Allelopathy in Rice

Edited by M. Olofsdotter

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Errata

Page 58, line 36, should read: “poor estimate of the genotype” instead of “poor example of the genotype.”

Page 59, line 27, should read: “...within strata.There are two cultivated species of rice: *O. sativa,* with worldwide distribution, and *O. glaberrima,* which is limited to West Africa, but is a good competitor..,”

Page 60, line I, should read: “...the strategy was refined and a selected sampling was prepared.”

Page 62, line I, should read: “...through correlation between these factors and the principal component axis scores.”

Page 63, line 13, should read: “..according to our present knowledge of the variability of the species.”

Page 64, line 7, should read: “A QTL location is obtained by...”

Page 64, line 3 I, should read: “...of identifying spurious QTLs.”

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P.O. Box 933, Manila 1099, Philippines

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Cover: Some rice cultivars can inhibit the germination andgrowth of weeds (right), whereas most rice cultivars do not affect weed growth (left). This phenomenon is called allelopathy and it could become important in weed management strategies. Photographs by Robert Dilday.

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Contents

Foreword

Weeds have been a persistent problem for farmers ever since agriculture began. Rice farmers, however. had one advantage over farmers growing other crops. Rice has a special ability to grow in water, whereas many weeds cannot. So, very early in the cultivation of rice, farmers used water and transplanted rice seedlings as a control measure for weeds. Farmers now perceive weeds as an increasingly important problem because labor for hand weeding and water for irrigation are becoming scarce.

Rice farming is undergoing rapid change. Labor costs are increasing and labor supplies in rural areas are decreasing as farmers turn to direct seeding of rice to lower costs. Also, competition is increasing for limited water, and agriculture in general (and rice in particular) will have to find new ways to use water more efficiently.

These trends, moving at a rapid pace, mean that research must find ways for the sustainable management of weeds. Farmers are already relying heavily on herbicides—a practice that may have many of the consequences of earlier experiences with other pests such as insects in rice. We must therefore ensure that our research today targets integrated weed management.

As farmers move to new methods of land preparation and seeding, they require new approaches and concepts for weed management. An important component of integrated pest management—for insects or weeds—is not to rely too heavily on any one tactic. For example, varietal tolerance of the pest can be an important component of an integrated approach. Allelopathy may offer one way for rice cultivars to suppress weed growth without affecting the environment. But allelopathy as a science is in its cradle and we still have a long way to go before allelopathy will be ready for adoption by farmers. At this stage in research, because dialogue and collaboration are essential for rapid progress, a workshop that could pinpoint problems and focus research was necessary. This book represents the outcome of such a workshop on allelopathy in rice, held at IRRI in November 1996, where scientists from around the world working on rice allelopathy gathered to discuss their research. This book could therefore be seen as the state of the art in rice allelopathy research. It is my hope that it will provide guidelines for further research in this area, which could give Asian farmers a tool for sustainable weed management.

KENNETH S. FISCHER
Deputy Director General
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Acknowledgments

The outcome of this workshop represents the contributions and dedication of many individuals, including all of the participants and IRRI staff involved with both the preparation and coordination of the workshop itself, as well as the coordination and editing of this book. I would like to thank all of you for your contributions.

This workshop was the first time all scientists working on allelopathy in rice germplasm had an opportunity to be together and I, as the organizer of the workshop and editor of this book, would like to thank the Danish government and the Danish International Development Agency for providing the money for this workshop. It was a wonderful opportunity and many fruitful connections and plans for the future were made. A special thanks goes to Digna Izon-Salisi for her skills in coordinating all the arrangements at the workshop itself.

The outcome of a workshop is never realized until it reaches a wider public. This book represents one such outcome. In the work editing this book, I have had help from several external reviewers, whom I would like to thank. They are Stephen Duke (Oxford, Mississippi), Rick Willis (University of Melbourne), Jeff Weidenhamar (Ashland University), Yoshiharu Fujii (Tsukuba University), Robert Hoagland (USDA, Stoneville, Mississippi), and, finally, Frank Einhellig (Missouri State University). Thank you for your energy and enthusiasm in reviewing the papers published in this book. I would also like to thank Domenic Fuccillo and Bill Hardy for their patient editing of these papers.

CHAPTER 1

Allelopathy in rice

M. Olofsdotter

Weeds are a major constraint to rice production worldwide. In traditional Asian rice production, they are managed by hand weeding. But today's fast industrialization in many rice-producing countries offers the labor force an opportunity to earn more money outside the agricultural sector, and labor is in short supply for hand weeding. Water has always been used in combination with transplanting of rice as a tool to reduce weed pressure by timely flooding at weed emergence. Now water is also becoming scarce because other sectors pay more for it than does the agricultural sector.

Shortages of labor and water are putting pressure on farmers to change their cultivation practices from transplanting of rice to direct seeding. In direct-seeded rice systems, weeds can become a severe problem when weeds and rice emerge together and early flooding of fields is not a possible weed control action until rice seedlings are 2-3 wk old. The only available weed management strategy is therefore herbicides, and Asia has rapidly increased their use. Herbicides are easy to use and, if used properly, provide the cheapest and most reliable weed management in rice. Concerns about negative effects of herbicide use, however, such as environmental contamination, development of herbicide-resistant weeds, and human health problems, make it necessary to diversify weed management options. The use of allelopathic behavior of the rice crop is one of the new options for sustainable weed management.

Most allelopathy research has been done in natural ecosystems, where flora changes and succession have been explained by competitive allelopathic interactions. Only recently has the use of allelopathic potential as a tool in crop protection become an area of scientific interest. Research on allelopathy in rice started in the United States in the late 1980s (Dilday et al 1991), and after discovery of the phenomenon in that crop the number of groups working on rice allelopathy has increased rapidly. The magnitude of weed problems in rice and increased environmental concerns about pesticide use have been the driving forces behind this research. Progress in these studies will depend on development policies.

As weed problems increase in most rice-growing systems, the poorest farmers will benefit most from the development of allelopathic rice cultivars. A weed control method inherent in seed promises them the only affordable and feasible alternative to hand weeding. In slash-and-burn systems, fields are used until the weed pressure

becomes unmanageable — usually after only 3 yr of cultivation. If rice farming could be prolonged by using allelopathic cultivars, the whole system would stabilize.

More than half of the world's population lives in Asia, where rice is the major staple food, accounting for more than a third of daily calorie intake and a fourth of the protein. Population growth in rice-consuming nations, by conservative estimates, will demand a 65% increase in rice production from 525 to 800 million t over the next 30 yr (Naylor 1996). This increase in production must be provided using environmentally sound agricultural practices. To feed the coming generations of rice consumers, we must develop all possible tools to enhance the sustainability of agroecosystems. Allelopathy holds promise for achieving the goal of sustainable food production for everyone.

Plant interference results from the combined effects of allelopathic and competitive interactions among plants in a field or a population. In the field, the two components of interference cannot be distinguished. From a farmer's perspective, it doesn't really matter if weed reduction is caused by competition or by allelopathy, as long as weed reduction is a reality. From a scientific perspective, however, we must be able to distinguish between the two effects or we will fumble in the dark when trying to optimize both effects for maximum weed reduction. Although this book discusses only the potential use of allelopathy for weed management. we must still keep competitive ability in mind as an important factor in plant-to-plant relations in the field.

Allelopathy is the direct influence from chemicals released from one plant on the development and growth of another plant. The effect has been known for many years but has only recently been accepted scientifically as a legitimate area of research. Research shows that allelopathy plays a significant role in natural ecosystems and has the potential to become an important tool in cultivated ecosystems. One of the reasons why scientists have questioned the importance of allelopathy is the lack of identified allelochemicals. As chemical identification procedures have become more advanced, however, biologically active substances with phytotoxic potential, by themselves or in interaction with other chemicals, have been found to explain allelopathic behavior.

Rice accessions with allelopathic activity have different origins and are at different stages of improvement (Dilday et al, this volume, Chapter 2). This means that allelopathy is widespread and already unconsciously included in many breeding programs. To improve natural weed-fighting capacity in rice cultivars, however, we need conscious selection for allelopathy and competition. Knowledge about the inheritance of allelopathy would be helpful in designing a breeding strategy. Preliminary genetic studies indicate that allelopathy in rice is a quantitatively inherited feature. As such, it has to be included in the breeding process at an early stage and cannot be genetically modified in varieties that are already high-yielding. Activity against a broad spectrum of target weeds would be desirable in an allelopathic rice cultivar, and therefore continued collaboration between weed scientists and plant breeders is a prerequisite for such development. With allelopathic potential widely spread in germplasm, breeders can develop locally adapted allelopathic cultivars for taste and

the environment in a given geographical area. This research can be done through "farmer participatory breeding," with breeders and weed scientists working together.

Since the start of rice allelopathy research (Dilday et al 1991), groups at IRRI and in Egypt, Korea, Japan, India, Thailand, and the United States have undertaken research to understand and improve allelopathy in rice. Several of these groups are represented in this volume. Allelopathic potential against one weed species has been found in up to 3.4% of the screened material (Dilday et al, this volume, Chapter 2, Hassan et al, this volume, Chapter 3, Olofsdotter et al 1997). The percentage decreases when we start to look for cultivars with allelopathic effects against two or more weed species. Thus, a rice cultivar allelopathic against one weed species will not necessarily be allelopathic against other weed species. This selectivity indicates that several chemical compounds with specific action against weeds are involved in allelopathy. It also suggests that allelochemicals in rice are specific molecules probably not found among the most widespread secondary metabolites.

One of the rice cultivars (Taichung Native 1) included in several of the studies has shown an allelopathic effect against four important weed species (*Echinochloa crus-galli, Trianthema portulacastrum, Heteranthera limosa,* and *Ammannia coccinea* Rottb.) (Dilday et al, this volume, Chapter 2, Olofsdotter and Navarez 1996). This cultivar also carries the gene for semidwarfism that is present in all modern varieties of rice, a relationship that explains why many modern rice varieties have allelopathic potential. Some cultivars giving promising results at IRRI have also been tested under field conditions in Korea, with results comparable to those obtained in the Philippines (Kim and Shin, this volume, Chapter 4). These are promising results for breeders because it is important to them for a character to be stable over several seasons and environments.

The possibilities are exciting for the practical use of allelopathy for weed management, but many problems remain to be solved and questions asked. First, we need to screen more rice cultivars for allelopathy and to develop techniques to select for allelopathy at an earlier stage in the breeding program, when the number of seeds is limited. Second, we need to know which chemicals cause allelopathic effects. Many secondary metabolites in plants have phytotoxic effects, and such chemicals can be found in almost all plant species (Putnam 1986). For these chemicals to be classified as allelochemicals effective against weeds, however, we must know that they are released from a living plant. All research on allelochemicals should therefore concentrate on these released compounds.

Another question to address is the physiological cost of allelopathy. Production of allelochemicals might require energy from the plants and use resources that would otherwise be used for kernel production. So far, no results point in the direction of a high yield penalty caused by allelopathy, but this possibility needs to be verified experimentally. Actually, the normally distributed and relatively high frequency of allelopathy in breeding material suggests a weak correlation with traits already included in the selection process, such as yield and disease resistance. But the physiological costs, large or small, may determine the target ecosystems where allelopathic rice will be suitable.

The deliberate release of phytotoxic chemicals, such as allelochemicals, commonly happens. These natural products must be biodegradable, otherwise they would be evident in the environment. It is possible that the continuous growth of allelopathic cultivars might result in the development of resistant weed biotypes. Thus, we must find out whether allelochemicals play a role in resistance development and, if they do, develop a strategy to avoid resistant weeds. If allelochemicals act synergistically by different modes of action, allelopathic cultivars would not likely create a resistance problem, but we still need to confirm this theory.

The ecotoxicological consequences of deliberate allelochemical release should also be studied carefully. In a situation where allelopathic cultivars could be grown on large areas, allelochemical releases could become an environmental problem. Even though concentrations outside the rice field would be low, they could create changes in the fauna and flora in nontarget environments. As most rice is grown under irrigated conditions and contamination of water is a concern, it is important to study allelochemical effects on organisms such as fish, birds, and snails,

Finally, plant interference resulting from the combined effect of allelopathy and competition should be seen as a component of integrated weed management (IWM). We should investigate links between allelopathic rice and a shift in herbicide use from preemergence to postemergence applications that would give us the opportunity to spray if necessary, and not as a precaution. New knowledge about the possibilities for using allelopathy for weed control will also raise many questions about naturally occurring defense mechanisms in plants. Weed science might require a paradigm shift —where breeding is viewed as a possible strategy for selective weed management. Cultivars with "resistance" genes for one or several specific weed problems could be developed in the same way that disease-resistant cultivars have been developed. With continued investigations over the next few years, rice allelopathy could possibly become a useful component of IWM systems that could lead to the use of this phenomenon for weed control in crops other than rice.

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CHAPTER 2

Allelopathic activity in rice for controlling major aquatic weeds

R.H. Dilday, W.G. Yan, K.A.K. Moldenhauer, and K.A. Gravois

Of the more than 16,000 rice accessions or varieties from 99 countries in the germplasm collection of the U.S. Department of Agriculture's Agricultural Research Service, a substantial number have been evaluated for allelopathic effects on aquatic weeds — about 12,000 for ducksalad [*Heteranthera limosa* (Sw.) Willd.] and around 5,000 for redstem (*Ammannia coccinea* Rottb.). In field tests during 1988-90, researchers identified 412 rice accessions that produced an area of allelopathic activity around the rice plant greater than 10 cm for ducksalad and 145 accessions that produced the same area of activity for redstem. The accessions demonstrating allelopathic effects on ducksalad originated in 31 countries. A hybrid between PI 338046 (allelopathic) and Katy (nonallelopathic) had superior agronomic characteristics in field tests, and significantly fewer ducksalad plants were found growing with this hybrid in greenhouse tests. Preliminary genetic data indicate that allelopathic activity in rice is quantitatively inherited.

Introduction

Allelopathy was coined by H. Molisch in 1937 from allelon ("of each other") and pathos ("to suffer"), although the definition covered both detrimental and beneficial biochemical interactions among all classes of plants, including microorganisms. Rice (1984) defined allelopathy as "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment" (Rizvi and Rizvi 1992). The concept of allelopathy is ancient in agricultural science. Theophrastus (ca. 300 B.C.) referred to "phytotoxicity" among plants in *Enquiry into Plants*. Democritus reported the use of naturally occurring plant products as a practical method of controlling weeds, and said that trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice. Plinius (A.D. 1) cited examples of apparent allelopathic interactions in *Natural History,* including the use of chickpea, barley, fenugreek, and retch to scorch up (meaning to exhaust or destroy) cornland (Rizvi and Rizvi 1992).

The word today refers to the study of the production of allelochemicals, mostly secondary metabolites, by a plant that can either harm or benefit another plant. Research indicates that a biochemical interaction takes place when allelochemicals pro-

duced by one plant escape into the environment and influence the growth and development of another plant. Putnam and Tang (1986) stated that chemicals with allelopathic potential exist in virtually all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, and seeds. Allelochemicals are released by such processes as volatilization, root exudation, leaching, and decomposition of plant residues (Rice 1984, Putnam 1986).

Allelopathy is postulated as one mechanism by which weeds affect crop growth, and it occurs widely in natural plant communities (Bell and Koeppe 1972, Gressel and Holm 1964, Whittaker and Feeny 1971). The allelopathic potential of weeds, through the release of toxic substances into the environment either by root exudation or from decaying plant material, has been demonstrated in about 90 species (Putnam 1986). These include quackgrass [*Agropyron repens* (L.) P. Beauv.] (Gabor and Veatch 1981, Kommedahl et al 1959), yellow and purple nutsedge (*Cyperus esculentus* L. and *C. rotundus* L.) (Friedman and Horowitz 1971), Johnsongrass (*Sorghum halepense* (L.) Pes.] (Abdul-Wahab and Rice 1967), Canada thistle [*Cirsium arvense* (L.) Scop.] (Bendall 1975), leafy spurge (*Euphorbia esula* L.) (LeTourneau et al 1956, LeTourneau and Heggeness 1957), giant foxtail (*Setaria faberrii* Herrm.), yellow foxtail [*Setaria glauca* (L.) Beauv.], velvetleaf (*Abutilon theophrasti* Medic.) (Elmore 1980), and tall fescue (*Festuca arundinacea* Schreb.) (Peters 1968, Peters and Luu 1984). In addition, some crops also possess allelopathic activity or weed-suppressing activity, including rye (*SecaLe cereale* L.) and wheat (*Triticum aestivum* L.) (Shilling et al 1985), sunflower (*Helianthus annuus* L.) (Leather 1983), oat (*Avena sativa* L.) (Fay and Duke 1977), barley (*Hordeum vulgare* L.) (McCalla and Haskins 1964), tobacco (*Nicotiana tabacum* L.) (Patrick et al 1963), and rice (*Oryza sativa* L.) (Dilday et al 1989, 1991, 1994). Putnam and Duke (1974) suggested that "wild types" of existing crops may have possessed high allelopathic activity, and this character was reduced or lost as they were hybridized and selected for other characteristics.

More than 50 weed species infest direct-seeded rice and cause major losses in U.S. rice production (Smith et al 1977). Ducksalad [*Heteranthera limosa* (Sw.) Willd.], an aquatic weed that can reduce rice yield by 27-30% when competing with rice in a water-seeded culture (Smith et al 1977, Smith 1988), is second only to barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] as the most frequently reported weed in ricefields, followed by hemp sesbania [*Sesbania exaltata* (Raf.) Cory], bulrushes (*Scirpus* spp.), red rice (*Oryza sativa* L.), broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash], and sprangletop (*Leptochloa* spp.) (Chandler 1981).

The germplasm collection of the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS) contains more than 16,000 rice accessions or varieties from 99 countries. We are evaluating these materials for allelopathic effects on aquatic and dry-seeded weed species to identify accessions that can be used for varietal development. Thus far, about 12,000 accessions have been evaluated for allelopathic effects on ducksalad and about 5,000 for redstem, and promising germplasm has been identified. Developing and using this germplasm should help to reduce the

need for herbicides and result in improved water quality and less environmental contamination.

The specific objectives of this study were to (1) evaluate the USDA-ARS rice germplasm collection for allelopathic activity against aquatic weeds in rice, (2) report all the accessions that have demonstrated apparent allelopathic effects in the field on ducksalad and redstem, and (3) identify common ancestors within groups of germplasm that have demonstrated allelopathic properties.

Materials and methods

History and experimental work, 1985-86

R.H. Dilday first observed apparent allelopathic activity against ducksalad in rice in 1985 and 1986 field tests at the Rice Research and Extension Center, Stuttgart, Arkansas, while evaluating accessions in the USDA-ARS collection for tolerance of alachlor. As a later part of the USDA-ARS evaluation of rice germplasm, field and laboratory experiments were conducted from 1987 to 1996 to identify rice accessions showing allelopathic effects on ducksalad, redstem, broadleaf signalgrass, rice flatsedge (*Cyprus iria*), sprangletop, and barnyardgrass. Results of ducksalad and redstem experiments are reported here.

Approximately 12,000 accessions, including checks (about 5,000 in 1988 and 1989 and 2,000 in 1990), were seeded in hill plots in April each year in a 0.75 × 0.75-m grid, with 5–7seeds placed in each hill. The field tests were replicated twice on a Crowley silt loam (fine montomorillonitic, thermic Typic Albaqualfs). Because a natural and uniform infestation of ducksalad occurs regularly at Stuttgart and occurred in these tests, seeding the test plots with ducksalad was not considered necessary. Allelopathic effects on ducksalad were recorded each year in July at about the panicle initiation stage for most of the accessions. Two methods were used to record the activity: (1) the radial area (cm) from the base of the rice plant that was affected, and (2) the percentage of weed control within the affected area based on the number of ducksalad plants in a check plot around a control plant that had no apparent allelopathic effect on ducksalad. In the first method, actual measurements were made from the base of the plant to the outermost edge of the area of activity, defined as the area around the plant where no ducksalad growth appears or a reduced stand of ducksalad is present. If an accession had a mean radial area of activity of less than 10 cm, then the accession was rated as having no allelopathic activity. The second method measures the reduction in the ducksalad population (plants m^{-2}) compared with a control plant that has a slight or no affect on the ducksalad population.

A total of 82 kg ha^{-1} N as urea was applied when the seedlings were at the 4th true leaf stage. The remaining 41 kg ha^{-1} N were applied in two equal increments about 23 d after the first application each year and then 12 d later. The test plots were irrigated twice in May each year to ensure uniform seedling emergence, and permanently flooded in June. Plant height (cm), days to maturity, plant type, panicle type, hull cover or pubescence, hull color, lemma color, awning, lodging, and grain type

were recorded for each accession. Plant height was measured from ground level to the tip of the mature panicle. Maturity was determined by calculating the number of days from the date of seedling emergence to the date that 50% of the panicles had emerged. Plant type, hull cover, hull color, lemma color. and awning were recorded in the laboratory after threshing. The accessions were characterized as having short (4.50 mm), medium (5.51–6.60 mm), or long (6.61–7.50 mm) grain. In 1988, the 5,000 accessions (including checks) were also evaluated for allelopathic effects on redstem because of the uniform population of volunteer redstem plants.

Breeding and enhancement

Accession PI 338046, which had shown allelopathic activity, was hybridized with Adair, Alan, Katy, Lemont/RA73, M-201/Katy, and Newbonnet in 1991. The F_1 hybrids were grown in the greenhouse in the winter of I991-92, the F_2 population at Stuttgart in 1992, the F_3 populations in Puerto Rico in the winter of 1992-93, and the F_4 and F_5 populations at Stuttgart in 1994 and 1995, respectively. Seven F_6 lines were field-evaluated for field and milling yield, days from emergence to heading, plant height, lodging, and other agronomic characteristics in 1996. The center four rows of each plot (1.3 × 3.8 m) were harvested. The lines were evaluated for allelopathic effects on ducksalad in 37.5-cm-diam plastic pots in the greenhouse in the winter of 1995-96.

Genetics

Another accession, PI 312777, which showed allelopathic activity, was hybridized with Lemont in 1994. The F_1 and F_2 populations were grown in the field at Stuttgart in 1995 and 1996, respectively. About 400 F_2 plants were evaluated for allelopathic effects on ducksalad in 1996 using area of activity around the plant as described earlier. Natural infestation of ducksalad at Stuttgart is usually heavy enough to observe allelopathic activity around a single plant, but in I996 the PI 312777/Lemont F_2 population was an exception and it could be divided into six groups that showed allelopathic activity.

Results and discussion

Ducksalad and redstem activity

In 1988, a total of 190 accessions had a radius of activity around ducksalad of >10 cm and these accessions were significantly different from the control, Rexmont (Table 1). In 1989, another 155 accessions from a different set of 5,000 had a radius of activity around ducksalad of >12 cm (Table 2). In 1990, 67 accessions from a different set of 2,000 had a radius of activity around ducksalad of >12 cm (Table 3).

Accessions that demonstrated allelopathic activity in 1988, 1989, and 1990 originated in Afghanistan, Argentina, Australia, Brazil, Colombia, Dominican Republic, Egypt, France, India, Indonesia. Iran, Iraq, Israel, Italy, Japan, Malaysia, Mali, Mexico, Pakistan, People's Republic of China, Peru, Philippines, Portugal, Republic of Korea, Soviet Union, Spain, Taiwan, Thailand, Turkey, United States, and Vietnam.

