes much of the existing vegetation, leaving bare areas in the pasture. This may be in fact an advantage to the weed, as these are exactly the type of environments where parthenium weed prefers to grow.



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# GA<sub>3</sub> and KNO<sub>3</sub> break dormancy in curly dock (*Rumex crispus*) seeds under varying temperatures

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**Abstract:** Curly dock (*Rumex crispus*) is one of the worst weeds of wheat and sugarcane fields in Northwestern Frontier Province of Pakistan. Dormancy is the adaptive significance in many a weed species for their persistence. Laboratory studies were initiated in the Weed Science Department, Agricultural University Peshawar, Pakistan in 2004 to investigate the response of curly dock seeds to GA<sub>3</sub> and KNO<sub>3</sub> at 0 to 800 ppm with an increment of 200 ppm exposed either to 10 or 20°C temperatures. The experiment was laid out in completely randomized design with a split plot arrangement. Temperatures were assigned to main-plots, while chemicals and their concentrations were kept into the sub-plots. Each sub-plot comprised of a single petri dish planted with 20 seeds. The germinated seeds were subsequently converted to percentage. The germination percentage data were subjected to ANOVA and the means were separated by DMRT. The analyses revealed chemicals, concentrations, temperatures and their interactions except rates x temperature significantly affected germination. The partitioning of the total variance into its components revealed that the greatest part of variability was governed by temperature in inducing germination. GA<sub>3</sub> induced better germination (12.3%) than KNO<sub>3</sub> (7.03%). The highest germination of 13.92 and 12.92% was recorded in 600 and 400 ppm, respectively, as compared to 5% germination in the untreated check. Higher (17.17%) germination was recorded at 10°C as compared to 2.2% at 20°C. The chemical x rate interaction revealed the highest germination (22.18%) under GA<sub>3</sub> applied at 600 ppm, as compared to about 6 times lower germination in the untreated check. The chemical x rate x temperature interaction exhibited the highest germination (38.33%) in GA<sub>3</sub> at 600 ppm under 10°C. These findings have implication of their commercial exploitation in inducing germination in the seed bank of the test species prior to planting.

**Key words:** Chemicals, concentrations, dormancy breaking, germination, noxious weed

## INTRODUCTION

*Rumex crispus*, the curly dock, family Polygonacea is a monoecious perennial from stout, erect rootstocks. It is self compatible and wind pollinated (Allard 1965; Proctor et al. 1996). Stems 3 to 10 dm are tall, erect, simple, glabrous. Leaves are alternate, while the stipules are modified into permanent sheaths. *R. crispus* is a native of Eurasia, but has naturalized throughout North and South America, Southern Africa, and Hawaii (Chapman 1991; Clapham et al. 1982; Gleason and Cronquist 1991; Holm et al. 1977). In Pakistan, *R. crispus* is found in waste places, ditch banks and cultivated fields. Curly dock is one of the worst weeds of wheat fields in irrigated agriculture in North Western Frontier Province, but its worst damage is seen in the fall planted sugarcane where, along with *R. obtusifolius* (broadleaf dock), it forms solid stands in sugarcane due to its arrested growth in winter months due to colder temperature. It is competitive thereby capturing all the soil resource until late February to mid March, when farmers uproot it with the inter-cultivation of sugarcane. The allied species, *R. obtusifolius* is a serious weed of alkaline wheat fields in Dera Ismail Khan, Pakistan. Several farmers have been heard complaining on broadleaf dock infestation in wheat under the saline soils of the command area of the Chashma Right Bank Canal in Dera Ismail Khan, Pakistan (personal communication). It is a noxious weed worldwide (Holm et al., 1977). Seeds of *R. crispus* remain viable for at least 7 years under field conditions (Cavers and Harper, 1964; Walmsley and Davy, 1997). Sometimes viability extends to 50-80 years (Darlington 1931; Toole and Brown 1946). Seed polymorphism for germination depending on the seed position



on mother plant was reported on its sensitivity to light and temperature (Cavers and Harper 1964, 1966; Williams 1971).

A seed represents the end of flowering process and the beginning of a new generation. It contains the new plant in miniature, means for dispersal, survival, renewal and germination. Failure of the seeds to germinate even if required conditions for germination are present, render them dormant. The factors responsible for dormancy are either innate or environmental (Radosevich and Holt, 1984). Harper (1957; 1977) suggests that some seeds are born dormant (innate), some achieve dormancy (induced) and some have dormancy thrust upon them (enforced or quiescence). There are several features, which render some of the weed species successful, but the most important one is the seed dormancy or rest period, which enables the seeds to persist in the soil and survive under the conditions not suitable for plant growth (Karssen 1982; Harper 1977; Holt 1987). Numerous investigations elucidate the basic and practical aspects of the problem (Crocker and Barton 1953). Popay and Roberts (1970) and Benvenuti and Macchia (1995) showed that the high CO<sub>2</sub> and low O<sub>2</sub> (hypoxia) induced dormancy, while Taylorson and Hendricks (1980) reported otherwise. The studies of Holm (1972) showed that decreased O<sub>2</sub> in the soil microenvironment accumulated acetaldehyde, ethanol and acetone. Several studies exhibit that the buried seeds of annual weeds undergo dormancy-non-dormancy cycles and even light does not stimulate germination (Karssen 1982; Schafer and Chilcote 1970; Taylorson 1970). Baskin and Baskin (1985) and Benvenuti and Macchia (1994) have further added that dormancy-non-dormancy transition may be related to changes in the membrane properties. Carmona and Murdoch (1995) germinated dormant weed seeds at constant and alternating temperatures using five chemicals. Germination of *Chenopodium album* when expressed in normal equivalent deviates, increased linearly with the logarithm of chemical dose up to an optimal concentration. Similar was the response of *Avena fatua*, while none of the chemicals including KNO<sub>3</sub>, sodium azide, thiourea, hydrogen peroxide and ethephone used in this investigation stimulated the germination of *Rumex crispus* seeds. Subsequent studies of Carmona and Murdoch (1996) deciphered that exposure to light, when pre-applying sodium azide and peroxide, increased germination of *C. album* and *R. crispus*, but did not affect the action of the chemicals. The compounds tested had little or no effect on *A. fatua* at constant or alternating regimes with a mean temperature over 10°C, but dormancy was relieved by sodium azide and potassium nitrate at low temperatures (3-10°C). Carmona (1997) in later studies deduced that the response to ethephone was not affected by pH from 3 to 9. Sodium azide had the greatest effect on seed decline, but its effect was pH-dependent. Caudra et al. (1996) reported increased germination in GA<sub>3</sub> incubated seeds.

Keeping the importance of the subject in view, experiments were carried out under laboratory conditions with these objectives a) to provide information about occurrence of dormancy in *R. crispus* seeds b) to investigate the behavior of dormancy related to different dormancy breaking chemicals, their concentrations, temperature regimes and their interaction.

## MATERIALS AND METHODS

Laboratory studies were initiated in the Department of Weed Science, NWFP Agricultural University Peshawar, Pakistan during 2003 to investigate the response of *R. crispus* seeds collected from the previous season as subjected to different levels of GA<sub>3</sub> and KNO<sub>3</sub> and at 10, 20 or 30 °C temperature regimes. The GA<sub>3</sub> and KNO<sub>3</sub> rates included in the studies were 0 to 800 ppm with an increment of 200 ppm. The seeds were incubated in a seed germinator (Growth Chamber, Model No.2020-2E, Sheldon Manufacturing Inc., 300 N, 26<sup>th</sup> Cornelius, OR 97113) for 4 weeks and the data on the germination were recorded. Experiment was laid out in completely randomized design with a split plot arrangement. Temperatures were assigned to main-plots, while chemicals and their concentrations were kept into the sub-plots. Replicated twice, each sub-plot comprised of a single petri dish planted with 20 seeds. The protrusion of radicle from the test declared the seed as



germinated. The germinated seeds were subsequently converted to percentage germination. The germination percentage data were subjected to Analysis of variance technique and the means were separated by the Duncan's multiple range test (Steel and Torrie, 1980). No seeds of curly dock germinated at 30°C, hence the data were excluded from the analysis as well as further report.

## RESULTS AND DISCUSSION

The investigations undertaken at the Department of Weed Science, NWFP Agricultural University, Peshawar, Pakistan exhibited that not a single seed of *R. crispus* germinated across any of the treatments at 30 °C. The ANOVA showed significant ( $P<0.05$ ) variability between temperatures, temperature and chemicals, rates of chemicals, chemical x rate and temperature x chemical x rate. The differences for rate x temperature were recorded as non-significant ( $P>0.05$ ) statistically. Overall partitioning of total variability into its components revealed the greatest part to the temperature followed by the chemicals. Higher germination (17.17%) was recorded under 10°C as compared to only 2.20% in 20°C. Exposure to GA<sub>3</sub> (12.30%) germinated more seeds as compared to 7.07% in KNO<sub>3</sub>. Chemicals interacted with temperature regimes in inducing germination. Lower temperature favored the germination in both chemicals. GA<sub>3</sub> and KNO<sub>3</sub> had 22.00 and 12.33% germination, respectively under 10°C as compared to their respective 2.6 and 1.8% germination under 20°C (Table 1). The rates of chemicals had an impact on germinating *R. crispus* seeds. The highest germination (13.9) was recorded under 600 ppm, which however, was statistically comparable with 400 ppm (12.92%) (table 2). The germination of 400 ppm, in turn was statistically at par with either 200 or 800 ppm (Table-2). The chemical x rate interaction (Table 2) revealed the highest germination (22.17%) in GA<sub>3</sub> applied at 600 ppm. It was followed by the application of the same chemical KNO<sub>3</sub> at 400 ppm (Table 2). GA<sub>3</sub> favored germination better as compared to KNO<sub>3</sub> (Table-2). All the rates of KNO<sub>3</sub> failed to surpass the untreated check (0 ppm). For the temperature x rate interaction, it was observed that the concentrations averaged across both chemicals had no effect on inducing germination (Table 3). The general trend in data manifested increase in numerical values with the rates when *R. crispus* were subjected to 10°C, whereas almost a static behavior across the rates was depicted under subjection to 20°C (Table-3). The influence of temperature has been the strongest in inducing germination of curly dock seeds. The temperature overcame the chemical and concentration involvement. The three-way interaction of chemical x rate x temperature showed highest germination (38.33%) in GA<sub>3</sub> at 600 ppm exposed to 10°C. It was followed by the same combination of the chemical and temperature when treated at 400 ppm (Table 4). Germination percentage in all rates of GA<sub>3</sub> was comparable to the check. No seeds germinated with GA<sub>3</sub> applied at 800 ppm under 20°C temperature regime and was statistically at par with all other 3-way interactions involving both herbicides at 20°C (Table 4). These findings are similar with the work of Corns (1960) and Caudra et al. (1996) who reported increased germination under GA<sub>3</sub>. Caudra et al. (1996) reported increased germination in GA<sub>3</sub> incubated seeds. The work reported by Paul et al. (1976) showed that the lower concentrations of both KNO<sub>3</sub> and GA<sub>3</sub> at 10 and 100 ppm were ineffective in inducing germination of Florida Pusley seeds. At 1000 ppm, both chemicals induced maximum germination. These findings are in line with Hassan et al. (2004) and our un-published data on the studies of dormancy breaking by KNO<sub>3</sub> and GA<sub>3</sub> under different temperature regimes in wild oats. Our findings however are contrary to the results of the work done by Carmona and Murdoch (1995) who found that KNO<sub>3</sub> and GA<sub>3</sub> were ineffective in inducing germination of *R. crispus*. The difference could be due to the difference in test environments. Subsequent studies of the learned workers however substantiate our findings. Carmona and Murdoch (1996) observed that dormancy was relieved by sodium azide and potassium nitrate at low temperatures (3-10°C). Our findings have agronomic implications of their future commercial exploitation in inducing germination in the seed bank of the test species prior to planting.



Table 1. Chemical x temperature interaction for seed germination pattern in curly dock.

Chemical	10°C	20°C	Chemical Means
GA <sub>3</sub>	22.00a	2.60c	12.30a
KNO <sub>3</sub>	12.33b	1.80c	7.07b
Temperature Means	17.17a	2.20b	

Means sharing a letter in common in the respective category do not differ by Duncan's Multiple Range Test at 5% level.

Table 2. Chemical x rate interaction for seed germination pattern in curly dock.

Chemical	0ppm	200ppm	400ppm	600ppm	800ppm	Chemical Means
GA <sub>3</sub>	3.83c	10.50bc	15.00b	22.17a	10.00bc	12.30a
KNO <sub>3</sub>	7.17c	5.838c	10.83bc	5.67c	5.83c	7.07b
Rate Means	5.50c	8.17bc	12.92ab	13.92a	7.92bc	-

Means sharing a letter in common in the respective category do not differ by Duncan's Multiple Range Test at 5% level.

Table 3. Temperature x rate interaction for seed germination pattern in curly dock.

Chemical	0ppm	200ppm	400ppm	600ppm	800ppm	Temperature Means
10°C	10	15.83	23.33	23.33	13.33	17.17a
20°C	1	0.5	2.5	4.5	2.5	2.20b
Rate Means	5.50c	8.17bc	1.92ab	13.92a	7.92bc	-

Means sharing a letter in common in the respective category do not differ by Duncan's Multiple Range Test at 5% level.

Table 4. Three-way interaction of temperature x chemical x rate for seed germination pattern in curly dock.

Temperature (°C)	Chemical	Rates (ppm)				
		0	200	400	600	800
10	GA <sub>3</sub>	6.67def	20.00bc	25.00b	38.33a	20.00bc
	KNO <sub>3</sub>	13.33cd	11.67cde	21.67bc	8.33def	5.67def
20	GA <sub>3</sub>	1.00f	1.00f	5.00def	6.00def	0.00f
	KNO <sub>3</sub>	1.00f	0.00f	0.00f	3.00ef	5.00def

Means sharing a letter in common in the respective category do not differ by Duncan's Multiple Range Test at 5% level.

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# Predicting seed production of redroot pigweed (*Amaranthus retroflexus*) by its biomass at harvest

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**Abstract:** Predicting weed seed production is one of the key steps in the study of seed bank dynamics. The seed productivity of a weed species in fields varies with agricultural practice, weed control measure, plant density etc. Determining seed productivity based on weed density only is not reliable. In order to find a reliable and practical approach to predict weed seed production, a series of experiments was conducted using redroot pigweed in Beijing, China, during 2002-2003. The results showed that the relationship between redroot pigweed seed production and shoot dry weight per plant at harvest was linear regardless of nitrogen level, plant density, and degree of herbicide injury. The findings indicated that weed seed production could be predicted based on weed biomass at harvest.