Table 1. Rice accessions (190) from the USDA-ARS germplasm collection that showed allelopathy against ducksalad in a field experiment at Stuttgart, Arkansas, in 1988.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
India AC 1423	85	18	297816	India
Tono Brea 439	85	18	420243	Dominica
Unkn LGVR (not CI 8720) NSSL 10/78	85	18	502794	USA
PHIL MGVR	90	15	4063	Philippines
UNMD MGVR-China PR	90	15	7109	China
Tsai yuan chon	90	15	294400	Taiwan
IR781-138-1-1-1	90	15	348793	Philippines
Unkn MGVR (not CI 8669) NSSL 10/78	90	15	502791	USA
Unkn LGVR (not CI 9131) NSSL 3/77	90	15	502812	USA
RXRE//250/MGNL	85	15	9553	USA
NATO/9209SEL//AROS/3/NROS	85	15	9844	Unknown
Pai jh tsao	85	15	160596	China
Kolamba K184	85	15	240636	India
Taichung Native 1 (TN-1)	85	15	271672	Taiwan
Donduni kunluz	85	15	277407	Afghanistan
Red khosha cerma	85	15	277414	Afghanistan
IR32 64 1 2	85	15	312571	Philippines
T65/2X T(N)1	85	15	312777	Philippines
CP231/SL017//Nahng mon	85	15	321184	Philippines
IR644 1 63 1 1	85	15	338046	Philippines
IR782 131. IR8X	85	15	338065	Philippines
I-Kung-Bau 4-2	85	15	346414	Taiwan
IR667-80-6-1-2	85	15	348786	Philippines
IR1323-115-2-1	85	15	350354	Philippines
Mon Z Wuan	83	15	389680	China
IR80 54 2 2. T VUOT X PI215936	80	15	321369	Philippines
IR943 18	80	15	338050	Philippines
IR527 1 57 2. TN-1 X ADT 27	70	15	338123	Philippines
UNMD MGVR-China PR	90	13	7071	China
UNMD MGVR-China PR	90	13	7074	China
BR41//Akaho/SBLR	90	13	9066	USA
C4-157/RXOR	90	13	9078	USA
LACR/MGNL//Cent/RXOR	90	13	9595	USA
Caloro I1 Y 316	90	13	160513	China
S 12 DZK-India	90	13	247887	India
Changhwa DG X T(N)1 5199	90	13	312646	Philippines
IR5321653	90	13	331483	Philippines
IR533823	90	13	331501	Philippines
SH 30 21 (Taiwan)	90	13	337362	Taiwan
Kaohsiung shen 2	90	13	338550	Taiwan
Kuanmin gea goo	90	13	389699	China
Hukoku mochi seln (CI 8685-1)	90	13	502698	USA
Huku kaisen mochi seln (CI 8686-1)	90	13	502699	USA
UNMD MGVR-China PR	85	13	5486	China
Peh hkhak	85	13	8352	China
Century Patna 52 (CP52)	85	13	9002	Unknown
Brun seln/ZNTH (same as CI 9209)	85	13	9113	Unknown
CI 9122/RXOR	85	13	9468	USA
LACR/S426A//ZNTH	85	13	9579	USA
CI 9209SEL/9210SEL//CI 9408	85	13	9947	Unknown

Continued

Table 1 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
Calrose 76	85	13	9966	USA
PR 428 Seln (CI 9224-1)	85	13	12326	Unknown
UNMD MGVR-Spain (CI168934-3)	85	13	12419	
Catetos seln (8922-2)	85	13	12512	Unknown
Hung	85	13	160451	China
Yang ku tsi	85	13	160590	China
Jh pen chin chih tao	85	13	160667	China
Yen fang chu 1 hao	85	13	160752	China
Chuan	85	13	160824	China
Yu hsuan	85	13	160827	China
Hsuan jh	85	13	160829	China
Chiu chiu ku/nan tao hao	85	13	160901	China
Yen tu T 18	85	13	161050	China
Javanese 58	85	13	164982	Turkey
Nante	85	13	165646	China
Dhan	85	13	165981	India
Egyptian rice	85	13	167009	Turkey
Mamoriaka	85	13	201838	Madagascar
UNMD LGVR Portugal	85	13	225744	Portugal
Chikara senbon	85	13	226158	Japan
India T 43	85	13	229272	India
S 67 - India	85	13	233894	India
Sathra 278 (very early)	85	13	247879	India
NC 7/37 - India	85	13	247880	India
Taichung hung shou guo zee	85	13	248485	Taiwan
Rover cella	85	13	248521	Italy
Afghanistan NO. 2	85	13	256340	Afghanistan
Taichung 16	85	13	277253	Taiwan
Cesariot	85	13	281758	France
India AC-2250	85	13	282120	India
H 207 (X10) - Taiwan	85	13	303680	Taiwan
I Kung pao 5 3 4	85	13	303681	Taiwan
IR52 30 6 2	85	13	312580	Philippines
I Geo tze	85	13	312645	Philippines
IR 238-	85	13	321183	Philippines
Bin ta pan	85	13	325820	Thailand
IR474 25 1	85	13	331543	Philippines
IRRI Seln	85	13	331652	Philippines
IR1061 41	85	13	338038	Philippines
IR527 40. TN-1X ADT 27	85	13	338124	Philippines
IR503 25 PD21 C2. PAK 2644	85	13	338941	Pakistan
IR506 12 PD12 C1. PAK 2670	85	13	338944	Pakistan
IR667-89-2	85	13	345807	Philippines
IR667-1-66-3-3-2	85	13	348784	Philippines
IR667-98-2-3-2	85	13	348788	Philippines
IR781-92-2-2-1	85	13	348791	Philippines
IR1323-73-1-2	85	13	350352	Philippines
IR1323-82-3-2	85	13	350353	Philippines
IR667-112-2-3-2-3-2	85	13	350440	Philippines
Dulatom 250 (Dular 250-3 MUT)	85	13	356011	Pakistan
IR1163-153-2-3	85	13	372992	Philippines
Early Rose (Brazil)	85	13	388444	Brazil

Continued

Table 1 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
Low li hung	85	13	389668	China
IR1480-125-2-3	85	13	402541	Philippines
Unkn MGVR (not CI 8665) NSSL 3/77	85	13	502790	USA
Unkn LGVR (not CI 8823) 83 STG MI-102	85	13	502796	USA
Unkn LGVR (not CI 9124) NSSL 10/78	85	13	502811	USA
MSHB/KINAI//Caly/SHMD	80	13	9056	USA
Desvaux II	80	13	231643	Japan
Taichung pai co	80	13	279153	Taiwan
IR238 149 2	80	13	321351	Philippines
IR532 1 65 3 2	80	13	338039	Philippines
IR532 1 128 3 1	80	13	338041	Philippines
IR626 1 28 2 2	80	13	338052	Philippines
CH 242	80	13	338113	Philippines
SAN-IN 73	80	13	346408	Japan
UZROS F-13	80	13	346928	Uzbekistan
IR1529-521-2	80	13	373089	Philippines
Pierrot	75	13	244082	Italy
Nung yu 1830	75	13	279150	Taiwan
IR528 1 57	70	13	338125	Philippines
H 1 (Taiwan)	93	10	338511	Taiwan
Tsao sheng hsu	90	10	160730	China
BB50/CROS XB 7 (Australia)	90	10	298959	Australia
IR548 9 1 3	90	10	338127	Philippines
IR643-75-1-1	90	10	345920	Philippines
IR665-1-132-3	90	10	345923	Philippines
IR781-497-2-3	90	10	348798	Philippines
IR1317-385	90	10	348835	Philippines
IR1317-386	90	10	348836	Philippines
IR1317-392	90	10	348837	Philippines
IR1321-12	90	10	348842	Philippines
CR52-3 (India)	90	10	373020	India
IR788-16-1-1-1	90	10	373026	Philippines
KROS/BROS//BR41 Seln	85	10	9067	Unknown
Short strawed NATO	85	10	9547	USA
BB50/cros 1-7-18-3-2 seln (298965-2)	85	10	12496	Unknown
BB50/cros 1-7-18-3-2 (CI 298965-2)	85	10	12497	Unknown
Dae kol bu	85	10	162189	China
Lambaygue 1	85	10	180177	Peru
Turkey 10657	85	10	182257	Turkey
Muga	85	10	187079	Portugal
Precosur 1	85	10	223494	Argentina
Victoria	85	10	223495	Argentina
Hattan 10	85	10	224815	Japan
Reata (Riata)	85	10	230105	Mexico
Melanothrix	85	10	231649	Japan
Victoria tardio seln 2	85	10	238496	Argentina
Johna 349 (early)	85	10	247882	India
Mahlar 346	85	10	247883	India
BB50/cros 17 24 1 2 (Australia)	85	10	298966	Australia
Lui chou 25 108 30	85	10	303683	Taiwan
IR36 27 1 1	85	10	312524	Philippines
IR34-56-3-2	85	10	312573	Philippines

Continued

Table 1 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
IR53-6-1-1	85	10	312581	Philippines
IR238-	85	10	321179	Philippines
IET 60. HR 12X T(N)1	85	10	338711	India
IR1317-396	85	10	348839	Philippines
IR667-112-3-3-2-2-3	85	10	350442	Philippines
IARI 7447	85	10	353745	India
IR262-(19723-4)-B-3-1	85	10	355785	Philippines
IR1317-266-2	85	10	355799	Philippines
SH-30-21 (Taiwan)	85	10	366150	Taiwan
Masrai	85	10	372764	Malaysia
Mehr	85	10	372920	Iran
BPI-121.407 (Phil)	85	10	372944	Philippines
IR480-5-9-3-3	85	10	372970	Philippines
IR790-28-1-3-3-2	85	10	372976	Philippines
IR1561-228-3-3	85	10	373106	Philippines
IR1561-243-5-6	85	10	373107	Philippines
Padma	85	10	373143	India
IR547 54 1 2	80	10	331504	Philippines
IR1044 15	80	10	338091	Philippines
Allorio lambda	80	10	346409	France
IR1321-14	80	10	348843	Philippines
IR1321-14-1-2	80	10	350346	Philippines
IR1321-18-3-2	80	10	350348	Philippines
IR1321-21-3	80	10	350349	Philippines
IR667-80-6-1-2-1-2	80	10	350431	Philippines
IR781-436-3-3-3-3	80	10	350454	Philippines
IR781-443-2-2-1-2	80	10	350455	Philippines
IR781-133-2-1-1-3-3	80	10	350475	Philippines
IR781-139-2-2-2-1-3	80	10	350478	Philippines
IR781-152-1-1-1-1-2	80	10	350481	Philippines
IR781-159-2-2-1-2-2	80	10	350482	Philippines
IR944-85-1-2-2-2-2-2	80	10	373011	Philippines
Masmati 443	80	10	385456	Pakistan
Masmati 140	80	10	385471	Pakistan
Lomello	75	10	233663	Korea
IR781-89-2-2-1-3-1	75	10	350467	Philippines
Rexmont (check)	0	0	502968	USA

Table 2. Rice accessions (155) from the USDA-ARS germplasm collection that showed allelopathy against ducksalad in a field experiment at Stuttgart, Arkansas, in 1989.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
Shuang-chiang-30-21	85	18	303684	Taiwan
Chun nan tsan	85	18	400456	Philippines
UNMD VAR-PAK	85	18	431228	Pakistan
Lawangin	85	18	431298	Pakistan
Yen shan ma chiu ku	80	18	161019	China
Woo co chin yu	80	18	279171	Taiwan
Jawari suakh-PAK 328	80	18	385867	Pakistan
Maliabhangar	75	18	392549	Unknown
Ta tou kuei	75	18	401454	China
Luk	75	18	431299	Pakistan
CICA 4	70	18	400280	Brazil
Taichung Native 1 (SI 418)	44	18	400158	Philippines
Tam lua	95	15	389259	Vietnam
Kasarwala and mundara PAK 116	90	15	385513	Pakistan
Mudgo	90	15	431302	Pakistan
Tung yan chin	88	15	389438	China
Lang chung yi lung ma ku	85	15	161017	China
Baszetze	85	15	165645	China
Kao luang	85	15	180169	Thailand
IR160-27-4	85	15	373881	Philippines
IR1665-8-1-B	85	15	376605	Philippines
Coarse PAK 76S	85	15	385561	Pakistan
Sathra PAK-323	85	15	385888	Pakistan
Tikal 2	85	15	420965	Colombia
Pakistan IP 18	85	15	430971	Pakistan
IET 4700	85	15	458479	Unknown
UNMD MGVR-China PR	80	15	7021	China
TN-1/H4	80	15	9963	Spain
Ai lu ta hei ai sh you mang	80	15	160902	China
Ai yeh lu-China PR 351981	80	15	160937	China
Bul do	80	15	162176	Korea
BJ 1 (India)	80	15	221109	India
Kaohsiung ta li chin yu	80	15	294396	Taiwan
IR 759-86-1 IR 8 X	80	15	345903	Philippines
IARI 6595	80	15	353716	India
Mentik penjon	80	15	373788	Indonesia
Kasarwala and mundara no7 PAK 111	80	15	385512	Pakistan
Bungua PAK 147	80	15	385646	Pakistan
UNMD VAR-PAK 24	80	15	385682	Pakistan
Sufaid PAK-246	80	15	385819	Pakistan
Bico branco peludo	80	15	388360	Brazil
Shoa bir tsan	80	15	389611	China
Leishi makrei (M-519)	80	15	400108	Philippines
IR1514A-E597	80	15	408642	Philippines
Ti chueh wu chien	80	15	415764	Taiwan
Juma 10	80	15	420239	Dominica
UNMD VAR-PAK	80	15	430949	Pakistan
Ardito	80	15	431104	Pakistan
Gukuku/Maichlati (PAK 924)	80	15	431133	Pakistan
Sarjoo 49	80	15	439094	India
Sadri-type (PI 431303?)(not IR5) BLTS	80	15	502669	USA

Continued

Table 2 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
NATO Seln (CI8998-5)	75	15	12394	USA
Tsao sheng shen li 1 hao	75	15	160714	China
Amarelo	75	15	189463	Portugal
Shali i mahin 1372	75	15	223517	Afghanistan
India T 21	75	15	229268	India
Bir me fen	75	15	275421	Taiwan
IR6 114 2 2	75	15	312681	Philippines
W 1193 (*O. perennis-Japan*)	75	15	346371	Brazil
IR568 20 1 1	75	15	348780	Philippines
Vella peruvazha	75	15	365230	India
IR841-67-1-1-1	75	15	372964	Philippines
IARI-5824 India	75	15	373867	India
Tiri 3-PAK 429	75	15	385659	Pakistan
Tijucas claro	75	15	388330	Brazil
Tan den	75	15	389175	Vietnam
Chu to	75	15	389334	China
Chunlun u le thou	75	15	389595	China
Tien sen bir	75	15	389625	China
Vary vato 275	75	15	400773	Philippines
B441B-24-4-5-1 (Indonesia)	75	15	408385	Indonesia
UNMDVAR-PAK	75	15	431229	Pakistan
Taichung Native 1	75	15	431249	Pakistan
Akabona	75	15	431292	Pakistan
Ratnagiri 24	75	15	439088	India
Mutant 12/42 PAK	70	15	385824	Pakistan
PAK C3-6 P1	70	15	385920	Pakistan
Muskkan red-PAK 616	70	15	392155	Pakistan
Sella sare	70	15	412795	Pakistan
IR1055(N) (CIAT)	70	15	420962	Colombia
IR1541-AF-597	70	15	431430	Pakistan
IR1541-76-2-65	70	15	431432	Pakistan
IR52 16 7 3	60	15	312576	Philippines
ARC 7345 India	60	15	373428	India
CO 34 (IET-400) India	90	13	373691	India
P758-30-2-1 (CIAT)	90	13	377568	Colombia
Hung ko man	90	13	389565	China
IR8-188-1	85	13	312620	Philippines
Ratura mushkan PAK-53	85	13	385772	Pakistan
UNMD VAR PHIL	85	13	392751	Philippines
Ratna 81	85	13	400136	Philippines
UNMDVAR-PAK	85	13	431234	Pakistan
UNMD MGVR (China PR)	80	13	161005	China
Jappein tunkungo	80	13	282767	Senegal
Chen chu yai	80	13	373356	Pakistan
Burma B35/1	80	13	373800	Malaysia
IR532-E369	80	13	373887	Philippines
Jhona PAK 82C	80	13	385564	Pakistan
IR634-24-1	80	13	388574	Brazil
Bansen (Uruchi)	80	13	389314	China
Chow geo shun	80	13	391146	Philippines
Nung shin 25	80	13	392879	Philippines
Takao 26	80	13	400562	Philippines

Continued

Table 2 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
Nung shin 21	80	13	400605	Philippines
Yuan hsing 1	80	13	400753	Philippines
Colombia 3	80	13	406040	Colombia
B459B-PN-4-5-6-1 (Indonesia)	80	13	408391	Indonesia
IR2058-78-1-3-2-3	80	13	408600	Philippines
CR94-13 (IRRI)	80	13	408644	Philippines
Jajathla 27	80	13	431232	Pakistan
Vikram	80	13	439105	India
Mala	80	13	452277	Bangladesh
Unkn MGVR (not PI 167927) NSSL 3/77	78	13	502871	USA
Calrose	75	13	8988	USA
Feronio	75	13	274578	Portugal
IR8-56-2-3	75	13	312616	Philippines
IR643 1 50 2 2 IR 8 X Dawn X	75	13	338072	Philippines
IR61-7-3	75	13	373880	Philippines
Shinali	75	13	373943	Afghanistan
Masmati 122	75	13	385418	Pakistan
Mutant 11/9 PAK	75	13	385823	Pakistan
Ziri jowain PAK-245	75	13	385911	Pakistan
PAK C3-6P3A	75	13	385922	Pakistan
PAK C3-6P5	75	13	385925	Pakistan
Pai vi ping	75	13	388299	Brazil
Kingmen toumen hung mi	75	13	391185	Philippines
Kangni-27	75	13	392201	Pakistan
Thapachinaceae	75	13	400043	Philippines
IR442-2-58	75	13	408685	Philippines
Zarai	75	13	412839	Pakistan
Russi Palman	75	13	412864	Pakistan
Taichung shien 2	75	13	415747	Taiwan
UNMD VAR-USSR	75	13	431235	Pakistan
HZ ROS 637	75	13	431267	Pakistan
IR528-PK-19-EL	75	13	431279	Pakistan
Basmati	75	13	431297	Pakistan
V20B-IRRI	75	13	452281	Unknown
RXOR/7689//RXOR/Nira	70	13	9255	USA
Ken yen seln (CI 161053-1)	70	13	12310	Unknown
IR23 9 3 3	70	13	312563	Philippines
IR1661-9-3-B	70	13	376549	Philippines
IR1661-30-2-B	70	13	376558	Philippines
IR1662-12-3-B	70	13	376576	Philippines
IR1995-121-S1-B	70	13	376661	Philippines
Mahler PAK 335	70	13	385486	Pakistan
I Kan poo chee	70	13	389025	Taiwan
WW 3/200	70	13	400669	Philippines
Balid	70	13	402741	Philippines
TG 37 (Indonesia)	70	13	408386	Indonesia
Taitung wu li	70	13	415758	Taiwan
Late Plata Mocorata (PAK 968)	70	13	431171	Pakistan
No-lku	65	13	8513	Japan
Baek kiong zo	65	13	162171	Korea
IR22-104-1-1	65	13	312602	Philippines
Manga Kely 694	70	10	400780	Philippines
Rexmont (check)	0	0	502968	USA

Table 3. Rice accessions (67) from the USDA-ARS germplasm collection that showed allelopathy against ducksalad in a field experiment at Stuttgart, Arkansas, in 1990.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
Basmati PAK 134	85	20	385421	Pakistan
Kingmen toumen chiu mu	90	18	389551	China
USSR Y2178 6	88	18	431042	Pakistan
IARI 10560	85	18	373870	India
Gin shun	85	18	391156	Philippines
Hwei ju	80	18	161013	China
San chiao tswen	80	18	161026	China
Santhi PAK 209	80	18	385871	Pakistan
Dou u lan (red stem)	80	18	391175	Philippines
Mamoriaka	80	18	400286	Brazil
B 1293 B-PN-24-2-1 (IRRI)	80	18	417822	Philippines
Rec. as *O. glaberrima*	80	18	431330	Pakistan
Ming shan wan tao ku	75	18	161032	China
Kuan yen chan PI 160586	75	18	388497	Brazil
E che goo	75	18	389570	China
Djim	75	18	393180	Philippines
Tsi chin tsa seln (160968-1)	70	18	12389	China
Torh-PAK 637	70	18	392173	Pakistan
Mahler PAK 335	65	18	385486	Pakistan
PA JU 40015-China PR 352072	85	15	161012	China
Fine PAK 286	85	15	385432	Pakistan
Fine red	85	15	385852	Pakistan
Peroz	85	15	431227	Pakistan
Aronlea	80	15	160493	China
IARI 10589	80	15	353778	India
Basmati PAK 938	80	15	385781	Pakistan
Lien cheng ta kuan pai	80	15	389554	China
Thou bir shun	80	15	389566	China
E ta chwan	80	15	389569	China
Shimokita	80	15	392328	Japan
Serendah kuning 60	80	15	392704	Unknown
Ingngoppor/Tinawon	80	15	393030	Philippines
Garunbalay	80	15	393033	Philippines
Basmati 5854	80	15	393146	Philippines
Kara sar	80	15	412844	Pakistan
Desi jhona	80	15	412867	Pakistan
Daud zai (PAK 949)	80	15	431157	Pakistan
Vulgazis ko ch azpasaly (P1000)	80	15	431195	Pakistan
Chinese variety (Weiss)-BMT 53	75	15	9096	China
NARB white seln (8329-1)	75	15	12266	China
Munji sufaid PAK 23B	75	15	385554	Pakistan
Ram tulasi seln	75	15	391705	Philippines
Nagai kuck look saap yat chu	75	15	391799	Philippines
Torh-PAK 638	75	15	392174	Pakistan
Karkati 87	75	15	392683	Unknown
Basmati 5702	75	15	393145	Philippines
Huallaga	75	15	408410	Peru
Zarai	75	15	412839	Pakistan
Ti chuch wu chien	75	15	415764	Taiwan
IR2071-586-5-6-3	75	15	417851	Philippines
Kashmir basmati	75	15	429861	Pakistan

Continued

Table 3 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
USSR Y2069	75	15	431035	Pakistan
IR841-85-1-1-2 Seln	75	15	439019	Philippines
CO 29 A 4545	75	15	439660	India
NARB Upland seln (8330-1)	70	15	12267	China
Bengali marima 120	70	15	400782	Philippines
CR 395-1-2-3 (Egypt)	70	15	439124	Egypt
Torh-PAK 636	65	15	392172	Pakistan
BMT 53 R-3568 (FR Siam)	80	13	9101	China
LU 1 29 (Shanghai)	80	13	401458	China
China-PR P151 Seln (160764-4)	75	13	12373	China
IR634-34-2-6	75	13	388575	Brazil
Chun lun 34	75	13	389550	China
IR841-67-1-1-1	75	13	400889	Philippines
Rikuto Norin 22	70	13	291662	Japan
Zhukovskii	70	13	439639	Iran
Polman India	65	13	388298	Brazil
Rexmont (check)	0	0	502968	USA

In a 1988 field experiment, 145 accessions had a radius of activity of >10 cm around redstem and were significantly different from the control, Rexmont (Table 4). Accessions that demonstrated allelopathic activity around redstem originated in Argentina, Australia, Colombia, Dominican Republic, Guatemala, Guyana, Hungary, India, Indonesia, Japan, Malaysia, Mexico, Myanmar, Pakistan, People's Republic of China, Philippines. Spain, Sri Lanka, Surinam, Taiwan, and the United States. Sixteen of the I45 accessions that showed allelopathic effects on redstem also demonstrated allelopathic effects on ducksalad (Table 5) and originated in India, Malaysia, Philippines, and Taiwan.