**Key words:** Biomass, redroot pigweed, seed production.

## INTRODUCTION

Redroot pigweed is one of most important weeds in upland agricultural fields in China. It produces huge numbers of seeds in one season (Li 2004). One goal of weed management is to minimize seed rain of weed seed bank. Predictive models of weed population dynamics are receiving increasing attention in weed science (Cousens and Mortimer 1995). However, it is difficult to predict weed seed production in the study of seed dynamics because there is no clear relationship between weed infestation and its seed production. The seed production of a weed plant is affected by crop planting pattern and weed control measure (Champion et al. 1998; Medd et al. 1985; Mertens and Jansen 2002; Teasdale and Frank 1983; Teasdale 1998; Wilson et al. 1995; Young et al. 1999). Seed production per plant tends to increase with biomass (Samson and Werk 1986; Thompson et al. 1991). So it is not precise to predict weed seed production based on plant density. Canner and McMaster (2002) tried to estimate the weed reproduction from crop yield loss data. Although this approach sounds reliable, it is difficult in practice. Really seed input of weed seed bank in fields comes from plants that survive from control measures. The objective of this study was to investigate effects of fertilization, herbicide application, and plant density on redroot pigweed seed production and to determine the relationship between seed production and biomass of this weed at harvest. We presumed that weed seed production is a function of weed biomass, so we can predict seed production of a weed species through its biomass.

## MATERIALS AND METHODS

All experiments were carried out in Beijing, China during 2002-2003. In greenhouse experiments, redroot pigweed grew in plastic pots (20 cm diameter, 30 cm depth). Each pot contained three plants. In field experiments, redroot pigweed population was dominant. Other weed species were removed manually or by herbicide treatment and a monoculture situation was maintained during the experiments.

Effect of herbicide on seed production

Fluroxypyr was applied at the 4- to 5-leaf stages and at the rates of 0, 37.5, and 75.0 g a.i. ha<sup>-1</sup> for



the greenhouse experiment and at the rates of 0, 37.5, 75.0, and 150.0 g a.i. ha<sup>-1</sup> for the field experiment. Spray volume was 450 L ha<sup>-1</sup>. The plot size of the field experiment was 12 m<sup>2</sup>.

#### Effect of nitrogen level on seed production

This experiment was conducted in a greenhouse. The application of nitrogen fertilizer (40% urea) was split into basal (40%), 1 month (30%), and 2 month (30%) after seeding of the weed. Application rates were 0, 18.7, 37.5, 75.0, 150 kg ha<sup>-1</sup>.

#### Effect of plant density on seed production

This experiment was conducted in the field. Redroot pigweed densities were designed as 2, 4, 8, 16, 32, 64, 128 plants m<sup>-2</sup>. The weed plants were thinned to desired densities after emergence of all plants. Plot size was 4 m<sup>2</sup>.

#### Experimental design and parameters collected

In all experiments, plots were arranged in a randomized complete block design with 4 replications. Seeds of redroot pigweed were collected from individual plant at 1-week interval from initial seed maturity to complete mature. After all matured seeds were collected; the above-ground biomass of each plant was harvested, dried at 60 °C, and weighed. Seed number of each plant was calculated from the following equation:

$$\text{Seed number} = \text{total seed weight} / 10,000\text{-seed weight} \times 10,000$$

#### Data analysis

Data analysis used SAS. The regression analysis between seed number and biomass of each plant was done for different treatments and pooled data to determine the relationship between two variables.

## RESULTS AND DISCUSSION

The results showed that seed number per plant of redroot pigweed increased with increasing shoot dry weight and the relationship between the two variables was linear regardless of herbicide application rates (Figure 1-2), nitrogen levels (Figure 3), and plant density (Figure 4). The linear correlation coefficients were significant for all data sets. The analysis of pooled data over all experiments showed that the relationship between seed number and shoot dry weight of each plant was linear (Figure 5).

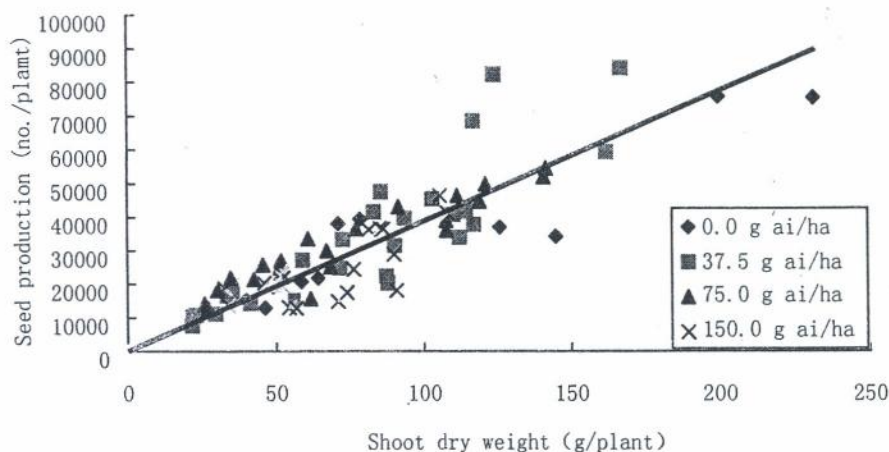


Figure 1. Effect of fluroxypyr on the seed production of redroot pigweed (field trial).  $Y = 386.81X$ ,  $r = 0.9707^{**}$ .

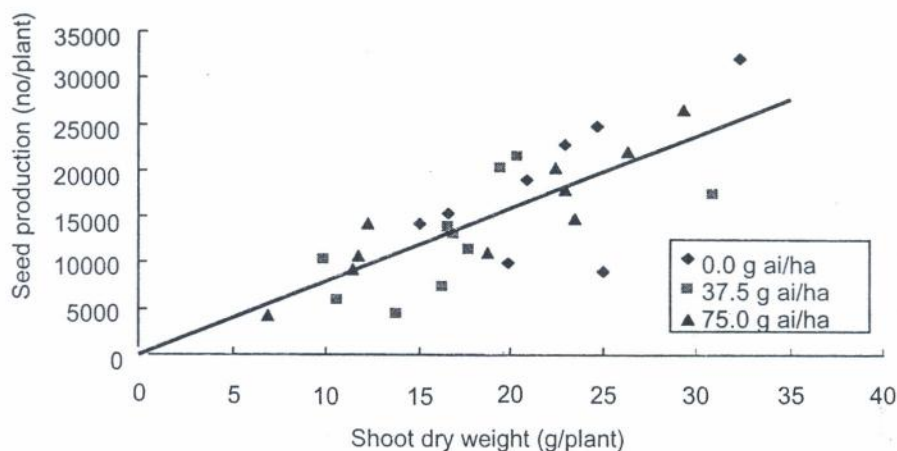


Figure 2. Effect of fluroxypyr on the seed production of redroot pigweed (greenhouse trial).  $Y = 790.99X$ ,  $r = 0.9653^{**}$ .