A total of 3,727 accessions from the International Rice Research Institute (IRRI) are available in the USDA-ARS germplasm resources information network (GRIN). The pedigrees of most of the accessions developed at IRRI are also available in GRIN. The pedigrees of the IRRI accessions that showed allelopathic activity around both ducksalad and redstem were analyzed and found to have at least four common parents, BPI 76, IR8, Peta, and TN-1. For example, the pedigree of IR52-16-7-3 (PI 312576) and IR52-30-6-2 (PI 312580) is BPI 76*2/KH 68; the pedigree of IR781-497-2-3 (PI 348798) is IR8*2//Yuhkara/TN-1; the pedigree of IR1317-385 (PI 348835) and IR 1321-12 (PI 348842) is Jin Heng/IR262-43-8-1//Senbon Asahi; the pedigree of IR262-43-8-1 (PI 400766) is Peta/TN-1; and the pedigree of IR788-16-1-1-1 (PI 373026) is IR8/NM 54. The pedigree of IR8, which is a parent of IR781-497-2-3 (PI 348798) and IR788- 16-1-1-1 (PI 373026), is Peta/DGWG.

Tables 1-3 list the 412 rice accessions that demonstrated apparent allelopathic activity around ducksalad, and Table 4 shows the 145 accessions that demonstrated apparent allelopathic activity around redstem. Listing each accession by plant intro-

Table 4. Rice accessions (145) from the USDA-ARS germplasm collection that showed allelopathy against redstem in a field experiment at Stuttgart, Arkansas, in 1988.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
IR800-17-1-3	85	18	348802	Philippines
IR614-6-2-1	85	18	350321	Philippines
IR75 69 3	80	18	312528	Philippines
IR52 16 7 3	80	18	312576	Philippines
IR52 30 6 2	80	18	312580	Philippines
IR1044 56	78	18	338093	Philippines
Il ziu	75	18	162230	Korea
Mushkan 41	75	18	247891	India
Taichung 178	75	18	313566	Taiwan
Cuba 65 V 58	73	18	260045	USA
UN GU 6	70	18	162340	Korea
Cuba 65 58A	70	18	260046	USA
Dulatom 298 (Dular 298-2)	70	18	356012	Pakistan
IR667 145 2, IR8X	83	15	331577	Philippines
Ta pai ku	80	15	160445	China
CP231/SL017//Nahng mon	80	15	321184	Philippines
IR140 136	80	15	331640	Philippines
IR934-210-3	80	15	348803	Philippines
IR1314-28-1-2	80	15	350338	Philippines
Gin bon tsan	80	15	389683	China
Mahlar 346	78	15	247883	India
Pai kan tao	78	15	282945	Taiwan
BB50/Cros 17 4 5 (Australia)	78	15	298964	Australia
IR238-	78	15	321183	Philippines
IR527 1 57	78	15	331646	Philippines
IR1317-82	78	15	348815	Philippines
IR1317-385	78	15	348835	Philippines
IRl321-12	78	15	348842	Philippines
IET 2254 (RP4-14)	78	15	408592	Philippines
Unkn SGVR	78	15	502897	USA
CA 4813A1-13	75	15	9505	Unknown
IR67 124	75	15	312515	Philippines
IR52 26 7 1	75	15	312579	Philippines
CP 231 X SLO 17	75	15	319504	Mexico
IR1317-190	75	15	348827	Philippines
IR1317-313	75	15	348831	Philippines
IR1317-406	75	15	348841	Philippines
CR10-5437(T 90 X IR8)	75	15	357054	India
Lusi 513	73	15	220742	Indonesia
Glutinous seln	70	15	9127	USA
Sellent	70	15	168949	Spain
Chogoei (A18)	70	15	234973	Japan
Johna 349 (early)	70	15	247882	India
S 12 DZK-India	70	15	247887	India
Fee la fon	70	15	248484	Taiwan
BB50/cros 17 4 2 1 (Australia)	70	15	298963	Australia
IR329 12 3 6 15	70	15	321246	Philippines
IR594-60-1-2, IR8X	70	15	345760	Philippines
IR800-19, F3	70	15	345804	Philippines
P881-13-6-8 Colombia	70	15	372047	Colombia
CR52-3 (India)	70	15	373020	India

Continued

Table 4 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
X69-56-12-19-6-3	70	15	408375	Myanmar
Zyouk sin ryouk	68	15	162387	Korea
Palawan	68	15	220424	Philippines
K8C/505/1/1- Surinam no. 23	68	15	263828	Surinam
IR781-424-3-3-2-2	68	15	350452	Philippines
P882-2-7-B (CIAT)	68	15	372051	Colombia
Masrai	68	15	372764	Malaysia
IR790-33-1	65	15	345775	Philippines
Ro wuan shun	63	15	389440	China
Unkn MGVR	63	15	502882	USA
Taichung Native 1 (TN-1)	60	15	271672	Taiwan
Perlita jalapa	58	15	271287	Guatemala
Ginmasari	80	13	294353	Japan
IR237 14 3 1 3	80	13	321203	Philippines
IR1317-39	80	13	348813	Philippines
IR22	80	13	399642	Philippines
IRRI seln (PI 430338?) BLTS 2306	80	13	502675	USA
Rexark/Asahi	78	13	9367	USA
K Kawoeng seln (C12474-2)	78	13	12069	Unknown
Kaohsiung line 164	78	13	282823	Taiwan
Custugulcule	78	13	282939	Taiwan
Kao chio lin chou	78	13	282940	Taiwan
Sukhwel 20	78	13	294324	India
T 21 (India)	78	13	294330	India
IR298 12 12, BPI 76 X	78	13	325917	Philippines
IR532 1 33 1	78	13	331481	Philippines
IR532 1 116 2	78	13	331484	Philippines
IET 429, T(N)1 X K 540	78	13	338710	India
H74-2-1 (Argentina)	78	13	346849	Argentina
IR 579-48-1-2	78	13	350654	Philippines
CI 8994/S415A	75	13	9405	USA
BB50/cros 1-7-18-3-2 seln (298965-2)	75	13	12496	Unknown
Early sutarsar	75	13	291499	Hungary
IR8 179 3	75	13	312619	Philippines
IR532 E 230 710	75	13	331627	Philippines
IR1176-40	75	13	345895	Philippines
IR934-234-2	75	13	348804	Philippines
Mutant from PI 349251	75	13	349252	Japan
Basmati PAK 375	75	13	385445	Pakistan
Lui chou 25 108 30	73	13	303683	Taiwan
IR77-7-2	73	13	312531	Philippines
IR77-27-3	73	13	312532	Philippines
IR253-19	73	13	312764	Philippines
IR503 1-45-1	73	13	331473	Philippines
IR1044-70	73	13	338094	Philippines
IR1044-79	73	13	338095	Philippines
IR952-59-3	73	13	345846	Philippines
IR442-2-50-2-2-3	73	13	345929	Philippines
Guyana 50778	73	13	346446	Guyana
IR1317-74	73	13	348814	Philippines
Iratom 24 mutant	73	13	356014	Pakistan
IR1544-284-2	73	13	373079	Philippines

Continued

Table 4 continued.

Cultivar name	Weed control (%)	Radius (cm)	CI/PI	Origin
IR1529-123-1	73	13	373083	Philippines
Juma 1	70	13	317316	Dominica
Shuho	70	13	343835	Japan
IR532-1-218-1	70	13	345928	Philippines
IR781-497-2-3	70	13	348798	Philippines
IR934-529-1	70	13	348807	Philippines
IR1307-1-3	70	13	350336	Philippines
IARI 319	70	13	353629	India
IR1163-132-1-2-2	70	13	372990	Philippines
IR841-67-7-1-1-1-1	70	13	373030	Philippines
BG 34-11 (Ceylon)	70	13	373039	Sri Lanka
IR334 13	68	13	321216	Philippines
IR334 14	68	13	321217	Philippines
IR334 55	68	13	321219	Philippines
IR478 3	68	13	321221	Philippines
IR580 E 452 248	68	13	338145	Philippines
IR503-20-PDI-C2	68	13	338933	Pakistan
Liso	68	13	340891	Spain
IR1063-22-1	68	13	345830	Philippines
IR943-12-3	68	13	345843	Philippines
IR781-419-2-2	68	13	348796	Philippines
IR934-133-3-2-2	68	13	350520	Philippines
IR789-63-1-1-1-1-1	68	13	373025	Philippines
IR788-16-1-1-1	68	13	373026	Philippines
IR842-40-1-3-1	68	13	373027	Philippines
IR498 1 78 1	65	13	331469	Philippines
IR532 1 218	65	13	338122	Philippines
IR1316-I6	65	13	348810	Philippines
Basmati PAK 406	78	10	385806	Pakistan
Hoyoku	75	10	318643	Japan
IR643-1-36-3-1-3	75	10	345868	Philippines
IR759-87-1	75	10	345904	Philippines
IR759-87-2	75	10	345905	Philippines
IR630-12-1-2	73	10	345856	Philippines
IR968-54-4	73	10	345859	Philippines
IR795 10 2	70	10	338099	Philippines
IR1317-155	70	10	348821	Philippines
IR39-14, Peta X TN-I	65	10	325890	Philippines
IR510 11 3	65	10	338116	Philippines
IR759-140-5	65	10	345906	Philippines
IRRI seln	65	10	345926	Philippines
IR1317-219-2-1	65	10	350364	Philippines
Rexmont (check)	0	0	502968	USA

Table 5. Rice accessions (16) from the USDA-ARS germplasm collection that showed allelopathy against both redstem and ducksalad in a field experiment at Stuttgart, Arkansas, in 1988 and 1989.

Cultivar name	Weed control (%) redstem/ ducksalad	Radius (cm) redstem/ ducksalad	CI/PI	Origin
BB50/Cros 1-7-18-3-2 seln (298965-2)	75/85	13/10	12496	Unknown
Johna 349 (early)	70/85	15/10	247882	India
Mahlar 346	78/85	15/10	247883	India
S 12 DZK-India	70/90	15/13	247887	India
Talchung Native 1 (TN-1)	60/85	15/15	271672	Taiwan
Lui chou 25 108 30	73/85	13/10	303683	Taiwan
IR52 16 7 3	80/60	18/15	312576	Philippines
IR52 30 6 2	80/85	18/13	312580	Philippines
IR238-	78/85	15/13	321183	Philippines
CP231/SL017//Nahng mon	80/85	15/15	321184	Philippines
IR781-497-2-3	70/90	13/10	348798	Philippines
IR1317-385	78/90	15/10	348835	Philippines
IR1321-12	78/90	15/10	348842	Philippines
Masrai	68/85	15/10	372764	Malaysia
CR52-3 (India)	70/90	15/10	373020	India
IR788-16-1-1-1	68/90	13/10	373026	Philippines

duction (PI) or crop introduction (CI) number, cultivar name, country of origin, radius of activity, and weed control (%) allows scientists from different countries to conduct their own pedigree analysis of varieties or accessions from their country or geographic region. A pedigree analysis will help a scientist in selecting germplasm (parents) with potential allelopathic activity that is adapted to a particular region.

Breeding and enhancement

Individual F_2 populations from hybrids involving PI 338046 with Adair, Alan, Katy, Lemont/RA 73, M-201/Katy, and Newbonnet were evaluated in 1992 in Stuttgart fields. IR8 and TN-1 are two parents in the pedigree of PI 338046. Therefore, PI 338046 or lR8*2//85894 A4-18-1*2/TN-1 has two (IR8 and TN-1) of the four common parents previously identified from the IRRI material, which possess allelopathic potential for both ducksalad and redstem. Seven F_6 lines from the crosses PI 338046/ /Lemont/RA 73, PI 338046/Alan, PI 338046//M-201/Katy, and PI 338046/Katy were field-evaluated in replicated tests in 1996. One line from the cross PI 338046/Katy (Stg 94L42-130) was the highest yielding entry (9,882 kg ha^{-1}) in the 1995 Arkansas Preliminary Test. The Stg 94L42-130 cross surpassed the yield of Katy (7,868 kg ha^{-1}) by almost 2.000 kg ha^{-1}. Also, in a 1995-96 greenhouse test, Stg 94L42-130 had significantly fewer ducksalad plants (35) than the nonallelopathic check, Rexmont (97), with LSD (0.05) = 31.

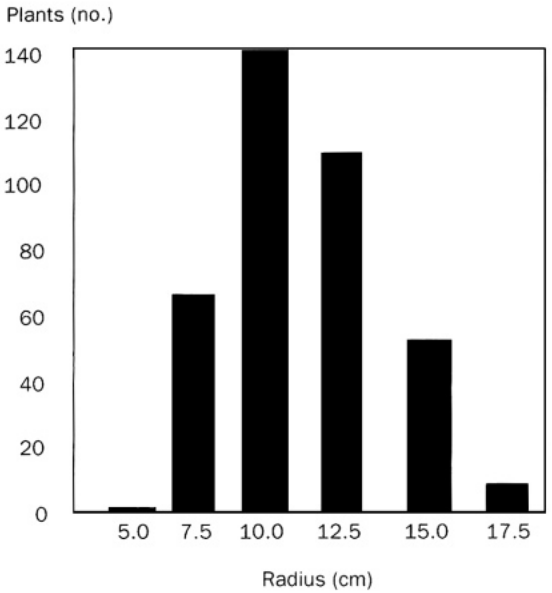

Fig. 1. F_2 distribution of allelopathic activity around ducksalad involving the cross combination PI312777/Lemont.

Genetics

The F_2 population involving PI312777/Lemont was distributed normally for allelopathic-activity (Fig. 1). This result indicates that allelopathic activity is quantitatively inherited. Also, our experience with environmental influences on allelopathic activity supports quantitative inheritance. For example, year-to-year variation, soil type, weed density, rice plant number, rice root development, and root density can affect the size of the area of allelopathic activity, especially for ducksalad. The conclusion also appears to agree with that of Rizvi and Rizvi (1992), who commented that "much of the evidence indicates that several chemicals are released together and may exert toxicities in an additive or synergistic manner. To date all cases of alleged allelopathy that have been thoroughly studied appear to involve a complex of chemicals. In no case has one specific phytotoxin been proved to be solely responsible for, or produce as a result of, interference by a neighboring plant." A genetic analysis of F_3 lines will be conducted in the laboratory in 1996-97 and in the field in 1997 to confirm polygenic control of allelopathic activity in rice around ducksalad.

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Notes

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CHAPTER 3

Weed management using allelopathic rice varieties in Egypt

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During 1993-96, approximately 1,000 rice varieties were screened and evaluated in the field, greenhouse, and laboratory to identify those with allelopathic effects on *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass) and *Cyperus difformis* L. These are the most troublesome weeds of rice in Egypt. More than 30 varieties, including RP 2269-424, LD 183-3, LDS 183-7, IET 1444, Dular, CI-Selection 63, UPR 82-1-7, GZ 1368-5-2, and OR 131-58, provided 50-90% control of *E. crus-galli* in the field. Incorporating the residues of some of these plants into the soil reduced seed germination of *E. crus-galli.* More than 10 varieties, including RP 2271-433-231, IET 11754, Dular, and OR 131-5-8, suppressed *C. difformis* by 50-75%. The varieties expressed allelopathic properties at the 3- to 4-leaf stage. They inhibited root development and emergence of the first or second leaf of both weeds. Residues of these varieties did not affect subsequent winter crops. *Ammannia* sp. stimulated rice growth and reduced weed growth.

Introduction

Weeds, ever-present in agroecosystems, are chemically suppressed to favor crop growth. Achieving sustainable rice production, however, will depend on our ability to decrease the use of chemicals while increasing yield, reducing production costs. improving farm profits, reducing risk, and harnessing the productivity of soil and water resources (Harwood 1990). Weed management in this context involves using improved cultivars, seeding methods, land preparation, irrigation, time of seeding, crop rotation, harvesting methods, biological control agents, allelopathic substances, preventive weed control methods, and appropriate applications of chemicals (Smith 1993).

Allelopathy results from biochemical interactions between plants, and is caused by toxic chemicals released through volatilization, leaching, and root exudation or produced during the decomposition of plant residues in the soil (Chou 1995). Such chemicals only temporarily suppress plant growth and regulate species diversity because they occur naturally and degrade rapidly into nontoxic substances that have little residual effect. Allelochemicals inhibit seed germination by blocking hydrolyzation of nutrient reserves and cell division, among other effects. Alleloche-

micals enter agroecosystems through crops, weeds, or decomposition of plant material. Weeds are always present in agricultural fields in association with crops (Putnam and Weston 1986).

Purvis (1990) reviewed the potential of allelopathy for weed control. Allelopathic rice cultivars could supplement the use of herbicides in direct seeding (Olofsdotter and Navarez 1996). Cultivars showing allelopathy against important rice weeds have been identified in the United States (Dilday et al 1991), Japan (Fujii 1992), Egypt (Hassan et al 1995), and the Philippines (Olofsdotter and Navarez 1996). Some allelopathic cultivars strongly inhibit root elongation of barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], but weakly affect the shoot (Navarez and Olofsdotter 1996). In Egypt, Hassan et al (1995) identified rice varieties that expressed allelopathic effects after plants reached the 3-leaf stage, and such varieties inhibited root development and emergence of the first or second leaf of *E. crus-galli.*

The phytotoxic potential of crop residues could be exploited in managing various weeds in agroecosystems (Moody 1995). Dilday et al (1992) reported that allelochemicals were present in rice straw of accessions that showed allelopathic activity in the field around *Heteranthera limosa* (Sw.) Willd. The use of rice germplasm that contains high allelopathic activity, combined with incorporating straw into the soil, controlled *Cyprus iria* L. almost as effectively as a tank mixture of propanil + bentazon (Lin et al 1992). Khan and Vaishya (1992) reported that residues of Sarjoo-52 rice incorporated 5–6cm deep at 5 t ha^{-1} reduced the population and biomass of *Echinochloa colona* (L.) Link and broadleaf weeds (*Ammannia bacifera* L., *A. multiflora* Roxb., and *Phyllanthus fraternus* Webster), whereas the residues stimulated germination and biomass production of *Fimbristylis dichomato* (L.) Vahl and *F. ovata* (Burm. f.) Kern. Allelopathic potential has been reported in some of the world's worst weeds (Einhelling 1995). In Thailand, Harada (1992) found that *A. bacifera* controlled weeds when incorporated into the soil before seeding.

The challenge to weed research is to develop control strategies that maintain or enhance farm profits while safeguarding the environment and human health (Moody 1995). The objectives of this study were (1) to identify allelopathic rice varieties and (2) to develop appropriate and safe methods of suppressing weeds and their seeds in the soil.

Materials and methods

Field screening

About 1,000 rice varieties were screened in two sets of field experiments from 1993 through 1996 to identify varieties possessing allelopathic properties around *Echinochloa crus-galli* and *Cyprus difformis* at the Rice Research and Training Center, Sakha, Kafr El-Sheikh. Pregerminated seeds of each variety were planted in mid-June each year in two rows spaced 20 cm apart in 1 × 0.6-m plots in a randomized complete block design with three replications. Each plot was infested with the selected weed seed before seeding rice. Other weeds were controlled with herbicide

applications followed by hand weeding. Plots were drained 5 d after seeding, flooded every 3–4 d, and permanently flooded 30 d after seeding. Allelopathic activity was recorded 30–40 d after seeding based on reduction in dry weight of the weeds between rows.

Evaluation of allelopathic rice varieties in broadcast-seeded rice

1995 experiment (*2a*). Eleven rice varieties were evaluated in a randomized complete block design with four replications in 1995 against *E. crus-galli* and *C. difformis* in 15-m^2 plots. Weed seeds were incorporated into water-leveled plots. Pregerminated rice seeds were broadcast at the rate of 140 kg ha^{-1} (dry basis) onto floodwater on 10 June 1995. The plots were drained 5 d after seeding for 3 d and then flooded every 3–4 d. Dry weights of *E. crus-galli* or *C. difformis* were taken 40 d after seeding, and the percentage of control was calculated.

1996 experiment (*2b*). A field experiment begun in I996 evaluated allelopathy of 13 rice varieties against *E. crus-galli* in a 20-m^2 strip-plot design. The strips were naturally seeded or infested by overseeding with *E. crus-galli,* and the rice varieties were distributed in the plots. Weed seed was planted at the rate of 40 kg ha^{-1} seed. Pregerminated rice seed was broadcast at the rate of 140 kg ha^{-1} (dry basis) onto water-leveled plots. The plots were drained 5 d after seeding for 3 d and then flooded every 3–4 d. Broadleaf and sedge weeds were controlled by applying bensufluron-methyl (a.i. 0.05 kg ha^{-1}) at 10 d after seeding.

Plant height, dry matter production, and leaf area index of rice varieties were measured 60 d after seeding. Dry weight of *E. crus-galli* and time required for hand weeding were recorded 40 d after seeding.

Effect of allelopathic rice cultivar residues on *E. crus-galli* in the soil

A pot experiment assessed the reduction of weed seeds in the soil caused by allelopathic rice varieties. After harvesting field experiment 2a, soil containing rice residues was sampled to a depth of 20 cm. and the samples air-dried and pulverized by grinding. The experiment was done in 30 × 20 × 50-cm plastic pots containing 10 kg of the soil samples in four replicates. The pots were watered every 3–4 d, and the number of surviving *E. crus-galli* in each pot was calculated 21 d after the first irrigation.

Response of successive winter crops to allelopathic rice varieties

Other soils sampled like those in the third experiment were planted to wheat, clover, barley, and faba bean. Dry weight of each crop was taken 30 d after planting.

Effectiveness of allelopathic rice varieties in nursery and transplanted rice

An experiment begun in 1996 determined the allelopathic effect of four rice varieties on *E. crus-galli* in the seedling nursery and after transplanting. Varieties were selected from greenhouse studies (data not presented). Plot size was 4 m^2 in the nursery and 15 m^2 in transplanted rice. The nursery plots for each rice variety were grown for

27 d and then transferred to the transplanted plots using a randomized complete block design with four replications. The dry weight m^{-2} of *E. crus-galli* was recorded 25 d after sowing the nursery or 40 d after transplanting, and percentage of control determined.

Effects of incorporated *Ammannia* sp. on rice and weeds

A greenhouse experiment done in 1996 used 30 × 20 × 50-cm plastic pots containing 10 kg of soil. Foliage of *Ammannia* sp., collected from fields when the plants were 20-30 cm tall, was air-dried and cut into small pieces. This material was incorporated at the rate of 100 g m^{-2} into the upper 3–5 cm of the soil, and the pots water-leveled. Seeds of *E. crus-galli* or *C. difformis* were incorporated into the soil surface. Then, pregerminated seeds of Giza cv. 177 were broadcast onto water in a randomized complete block design with four replications. Thirty days after planting, the height, dry weight, and leaf area of each species from 25 plants were recorded.

Results and discussion

Field screening

Forty of 1,000 varieties showed from 20% to 90% allelopathic activity around *E. crus-galli* (Table 1). Thirty of the 40 suppressed *E. crus-galli* by 50–90%. The following cultivars originated in different countries and produced 80% or higher allelopathic activity over the control: IET 1444, UPR 82-1-7, BG 1165-2, LD 183-3, CI-Selection 63, OR 131-5-8, RP 2271-433-231, AC 1225, IRBL 775-30-3-2-2, Yunlen 5 and 6, IRT 2037-93-1-3-1–1, and IR62155-138-3-3-2-2-2. Reduced *E. crus-galli* growth and germination rate occurred after the 1- to 3-leaf stage. Fifteen rice varieties showed high allelopathic activity (30–75%)around *C. difformis* (Table 2). The highest activity was found in IET 11754 (50–75%)and Kim Rad F 87 (60–75%).Radial activity of all allelopathic rice varieties ranged from 5 to 10 cm. Results were confirmed in laboratory and greenhouse studies (data not presented).