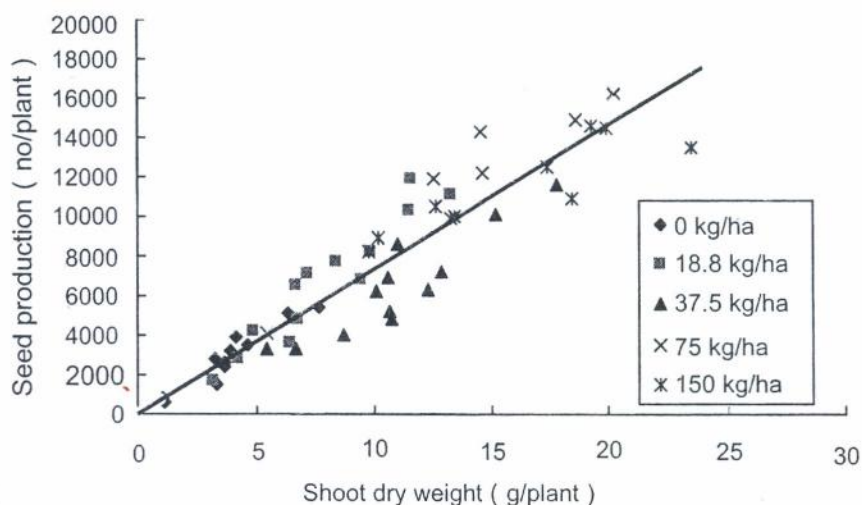


Figure 3. Effect of nitrogen level on the seed production of redroot pigweed.  $Y = 734.93X$ ,  $r = 0.9843^{**}$ .

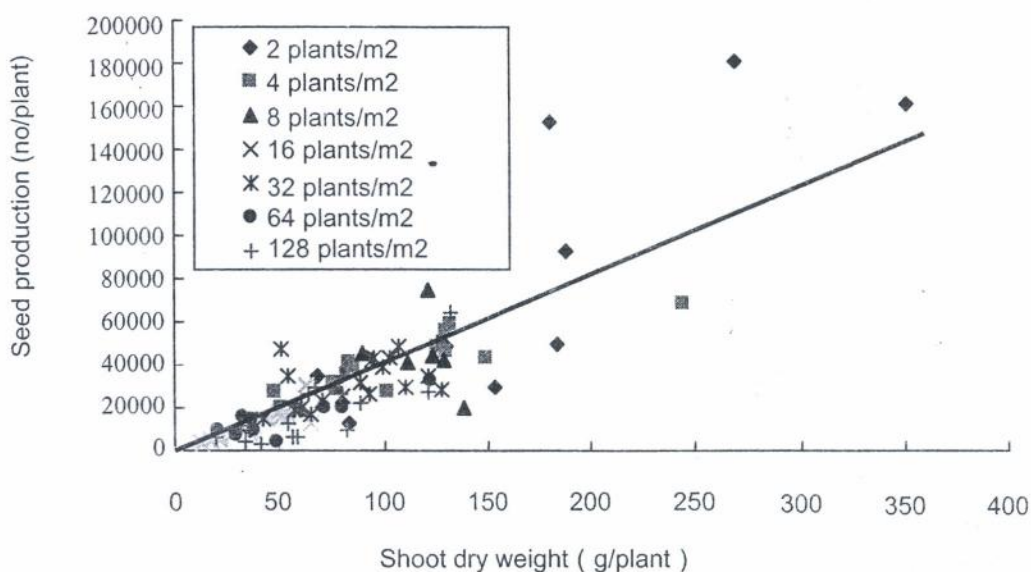


Figure 4. Effect of density on the seed production of redroot pigweed ( $Y = 409.41X$ ,  $r = 0.9196^{**}$ )



The findings of the experiments indicated that there was a linear relationship of seed production of redroot pigweed with its biomass per plant regardless of injury degree due to herbicide application, density, and nitrogen level. Thus seed production per unit of shoot biomass of the weed was constant under the different conditions so that we can predict seed production of the weed through its biomass at harvest. Further studies need to determine whether this relationship is suitable for other weed species. However, the findings in this paper show us a simple way to predict weed seed production.

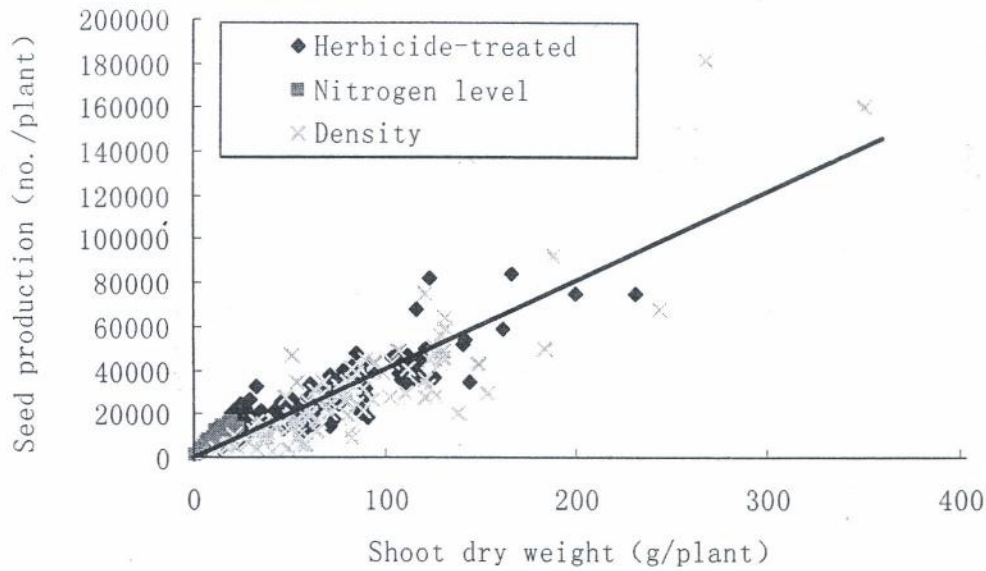


Figure 5. The relationship between the seed production and shoot dry weight of redroot pigweed per plant under different conditions ( $Y = 405.00X$ ,  $r = 0.9334^{**}$ )

### ACKNOWLEDGEMENTS

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# Lambsquarter (*Chenopodium album*) and barnyard grass (*Echinochloa crus-galli*) competition with sugar beet (*Beta vulgaris*) under semi-arid climatic condition

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**Abstract:** This investigation was conducted to study the competitive ability of two weed species, i.e., *Chenopodium album* and *Echinochloa crus-galli*, with sugar beet crop under different pressure of competition experiencing semi-arid conditions. The experiment was carried out at the farm of Khorasan Agricultural Research Center during 2001-2002 growing season. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications; including weed-free treatment. Additive method was adopted where treatments comprised of four different densities of each weed species, viz., 2, 4, 8 and 16 lambsquarters (*C. album*) and 10, 20, 40 and 80 barnyard grass with a fix number of 10 sugar beets per square meter. Various regression methods were tested to find the best model explaining competition relations. Results revealed that lambsquarters more severely damaged final yield than barnyard grass. Even at the lowest density of lambsquarters, i.e., 2 plants m<sup>-2</sup>, a significant reduction in yield of about 15% was observed. Barnyard grass could not make significant damage on yield due to late germination. Sigmoidal model could explain observed data sophistically compared to other models.

**Key words:** *Amaranthus retroflexus*, competition, *Echinochloa crus-galli*, sugar beet

## INTRODUCTION

Sugar beet does not have much competition ability due to its initial sluggish growth. Leaving it alone to grow with weedy species in the field avoiding any support would result in a significant yield reduction. About 80-100 per cent yield losses were reported as a result of weed competition with sugar beet (Cook 1993). For any rational and successful weed management program, it is necessary to determine competition relations, which govern and influence final yield, as competition is the most important identified bilateral interaction in crop production (Cruse et al. 1995). Various experimental and regression models have been proposed for studying competition relations between field crops and weed species, each of which encompasses merits and demerits. Additive, micro-plot and replacement series are among those experimental designs. Relative leaf area and Cousens regression, which express the relation between area and number of leaves with yield, may be mentioned as the most applied regression functions (Kropff 1988, Barrentine 1977).