Evaluation of selected allelopathic rice varieties in broadcast-seeded rice

1995 experiment (2a). The greatest suppression of *E. crus-galli* (81–85%) was obtained from IET 1444, IET 11754, and CI-Selection 63 (Table 3). Fair suppression was observed with Ratna (62%), IR1108 (62%). Dular (66%), GZ 4255-9-1 (6l%), and RP 2269-424-298 (63%). Rice varieties IR2006-B3-33-2 and LD 183-7 suppressed *E. crus-galli* 71% and 75%, respectively. The best suppression of *C. djfformis* was obtained using IET 11754 (77%). IET 1444, Kim Rad F-87, Dular, and CI-Selection 63 caused 50%, 63%, 69%, and 53% suppression, respectively.

1996 experiment (2b). Plant height, dry matter production, and leaf area index at 60 d after planting were recorded (Table 4). Measurements were higher for some varieties (IET 1444) than for others (such as AC 1225 and GZ 1368-5-8). Allelopathic rice varieties offered good tools for suppressing the *E. crus-galli* population and reducing the time and labor required to eradicate the weed (Table 5). Under natu-

Table 1. Allelopathic activity of rice varieties around *Echinochloa crus-galli* in the field (1993-96).

Rice variety	Origin	Control (%)
Giza 176 (control)	Egypt	0
Sakha 2	IRRI	20-40
IR1108-16-1	India	30-60
UPR 82-1-7	India	50-80
Bala	India	60-70
IET 1444	India	70-80
IET 11754	India	60-70
Dular	Egypt	60-70
GZ 4255-9-1	Sri Lanka	40-50
BG 1165-2	Brazil	60-80
CNA 6446	IRRI	50-70
IR53453-107-2-2	India	40-60
RAU 4004-127	India	40-50
RP 2269-424-298	Korea	40-50
IRI 372	Korea	50-70
SR 11327-22-3-2	Japan	40-60
Kanto 51	Sri Lanka	50-60
LD 183-3	Sri Lanka	70-80
LD 183-7	Philippines	60-70
CI-Selection 63	Bangladesh	70-90
BR 4608-R1-R2	Zaire	60-70
PR 1699-26-1-1	Egypt	60-70
GZ 1368-5-4	India	40-50
RP 1670-1418	India	50-70
OR 131-5-8	India	70-90
RP 2271-433-231	Argentina	70-90
H-175-13-11	Egypt	60-70
GZ 5341-5-3-1	Egypt	40-50
GZ 5121-5-2-1	Egypt	30-50
AC 1225	Iran	60-80
Zarrak	China	50-70
Chente No. 232	Korea	60-70
HR 6852-78-4-2-3	IRRI	60-70
IR31775-30-3-2-2	Korea	60-80
SR 14338-27-4-1-3	China	60-70
TE-SAN-A1.2	India	60-70
BARKAT (K 78-13)	China	60-80
Yunlen 5	China	70-90
Yunlen 6	IRRI	70-90
IR2037-93-1-3-1-1	IRRI	60-80
IR62155-138-3-3-2-2-2		70-90

Table 2. Allelopathic activity of rice varieties around *Cyperus difformis* in the field (1993-96).

Rice variety	Origin	Control (%)
Giza 176 (control)	Egypt	0
IR53453-107-2-2	IRRI	50-60
RAU 4004-127	India	50-60
LD 180-10 B	Sri Lanka	40-60
IR19819-9	IRRI	40-50
Pusa Basmati	Pakistan	40-50
IET 11754	India	50-75
Kim Rad F-87	Japan	60-75
Dular	India	50-70
IR2006-P-3-33-2	IRRI	50-70
GZ 1368-5-4	Egypt	30-40
RP 1670-1418	Zaire	40-50
OR 131-5-8	India	40-60
RP 2271-433-231	Zaire	40-60
CI-Selection 63	Philippines	40-50
AC 1225	Egypt	30-50

Table 3. Allelopathic activity of rice varieties around *Echinochloa crus-galli* (4,075 g m^{-2}) and *Cyperus difformis* (425 g m^{-2}) in the field (1995).

Rice variety	Control(%)	
	E. crus-galli	*C. difformis*
Bala	75	0
IET 1444	83	50
IET 11754	81	77
Kim Rad F-87	67	63
IR2006-B 3-33-2	71	0
Ratna	62	0
IR1108	62	0
Dular	66	69
GZ 4255-9-1	61	0
RP 2269-424-298	63	0
LD 183-7	75	0
CI-Selection 63	85	53
Giza 176 (control)	0	0

ral infestation, the potential for suppressing *E. crus-galli* was very high (92–96%) in IET 1444, Zarrak, LD 183-7, RP 2269-424-298, AC 1225, GZ 1368-5-8, OR 131-5-8, UPR 82-1-7, and RTP 2271-433-281. Weeding *E. crus-galli* under natural infestation took between 5 and 9 h ha^{-1} with these varieties. Also, under artificial infestation, when losses reach 100% in the check variety Giza 176, allelopathic rice varieties UPR 82-1-7 and RP 2271-433-281 suppressed *E. crus-galli* by 80% and 79%, and reduced the time required for weeding by 79% and 78%, respectively.

Table 4. Plant height, dry matter of shoots, and leaf area index (LAI) at 60 d after seeding allelopathic rice varieties (1996).

Rice variety	Plant height (cm)	Dry matter ($g\ m^{-2}$)	LA I
Giza 176	74	1213	5.6
IET 1444	90	1568	10.8
IET 11754	95	1380	9.6
Balal	67	1392	8.3
Zarrak	78	1312	8.6
LD 183-7	83	1520	9.6
RP 2269-424-298	83	1552	8.8
AC 1225	64	1248	6.4
Dular	103	1370	14.0
GZ 1368-5-8	73	1260	7.8
OR 131-5-8	84	1390	9.3
UPR 82-1-7.	94	1480	9.2
RP 2271-433-281	86	1490	8.6
GZ 4255-9-1	76	1265	6.4

Table 5. Allelopathic activity of rice varieties around *Echinochloa crus-galli* under natural or overseeded plots (1996).

Rice variety	Natural infestation[a]		Overseeded	
	Dry weight ($g\ m^{-2}$)	Hand-weeding time ($h\ ha^{-1}$)	Dry weight ($g\ m^{-2}$)	Hand-weeding time ($g\ m^{-2}$)
E. crus-galli (control)	458 a	88 a	1940 a	633 a
Giza 176	440 b	84 a	1860 a	614 a
IET 1444	23 g	6 ef	485 efg	161 de
IET 11754	46 ef	8 de	520 e	183 cd
Bala	98 c	23 b	840 b	293 b
Zarrak	38 fg	7 def	505 e	195 c
LD 183-7	33 fg	7 def	490 e	166 d
RP 2269-424-298	21 h	8 de	465 g	154 e
AC 1225	68 d	13 c	630 d	180 cd
Dular	53 de	12 c	680 d	210 c
GZ 1368-5-8	33 fg	9 d	730 c	269 b
OR 131-5-8	31 fg	6 e	495 ef	165 d
UPR 82-1-7	18 h	5 f	390 I	132 e
RP 2271-433-281	22 gh	6 ef	410 h	139 e
GZ 4255-9-1	100 c	25 a	866 b	287 b

[a]Means followed by the same letter within a column are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Effects of residues of allelopathic rice varieties on seeds of *Echinochloa crus-galli* in the soil after rice harvesting (1995).

Rice cultivar	Barnyardgrass plants[a] (no. m^2)	Reduction (%)
Check (barnyardgrass)	453 a	0
Check (Giza 176)	448 a	1
IET 1444	260 c	43
IET 11754	292 b	36
Bala	256 c	43
LD 183-7	243 c	46

[a]Means followed by the same letter within a column are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. Response of successive winter crops to allelopathic rice variety residues in the greenhouse (1995).

Rice variety	Dry weight of successive crops (g pot^{-1})			
	Wheat[a]	Barley	Faba bean	Clover
Check	39 a	31 a	42	36
Giza 176	41 a	30 a	40	38
Giza 177	38 a	33 a	41	36
IET 1444	37 a	30 a	43	39
IET 11754	36 a	34 a	41	35
CI-Selection 63	38 a	30 a	42	36
Dular	29 b	29 b	40	37
Bala	41 a	31 a	43	38
LD 183-3	38 a	32 a	41	36

[a]Means followed by the same letter within a column are not significantly different at the 5% level by Duncan's multiple range test.

Effect of allelopathic rice residues on *E. crus-galli* in the soil

Residues from allelopathic rice varieties reduced the seed bank of *E. crus-galli* significantly shortly after harvesting and land preparation (Table 6). The reductions were 43%, 36%, 43%, and 46%, respectively, after growing IET 1444, IET 11754, Bala, and LD 180-7 rice varieties. Consequently, one should study varieties for allelopathic potential both through and after the growing season.

Response of successive winter crops to allelopathic rice varieties

Dular did cause a reduction in growth of wheat and barley (Table 7), but faba bean and clover were not affected by the allelopathic varieties. No other statistically significant effects could be seen from any of the allelopathic varieties.

Table 8. Allelopathic activity of different rice varieties around *Echinochloa crus-galli* in the nursery and in transplanted rice fields (1995).

Rice variety	Control (%)	
	Nursery	Field
E. crus-galli (g m^{-2} dry weight)	148 g	1140 g
Giza 176	0	0
IET 14446	89	73
IET 11754	75	84
LD 183-7	95	85
RP 2269-424-298	84	80

Table 9. Effect of incorporated shoots of *Ammannia* sp. (Am) on growth of Giza 177, *Echinochloa crus-galli* (ECG), and *Cyperus difformis* (CD) in the greenhouse (1996).

Plant species	Plant height (cm)			Fresh weight (g m^{-2})			Leaf area (cm^2 per 25 rice plants)
	Rice	ECG	CD	Rice	ECG	CD	
Rice	42			32		320	
Am + Rice	49		49			440	
ECG		56			63		
Rice + ECG	39	54		24	52		280
Am + Rice + ECG	45	22		48	21		480
CD			13			7	
Rice + CD	40		8	30		8	310
Am + Rice + CD	48		2	47		2	440
LSD =	3			8			43

Effectiveness of allelopathic rice varieties in the nursery and in transplanted rice

All rice varieties tested for allelopathic activity showed biologically active suppression of *E. crus-galli* by 75–95%in the nursery and 73–85% in permanent fields (Table 8). Variety LD 183-7 was the best in the nursery (95%) and in the field (85%). These results indicate that nursery studies can predict allelopathic activity in the field.

Effects of incorporated *Ammannia* sp. on rice and weeds

A strong allelopathic effect of incorporated foliage of *Ammannia* sp. on *E. crus-galli* and *C. difformis* was observed in the greenhouse (Table 9) and laboratory (data not presented). The height of *E. crus-galli* was reduced by 59%, whereas biomass was reduced by 60% as a result of incorporating *Ammannia* sp. foliage. Greater reduc-

tions in *C. difformis* height (63%) and fresh weight (75%) were observed when *Ammannia* foliage was incorporated in the plots. These data indicate that the population and growth of *E. crus-galli* and *C. difformis* can be reduced by incorporating *Ammannia* foliage in the top layer of soil before seeding. The growth of rice plants was clearly stimulated in soils containing *Ammannia* sp. foliage (Table 9). The height of rice plants increased 17%, whereas fresh weight and leaf area index increased 35% and 38%, respectively.

In conclusion, we found a strong allelopathic effect of different types of rice varieties on specific weed species. Some varieties can be broadcast-seeded and others both broadcast-seeded and transplanted. The broadleaf weed *Ammannia* sp. can be used to suppress weed species and stimulate rice crops at the same time.

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CHAPTER 4

Rice allelopathy research in Korea

K.U. Kim and D.H. Shin

In Korea, direct seeding of rice is receiving much attention because of its low-input nature. Farmers who shift to direct seeding are experiencing problems with the weed *Echinochloa crus-galli.* Development of allelopathic rice would be an alternative to chemicals and other practices in reducing occurrence of this serious weed that thrives in direct-seeded rice. Studies on screening of allelopathic rice have been conducted recently under Korean conditions, using accessions obtained from IRRI and improved in Korea as well as traditional cultivars. Seven cultivars—AC 1423, Tang Gan, Kouketsumuchi, Musashikogane, Takanenishiki, PSDRC 10, and Dandura—showed promise under Korean conditions. Isolation and identification of allelochemicals from rice through bioassay-directed isolation and chemical analyses are in progress. The inheritance of allelopathic potential in rice is being investigated to provide information needed to develop an allelopathic rice for Korea as well as to generate isogenic lines.

Introduction

Rice is Korea's most important crop. But rice cropping characterized by the heavy use of fertilizers, herbicides, and other pesticides may cause environmental problems in the future in both water and the soil in the paddy ecosystem. Therefore, low-input sustainable agriculture may be an alternative way to minimize environmental costs. Allelopathy, the direct influence from a chemical released from one plant on the development and growth of another, could be an alternative weed control method. Since Dilday and his group in Arkansas (USA) found allelopathic potential in rice accessions (Dilday et al 1991). several others have reported allelopathic activity in rice (Hassan et al 1994, Olofsdotter et al 1995). In Korea, several papers reported on the allelopathic potential of crop residues (Kim and Back 1990, Park et al 1992, Kim et al 1994). Research on rice allelopathy in Korea, however, started only recently.

Direct seeding of rice is receiving much attention because it reduces production costs. As cultural practices for rice shift from transplanted to direct-seeded rice, weed problems will increase because rice and weeds can emerge together. Barnyardgrass (*Echinochloa crus-galli*). one of the worst weeds in irrigated rice systems, is expected to become a more important weed in dry direct-seeded rice because it is adapted for better growth under dry than under wet conditions. An increase in direct-seeded

areas is expected to produce a greater reliance on herbicides. Therefore, allelopathic rice varieties could complement herbicides in direct-seeded rice.

This study was conducted to evaluate the allelopathic potential of 24 cultivars obtained from Olofsdotter's International Rice Research Institute (IRRI) study, 30 improved Korean cultivars, and 80 traditional cultivars grown under Korean conditions.

Screening of allelopathic rice

Field experiments were conducted in the I996 summer season. Twenty-four cultivars obtained from IRRI in collaborative work were tested in a randomized complete block design with three replications. Each rice cultivar was dry-seeded in three 75-cm rows with 25 cm between rows. *Echinochloa crus-galli* seeds (2 kg ha^{-1}) were sown in 4 rows across the rice rows. Grass weeds other than *E. crus-galli* were hand-weeded. Broadleaf weeds and sedges were controlled with bentazon.

Among the 24 cultivars, AC 1423, Tang Gan, Kouketsumuchi, Musashikogane, and Takanenishiki were selected as promising (Table 1). Park and Lee (1996) also reported that cultivars Tang Gan, Kouketsumuchi, and PSBRC 10 provided at least

Table 1. Performance of allelopathic rice cultivars screened at IRRI under dry direct-seeded fields in Korea.

Cultivars	Shoot length reduction of barnyardgrass[a]
Woo Co Chin Yu	+++
AC 1423	+++
Musashikogane	+++
Taichung Native 1	+++
Takanenishiki	+++
Tang Gan	+++
Sakna 2	+++
YH-1	+++
Kouketsumuchi	+++
IR32	++
UPR-82-1	++
Lubang Red	++
Jena 015	++
AUS 257	++
Chiu Chiu Kulnan Tao Ha	++
IR29	++
IR56	++
Cica 4	+
IR52341-60-1-21	+
IR46	+
Vandana	+
IR62751-06	+
Buramada	+
AUS 196	+
No rice check	-

[a]Control (%) was measured 8 wk after seeding, +++ = above 35%, ++ = 30–35%. + = less than 30%.

70% weed control in transplanted rice under Korean conditions (Table 2). The results suggest high potential for breeding allelopathic rice that can complement herbicides in rice cropping.

Laboratory tests were conducted with a relay seeding method developed by Navarez and Olofsdotter (1996). Among 30 improved and 80 traditional Korean cultivars. Danduna showed the most allelopathic potential (Table 3).

Table 2. Effect of allelopathic rice germplasm on weed control.

Germplasm	Weed control (%)[a]	Germplasm	Weed control (%)
Taichung Native 1	39	IR41996-50-2-1-3	59
Woo Co Chin Yu	2	Musashikogane	54
Tsai Yuan Chou	8	Sanghaehangheyina	75
Norin 29	49	Moroberekan	4
Takanenishiki	41	Speaker	53
Canabongbong	30	Dinorado	62
Kahei	57	IR63429-23-1-3-3	67
Shuang-Chiang-30-21	30	Nepal No.8	53
Tono Brea 439	62	Jena 015	59
Mack Kheua	52	Buramada	53
Dam Ngo	30	Uamayutaka	47
Deng Mah Tek	48	Tang Gan	73
Cica 4	77	Kouketsumuchi	71
Bodat Mayang	47	PSBRC 10	72
Sathi	45	Dular	50
Daudzai	45	Sekiyama	43
Asiminori	56	Jikkoku/Jukkoku	31
IR52341-60-1-2-1	50	UPR 82-1	63
IR50363-61-1-2-2	31	IET 1444	0
Check	–		

[a] Based on weed dry weight measured 35 d after transplanting.
Source: Park and Lee 1996.

Table 3. Allelopathic potential of traditional Korean cultivars tested in laboratory conditions.

Cultivars	Reduction of barnyardgrass root growth (%)[a]
Danduna	81
Kujungdo	77
Namwonbyeo	75
Hwacheongbyeo	75
Mangumbyeo	74
Daeyabyeo	72
Sangjubyeo	70
Palgongbyeo	64
Sobaekbyeo	51
AUS 196	21
No rice check	0

[a] Determined 10 d after incubation.

Identifying allelochemicals in rice

Plant extracts and hydroponic culture were used to isolate allelopathic substances from rice. The hydroponic culture method is designed as a system that continuously circulates nutrient solution pumped through the hydroponic system and connected to an Amberlite XAD-4 column chromatography unit in order to adsorb the allelochemicals released from plants on the adsorbent. Compounds were isolated from the ethyl acetate fraction of the different cultivars showing allelopathic potential and were analyzed by a gas chromatograph/mass spectrometer. The ethyl acetate extracts were found to be phytotoxic to barnyardgrass. Fatty acid ester, unsaturated ketone, polycyclic aromatic compounds, and alkaloids were isolated from the extracts.

Development of allelopathic rice

Six cultivars (Tang Gan, Kouketsumuchi, Takanenishiki, Woo Co Chin Yu, Dongjinbyeo, and Hwayoungbyeo) were chosen from previous rice allelopathy studies and used as parents in a full diallel set of crosses for inheritance analysis. The 30 F_1 hybrids obtained will be used for the F_2 generation. In addition, crosses were made to generate an isogenic line that can be used to develop allelopathic rice cultivars.

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CHAPTER 5

Using and improving laboratory bioassays in rice allelopathy research

Inderjit and M. Olofsdotter

Weeds are a major biological constraint to rice production worldwide. One option to reduce herbicide dependency is to use allelopathic effects that rice may have on certain weeds. To prove the existence of allelopathy in rice, compounds produced by rice varieties or their residues must be shown to interfere with the associated weed species. Laboratory bioassays permit researchers to screen large amounts of plant material in a short time. By design, they also allow researchers to eliminate interference factors other than the one under study. Laboratory experiments conducted under controlled environments, including the use of bioassays, however, must always be validated in the field to render convincing evidence of the allelopathic effect of rice on weeds. The main objectives of these bioassays are to demonstrate allelopathic interactions and to validate allelopathic strength in a large plant collection. These objectives create a different set of quality criteria and, as such, different sets of bioassays. This paper reviews some of the concerns about laboratory bioassays and makes some modest proposals for improving the bioassay as a tool in rice allelopathy research.

Introduction

Weeds are the most important biological constraint to rice production worldwide. In spite of the heavy use of chemical herbicides to control noxious cropland weeds, crop yield losses from weeds remain high (Zimdahl 1993). As herbicide use increases throughout the developing world, concerns about the environment and farmers' health put more pressure on the agricultural complex to find alternative solutions for weed control. Synthetic chemical herbicides will probably continue to be a key component in most integrated weed management systems, but the set of tools available for weed control has to be enlarged. One option to reduce herbicide dependency would be to use the allelopathic potential of a species. Allelopathy can be used in weed control in two ways: (1) selecting an appropriate crop cultivar or incorporating an allelopathic character into a desired crop cultivar, and (2) applying residues and straw as mulches or growing an allelopathic cultivar in a rotational sequence that allows residues to remain in the field (Putnam and Duke 1974, Rice 1995).

Rice allelopathic potential has been used successfully to control weeds via rice cultivars and rice residues and straw used as mulches (Dilday et al 1991, Olofsdotter and Navarez 1996). In ricefields, however, plant interference components operate sequentially and simultaneously, so that one mechanism of interference does not eliminate the likely involvement of the other. Before we can exploit the allelopathic potential of a species to control weeds, we must therefore demonstrate its allelopathic potential in the laboratory, greenhouse, and field. Demonstrating the allelopathic phenomenon is difficult because of the complexity of events involved. This paper focuses on methods to investigate allelopathic interactions between the rice crop and its associated weeds.

Compounds produced by rice cultivars or residues must interfere with the associated weed species to prove the existence of allelopathy successfully. Laboratory bioassays are an important part of rice allelopathy research because they allow researchers to study large amounts of plant material in a short time. Bioassays, by design, also allow researchers to eliminate interference factors other than the one under study. Laboratory experiments conducted under controlled environments, however, must always be validated in the field to present convincing evidence of allelopathic effects of rice in nature. Allelopathy, in general, still lacks experimental setups that convincingly demonstrate interference by chemical compounds in natural systems (Connell 1990, Willis 1985). One major criticism stems from the fact that most allelopathy bioassays are conducted under artificial conditions that correspond little to natural conditions (May and Ash 1990). This criticism is often valid, but the hypotheses and objectives of the studies have to be considered before dismissing the bioassay as a tool in allelopathy research. The main objectives of allelopathy bioassays are to demonstrate allelopathic interactions and to validate relative allelopathic strength in a large plant collection. These objectives create a different set of quality criteria and, as such, different kinds of bioassays. This paper discusses some of the concerns about laboratory bioassays, and makes some modest proposals for improving the bioassay as a tool in rice allelopathy research.

Bioassays to demonstrate allelopathy

Willis (1985) suggested seven points to demonstrate the phenomenon of allelopathy:

1. A pattern of growth inhibition of the target plant.
2. Donor plant production of a chemical compound.
3. A mechanism for releasing a compound into the environment.
4. A mechanism for accumulating or transporting a compound in the environment.
5. A mechanism for toxin uptake by the target plant.
6. Sensitivity of the afflicted plant to the toxin.
7. An observed pattern of inhibition unexplained solely by abiotic or biotic factors, especially resource competition, herbivority, and disease.

Confirmation of these points does not prove that allelopathy is operative, only that allelopathy offers a reasonable explanation of the observed pattern. We have two concerns, however, regarding the Willis protocol. First, allelopathy involves both in-

hibitory and stimulatory effects (Rice 1986). All organic molecules can be inhibitory, stimulatory, and/or neutral, depending on the concentration, which makes any growth response with extract bioassays ecologically irrelevant. Second, it is not always necessary to demonstrate the uptake of an allelopathic compound by the target species. A compound may influence the growth and distribution of species indirectly through its effect on microbial ecology (Rice 1984) and nutrient availability (Appel 1993. Inderjit 1996).