Determining economic threshold of weeds is one of the most popular and practical use of competition studies. Schaefe (1992) suggested economic threshold must be determined not only during critical period but also during the whole growing season. Norris (1992), in his study on competition between *E. crus-galli* (L.) Beauv. and sugar beet, found that increasing number of this weed beyond 20 stands m<sup>-2</sup> could reduce sugar beet yield up to 80%. He applied densities of 0-200 barnyard grass m<sup>-2</sup> in his studies. Kropff (1987) reported that a density of 5.5 lambsquarter m<sup>-2</sup> made 37% reduction in sugar beet yield. Odonvan (1985) showed that growing one plant of redroot pigweed along with 4 plants of sugar beet simultaneously causes 21% reduction in sugar beet yield. Densities of 50-100 plants m<sup>-2</sup> and 200-300 plants have been practiced for broad and narrow leaf weed species, respectively, in competition studies (Lawrence 1986). Postma (1990) expressed on magnificence of weed threshold as an effective approach to reduce herbicide application. Gerowitt (1992) and Entrup (1991) investigated changes in seed bank in a wheat-sugar beet rotation system and alleged that weed biomass production as well as seed bank could not make significant effects



on weed management in subsequent years. Heyland and Kaiol (1992) believed that by adopting simple simulation models for competition, it is possible to restrict weed management practices and herbicide application. Barrentine and Oliver (1977) combined economic threshold and critical period to determine yield loss in soybean during various growth stages and different densities. All these studies may not proceed unless physiological associations are determined.

## MATERIALS AND METHODS

The present study was conducted at the farm of Khorasan Agricultural and Natural Resource Research Station during the growing season of April-October 2002. The experiment was laid out in a Randomized Complete Block Design adopting split plot method where a combination of two weed species i.e. *Chenopodium album* L. and *Echinochloa crus-galli* as well as their densities, viz., 2, 4, 8, 16 and 10, 20, 40 plant m<sup>-2</sup>, respectively, formed main plots (additive method). Sampling stage was considered as sub plot. Each treatment was replicated thrice. Plots consisted of 6 rows of sugar beet spaced 60 cm apart within 6 m in length with a fixed plant density of 100,000 sugar beet ha<sup>-1</sup>. Hand weeding was included in the experiment. Soil samples were collected from 30 cm depth at different parts of the field before conducting the experiment. Ammonium phosphate and urea at the rate of 100 and 80 kg ha<sup>-1</sup>, respectively, were applied prior to sowing sugar beet. Another 200 kg ha<sup>-1</sup> of urea was applied to the field at the 4-6-leaf stage of sugar beet. To stimulate seed germination, *E. crus-galli* seeds were treated with 98% sulfuric acid for 5 minutes and *C. album* seeds were soaked in tap water for 24 h. Sugar beet was sown on April 28 using precision planter followed by the planting of weed species on the sugar beet rows to obtain desired weed densities. Additional weed and crop seedlings were thinned out at 2-4 leaf stages of sugar beet to maintain default densities. The experimental field was irrigated every 7 days. No pest and disease management took place, as there was no significant infection. Leaf number, weight and dry matter of sugar beet roots as well as weed number and their dry matter were sampled from a 0.5 m<sup>-2</sup> quadrat during the growing season. Sampling was carried out every week during the critical period of competition, i.e., first 2-8 weeks, followed by monthly sampling until the end of the study. Cousens three parameters regression as well as sigmoidal model was fitted to the collected data to estimate competition relations and predict yield losses.

Cousens:

$$Y = Y_{wf}(1 - ID/100(1 + ID/A))$$

Where:

Y: Estimated yield

Y<sub>wf</sub>: yield in weed free condition

I: Yield reduction per cent due to every weed stand while its density moves toward zero.

A: Yield reduction per cent due to every weed stand while its density moves toward infinity.

D: Weed density

Relative competitive Intensity (RCI) was determined as follows:

$$RCI = (B_{mono} - B_{mix}) / B_{mono}$$

Where:

B<sub>mono</sub>: single plant biomass in mono-cropping and

B<sub>mix</sub>: single plant biomass in inter-cropping.

Sigmoidal:

$$Y = a_0 + a_1 / (1 + \exp(-(D - a_2)/a_3))$$

Where:

Y: Estimated yield

a<sub>0</sub>+a<sub>1</sub>: Y<sub>wf</sub> (yield in weed free condition)



- a2: equation coefficient deciding yield reduction in 2nd phase of the model, lower a2 values means faster reduction in yield after lag phase.
- a3: a negative value describing intra species competition. Moving a3 toward zero means higher intra-species competition.
- a1/a0: higher the ratio, higher the yield reduction when weed density  $\rightarrow 0$

## RESULTS AND DISCUSSION

Skewness and kurtosis indexes were calculated prior to any analysis to ensure that the recorded data follow a normal distribution. Original data were analyzed directly as they showed a normal distribution. Results revealed that both *C. album* and *E. crus-galli* could significantly decline root yield of sugar beet ( $p < 0.01$ ). Yield losses due to different densities of *C. album* ranged from 7 to 79.5 %. However such yield reduction was significantly lower in *E. crus-galli*, whose yield losses ranged from 5.5 to 20.6% (Fig. 1)

It was found that Cousens regression model cannot properly explain observed data but they were well fitted to a sigmoidal one. Such outcomes were due to the fact that Cousens model simply ignores insignificant low-yield losses at very initial densities. The lag phase prior to steep yield reduction depends on weed species. However, applying Sigmoidal regression model can describe even variations in this small range of density.

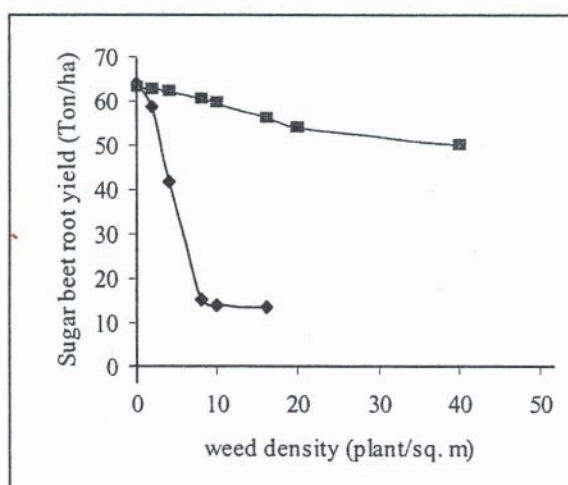


Figure 1. Sugar beet root yield as affected by different densities of lambsquarter and barnyard grass.

Table 1. Estimated yield of sugar beet in lambsquarter and barnyard grass treatments applying the Sigmoidal model.