Some workers demonstrating the allelopathic potential of rice cultivars and their residues have used extract bioassays. Chou and Lin (1976) reported that aqueous extracts of decomposing rice residues in soil inhibited root growth of lettuce and rice seedlings. There is a problem in relating allelopathic effects to those of plant extracts, because compounds that affect plant growth may not even leach or exude from the plant in nature. Therefore, the effect might not be significant physiologically although it may be significant in laboratory bioassays. On the entry of chemicals into the soil, processes such as retention, transformation, and transport may affect the active concentration and availability of chemicals, and thus allelopathy (Cheng 1995). We will not further discuss the unsuitability of extract bioassays in rice allelopathic studies. but we strongly discourage using them to demonstrate rice allelopathy in laboratory bioassays.

Phenolics, such as *p*-hydroxybenzoic, vanillic, ferulic, *p*-coumaric, and *o*-hydroxyphenylacetic acids, were identified from rice residues under waterlogged conditions (Chou and Lin 1976). It is not clear, however, whether these compounds are leached out from rice residues or are microbial degradation byproducts of compounds leached out by rice residues. In paddy fields, irrigation is frequent. Under flooded conditions, free phenolic acids undergo anaerobic degradation. With an increase in soil water content, the microbial population shifts to facultative anaerobic organisms that degrade phenolic compounds through fermentation (Dao 1987). When conducting an extensive phytochemical analysis on rice plants, one should identify compounds from their rhizosphere. Microbial-degraded products may have greater allelopathic potential. Ceratiolin, an inactive dihydrochalcone. released from fresh leaves/litter of *Ceratiola ericoides.* undergoes light-dependent degradation and results in the active compound hydrocinnamic acid (Tanrisever et al 1987). This acid undergoes microbial degradation to produce acetophenone, which is also inhibitory toward target species. When designing bioassays, particularly to understand rice allelopathy, one must pay careful attention to the rhizosphere microclimate and physical and chemical soil factors.

After identifying compounds from an allelopathic cultivar, growth bioassays should be performed with mixtures of isolated compounds. Allelopathic interactions—additive, antagonistic, or synergistic—can be the result of several chemicals that affect the target species. Einhellig (1995) stated that, "In my opinion almost all cases of allelopathic inhibition in a plant community result from the combined effect of several compounds." It is therefore important to do bioassays with a mixture of identified and unidentified compounds.

Allelopathic compounds, directly or indirectly, are released into the rhizosphere by leaching, residue incorporation and decomposition, volatilization, root exudates, etc. In rice allelopathy research, however, we should focus on effects from living plants and decomposing plant material. In tropical soils, very little plant material is left from one growing season to the next except in intensive systems with continuous growth of a rice crop (3 crops $year^{-1}$). The significance of soil texture, associated species as test species, bioassay design, growth parameters, the critical age of the donor plant, and the critical stage of allelochemical release have been discussed by Inderjit and Dakshini (1995a) and Olofsdotter et al (1995). While investigating the allelopathic potential of the monocarpic annual weed *Polypogon monspeliensis,* Inderjit and Dakshini (1995b) reported that *P. monspeliensis* has allelopathic potential expressed through incorporation of its straw and does not affect the same season crop, but interferes with the next season crop after incorporation of its straw. They concluded that if annual weeds appear at the time of crop seed sowing or after crop seed sowing, the chances of annual weeds being allelopathic to the same season crop are remote. When using allelopathic rice cultivars to control associated weed species, we must specify the time of crop sowing, weed emergence, crop harvesting, and weed maturation.

Most weeds have a critical period when they are most susceptible to control actions, which is at the beginning of the growing season. As weeds grow larger, they reach a stage of development at which they will unlikely be affected by allelochemicals released by cultivars in their rhizosphere. If allelopathic rice cultivars start releasing allelochemicals in bioactive concentrations when weeds have already passed this critical stage, a direct allelopathic effect of the cultivars is unlikely. One should study at what stage of the life cycle a rice cultivar begins releasing allelochemicals in bioactive concentrations. Bell and Koeppe (1972) reported that interference with maize growth by *Setaria faberi* resulted only after the weed attained a significant growth advantage over the crop, indicating that a sufficient pool of allelochemicals became available to the germinating maize seeds to cause interference. Similarly, the observations of Schumacher et al (1983) that wild oats become allelopathic against spring wheat at the 4-leaf stage suggest the importance of the plant age at which release of allelochemicals starts.

Furthermore, it is important to know the sensitive stage of susceptible plants. Maximum release of allelochemicals from roots of an annual weed, *Parthenium hysterophorus,* was observed at the rosette and flowering stage of the weed (Kanchan and Jayachandra 1979). Allelopathic rice cultivars must release allelochemicals in bioactive concentrations. The compounds must have persistence in the soil environment or their degraded products must be allelopathically active. The mere presence of allelochemicals in plants is not enough to prove allelopathy. Studies on the release of allelopathic compounds in bioactive concentrations from rice plants are important to prove allelopathy in rice. Therefore, we need to address two questions: (I) What is the critical stage of the rice plant after which it starts releasing allelochemicals into

the soil? (2) What is the critical stage of development of the weed species after which it is less probable that it will be affected by allelochemicals released by rice?

Role of edaphic and chemical factors in rice allelopathy

An allelopathic response largely depends on the qualitative and quantitative composition of allelochemicals in the soil and their degraded by-products caused by abiotic and biotic soil factors (Dalton 1989, Cheng 1995, lnderjit et al 1997). Various edaphic and chemical components may play a crucial role in the expression of allelopathy by rice cultivars. Cheng (1995) discussed the significance of the transport, transformation, and retention of allelochemicals in the rhizosphere. To demonstrate the allelopathic potential of different rice varieties, natural soils should be used in field-simulated bioassays. Bioassays with leachate or extracts, agar gel, or sand are not of much relevance. A better alternative would be to grow donor and receiver plants together, as demonstrated by Navarez and Olofsdotter (1996). We used a petri dish bioassay, however, and did not simulate bioassays under field conditions. This kind of expermental design might eliminate the likely involvement of competition, because young seedlings withdraw nutrients from the seed and moisture is not a limiting factor. A better experiment would use both donor and receiver plants in field soils.

Chou et al (1981) reported the effects of temperature on the production of phenolics during rice straw decomposition in the soil. Their results are of significance in situations with more than one rice crop per year. Soil texture is an important factor in the expression of allelopathy. Inderjit and Dakshini (1994) reported the effect of soil texture on total phenolic content after amendment with leachate or leaves of *Pluchea lanceolata.* They concluded that (1) plant debris should be added to the soil in different amounts followed by chemical analysis, and (2) a statistical comparison of amended soils with natural soils should be made and the dilution level having the least significantly different chemical characteristics from those of natural soils should be selected for bioassays. In a broader perspective, this means that if farmers are cultivating their rice crop in clay-loam soils, the use of sandy loam or silty soil in bioassays to understand rice allelopathy will be ecologically irrelevant.

When amending soils with rice residues and straw to study allelopathic effects, a particular ratio of soil:residues must be worked out. After amendment, soil chemical characteristics (such as pH, organic matter, electrical conductivity, and nutrients) are likely to be altered. Aside from investigating qualitative and quantitative changes in the allelochemical pool, researchers should quantify chemical changes in the soil after residue amendment. Chou et al (1977) reported that soil with rice residues had higher phenolics and lower NH_4^+ and NO_3^- levels than soil without rice residues. This is an important study because it demonstrated the effects of rice residues on the availability of NH_4^+ and NO_3^- in the soil. But it is not clear whether this reduction in NH_4^+ and NO_3^- availability in soil with rice residues was attributable to phenolic compounds leached out by the rice residues or simply to residue effects. Chou and Chiou (1979) reported a decrease in levels of available N, Ca, Zn, Cu, and Mn after rice

straw was added to the soil. Incorporating residues in the soil results in higher microbial activity, which results in lower availability of NH_4^+ and NO_3^- (Harper 1977). Phenolics, however, are also known to form a complex with nutrient ions and influence their availability (Appel 1993, Inderjit 1996, lnderjit and Mallik 1997). Northup et al (1995) reported that polyphenols leached from litter of *Pinus muricata* influence the levels of dissolved organic nitrogen in the soil. These aspects should be investigated to better understand rice allelopathy. Such an experimental approach will help to identify whether toxic effects of rice residues are attributable to the chemicals they release or to physiochemical changes caused by rice residues (Facelli and Pickett 1991).

The following points should also be considered in addition to an earlier proposed protocol (Willis 1985):

1. Donor and receiver plants grown together in a simple bioassay.
2. The use of soils not previously infested with the allelopathic donor plant, but from the same area, to keep microflora and other physiochemical characteristics of soil similar.
3. The effects of allelochemicals on soil chemistry.
4. How soil chemistry influences allelochemicals quantitatively and qualitatively.
5. Whether the observed effects are caused directly by allelochemicals released or indirectly by their effects on soil biota and nutrient cycling.
6. The life cycle pattern prior to planning debris soil bioassays.

The bioassay in comparative studies

In rice, allelopathic potential differs among cultivars (Dilday et al 1991, and this volume, Chapter 2, Olofsdotter and Navarez 1996). Cultivars AC 1423, Aus 257, IR56, Lubang Red, Musashikogane, Vandana, Taichung Native I, and YHI are allelopathic, whereas Aus 196, Dinorado, and IR62751-06 do not show any allelopathic effects (Courtois and Olofsdotter, this volume, Chapter 6). If abiotic and biotic soil components and environmental factors (such as precipitation, temperature, humidity, etc.) for these cultivars are similar, we can assess their allelopathic potential by growing them together. Problems in choosing a bioassay will therefore turn from demonstrating allelopathic ability toward being able to distinguish allelopathic cultivars from nonallelopathic ones in laboratory screening. The goal of such an exercise will always be to understand allelopathic potential in terms of selectivity and strength and to incorporate this feature into modern high-yielding rice cultivars. The most important factor in determining the design of a bioassay for comparative studies is that the results should be correlated with cultivar performance under natural conditions. The bioassay chosen should also be easy to perform. cheap, and reproducible over time, space, and location.

To create a bioassay correlated with field performance, the test species is one of the first choices to make. Lettuce or radish has been used in many allelopathy bioassays as a test species (Putnam et al 1983, Fujii 1993). For comparative experiments,

results obtained with artificially sensitive species as test plants rarely correspond to field performance. The optimal test species will always be one already associated with the field crop (Olofsdotter et al 1995). There are problems in growing weeds for use as test species under laboratory conditions. Germination is low and inconsistent, and weeds rarely germinate simultaneously. Therefore, weed-related domesticated test species are often chosen. Researchers must try to standardize bioassays by using at least one or two associated weeds as test species. An alternative is to germinate weed seeds separately and use weed seedlings with approximately 1-mm plumules for bioassay purposes. Because inhibition or promotion ofreceiver seedlings is mostly relative, the choice of a control accession becomes crucial in comparative bioassays with rice cultivars.

When growth inhibition of receiver species growing with rice cultivars is compared with that of a control growing without a rice cultivar, allelopathy cannot be suggested as the probable cause of inhibition in the absence of data on soil components. If only a receiver test species is growing in the control, and both the receiver and rice cultivar are growing in the treatment, it is almost impossible to eliminate the likely involvement of competition. In well-replicated studies, investigators must carefully demonstrate the release of a chemical compound by the rice cultivar and must demonstrate its bioactive concentration and persistence in the rhizosphere. It is important to demonstrate that the interference of the compound released by the rice cultivar influences the growth of the receiver plant. We must keep in mind that the presence of competition does not eliminate the likely involvement of allelopathy and vice versa.

Density-dependent experiments may help to demonstrate the likely involvement of allelopathy. If the density of the receiver plant increases and the density of rice cultivars is kept constant, allelopathic growth inhibition should decline (Thijs et al 1994). A high population of receiver species may not be as effective as a low population. At low density, greenhouse-grown oat and soybean were less sensitive to atrazine than plants grown at high density (Hoffman and Lavy 1978). Winkle et al (1981) reported the effect of weed density on phytotoxic effects of atrazine on white mustard (*Brassica hirta*) and alachlor on foxtail millet (*Setaria italica*). They found that the fresh weight of both plants was reduced by the herbicide treatment; however, as the weed density increased, the application rate required to cause equivalent effects increased. It would be interesting to demonstrate experimentally whether rice cultivars exhibit a similar density-dependent phenomenon.

In rice residue bioassays, a realistic amount of rice residue should be added to the soil in an amending experiment. An unrealistically high amount of residue (in fact, of any plant irrespective of allelopathic effect) may inhibit plant growth but is of no value in term of field interactions. Furthermore, any growth inhibition after amending soils with rice residues does not necessarily equate to allelopathy. We must also collect data on soil chemistry (Inderjit and Dakshini 1994).

Conclusions

Weed management using allelopathic rice varieties has been suggested in Egypt (Hassan et al, this volume, Chapter 3), Sri Lanka (Marambe, this volume, Chapter 12), and the United States. Besides demonstrating the allelopathic effects of rice on weeds common to ricefields, it is important to study its effects on abiotic and biotic soil components. Data must be collected on (1) how rice residues or allelopathic rice varieties affect crop growth and yield other than adversely affecting weed growth, (2) their effect on soil microbial ecology, and (3) whether incorporation of rice straw and residues adversely affects availability of certain nutrient ions, and thus soil fertility. Data on the allelopathic potential of rice residues compared with living rice plants will be of interest. Rice is grown in soils ranging from well-drained to poorly drained lowlands with varying soil moisture levels (Marambe, this volume, Chapter 12). We must ask how the allelopathic potential of rice varies with soil texture from well-drained to poorly drained soils under these diverse soil moisture conditions.

When designing a bioassay for rice allelopathy, we must consider ecophysiological, biological, and chemical components of soil, and environmental factors. The allelopathic potential of a particular rice cultivar depends on agricultural practices, soil characteristics, and environmental conditions. When studying interference mechanisms of a rice cultivar or any other plant, we must consider these factors rather than designing unrealistic bioassays to demonstrate allelopathy. Prior to undertaking detailed phytochemical, physiological, and biochemical studies to understand allelopathy, we must demonstrate that allelopathy actually exists. A scientist engaged in demonstrating allelopathy in nature (probably the first step) should not be identified as an allelopathic or competition scientist but as an ecologist. This name is appropriate because competition, allelopathy, or both may be responsible for the observed pattern.

Olofsdotter and Navarez (1996) reported the allelopathic effects of rice on the noxious weed *Echinochloa crus-galli,* which is also known to show allelopathic activity (Rice 1984, 1995). We need to address the question of why rice is not affected by the allelochemicals released by *Echinochloa.* We looked for allelopathic rice cultivars that can perform satisfactorily under weedy conditions by suppressing the weeds and maintaining a high yield in the presence of weeds that are not affected by rice cultivars. Allelopathic cultivars may create physiological-cum-chemical stress effects on the associated weed species and, because of these effects, these weeds could express their allelopathic potential. Further experimentation is needed, however, to prove this hypothesis.

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Notes

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CHAPTER 6

Incorporating the allelopathy trait in upland rice breeding programs

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Promising results in rice allelopathy research offer the possibility to use this phenomenon in breeding programs to enhance weed control in upland rice. To incorporate the allelopathy trait in improved rice varieties destined for the uplands, several elements are necessary: (1) a good screening technique, (2) the existence of genetic variability within *Oryza sativa, O. glaberrima,* and the close wild relatives of rice, and (3) an understanding of the structure and genetic control of the trait. This paper outlines current gaps in knowledge, ways to fill in these gaps, and a perspective on possible breeding strategies.

Introduction

Rice can be grown under diverse agroecological conditions. Weeds are present in all ricefields, but the magnitude of their problem depends on the ecosystem. Under upland conditions, farmers and scientists agree that weeds are the major constraint to yield. In slash-and-burn and permanent rice-based cropping systems, which are the most widespread in the uplands of South and Southeast Asia, management options for weed control are limited because farmers do not have access to inputs, cannot afford them, or do not want to invest in them because of high risks of crop failure from drought. Hand weeding is therefore still the dominant weed control strategy in these areas. Consequently, farmers value cultivars that show good weed interference ability and significantly reduce the time necessary for weeding operations. Although a breeding approach alone cannot overcome weed problems, an increase in the allelopathic potential of rice varieties will likely have a major impact on low-input cropping systems. Moreover, a low-cost seed-based technology is also more easily transferable to farmers than knowledge-intensive weed management technologies such as the use of herbicides.

In the irrigated and rainfed lowland ecosystems, weeds are also the major biotic constraint, but farmers have more options for their control. With the shift in cultivation practices from transplanting to direct seeding, however, and the decrease in water availability for weed suppression and the possible shift to a less vigorous new plant type, the magnitude and complexity of the weed problem will grow.

This paper focuses on upland rice because our work started in this ecosystem, where the weed problem is acute. The research principles and agenda would be fundamentally the same in other ecosystems.

To improve a given trait, breeders need a reliable screening technique along with an understanding of the trait's variability and genetic control. From these elements, a sound breeding strategy can be established. Allelopathic potential has been demonstrated in rice, and screening techniques are available to test for the allelopathic potential of cultivars against important weed species, although our knowledge of the mechanisms of action is still relatively shallow. Our aim is to develop a strategy to increase the allelopathic potential in rice cultivars.

Existing literature is of limited help. Besides evaluating variability, little work has been done on genetic improvement, although the possibility of increasing the allelopathic potential of varieties is often mentioned. This paper summarizes existing knowledge on breeding-related problems in allelopathy research and suggests breeding strategies for the next five years.

Screening technique

A good screening technique is the first essential tool. Such a technique should be reliable, cheap, simple and fast, and preferably usable for mass screening, possibly on a plant-by-plant basis. Classically, laboratory bioassays have been used for screening because they allow detection of individual allelopathic effects, whereas under field conditions it is difficult to distinguish between allelopathic effects and competition. Several bioassays involving different test species and different indices were presented by Leather and Einhellig (1986) and Olofsdotter et al (1995). We will review here only those few elements that are important for breeders.

Most of the bioassays are based on rate and speed of' seed germination and/or root and shoot growth of the target species at the early development stages. They generally assume 3–6replications and measurements averaged on 10–20seeds within each replication. For mass screening purposes, replications can be reduced to two. Plant-by-plant screening is not yet available, which limits screening to already relatively homozygous material. Screening techniques based on individual plant material could be beneficial for breeding purposes.

One element in evaluating the reliability of a screening technique is to determine the broad-sense heritability of the studied trait in the context of the test. Broad-sense heritability measures the degree of confidence breeders can have that the phenotype represents the genotype. A low heritability means that the phenotype constitutes a poor example of the genotype, and genetic progress is likely to be slow when breeders have access only to the phenotype. For genotype comparisons, broad-sense heritabilities at the genotypic mean level, in the context of the test, can be computed as

$$h^2_F = \sigma^2_G \, (\sigma^2_G + \sigma^2_e / n)$$

where σ^2_G and σ^2_e are estimates of genetic and residual variances, respectively, derived from the expected mean squares of the analysis of variance, and n is the number of replications (Gallais 1990). Navarez and Olofsdotter (1996) developed a relay seed-

ing technique that consisted of seeding barnyardgrass (*Echinochloa crus-galli*) in plant boxes where 7-d-old rice seedlings were established and measuring root and shoot length ofthe weed 10 d later. They calculated a heritability of 0.85 for reduction in barnyardgrass root growth, a high value that satisfies the need for reliability.

Another important concern is technique efficiency, which we estimate at 200–250 lines in 3 wk at International Rice Research Institute (IRRI) laboratories. This efficiency allows us to perform genetic studies and does not seriously limit breeding options. The plant box method developed by Fujii (1992) requires almost 2 mo to obtain the same results, but is also acceptable. We can expect the method to be further refined and its efficiency increased, but it is already possible to screen the several hundred lines coming from conventional breeding programs each year. Because of replication requirements, screening for allelopathy has to be delayed to a late stage, after the number of lines has been reduced to a manageable size through selection for other important agronomic traits.

Genetic variability

Another requirement is the existence of genetic variability within the target crop or within its close wild relatives. Because it would be extremely costly to test all 80,000 accessions (not including breeding lines) in the Rice Germplasm Bank held at IRRI, an economically and scientifically sound sampling strategy must be defined. Because of reproductive barriers between cultivated and wild species, it is easier to start screening within cultivated species, testing varieties as close as possible to the target. If this approach is unsuccessful, it will then be necessary to screen wild relatives of the secondary gene pool.

Vaughan (1991) compared possible strategies to choose *Oryza sativa* germplasm for evaluation. Our breeding objective is to develop *O. sativa* upland varieties with high interference ability; thus, we stratified germplasm based on isozymic classification and ecogeographical distribution, and sampled within strata. Two cultivated species of *O. sativa* have worldwide distribution. and *O. glaberrima* although limited to West Africa, is a good competitor because of its rapid and profuse vegetative growth (Jones et al 1996).

Oryza sativa contains vast genetic variability. Because of a diphyletic pattern of domestication (Second 1985), this variability is strongly bipolar with two major subgroups—indicas (group 1 in the isozymic classification) and japonicas (group 6) —and four additional groups (groups 2 to 5) of lesser numerical importance, possibly intermediate between the two main groups (Second and Ghesquiere 1994) or resulting from introgressions from wild species of the Himalayan border (Glaszmann 1987). Our sampling provided a reasonable representation of these groups and, within each group, of the different upland rice-growing countries. For ecosystems, the upland ecosystem was over-represroted. *O. glaberrima* has a much more limited variability based on genetic or agronomic data (Pham 1992). Because *O. glaberrima* has only two morphological types, floating and erect, testing a few accessions produced a reasonably correct idea of variability for the trait.

From the results of the initial evaluation, the strategy was refined and selective samples tested. Variability of the allelopathic potential in *O. sativa* and in a few accessions of *O. glaberrima* was studied by Dilday et al (1991), Fujii (1992), Hassan et al (1995), and Olofsdotter and Navarez (unpublished results). Among the 10,000 rice varieties tested by Dilday et al (1991) under field conditions, 3.9% were found with some degree of allelopathic effects on one or more weed species. Fujii found that 12.7% of 189 varieties in the laboratory were allelopathic against lettuce (*Lactuca sativa*), a common test species. Hassan et al identified 3.4% of 700 accessions as allelopathic against *Echinochloa crus-galli* and 1% against *Cyperus difformis*. The varieties now identified as allelopathic belong to the different isozymic groups identified by Glaszmann (1987), and to different ecosystems. There tended to be a higher frequency among tropical japonicas within *O. sativa* and among *O. glaberrima* accessions (Fujii 1992). These results were confirmed at IRRI using upland cultivars (Table 1).

The continuum of allelopathic potential suggested polygenic control. The trait was more prevalent in traditional varieties than in the improved varieties in Fujii's sample, but was more prevalent in improved varieties than in traditional varieties in the IRRI sample. This difference is attributed to the dominant japonica base of the improved upland varieties. The parentage of allelopathic improved varieties can explain part of these results. Results of Dilday et al (1991) and Hassan et al (1995), for

Table 1. Average and standard deviation of the allelopathic potential of upland rice varieties measured as percentage of barnyardgrass root reduction, using the relay seeding technique. Distribution is between isozymic group, varietal type, and geographical origin.