Weed species	a0	a1	Ywf(a0+a1)	a1/a0	a2	a3	R2	P
<i>C. album</i>	13.62	51.42	65.04	3.77	4.16	-1.15	0.98	<0.001
<i>E. crus-galli</i>	49.75	14.59	64.34	0.29	14.40	-6.29	0.99	<0.001

It can be concluded that *E. crus-galli* has lower competitive ability than *C. album* in various densities. Estimated coefficients confirm this conclusion as a1/a0 ratio is much higher in case of lambsquarter than barnyard grass (Table1). Low value of a2 coefficient in lambsquarter explains why yield has reduced significantly in density just after 2 plants m<sup>-2</sup> but this trend of fast reduction can not be observed in barnyard grass. As it can be observed in Figure 1, yield loss percentage had very minor changes after about 10 lambsquarters m<sup>-2</sup> while in barnyard grass yield losses

percentage was going on in densities under study. It can be understood that intra-species competition in lambsquarter is more intense than barnyard grass. The  $a_3$  coefficients in barnyard grass and lambsquarter (-6.28, -1.14, respectively) support these conclusions. Such differences between these two weed species can be interpreted by the fact that lambsquarter germinated quickly 10 days after sowing but germination of barnyard grass prolonged to about 30 days. On the other hand, lambsquarter had higher growth rate in terms of dry matter accumulation than barnyard grass (Fig. 2). Meanwhile, lambsquarter as a broad-leaved weed is expected to encompass higher competitive ability than barnyardgrass as a narrow-leaved weed species (Cown et al. 1998). In both weed species, individual plants capture higher proportion of growth resources at initial low densities but impose little pressures either as inter or intra-species competition because at these densities growth resources are not limited. This is why Sigmoidal model with three different phases sophisticatedly interpret this type of data. Although biological yield of plants in a inter-cropping system represents their ability in resource utilization (Grace 1995), however, this characteristic may not give a suitable idea regarding competition effects on sugar beet since leaves of sugar beet are continuously produced and senesced simultaneously. Leaf Area Index (LAI) of sugar beet was studied as well. Results showed that LAI dramatically decreased from 4 in weed-free plots to 1 in plots with 16 lambsquarter  $m^{-2}$  95 days after sowing (Fig. 3). Yet such a decline in LAI was not registered even in 40 barnyard grass  $m^{-2}$  (Fig. 3). These results show again that barnyard grass species is not a strong competitor compared to lambsquarter. Barnyard grass is well adapted to humid climatic condition where there is no moisture deficiency, which is not met in semi-arid condition of Khorasan. Keeping in view the differences between these two weed species, one may conclude that critical period of weed competition is not a fixed period of time during the growth season of a field crops. It can vary depend on weed species as well as climatic condition and even other factors affecting plants growth and development.

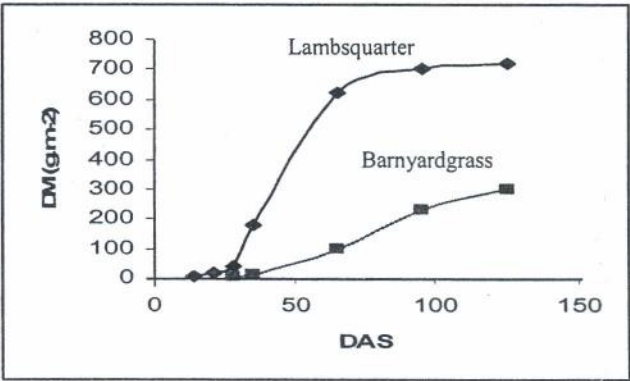


Figure 2. Dry matter accumulation of single plant.

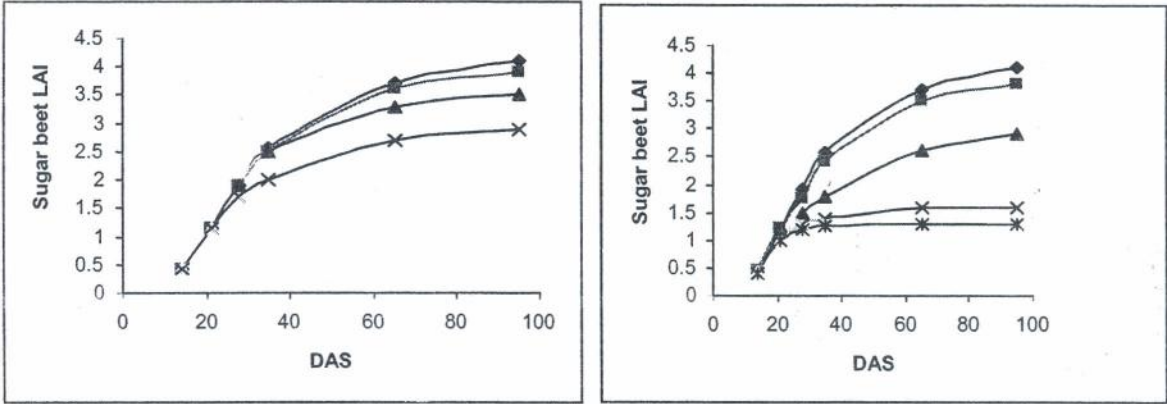


Figure 3. LAI of sugar beet as affected by different densities of lambsquarter and barnyard grass



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**Growth, seed production, and germination of tropical spiderwort  
(*Commelina benghalensis* L.) depending on emergence period and shading conditions**

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**Abstract:** Tropical spiderwort (*Commelina benghalensis* L.) was observed in pot experiments to determine the numbers of spathes and seeds produced by the aerial and underground parts of the plants in different emergence periods. Of the plants from aerial seeds that emerged from March to September, those that emerged in June had the longest plant length and the most numerous first branches, which reached a maximum of about 98 cm long and 12 per plant. Aerial seed production was the highest in the plants that emerged in April, as they produced about 720 large and 2,300 small seeds per plant. Spathes and large and small seeds borne below the ground were also the most numerous in plants that emerged in April. Both large and small aerial seeds began to germinate five weeks after flowering. The plants grown under 18 % shading had the longest plant length and most numerous first branches of all of the plants grown under shading or non-shading conditions, reaching about 54 cm long and 6 per plant. Plants grown under 18 % shading produced the most aerial seeds, as each plant produced about 71 large and 190 small seeds. Spathes and large and small seeds borne below ground were also the most numerous in plants grown under 18 % shading.

**Key words:** Emergence, flowering, seed germination, seed production, shading, tropical spiderwort.

## INTRODUCTION

Tropical spiderwort (*Commelina benghalensis* L.) is a species of the family Commelinaceae and is native to the tropical region of Asia (Takematsu & Ichizen 1997). The species has been designated as noxious weed in 25 different crops in 28 countries including Africa, America, Asia, and Australia (Wilson 1981; Holm et al. 1991). It seems to be the most troublesome in the fields of glyphosate-resistant cotton (*Gossypium hirsutum* L.) in America (Culpepper et al. 2004). In Japan, the species has been found in sandy soil along the seashore in the western area (Kasahara 1968). Recently, however, it has extended its area of distribution, infesting citrus orchards on Kyushu and Shikoku islands.

Previous study has found that seedlings from large and small aerial seeds of tropical spiderwort emerged intermittently over a long period from March to September, under natural conditions (Matsuo et al. 2004). Therefore, we must determine the optimum time of the year to effectively control the species. However, there are no reports on the growth and seed production in tropical spiderwort that emerges from March to September. Also, seed germination after flowering has not yet been studied. Furthermore, the reason of extension to citrus orchards in Japan is still unclear. The objectives of the present study were to evaluate growth and seed production of tropical spiderwort in emergence periods from March to September, seed germination, and growth and seed production under shading conditions.