Group	Varieties tested (no.)	Weed root length (cm) (av ± SD_{n-1})
By isozymic groups[a]		
1	52	72.1 ± 10.8
6A (tropical)	83	57.0 ± 11.5
66 (temperate)	15	63.3 ± 11.3
2	9	79.6 ± 6.3
5	2	81.4 ± 3.4
O. glaberrima	4	54.1 ± 13.4
By varietal type		
Traditional	24	71.0 ± 13.7
Improved	145	62.3 ± 13.0
By geographical area		
Latin America	31	56.4 ± 13.6
West Africa	32	55.1 ± 11.2
Southeast Asia	61	63.3 ± 9.5
South Asia	47	76.0 ± 8.9

[a]According to *Oryza sativa* isozymic classification (Glaszmann 1987).
Source: Olofsdotter and Navarez, unpublished results.

example, seem to trace the origin of allelopathic activity of some semidwarf varieties (such as Taichung Native l, IR8, and Bala) to their common parent Dee Geo Woo Gen (not yet tested). Only one of the four accessions of *O. glaberrima* tested by Olofsdotter and Navarez was allelopathic. Even though the variability of the species is not very wide, more accessions should be studied. Links were established with the West Africa Rice Development Association (WARDA), a sister research organization, which is well advanced in its program of hybridization between *O. sativa* and *O. glaberrima* (Jones et al 1996). One cross involved the accession found to be strongly allelopathic, and it would be interesting to see if the potential was transmitted to the progeny.

Among the close relatives of *O. sativa,* only a few *O. rufipogon* accessions have been tested (Chou et al 1991). They showed a high frequency of allelopathic effects on root length of cabbage (*Brassica oleracea*) under laboratory conditions. Although these authors found no correlation between allelopathic activity and the expression of perenniality, this subject should be examined in more detail. Putnam and Tang (1986) indicated that some aggressive perennial grasses, such as *Sorghum halepense,* had strong allelopathic ability and they explained it by competition during evolution. Within the group of species bearing the same A genome as *O. sativa—O. longistaminata* and *O. rufipogon*—the two perennial species have to be sampled more extensively although seed availability could be a problem for *O. longistaminata.* Of course, a variety with narrow-spectrum allelopathic activity is likely to be of too limited an interest to justify the cost of a breeding program. Varieties with allelopathic effects on a reasonable array of weed species should be identified.

Genotype by environment interactions

Increasing the complexity of breeding is the existence of genotype by environment interactions (G × E), which introduce uncertainty when measuring the superiority of genotypes (Cooper et al 1993). Environment is used here broadly to include weeds.

Knowledge of the magnitude of G × E interactions in rice allelopathy is limited. We found that the water status of the plant (aerobic in the upland ecosystem versus anaerobic in the irrigated ecosystem) did not change the grouping of varieties into qualitative classes (strongly allelopathic, moderately allelopathic, and nonallelopathic) and that the grouping in the field was consistent with the one under laboratory conditions. It has been clearly shown, however, that factors such as solar irradiation (quality as well as intensity), mineral deficiencies, water stress, temperature, and rhizosphere organisms can influence the expression of allelopathy in other species (Rice 1984). We do not know whether these factors interact differentially with genotypes. Soil organic matter content could also be an important factor. Once we identify a set of varieties that is reasonably well adapted to the target ecosystem and is allelopathic to a range of weeds, we will need to quantify the proportion of the variability for the allelopathic effect that is due to genotype, environment, and their interaction. The additive main effects and multiplicative interaction model (AMMI) developed by Zobel et al (1988) and refined by Crossa et al (1990) and Gauch (1992) would allow

us to identify the factors most responsible for G × E interactions through correlations between principal component-axis scores.

The magnitude of G × E effects will determine the conditions needed to do breeding work and the likelihood of gain. A strong G × E effect for allelopathy with crossover interactions would slow breeding progress and indicate a need for decentralized breeding more than would a weak G × E effect. Identifying the main factors responsible for the interaction would allow us to subdivide the ecosystem into homogeneous subregions and then allow us to choose breeding and testing sites that adequately represent the target environments.

Understanding genetic control of allelopathy

A good understanding of the genetic architecture of a trait is helpful in defining a sound breeding strategy. Dilday et al (this volume, Chapter 2) have done the only rice studies and only limited work has been done in other species. Panchuk and Prutenskaya (1973) and Grodzinsky and Panchuk (1974, quoted by Rice 1984) studied the transfer of the allelopathic potential of wheatgrass (*Agropyron glaucum*) to wheat (*Triticum aestivum*). The allelopathic effect on seed germination of radish (*Raphanus sativus*) and on root growth of *Lepidum sativum,* which was linked to the presence of a great number of different inhibitors, was controlled by dominant genes and was directly proportional to the number of genes originating from wheatgrass in the progeny.

We should consider several important points when looking at the potential for genetic improvement. First is the number of genes controlling a trait. Our assumption is 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 are assumed to act in an additive or synergistic way rather than in an isolated way. This essential point needs to be checked. Dilday et al (this volume, Chapter 2) showed that oligogenic control was not a hypothesis to eliminate a priori.

Second, nonallelic interactions between the genes involved in the control of a trait can substantially skew the distribution.

We must then assess the risk of linkage drag, meaning the transfer with the gene(s) of interest of pieces of chromosomes physically linked to it, and bearing genes that might increase or decrease the agronomic fitness of the variety. The distance (cM) between two genes is a direct measurement of the probability of observing crossing-over between these genes and of breaking the favorable or unfavorable linkages.

Last, we must consider the cost in yield associated with the transfer of this trait. Elaborating allelopathic compounds has a cost because it diverts some carbohydrates from yield accumulation. Breeders are experts in manipulating tradeoffs, but it is always easier to find a compromise when cost is not too important. It should be easy to quantify tradeoffs once near-isogenic lines carrying the different genes are obtained. In a plant that is included in a rotation, such as upland rice, the potential yield reduction of other crops attributable to residual effects should probably also be evaluated.

Presently, the best method for examining this array of features is by using molecular markers. This tool would allow us to evaluate the number of genes involved and their location on the rice chromosomes, and identify closely linked markers. We could then evaluate their individual contribution to the variability of the trait, analyze their interaction, determine how much of this variability is additive and how much is due to epistatic effects, and, by comparing the location of the quantitative trait loci (QTLs) with those controlling others traits, evaluate the risks of linkage drag.

The principle for genetic studies is simple—two parents with contrasting behavior are crossed and recombinant inbred lines (RILs) are derived through single-seed descent at a rate of two generations per year, which is slow but secure. This goal could also be achieved through anther culture, a faster but riskier method because of unpredictable anther-culture responses of the material. Table 2 lists potential parents, established according to our present knowledge of their variability. Once a reasonable degree of fixation is obtained, the allelopathic potential of the RILs can be evaluated. A field evaluation should complement laboratory assays. Line fixation will allow replications across years and environments to give more precise results. Using the F_2 population or backcross as the material for genetic analysis, though giving faster results, is less likely to be successful because of the inability to evaluate individual plants.

The next step is to establish the molecular map. Polymerase chain reaction (PCR)-based markers are easier, cheaper, and faster to manipulate. It takes only 2 mo to establish a map for a population of 150 lines versus the 2 yr previously required (N. Huang, personal communication). Therefore, amplified fragment length polymorphism (AFLP) markers are now preferred over restriction fragment length polymorphism

Table 2. Varieties used in crosses aimed at determining the genetic control of allelopathy in rice (strongly allelopathic variety x nonallelopathic variety).

Variety	Origin	Genetic group[a]
Strongly allelopathic		
AC 1423	India	1*[b]
Aus 257	Bangladesh	2
IR56	IRRI	1
Lubang Red	Philippines	1
Musashikogane	Japan	1
Taichung Native 1	Taiwan	1
Vandana	India	1*[b]
YH1	Taiwan	1
Nonallelopathic		
Aus 196	Bangladesh	2
Dinorado	Philippines	6
IR62751-06	IRRI	?

[a]According to Glaszmann's classification (1987). [b]1 = indica, 1* = close to indica group, 2 = aus, 6 = japonica, ? = unclassifiable because of segregation.

(RFLP) markers. A few RFLP anchor markers spaced on the chromosomes, however, can be put on the map to allow comparisons with older maps and to make use of the existing information on QTLs for other traits.

The parents have to be surveyed to identify polymorphic markers. The level of polymorphism could dictate which crosses to work with. For example, the level will be high in indica × japonica crosses, but could be limiting in aus × aus crosses. Next, the molecular map for each of the RILs has to be established. A QTL is obtained by correlating molecular data and phenotypic data. Recently developed software (McLaren 1996) allows easy and fast data manipulation.

This program, although straightforward, will be relatively long because of the time needed to fix the material. A fast and extremely cheap solution would be to use the maps developed for other purposes or by other teams. Four mapped populations are now available at IRRI, and a few more are being developed for the rainfed lowland ecosystem. The parents of crosses are being evaluated to determine whether they are good candidates for genetic studies. Because of the polymorphism requirement, however, most of them are indica × japonica and are not easy materials with which to start a breeding program.

Once the first set of QTLs is identified, fine mapping has to be done in order to find more closely linked markers (less than 5 cM) that can be used as marker genes for the QTLs.

To justify the investment in a marker-aided breeding program, we should be certain of the value of the QTLs identified. This ability presupposes checking on whether or not the same QTLs are detected consistently across environments and across genetic backgrounds. Results for maize (*Zea mays* L.) yield showed surprisingly low QTL × E interactions, even where G × E interactions took place (Stuber et al 1992), but the results were trait-specific. The same population, therefore, has to be tested at multiple sites under different laboratory or field conditions. International collaboration would help speed up this step.

Using chemical knowledge of allelopathic compounds

Marker-aided genetics is a potentially frustrating tool because of statistical correlations between genotype and phenotype that introduce a risk of identifying false QTLs. Breeders determine this risk during data analysis and can minimize it. Nevertheless, breeding work would benefit from a better understanding of what molecules the QTLs are actually coding for.

A huge number of molecules contributing to allelopathy have been identified in crops other than rice. Rice (1984), for example, demonstrated that most of them were phenolic acid compounds derived through the shikimic and acetate pathways. Some were water-soluble and others were volatile. Their primary effect appeared to be membrane disruption (notably permeability) or interaction with hormones. Some allelopathic compounds identified in rice were summarized by Olofsdotter et al (1995). Extending an idea proposed by H. Leung (personal communication) for disease resistance, an alternative approach to the use of randomly chosen probes as markers would

be to perform a systematic search of the databases for sequenced genes involved in the shikimic or acetate pathways, and the molecules involved in membrane functions. Because of sequence homologies between genes of different species, the search does not have to be restricted to rice genes. If some of the intermediate products are already sequenced, PCR primers can be readily constructed and the genes used as probes. The probability is low of finding a QTL and sequenced candidate genes mapping at the same position on a chromosome, but a match would be a major breakthrough. Such a finding would provide an understanding of the role of the QTL, validate the QTL results, and open the door to the possibility of transformation. Several transformation systems are available in rice as well as constitutive root-specific promoters and techniques to induce overexpression of certain genes. Transformation of released elite varieties with such genes can provide a quick impact if the number of genes involved is small or if some of them have a major effect.

Breeding strategy

Unanswered questions on upstream breeding steps have never prevented breeders from improving germplasm by empirical means. We usually define the broad lines of a breeding strategy knowing that the approach will be revised as we come to understand a phenomenon, especially its genetic control.

Because, with some exceptions, parents with the highest allelopathic potential thus far appear to have a low agronomic value, the first breeding strategy is to introgress the chromosomal segment bearing the gene of interest into good agronomic backgrounds. As long as the gene coding for the QTL has not been identified, a marker-assisted backcross program seems to be the best option. This approach allows us to recover the elite genotypes with QTLs introgressed as quickly and tidily as possible. The probability of success of such an approach, however, depends on the number of QTLs we will be able to detect and the proportion of phenotypic variability they explain. Should a few QTLs (fewer than five) explain a major part of the variation, a marker-aided backcross program can be considered. This is not an unrealistic restriction because, contrary to the assumptions of quantitative genetics, most results in rice show that a few QTLs with important effects are associated with several QTLs with minor effects.

If a high number of QTLs with little effect are involved, then a classical breeding program is a reasonable option. From a breeding perspective, the distinction between allelopathy and competition is somewhat academic because, in a farmer's field, interference is the phenomenon that really counts. A classical breeding program should try at once to both improve allelopathic potential and competitive ability by giving field testing high priority during the selection phase. Because of the constraints of the screening technique, selection should be delayed until some degree of homozygosity is reached either through anther culture or single-seed descent. Early generation screening for other traits is possible, but this procedure may carry the risk of losing the variability in which we are interested if the linkage situation is unfavorable.

Recurrent selection that progressively accumulates favorable alleles in a population might be another method to explore. The use of the recessive male-sterility gene of IR36 would facilitate the intermating process. This approach has been successful in breeding rice with less complex traits (Chatel et al 1996). To keep the recurrent selection cycle timely, however, progress is needed on the technique that allows selection among S1 or S2 plants.

Conclusions

The study of allelopathy began only 10 yr ago in rice, and breeding for the trait is in its infancy. We have a long way to go before we can release varieties with improved allelopathic ability. We face many unknowns, but the challenge is exciting. We must remain flexible and be ready to modify breeding strategies according to our progress in understanding this complex trait.

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CHAPTER 7

What are allelochemicals?

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In this paper, we clarify and unify the terminology used by researchers who study allelopathy. In particular, we use the term "allelopathy" as defined at the 1996 meeting of the International Allelopathy Society (IAS) and propose definitions for the terms "allelochemical," "biocommunicator," and "plant growth regulator." Also, we look at the structural relations between plant hormones and allelochemicals, their bioactivity, and their mechanisms of action. And we discuss possible applications of allelopathy in weed control, pointing out the benefits that this environmentally friendly technology should contribute to agriculture and public health. A summary of the preliminary results we obtained with sunflower varieties is presented as an example of the weed control potential that allelopathy can offer to farmers.

Introduction

Allelopathic interactions derive from the production of secondary metabolites (allelochemicals) by higher plants. These chemicals induce a wide array of biological effects, and interdisciplinary studies using a team approach will accelerate our understanding of these effects to the benefit of agriculture and weed management.

The term "allelopathy" has undergone several changes over time (Molisch 1937, Rice 1974, 1984). In 1982, C.-H. Chou and G.R. Waller used the term "allelochemical" to mean that both inter- and intraspecific interactions between organisms were mediated through a chemical process (Chou 1993). The definition accepted by the International Allelopathy Society (IAS 1996) is "any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influences the growth and development of agricultural and biological systems." This definition considers all biochemical interactions between living systems—where plants, algae, bacteria, and fungi are included—and their environment.

Macías and Galindo (1995) and Galindo (1993) proposed the term "biocommunicator" to refer to "every single chemical or mixture of chemicals used by living organisms to exchange information." The introduction of the term "biocommunicator" into the definition of allelochemicals suggests the possibility of active mixtures, because of the increasing number of findings in which single compounds are not active or are not as active as a mixture. Synergistic effects between the components of such mixtures could explain observed behavior.

The use of both terms, "allelochemical" and "biocommunicator," covers a range of chemicals used by organisms to exchange information, from pheromones and allomones through poisons, toxins, deterrents, phytoalexins, or allelopathic agents. These chemicals govern most organism behavior, from defense-attack responses to social conduct or warning signals. Another term, sometimes used erroneously, is "plant growth regulator," which is "any chemical, natural or synthetic, that can influence the growth and development of plants." This definition covers plant hormones (natural products synthesized by the plant) used internally, and with a regulator role for plant growth and development, as well as herbicides. An allelopathic agent could be considered a plant growth regulator, the main difference being that an allelopathic agent has to be introduced into the environment to accomplish its protective role.

Plant hormones versus allelopathic agents

Plant hormones can be classified mainly into six types: auxins, with a structure of the 3-indoleacetic acid (IAA) type; gibberellins, based on the gibberellic acid skeleton and the most abundant plant hormone class; cytokinins. with a purine as the base structure; abscisic acid (ABA) and related compounds: ethylene; and brassinosteroids. Several allelopathic agents are structurally similar to these compounds and are good examples of the slight differences between an allelopathic agent and a plant hormone. Figure 1 shows some examples of these compounds.

The mechanisms of action of allelopathic agents resemble those of the synthesis of plant hormones, such as ferulic acid, which activates the synthesis of ABA (Hollapa and Blum 1991), and the dihydroflavonone naringenin. which promotes IAA degradation by stimulating the IAA oxidase (Stenlid 1970). The sesquiterpenes farnesol and b -selinene present antiauxin and antigibberellin activity (Komai et al 1981), and the sesquiterpene lactone argophylline A presents antiauxin activity (Watanabe et a1 1982) (Fig. 2).

The mechanisms that govern plant growth and the steps in plant development involve many metabolic processes. The synthesis and control of plant hormone levels also involve many steps. These steps represent good targets for defense-attack strategies. Little has been done using these strategies, and future research in this area should lead to a more complete knowledge of the role and mode of action of allelopathic agents.

Developments in weed control can be divided into three periods. Before 1945, organic and inorganic herbicides had very low activity and zero crop selectivity. By the mid-1940s, with the discovery of the phenoxy herbicides, followed by phenylureas, triazines, and glyphosates, the modern era of herbicides began. We successfully used the different steps in the biochemistry of plants as targets for herbicide action, and for the first time selectively used weed control in crops. The mid-1970s marked the era of low-dose herbicides, which corresponded to the discovery of the sulfonylurea herbicides, which allow crop-selective weed control (Macías 1995).

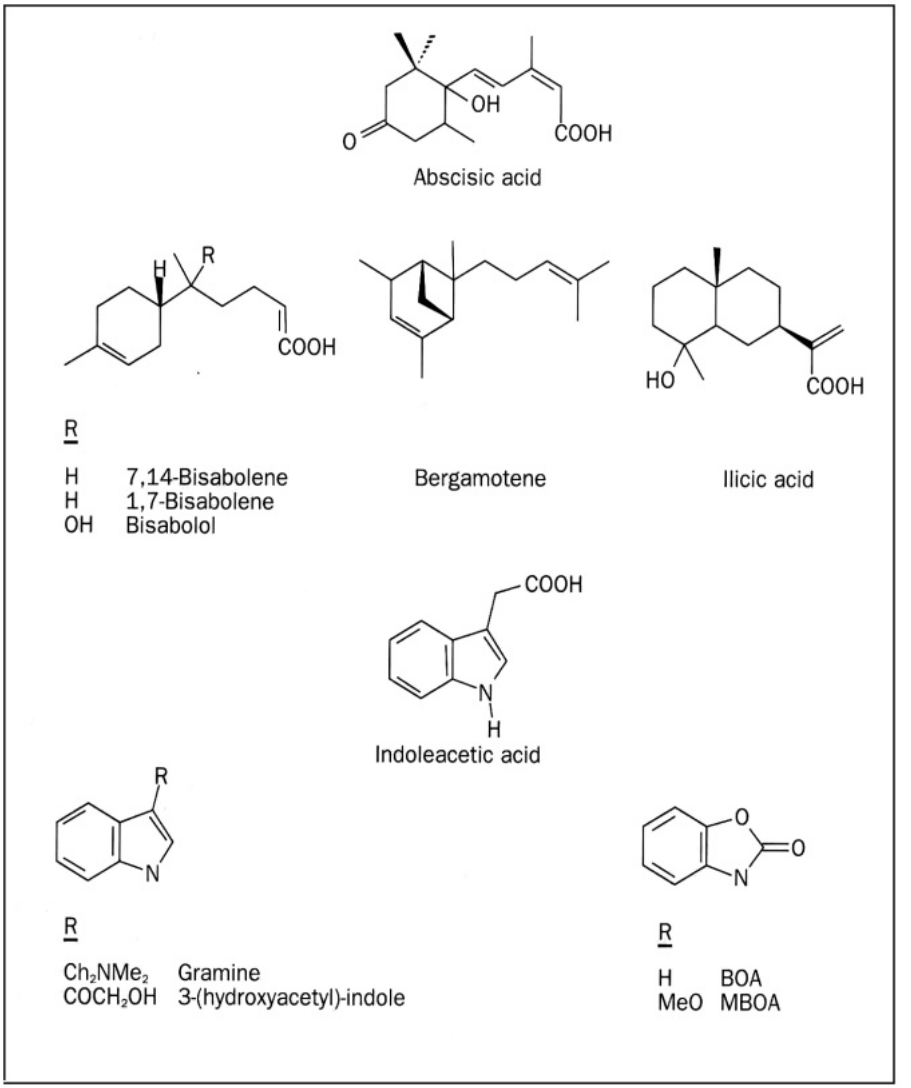

Fig. 1. Some allelopathic agents with an ABA- and IAA-like structure.

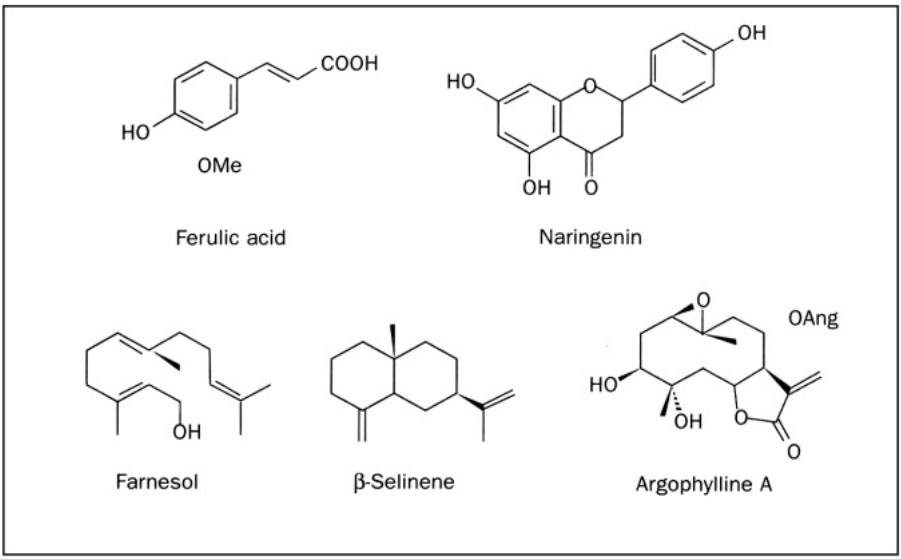

Fig. 2. Allelopathic agents that interact with hormone processes.

What should allelopathy contribute to weed control? Could the development of new herbicides be reaching its peak and do we need to examine new methods of weed management? Allelopathy, in all its different aspects (from agronomic to chemical points of view), should offer a new approach. Different strategies should be followed:

1. Using allelopathic plants in crop rotations, as companion plantings, and as smother crops. This technique is already being used while research proceeds. Japan, India, and South Korea are among the leaders in this technology.
2. Closely related to the above, using phytotoxic mulches and cover crop management, especially in no-tillage systems, is another good option. Again, this system is being developed mainly in Japan, India, and South Korea.
3. Transferring allelopathic traits into commercial crop cultivars. Geneticists in the United States are the ones mainly interested in this area of research.
4. Using natural or modified allelochemicals as herbicides. Along with the first point mentioned, this approach represents the target where a great part of the research is focused. Knowledge of the chemicals responsible for allelopathic interactions should help us to characterize new families of herbicides based on natural-product skeletons. Researchers worldwide are working on this objective.

What should be the advantages of herbicides developed from natural products with phytotoxic activity? Some benefits expected from this research are:

1. The introduction of new classes of herbicides would help to overcome resistance produced by the repetitive use of synthetic herbicides.