## MATERIALS AND METHODS

Experiments were conducted at the University of Miyazaki from October 2003 to November 2004. Aerial seeds of tropical spiderwort were collected from plants occurring naturally in the upland fields at the Field Science Center, Faculty of Agriculture, University of Miyazaki. Seeds collected in October 2003 were divided into large and small types and stored at room temperature until required.



## Growth and seed production of plants emerging at different periods

A tropical spiderwort seedling with the first completely developed leaf was transplanted every month from March to September on the soil surface in each pot (30 cm diameter by 40 cm depth). Each pot was replicated three times. The pots were placed in a vinyl house. The height of the plants and numbers of first branches, spathes, and large and small seeds on the aerial part of each plant were recorded every week after transplanting. Each plant more than 50 % dead was sampled from its pot, and the number and total length of the underground branches and the numbers of spathes and large and small seeds on the underground part of the plant were recorded. The transplanted date is described as the emerged date.

### Aerial seed germination after flowering on plants

Flowering aerial spathes were found on plants that developed naturally in upland soil in containers on October 19. Every week after that date, three spathes were collected from the upland plants and the large and small seeds were divided. The seeds were sown on wet filter paper in petri dishes (70 mm diameter by 17 mm depth) and were then incubated in a growth chamber set at 25°C under 12 h light conditions. Seeds germinating in each dish were counted every 2 days for 14 days after seeding. Non-germinated seeds were re-incubated for 14 days after their hilums were removed with a scalpel.

### Growth and seed production on plants grown under shading conditions

Three large aerial seeds were sown on the soil surface in each pot on July 6. After the seedlings were thinned to one per pot at the three-leaf stage, the pots were placed in boxes with light densities adjusted by cheesecloth to 18 %, 50 %, 86 %, and 96 %. The height of the plant and numbers of first branches, spathes, and large and small seeds on the aerial part of each plant, were recorded every week after treating. Each plant more than 50 % dead was sampled from its pot, and the number and total length of the underground branches and the number of spathes and large and small seeds on the underground part of the plant were recorded. Each treatment was replicated three times.

## RESULTS AND DISCUSSION

Tropical spiderwort plants that emerged in March and from April to June reached maximum length in August and in September, respectively. Plants that emerged in June grew the longest, reaching 98.1 cm by September (Fig. 1). The average main stem and plant length were almost equal for all plants that emerged in the study period (Data not shown). Plants that emerged from March to May produced almost the same number of first branches, reaching 5 to 7. However, the plants that emerged in June had the most first branches, reaching 12 by September (Fig. 1).

The spathes and large and small seeds on aerial and underground parts of the sampled plants were counted. The plants that emerged in April produced the most aerial spathes, reaching 658, and aerial seeds, reaching 725 large and 2,282 small per plant. They also produced the most underground spathes (31) and large seeds (17). The plants that emerged in May produced the highest number of small underground seeds (25) (Table 1).

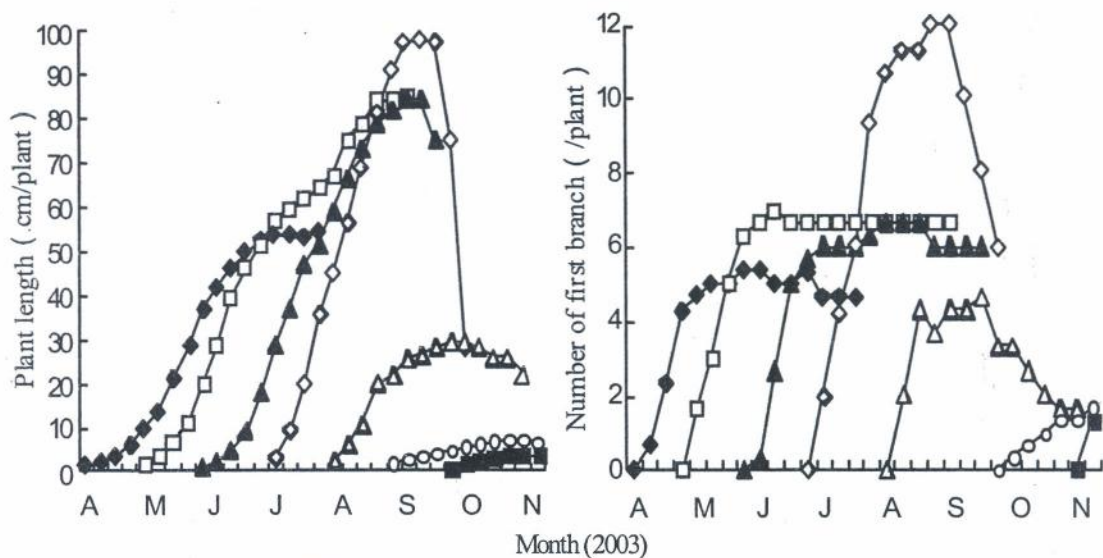


Figure 1. Effects of emergence periods on growth of tropical spiderwort.

Emergence periods : ◆ Mar. □ Apr. ▲ May ◇ Jun. △ Jul. ○ Aug. ■ Sep.

Table 1. Spathes and seed production of tropical spiderwort plants at different emergence periods.

Emergence period	Aerial			Underground		
	Spathes	Large seeds	Small seeds	Spathes	Large seeds	Small seeds
March	484 <sup>b</sup>	574 <sup>b</sup>	1686 <sup>bc</sup>	30 <sup>a</sup>	10 <sup>ab</sup>	23 <sup>ab</sup>
April	658 <sup>a</sup>	725 <sup>a</sup>	2282 <sup>a</sup>	31 <sup>a</sup>	17 <sup>a</sup>	20 <sup>ab</sup>
May	444 <sup>b</sup>	528 <sup>bc</sup>	1853 <sup>ab</sup>	18 <sup>ab</sup>	15 <sup>a</sup>	25 <sup>a</sup>
June	295 <sup>c</sup>	412 <sup>c</sup>	1290 <sup>c</sup>	14 <sup>ab</sup>	11 <sup>ab</sup>	16 <sup>ab</sup>
July	35 <sup>d</sup>	47 <sup>d</sup>	132 <sup>d</sup>	3 <sup>b</sup>	3 <sup>b</sup>	2 <sup>b</sup>
August	1 <sup>d</sup>	1 <sup>d</sup>	1 <sup>d</sup>	2 <sup>b</sup>	—	1 <sup>b</sup>
September	—	—	—	—	—	—

1) Each number indicates average of three plants.

2) The different letters indicate that there are significantly different at the 5 % level by Fisher's LSD test.

In Japan, tropical spiderwort can emerge from March to September (Matsuo *et al.* 2004), but we found that the plants that emerged in April grew the most and produced the most aerial and underground seeds. As the plants that emerged in August grew the least and produced the fewest seeds, emergence of tropical spiderwort plants could be suppressed next year by controlling those that emerge until July.

About 50 % of the large aerial seeds germinated 5 weeks after flowering without treatment. However, 25 % of the non-germinated seeds could germinate after removal of the hilum. After 6 weeks, more than 80 % of the large seeds germinated without hilum removal (Fig. 2a). Small aerial seeds with the hilum removed germinated at 25 % and 100 % within 5 and 6 weeks after flowering, respectively. However, untreated seeds germinated at 28 % in 7 weeks and 60 % in 8 weeks (Fig. 2b). It was found that large and small aerial seeds could mature and germinate as early as 5 weeks after flowering. Therefore, the aerial seeds produced in plants that emerged and grew in the early period could germinate in the same year to produce the next generation.



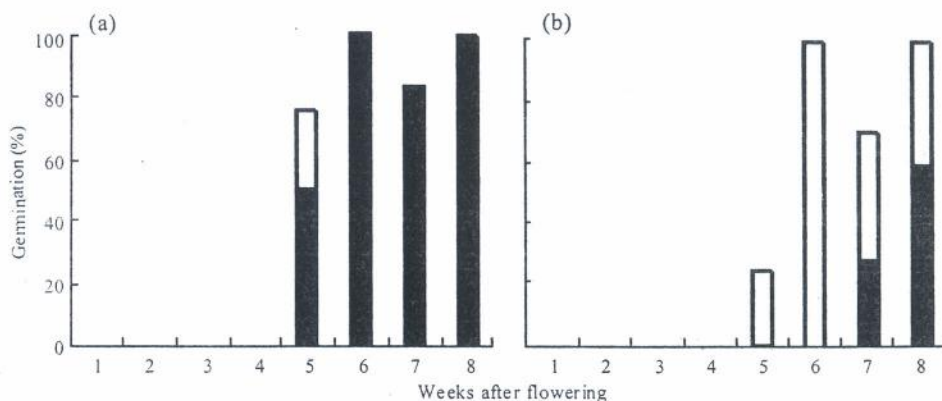


Figure 2. Effects of period on germination of tropical spiderwort. (a) Large aerial seeds. (b) Small aerial seeds. (■), untreated; (□), hilum removed

The tropical spiderwort plants grown under 86 % shading were the tallest until 29 days after treating. After that, the plants grown under 18 % shading became the tallest. The plants grown under 0 % shading were significantly shorter than those grown under shading conditions. The plants grown under 96 % shading grew until 57 days after treatment, and then died. The plants grown under 18 % shading grew the most first branches, having 6 first branches 71 days after treatment. However, there was no significant difference among all of the shading treatments (Fig. 3).

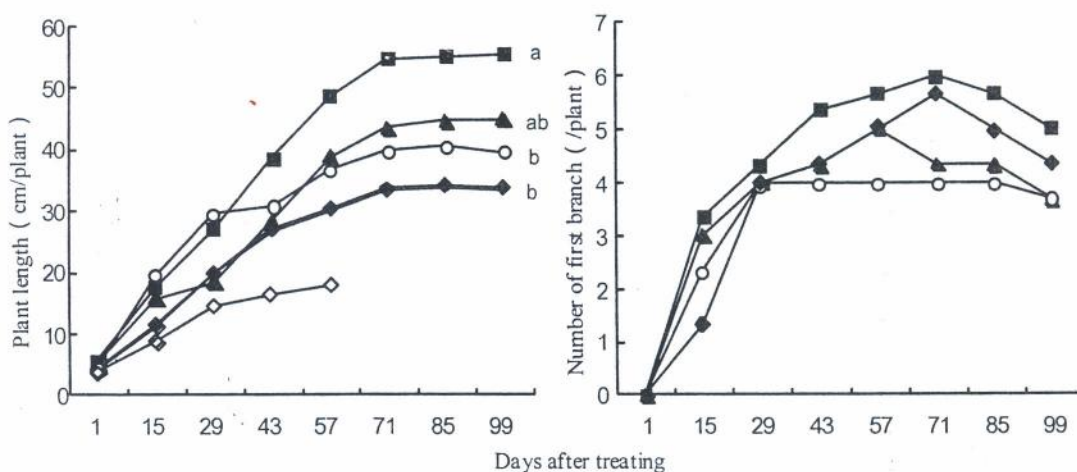


Figure 3. Effects of shading on growth of tropical spiderwort. Means followed by the different letter differ according to Fisher's LSD test ( $p=0.05$ ).

Shading: —◆— 0% (Control) —■— 18% —▲— 50% —○— 86% —◇— 96%

Of the sampled plants, aerial spathes were the most numerous in plants grown under 18 % shading, reaching 49. They also produced the most aerial seeds: 71 large and 190 small. The plants grown under 18 % shading had produced the most underground spathes (13), large seeds (11), and small seeds (11). However, there was no significant difference in the number of spathes and large and small seeds among the shading treatments (Table 2).

Table 2. Spathes and seed production of tropical spiderwort plants grown under shading conditions.

Shading (%)	Aerial			Underground		
	Spathes	Large seeds	Small seeds	Spathes	Large seeds	Small seeds
0	36 <sup>ab</sup>	46 <sup>ab</sup>	134	9	8	16
18	49 <sup>a</sup>	71 <sup>a</sup>	190	13	11	11
50	30 <sup>ab</sup>	43 <sup>ab</sup>	110	6	6	4
86	21 <sup>b</sup>	29 <sup>b</sup>	85	4	3	4
96	—	—	—	—	—	—

1) Each number indicates average of three plants.

2) The different letters indicate that there are significantly different at the 5 % level by Fisher's LSD test.

It was found that tropical spiderwort plants were bigger and produced more aerial and underground seeds under shading conditions than those grown under non-shading conditions. This result is consistent with Kim & De Datta's finding (1993) that tropical spiderwort could survive even under shade conditions. Therefore, controlling plants grown under the shade of citrus orchard trees in Japan will require extensive effort.

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## Notes on some alien invasive plants species in Indonesia\*

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**Abstract:** SEAMEO BIOTROP has been working on weeds for quite sometimes, at present not only weeds which inflict a considerable agricultural loss, degrade catchments areas and freshwater ecosystem but also plant invasive alien species which constitute one of the leading threat to natural ecosystems and biodiversity.

The invasive plant species, some of them have been well recognized as weeds in agricultural production system. Agricultural weeds problems have received more attentions than environmental weed problems.

Important alien invasive plants species have been documented in Indonesia, it is hoped that collections and dissemination of information on their spread alert people for their controls and managements.

Records and distributions of some important invasive alien plant species in Indonesia are discussed i.e.: *Acasia nilotica* (L.) Willd. ex Del., *Austroeupatorium inulifolium* (Kunth) R. M. King & H. Rob, *Chromolaena odorata* (L.) King & H. Rob., *Clibadium surinamense* L., *Eichhornia crassipes* (Mart.) Solms, *Eupatorium sordidum* Less, *Hydrilla verticillata* (L.f. Royle), *Mikania micrantha* Kunth, *Mimosa diplotrica* C. Wright ex Sauvalle, *Mimosa pigra* L., *Passiflora edulis* Sims, *Pennisetum polystachion* (L.) Schult., *Piper aduncum* L., *Salvinia molesta* D.S. Mitchell, *Stachytarpetta jamaicensis* (L.)

**Key words:** Invasive plant species.

## INTRODUCTION

As people move around the world, they bring together plants with them (Weber, 2003). Expanding global trade in agriculture, forestry and other industries that depend on raw materials has allowed the transport of species to various parts of the world including Indonesia.

Alien species are imported to Indonesia for cultivation such as food crops, horticulture, plantation, forest plantation, ornamental aquarium plants, collection of the botanical garden, experimental plants or other curiosities. Aside from plants purposely imported one there are also introduced plant propagules contaminating imported agricultural products.

Not all the alien species are harmful, most of them are crop or ornamental plants. Some species are naturalized and adapted well to the local environment, some become invasive.

SEAMEO BIOTROP has been working on weeds for quite sometimes, at present not only weeds which inflict a considerable agricultural losses, degrade catchments areas and freshwater ecosystem but also plant invasive alien species which constitute one of the leading threat to natural ecosystems and biodiversity.