2. The high specificity of allelopathic effects lets us expect to develop highly specific herbicides to be applied according to the problems we must solve.
3. The use of mulches, residues, and cover crops in an appropriate design would allow us to exploit the phytotoxic activity of allelopathic plants to minimize the impact of weeds. The correct application of these techniques would profit farmers in two ways: by adding value to crop by-products that have no economic value and by developing no-tillage or low-tillage systems that reduce costs.
4. Although we know that the persistence of a natural product in the soil depends on the soil's physical and microbiological characteristics, we do not know the half-life periods of these products. Where "soil sickness" has been reported, monocultures developed over many years with a continued accumulation of residues and phytotoxins are the cause in wheat (Putnam and Duke 1978, Lodhi et al 1987), coffee (Waller et al 1986), and asparagus (Young 1986), for example. Except for such cases, natural products stay in the soil only long enough to produce an effect and then are progressively biodegraded, through photochemical, oxidation, and biodegradation mechanisms. The use of natural chemicals, therefore, should be considered as "environmentally soft."
5. The development of new transgenic crop cultivars with allelopathic properties will allow us to use lower amounts of herbicides, with subsequent benefits for the environment.

In conclusion, advances in allelopathy research are likely to lead to new solutions in agriculture and solve new problems in public health, the environment, and weed management.

Sunflower: an example

Sunflower is a commercial crop of worldwide economic interest and a good example of the possibilities of exploiting allelopathy in weed control. Reports of its allelopathic properties date back to 1931, when Cooper and Stoesz mentioned the atypical distribution of "fairy ring" (characterized by a decrease in the number of plants, size, and inflorescences in the middle of the ring) in wild sunflower prairies of *Helianthus rigidus*. This phenomenon was also observed in 1968 for the common sunflower *H. annuus* (Wilson and Rice 1968) in a first step of weed succession in abandoned plots. This kind of behavior was proposed as allelopathic, and chlorogenic acid, iso-chlorogenic acid, and the coumarins ayapin and scopoletin were suggested as being responsible (Fig. 3). Because chlorogenic and iso-chlorogenic acids were not present in root exudates or leaf leachates, it was suggested that these acids came into the environment via the decomposition of plant residues. This suggestion also explained the success of sunflower in the first step of succession.

Thereafter, several phenolic acids and fatty acids isolated from roots and leaves of *H. annuus* and *H. tuberosus* were correlated with their allelopathic properties (Leather and Forrence 1979). The compounds characterized were gallic, protocatechuic, *p*-hydroxybenzoic, benzoic, vanillic, syringic, and salicylic acid, and several

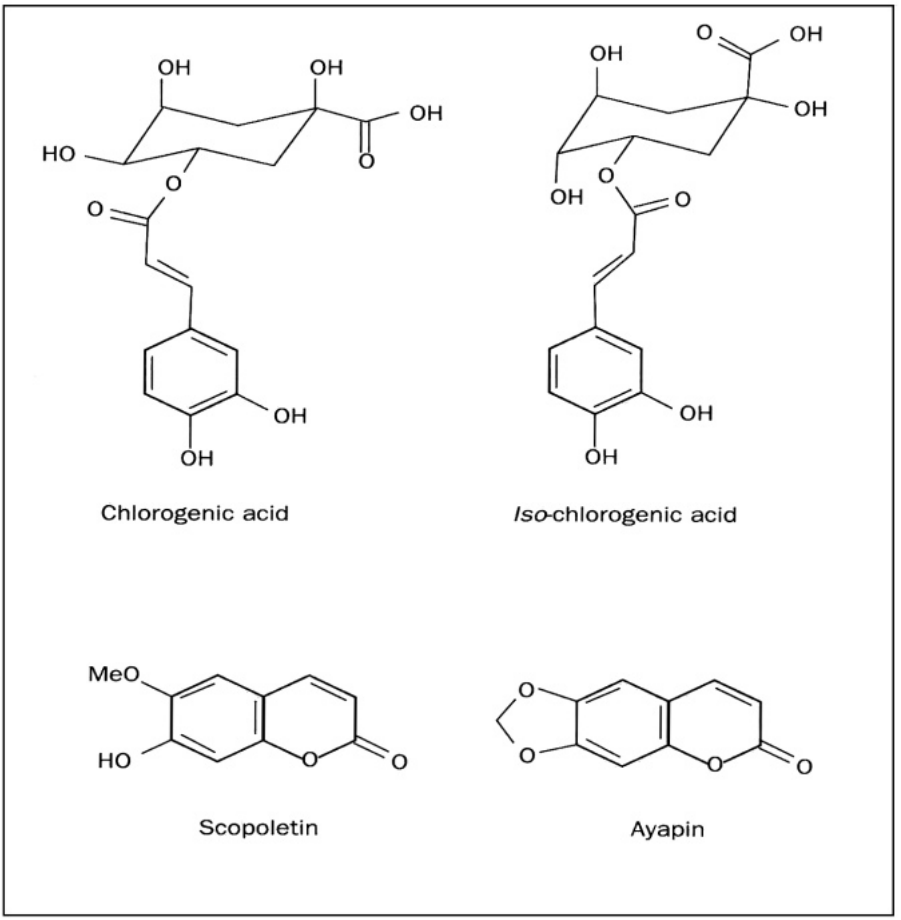

Fig. 3. First allelopathic agents isolated from *Helianthus annuus*.

C_{10} to C_{18} fatty acids. Neither the levels of phenolic acids detected in the plant and the surrounding soil nor the concentrations of these compounds necessary to show phytotoxic activity can explain the allelopathic properties of *Helianthus* spp. So, other classes of secondary metabolites were studied.

As a member of the family Compositae, *Helianthus* species are rich in sesquiterpene lactones, an interesting group of sesquiterpenes with a wide spectrum of bioactivity. The sesquiterpene lactones argophylline A and B and niveusin B and C were isolated from *H. argophyllus* (Macías 1995) and *H. annuus* var. Giganteus (Spring et al 198l), respectively. All of them interact with the hormonal system of the plant and show antiauxin activity (Macías 1995). In particular, niveusin B and C produce a growth inhibition by blocking the bound IAA complex (Spring et al 1982). These

compounds, as well as most terpenoid compounds of *Helianthus,* are located in the trichomes of the back of the leaf, so they are easily leached by rain, fog, and dew into the environment.

Pursuing this research, we isolated several phytotoxic sesquiterpene lactones with guaianolide and germacranolide skeletons from aqueous extracts of sunflower leaves of different varieties (Macías et al 1993a, 1996, 1997, Torres 1994, Varela 1996). These compounds were obtained using a bioassay-guided protocol from bioactive fractions, which have also yielded sesquiterpenes belonging to a new family named heliannuols (Macías et al 1993b, 1994) (Figs. 4 and 5). From these fractions, several sesquiterpenes, bisnorses-quiterpenes, diterpenes, triterpenes, chalcones, and other phenolics have also been obtained (Macías et al 1993a, 1996). The results of the bioassays indicate that:

1. These chemicals have a more pronounced activity on dicotyledon species than on monocotyledons. This result correlates well with earlier results from bioassays of aqueous extracts and chromatographic fractions.

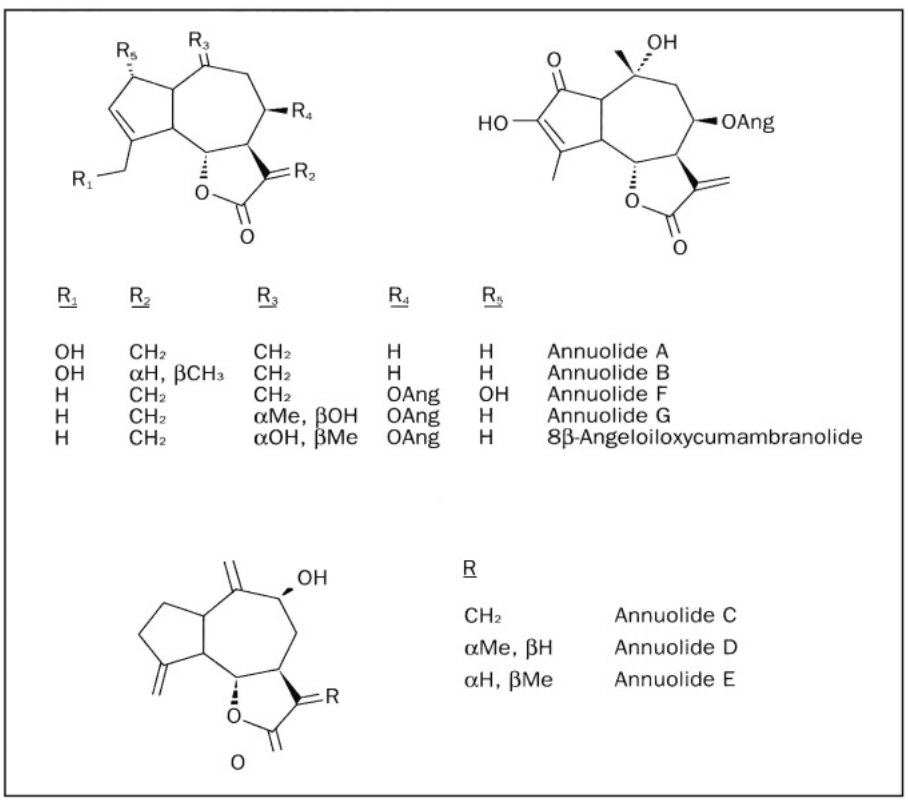

Fig. 4. Guaianolides from *Helianthus annuus.*

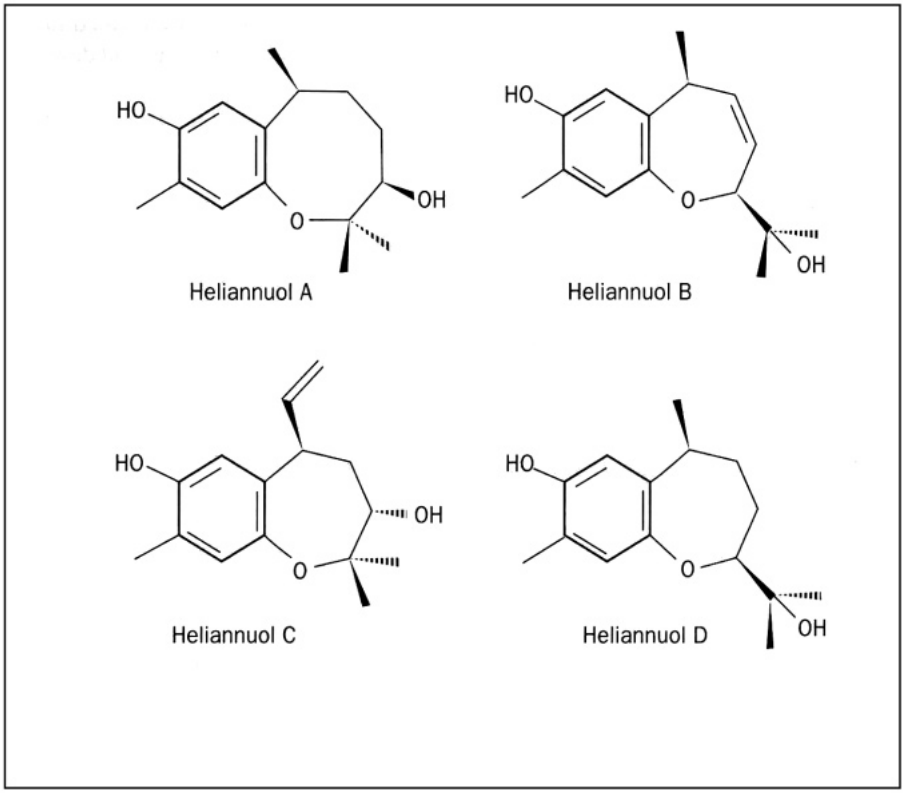

Fig. 5. Heliannuols isolated from *Helianthus annuus.*

2. Sesquiterpenes (lactones and heliannuols) show more pronounced activities than the rest of the compounds isolated (Macías et al 1997, unpublished results). For example, annuolides B and C exert levels of inhibition of germination above 40% at concentrations as low as 10^{-6} M and 10^{-9} M, respectively. Therefore, they are the primary compounds responsible for the allelopathic activity of sunflower.
3. Because all of these compounds have been isolated from aqueous extracts obtained by soaking leaves under mild conditions, it is also reasonable to think of them as allelopathic agents easily leached by rain, fog, or dew.

Rice (1984) and Leather (1983) reported the allelopathic effects of sunflower on different common and highly problematic weeds. It has also been reported that sunflower can have negative effects on successive crops because of the accumulation of residues and leachates. Sorghum is a good example (Schon and Einhellig 1982). Furthermore, Leather (1987) reported an integrated method of weed control using sunflower and the herbicide 5-ethyl-dipropylcarbamotinate. This method was effective only until the second year of treatment, when autotoxic effects reduced crop yields. Phenolics were thought to be responsible for such behavior (Einhellig 1986).

Conclusions

Throughout this paper, we have attempted to clarify terminology that is often used in a wrong, confusing, or even contradictory manner. Once the meaning of terms is agreed upon, a second objective is to draw a picture of the applicability of compounds, not only to explain the interactions between living systems but also to use them as models for developing new herbicides or new techniques of crop management. Every allelopathic study of any single system implies a knowledge of the biocommunicators involved and their mechanisms of action.

Finally, traditional agricultural practices based on ancient knowledge are useful as starting points for new allelopathic studies. This approach, similar to that used in the search for new medicines, is based on the belief that these practices result from the close contact between nature and humans throughout history, associations that have led to the development of certain "modus operandi" in which intuition and knowledge of nature go hand in hand. In this way, the study of allelopathy using ancient cultivars of rice, wheat, maize, barley, or grapes might add more interesting results and applications to those already existing.

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Notes

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CHAPTER 8

Searching for allelochemicals in rice that control ducksalad

J. Mattice, T. Lavy, B. Skulman, and R. Dilday

Forty-one compounds were analyzed by gas chromatography mass spectrometry (GC/MS). Single allelopathic or nonallelopathic rice plants were grown in either 500 or 50 g of soil and then transplanted to deionized water for 48 h. GC/MS analysis of extracts of the water showed statistically higher levels of 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 4-hydroxyhydrocinnamic acid, and 3,4-dihydroxyhydrocinnamic acid in water coming from soil growing allelopathic rice lines PI# 294400 or PI# 277414 than in water from soil growing the nonallelopathic rice variety Rexmont. The GC/MS analysis also tentatively identified 4-hydroxyphenylacetic acid. The aquatic weed ducksalad [*Heteranthera limosa* (Sw.) Willd.] was better controlled in soil where the two allelopathic lines were grown than in soil where the nonallelopathic variety was grown. When rice was grown in 5 g of soil that was analyzed before flooding, statistically larger amounts of 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 3-hydroxy-4-methoxybenzoic acid, 4-hydroxycinnamic acid, stearic acid, tetradecanoic acid, and a compound tentatively identified as n-valeric acid were found in allelopathic line PI# 312777 than in Rexmont or the blank control soil. The use of ethyl ether for the extraction of known and potential acidic allelochemicals was found to be superior to solid-phase extraction methods using C18, charcoal, styrene divinylbenzene, or XAD-4 resin.

Introduction

An agricultural crop that produces allelochemicals that inhibit the growth of weeds can lower production costs for herbicides and their application.

One of the most prevalent aquatic weeds in rice in the United States is ducksalad [*Heteranthera limosa* (Sw.) Willd.] (Dilday et al 1991, Chandler 1981). Over several years, Dilday at the Rice Research and Extension Center at Stuttgart, Ark. (USA), screened approximately 12,000 accessions from the USDA/ARS rice germplasm collection for allelopathy toward ducksalad. Allelopathic activity was measured in two ways. One was to measure the distance from the rice plant to the outer edge of the area of suppression of ducksalad. The second was to measure the reduction in the ducksalad population on a percentage basis compared with a control rice plant that had no effect on the ducksalad population. Researchers found 412 accessions with allelopathic activity. Nine accessions had a radius of activity of 17 cm or greater and

weed control of 70% or greater. The accessions with allelopathic activity were from 30 different countries and were genetically diverse for other plant characteristics. Apparent allelopathic activity toward at least four other weed species among other accessions was observed. These species are purple ammannia [*Ammannia coccinea* Rottb.], broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash], flatsedge [*Cyperus iria* L.], and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.].

Although these rice plants may have allelopathy toward ducksalad, they are not agronomically acceptable. But if the allelopathic traits can be incorporated into commercially useful cultivars while maintaining the yield and quality of the grain, farmers, consumers, and the environment could benefit.

Chou and Lin (1976) found significant amounts of phytotoxic substances, including 2-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, cis and *trans*-4-coumaric acid, ferulic acid, and vanillic acid in rice paddy soil containing decomposing rice straw. In a later study (Chou et al 1981), syringic acid was also identified.

In the situation we were investigating, the allelopathic effect on ducksalad develops as the rice grows; therefore, it is unlikely that the compounds are being produced by decomposing rice tissue. The compounds are more likely being exuded from the rice roots. This would be consistent with the observation that the effect is seen out to the edge of the roots but not further. It is still possible that the compounds observed by Chou and Lin (1976) are also released by roots, and these were some of the first compounds we were looking for.

Tang (1986) discussed some of the problems of collecting root exudates. Physical damage to the roots during the experiment could release intracellular compounds that would not be true root exudates. Also, if the plants are stressed, they may exude different amounts of compounds than they would if not stressed.

Tang and Young (1982) developed a continuous root exudate trapping system (CRETS) using XAD-4 resin as the trapping medium to overcome these problems. The resin trapped neutral compounds well but not acidic compounds (Tang 1986). Ion exchange resins could not be used because they would change the pH of the nutrient solution.

Our objective was to identify the chemicals responsible for the allelopathic effect on ducksalad. This may help in the incorporation of this trait into other rice lines. New herbicides may also be produced that are based on the compounds produced by the rice plant.

Materials and methods

Our approach was to compare chromatograms of extracts of rice tissue, soil, and water or nutrient solution from allelopathic and nonallelopathic samples. If the allelopathy were attributable to compounds produced by the allelopathic rice but not by the nonallelopathic rice, then we would likely see peaks in the chromatograms from the allelopathic lines that would not be present in those of the nonallelopathic lines. If, as is more likely, the effects were due to chemicals present in both lines, but present

in larger amounts in the allelopathic lines, then we should see larger peaks for those compounds in the chromatograms from the allelopathic samples.

Table 1 lists the compounds investigated for which standards were obtained. Initially, the first 15 compounds were chosen for investigation. Over time, as other compounds were identified in tissue, soil, or water extracts, they were incorporated into the study.

Analytical instrumentation and conditions

High-performance liquid chromatography (HPLC). The equipment consisted of an ISCO model 2350 pump, model V4 UV detector, and model 2360 gradient programmer, and an LDC Milton Roy autosampler.

Separation was developed for the original 15 compounds on a Rainin 25 cm × 4.6 mm Dynamax C 18 column. Two mobile phase systems were used, and both were optimized using DryLab Software (LCResources Inc., Lafayette, Calif., USA). The flow for both systems was 1.5 mL/min. System 1 used tetrahydrofuran, acetic acid, and water as follows:

Solution A: 5/1/95 tetrahydrofurad/acetic acid/water
Solution B: 95/1/5 tetrahydrofurad/acetic acid/water
Gradient: linear from 100% A to the mixture 65% A/35% B over 44 min

System 2 used acetonitrile, acetic acid, and water as follows:

Solution A: 5/1/95 acetonitrile/acetic acid/water
Solution B: 95/1/5 acetonitrile/acetic acid/water
Gradient: linear from 100% A to the mixture 65% A/35% B over 41 min

Detection was at 254 nm for all compounds except 2-hydroxycinnamic acid, *trans*-cinnamic acid, and 2-hydroxybenzoic acid, which were detected at 277 nm. Injection was 50 or 100 microliters.

Gas chromatography mass spectrometry (GC/MS). The system was a Varian Saturn2 Ion Trap mass spectrometer and model 3400 GC equipped with a septum programmable inlet (SPI) and a model 8100 autosampler. It was fitted with a J&W DB-5MS 30 m × 0.25 mm, 0.25 micron column, and the flow was 0.4 m sec^{-1} of helium. The temperature program for the column was 80 °C for 1.25 min, increase to 290 °C at 10 °C min^{-1} and hold at 290 °C for 8 min. The program for the injector was 70 °C for 15 sec, increase to 260 °C at 180 °C min^{-1} and hold at 260 °C for 2 min. The filaments were turned on after 5 min and data for electron ionization spectra were collected from 5 to 30 min over a mass range of 50 to 600 mass units.

Extraction of samples

Soil and roots. Soil samples were 5 g and root samples from small rice plants were typically between 20 and 100 mg. Roots were cut into small pieces and homogenized with the extractant for 1 min before shaking.

The soil or root sample was placed in a 125 × 20-mm screw cap culture tube (150 × 25 mm for roots) along with 10 mL of extractant (0.05 N NaOH in 50%

Table 1. Chemicals characterized on gas chromatography/mass spectrometry as trimethylsilane derivatives.

Chemical name	Common name
3-hydroxyphenol	Resorcinol
Phenylacetic acid	
2-hydroxyphenylacetic acid	
2-hydroxybenzaldehyde	Salicylaldehyde
4-hydroxybenzaldehyde	
Benzoic acid	
2-hydroxybenzoic acid	Salicylic acid
4-hydroxybenzoic acid	
4-hydroxy-3-methoxybenzoic acid	Vannilic acid
3,5-dimethoxy-4-hydroxybenzoic acid	Syringic acid
trans-cinnamic acid	
2-hydroxycinnamic acid	*o*-coumaric acid
4-hydroxycinnamic acid	*p*-coumaric acid
3,4-dihydroxycinnamic acid	Caffeic acid
4-hydroxy-3-methoxycinnamic acid	Ferulic acid
3-hydroxybenzoic acid	
2-phenylethanol	
3-phenylpropanol	
4-phylbutanol	
Octanoic acid	
Decanoic acid	
Tetradecanoic acid	Myristic acid
Hexadecanoic acid	Palmitic acid
9,12-octadecadienoic acid	Linoleic acid
9-octadecanoic acid	Oleic acid
Octadecanoic acid	Stearic acid
3-phenylpropanoic acid	
4–phenylbutanoic acid	
2,4-dihydroxybenzoic acid	
3,4-dihydroxybenzoic acid	
3,5-dihydroxybenzoic acid	
2,3-dimethoxybenzoic acid	
2,4-dimethoxybenzoic acid	
2,5-dimethoxybenzoic acid	
3,5-dimethoxybenzoic acid	
3-(2-hydroxyphenyl)propanoic acid	
3-(3-hydroxyphenyl)propanoic acid	
3-(4-hydroxyphenyl)propanoic acid	
3-(3,4-dihydroxyphenyl)propanoic acid	
3-(2,3-dimethoxyphenyl)propenoic acid	
3(2,4-dimethoxyphenyl)propenoic acid	
3-(2,5-dimethoxyphenyl)propenoic acid	
	Ursolic acid
	Abietic acid
	Dehydroabietic acid
5-hydroxy-2-indolecarboxylic acid	
1,7-heptanedicarboxylic acid	Azelaic acid

water:methanol). The tubes were capped and shaken for 30 min on a wrist-action shaker at 3-4 shakes sec^{-1}. A 250-µL portion of concentrated HCl was added, the tube was briefly shaken to mix the contents, and the samples were then centrifuged at 700 g for 10 min. Two mL were transferred to a vial for HPLC analysis. The remainder was resuspended and extracted with four 5-mL portions of ethyl ether, shaking for 1 min per extraction. The combined ether portions were placed in a clean, dry test tube and approximately 1 mL of anhydrous sodium sulfate was added and allowed to stand for approximately 5 min. The ether was then transferred to a clean, dry 150 × 15-mm screw cap culture tube and the sample was then immersed in a 35 °C water bath and the ether was evaporated to dryness under a stream of nitrogen. The samples were derivatized for GC by adding 50 µL of Regisil (99% N,O bis[trimethylsilyl]trifluoroacetamide + 1% trimethylchlorosilane) and 116 µL of pyridine. The tubes were capped and heated for 90 min at 100-105 °C. The tubes were then cooled, 100 µL of 100 ppm 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzeneamine (trifluralin) were added as an internal standard, and 2 mL of ethyl acetate were added by pipette. The samples were mixed and analyzed by GC/MS within 6 h.

Water: Depending on the study, the water samples varied in size from 10 to 300 mL. The 300-mL samples were acidified to pH 1 with HCl and extracted with 150 mL of ethyl ether followed by 100 mL of ether, shaking 1 min per extraction. The 10-mL samples were extracted with three 5-mL portions of ether. The combined ether portions were dried over anhydrous sodium sulfate and then evaporated to dryness and derivatized as for the soil samples.

Trapping and desorption of potential allelochemicals using XAD-4, charcoal, and C18. A 3-mL J.T. Baker C18 SPE cartridge was conditioned with 1 column volume of methanol followed by 2 column volumes of deionized water. The tubes were equipped with 75-mL reservoirs.

Carbopack B cartridges were prepared using 0.5 g of 60/80 mesh Carbopack B in a 3-mL plastic cartridge. They were conditioned with 5 mL of 80:20 CH_2Cl_2-MeOH followed by 3 mL of MeOH and 10 mL of deionized water.

XAD-4 resin that had been previously Soxhlet-extracted with MeOH and stored under MeOH was placed in a beaker and equilibrated with deionized water. It was then poured into a 13-mm column to a depth of 8 cm and maintained under water.

The samples were 100 mL of water containing the original 15 compounds in concentrations ranging from 0.05 to 1.25 ppm depending on the sensitivity of the detector to the compound.

The samples were drained through the columns and the trapped compounds were eluted and collected. The charcoal was eluted with 1 mL of MeOH followed by 7 mL of 4:1 CH_2Cl_2-MeOH. The C18 was eluted with 3 mL of MeOH and the XAD-4 was eluted by drawing through a small portion of MeOH and then allowing the resin to equilibrate with the MeOH. After equilibration, 25-30 mL of MeOH were drawn through. The collected eluents were evaporated to almost dry under a stream of nitrogen and then made into 3 mL with 1% acetic acid for HPLC analysis.

Stability in water and nutrient solution

The original 15 compounds were placed in 20 mL of each of the following: deionized water, Hoagland solution with ferric chloride, Hoagland solution with ferrous sulfate, Hoagland solution with sequestrene iron, and nutrients with no iron. The solutions were allowed to stand in the greenhouse for 44 h and were then extracted and analyzed by HPLC, when freshly prepared samples in water were also analyzed.

Plant-growing systems

Flow-through system. The first flow-through system was a CRETS as described by Tang (1986). The major modification to the original design was to include a 1-L reservoir connected to the outlet of the trapping resin tube (Fig. 1). The modification allowed for transpirational losses to occur without requiring constant supervision of the system. All tubing, reservoirs, and pots were covered with aluminum foil to restrict light and deter algae growth. Air for pumping the nutrient solutions was supplied by commonly available aquarium pumps. It was necessary to include several one-way check valves on the flow line to prevent backflow of the air into the reser-

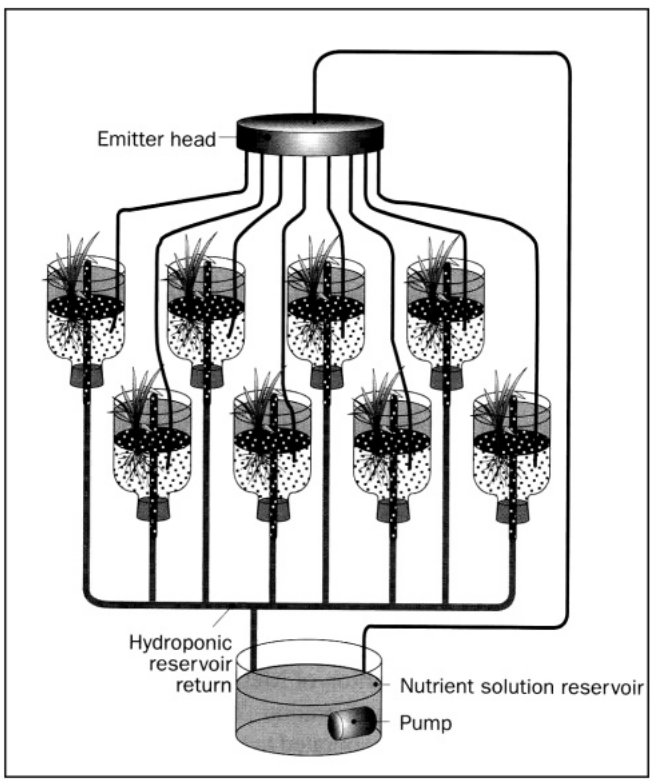

Fig. 1. Continuous root exudate trapping system (CRETS).

voir and to prevent nutrient liquid flow into the air pumps. As much as possible, all parts were constructed of glass or Teflon. Teflon tape was used to cover nonglass/Teflon components except for the check valves. The pots for growing rice were 4-L brown glass reagent bottles with the bottoms cut off. These bottles were inverted and held in a wooden frame with the trapping resin column inserted into the bottle neck through a Teflon-covered stopper. The reservoir and pump were located underneath each bottle on the wooden frame. Each pot was a separate self-contained system. Eight seeds of rice were planted in an 8-cm circle near the center of the pot, hand-watered until germinated, and grown to 10-15 cm in height before the CRETS system was started. At that time, the plants were thinned to 4 plants pot^{-1}, nutrient solution circulation started, and chemical trapping began.

Bench system. A second flow-through (bench) system was used that was equipped with a relatively large 60-L central reservoir that fed nutrient solution to eight separate rice pots of the same type as used for the CRETS (Fig. 2). In these experiments, the nutrient solution and reservoir were the common connection between the pots. The drains for this system were designed to allow continuous flood on the rice and

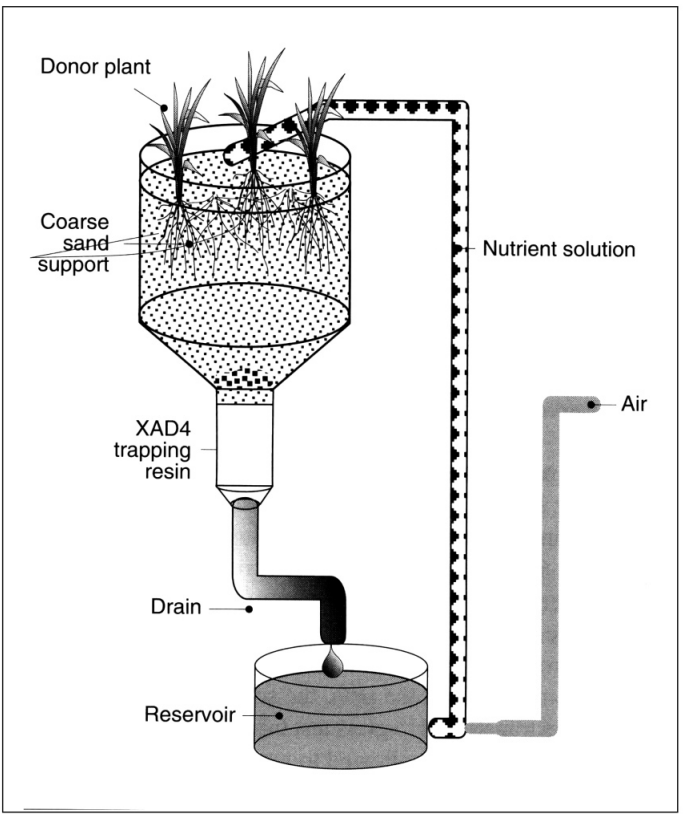

Fig. 2. Bench system.

thereby simulate field conditions. Nutrient solution was pumped up to an irrigator manifold from which individual lines fed the separate pots of rice. The pots then drained back to the central reservoir. The total system of eight pots and one reservoir constituted one bench. Eight seeds of rice were planted in an 8-cm-diam circle in the center of each pot and hand-watered until grown to 10-15 cm, at which time the flood was applied. The central reservoir was periodically sampled and analyzed. Attached pots contained either allelopathic or nonallelopathic rice. In some experiments, half the pots contained only ducksalad, whereas the other pots contained either allelopathic or nonallelopathic rice. The system allowed for continuous flushing of the rice root exudates into the common reservoir, where they could be sampled.

Static system. For the static systems, rice was grown in pots or tubs in order for allelochemicals to be exuded and concentrated in situ. Either nutrient solution with a sand support or water with a soil support was maintained as needed to simulate field conditions. Initially, 12-L pots equipped with a drain spigot were used. Periodically, water or nutrient solution was collected from the spigot and analyzed. Later, rectangular tubs (56 cm long × 39 cm high × 38 cm wide) were used with soils infested with ducksalad seed. The tubs were filled to a depth of 20 cm. Rice was planted in these tubs along the wide side approximately 10 cm from the tub wall and at a 10-cm spacing. The tubs were equipped with a bottom sampling drain covered with gravel over which was placed a layer of glass wool to prevent loss of the support media through the drain. Solution was removed periodically for analysis.

Several types of support media were used in these studies. Initially, coarse sand was chosen because of concerns about adsorption of the released allelochemicals by soil organic matter. When sand was the support medium, a Hoagland solution was used to provide nutrients to the growing plants. Later, a 1:1 mixture of soil:coarse sand provided for the modifying influences of soil microorganisms that might occur in the root zone, and still allowed adequate drainage for sampling through the bottom drain. A half-strength Hoagland solution was used with these systems. Nutrient levels and pH were maintained by appropriate additions of stock nutrients or 1 M hydrochloric acid to the reservoirs.

Soil water transplant experiments

500 grams. Some 500 ± 10 g of commercial potting soil were placed in a plastic pot 13 cm high with a bottom diameter of 11 cm and a top diameter of 17 cm. Two rice seeds were planted and 60 ducksalad seeds were spread over the top. The soil was watered as needed to germinate the rice. The samples were thinned to 1 plant pot^{-1}, and the pots were flooded when the rice was approximately 10 cm high. The plants were fertilized with approximately 0.1 g of urea every 10 d. At various times, selected plants were carefully removed from the pots, the soil was gently removed from the roots, and the plants were transferred to glass test tubes or Erlenmeyer flasks containing deionized water. The plants were allowed to grow for 48 h, with more deionized water added as necessary. The water was then collected and adjusted to 250 mL, and a 100-mL aliquot removed for extraction, derivatization, and GCMS analysis. The number of ducksalad plants in the pots was recorded on a weekly basis beginning 2

wk after flooding and also just before transplanting the rice plants to the deionized water. Additional data collected were number of stems and tillers, number of leaves tiller^{-1}, length of each leaf at time of transplanting, and days after planting and days after flooding at the time of transplanting. After the water samples were collected for analysis, the weights of the roots and tops were recorded. Plants used were PI# 502963 (Rexmont), PI# 277414, and PI# 294400. Blank soil was used as a control.

50 grams. The 50-g experiment was designed similarly to the 500-g experiment except that 50 g of soil were used in a plastic cup with a 6-cm bottom diameter. Both potting soil and soil infested with ducksalad seed from Stuttgart, Ark. (USA), were used. The following data were recorded: planting date, emergence date, flooding date, height and number of leaves at flood, transplant date, derivatization date and time, root and top weights at derivatization, pH of water, and smell of water and color of roots at the time of analysis.

The soil was retained and kept under flood to see whether ducksalad would grow and to record the number of plants.

Single-plant, 5-gram study. Five g of Stuttgart soil were placed in a 20 × 2.5-cm test tube and one rice seed was planted. At the time of analysis, the rice plant was separated from the soil and both soil and rice roots were analyzed. Planting date, date of emergence, date of analysis, root mass, and top mass at the time of analysis were recorded. The rice varieties used were Rexmont and PI# 312777. Blank soil was also included.

Results and discussion

Stability in water and nutrient solution

Low recovery of some of the original 15 compounds from fortified CRETS systems led us to investigate the stability of the compounds in different nutrient solutions and water. Table 2 shows the results for ether extraction of the acidified samples. System 6—freshly fortified water—indicates good recovery for all compounds except 2-hydroxybenzaldehyde and 3,4-dihydroxycinnamic acid, although the results for the latter are suspect because an analysis of water samples that were 44 h old resulted in 88% recovery. The 2-hydroxybenzaldehyde was either very unstable or extraction with ether was inefficient. A comparison of systems 1 and 6 indicates that the other compounds were stable over 44 h. The presence of nutrient solution minus iron had no effect on the recovery of most of the compounds. The exceptions were 4-hydroxybenzaldehyde, benzoic acid, and 3,4-dihydroxycinnamic acid, and to a lesser extent 2-hydroxycinnamic acid and 4-hydroxycinnamic acid. In some cases, iron caused a further decrease in recovery, with sequestrene iron having the largest effect.

Trapping of allelochemicals from water using solid-phase extraction columns and disks

The original 15 compounds were recovered from water using C18, charcoal, and XAD-4 resin (Table 3). As SPEC C18 disks and Empore styrene divinylbenzene (SDVB) disks became available, they were also examined (Table 4). Generally, recoveries were not as good as when using conventional liquid-liquid extraction with

Table 2. Stability of allelochemicals in water and nutrient solution for 44 hours.

Chemical name	System 1[a]		System 2		System 3		System 4		System 5		System 6	
	%	SD[b]	%	SD	%	SD	%	SD	%	SD	%	SD
3-hydroxyphenol	102	2	95	2	86	7	82	10	99	2	95	6
Phenylacetic acid	96	13	96	6	78	11	89	11	89	11	89	11
2-hydroxyphenyl-acetic acid	106	5	98	4	98	9	91	3	103	5	94	5
2-hydroxybenzaldehyde	nd[b]	0	nd	0	nd	0	nd	0	nd	0	22	15
4-hydroxybenzaldehyde	104	3	nd	0	nd	0	9	16	14	24	93	8
Benzoic acid	72	13	42	36	34	32	18	31	nd	0	74	10
2-hydroxybenzoic acid	81	6	83	11	79	4	74	13	75	10	77	9
4-hydroxybenzoic acid	108	3	19	2	16	5	157	65	120	38	101	6
4-hydroxy-3-methoxy-benzoic acid	106	3	97	7	89	6	93	3	108	6	97	3
3,5-dimethoxy-4hydroxy-benzoic acid	102	6	102	3	95	6	91	3	100	0	93	3
trans-cinnamic acid	99	6	89	14	84	16	89	16	109	13	93	6
2-hydroxycinnamic acid	97	7	77	5	76	4	68	4	77	5	96	5
4-hydroxycinnamic acid	44	9	65	10	65	10	68	3	62	7	98	3
3,4-dihydroxycinnamic acid	88	14	8	8	nd	0	37	33	3	5	37	32
4-hydroxy-3-methoxy-cinnamic acid	70	7	57	15	47	7	43	3	72	12	83	10
Total	85	29	62	39	56	37	67	44	69	44	83	24

[a]System 1: water, System 2: nutrients + ferric chloride, System 3: nutrients + ferrous sulfate, System 4: nutrients + sequestrene iron, System 5: nutrients only, System 6: fresh water. [b]SD = standard deviation, nd = not detected.

Table 3. Recovery of allelochemicals from water using solid-phase extraction columns.

Chemical name	Recovery (%)		
	C18	Charcoal	XAD-4
3-hydroxyphenol	8	70	87
Phenylacetic acid	20	40	20
2-hydroxyphenylacetic acid	17	nd[a]	31
2-hydroxybenzaldehyde	nd	nd	nd
4-hydroxybenzaldehyde	48	110	86
Benzoic acid	nd	48	24
2-hydroxybenzoic acid	nd	nd	24
4-hydroxybenzoic acid	11	89	16
4-hydroxy-3-methoxybenzoic acid	24	69	22
3,5-dimethoxy-4–hydroxybenzoic acid	53	nd	23
trans-cinnamic acid	93	9	46
2-hydroxycinnamic acid	100	nd	36
4-hydroxycinnamic acid	81	12	35
3,4-dihydroxycinnamic acid	42	nd	nd
4-hydroxy-3-methoxycinnamic acid	96	nd	44

[a]nd = not detected.

Table 4. Comparison of trapping of allelochemicals with SPEC C18 and Empore SDVB disks.

Compound	Recovery (%) (SD[a])	
	SPEC	SDVB
Benzoic acid	17 (1)	54 (10)
Octanoic acid	86 (3)	48 (10)
Phenylacetic acid	16 (1)	51 (9)
2-hydroxybenzoic acid	12 (0)	31 (1)
4-hydroxybenzaldehyde	15 (1)	20 (2)
3-hydroxyphenol	2 (0)	4 (1)
Decanoic acid	91 (3)	54 (6)
4-phenylbutanoic acid	79 (5)	60 (5)
trans-cinnamic acid	56 (4)	63 (6)
2-hydroxyphenylacetic acid	10 (1)	18 (1)
2,3-dimethoxybenzoic acid	35 (5)	57 (4)
4-hydroxybenzoic acid	7 (0)	12 (0)
2,6-dimethoxybenzoic acid	16 (4)	nd[b]
2,5-dimethoxybenzoic acid	nd	nd
3,5-dimethoxybenzoic acid	69 (5)	53 (14)
2,4-dihydroxybenzoic acid	6 (0)	13 (2)
2,4-dimethoxybenzoic acid	42 (6)	48 (5)
2,3-dihydroxybenzoic acid	27 (1)	20 (2)
4-hydroxy-3-methoxybenzoic acid	11 (1)	31 (2)
2,6-dihydroxybenzoic acid	29 (0)	nd
2,5-dihydroxybenzoic acid	33 (0)	17 (1)
2-hydroxycinnamic acid	26 (3)	69 (2)
3,5-dihydroxybenzoic acid	7 (0)	14 (0)
3,4-dihydroxybenzoic acid	nd	8 (1)
3,5-dimethoxy-4-hydroxybenzoic acid	14 (1)	44 (2)
3-(2,3-dimethoxyphenyl)propenoic acid	86 (4)	64 (4)
4-hydroxycinnamic acid	15 (1)	51 (3)
3-(2,5-dimethoxyphenyl)propenoic acid	83 (3)	68 (4)
3-(3,5-dimethoxyphenyl)propenoic acid	80 (4)	67 (4)
3-(2,4-dimethoxyphenyl)propenoic acid	89 (6)	65 (4)
4-hydroxy-3-methoxycinnamic acid	34 (6)	55 (6)
3-(3,4-dihydroxyphenyl)propanoic acid	6 (1)	nd
9,12-octadecadienoic acid	84 (5)	46 (7)
9-octadecanoic acid	80 (4)	41 (7)
Lenic	83 (5)	42 (6)

[a]SD = standard deviation, nd = not detected.

ethyl ether, as was used to obtain the results in Table 2. Recoveries with solid-phase extraction were usually better with C18 for the less polar compounds, but were poorer for the more polar ones. The XAD-4 resin was not very efficient at trapping the chemicals, but most were recovered to some extent.

Flow-through and static systems

Although the original 15 compounds were acidic to varying degrees, we first tried to use the CRETS with XAD-4 resin. We originally anticipated having much extraneous material from soil collecting on the resin or having allelochemicals adsorbed to soil particles, so the plants were grown in sand with nutrient solution. With the static systems, either sand or a sand-soil mixture was used. Although benzoic acid, 2-

hydroxybenzoic acid, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 2-hydroxycinnamic acid, and 4-hydroxy-3-methoxycinnamic acid were identified, there was no correlation between the amounts found and treatments (allelopathic or nonallelopathic rice).

The large variability found between replications within treatments made it difficult to find differences between treatments. We postulated that the allelopathic trait may be variable between plants within a line. If so, then replications having 6 plants pot^{-1} may have 5 plants with high activity in one replication, 2 in another, and 4 in a third, leading to a large variation in the results. In the field, where many plants are grown close together, the effect would be seen but it would be missed in the greenhouse, where only a few plants were used. This idea led us to look at individual plants as the test unit instead of several plants.

Single-plant studies

500-g soil water transplant experiments. The rationale for these experiments was that if the allelochemicals being produced by the rice plants' are present in small concentrations and if they are also normally present in the soil, then it would be more difficult to measure the amount in the soil that was actually produced by the plant. Also, if the compounds are very strongly adsorbed to soil particles or absorbed into organic matter, it may be difficult to extract them. If the plants were transplanted to deionized water and remained healthy for 2-3 d, and if they continued to produce the same chemicals, then the compounds would not need to be desorbed from the soil and any compound found would have come from the plant. Also, water would be a much cleaner matrix to work with because it produces less background interference.

Four Rexmont plants were removed from the soil and transplanted to water at 20-25 d after planting and immediately before flooding. All the other samples for all the rice lines were transplanted to water 6-8 wk after planting and 3-5 wk after flooding. Three samples were analyzed in duplicate. The overall relative standard deviation for these duplicate samples was 13%.

Table 5 shows the results for compounds for which differences were observed. For some cases without significant differences, there appeared to be a trend toward

Table 5. Analysis of water from a 500-g soil water transplant experiment, relative amounts.

Compound	Rexmont (n=17)	PI# 294400 (n=14)	PI# 277414 (n=16)
Ducksalad plants (no.)	13.8 A[a]	2.4 B	5.0 B
3-hydroxybenzoic acid	2A	50 B	29 B
4-hydroxybenzoic acid	2a	33 b	43 b
4-hydroxyphenylacetic acid[b]	5a	73 b	28 ab
3-(4-hydroxyphenyl)propanoic acid	4A	67 AB	95 B
3-(3,4-dihydroxyphenyl)propanoic acid	44 a	287 ab	569 b

[a]Numbers followed by different uppercase letters were significantly different at $P = 0.05$; numbers followed by different lowercase letters were not significant at $P = 0.05$ but were significant at $P = 0.1$. [b]Good match of the mass spectrum with computer library but has not been confirmed with an authentic sample.

larger amounts for the allelopathic lines. The large standard deviations for the results for some of the compounds usually occur because most samples contained small or no detectable amounts of the given compound, although several samples had high amounts. For example, most of the Rexmont samples produced chromatograms that had area counts of less than 10,000 for 3-(3,4-dihydroxyphenyl)propanoic acid, although one sample recorded 78,377 and another 609,156. For PI# 294400, seven samples produced chromatograms showing no detectable levels of 4-hydroxybenzoic acid, one produced a peak with area counts of 4,775, three were greater than 100,000, and the highest was 229,728. Other samples usually varied. In the analysis of PI# 294400 for 3-(3-hydroxyphenyl)propanoic acid, the distribution was as follows:

Number	Area count range
4	1–1,000
3	1,001–50,000
3	50,001-200,000
2	200,001–1,000,000
2	>1,000,000

Much larger variability was seen in the results between samples than for duplicates of the same samples. These results would be consistent with a variable allelopathic effect for each plant within a line.

The mean number of ducksalad plants pot^{-1} was 13.8 for Rexmont and 2.4 and 5.0 for PI# 294400 and PI# 277414, respectively. No difference was seen in the amounts of 4-hydroxy-3-methoxybenzoic acid, 3,5-dimethoxy-4-hydroxybenzoic acid, 4-hydroxycinnamic acid, and 4-hydroxy-3-methoxycinnamic acid, which have been identified as being produced in decomposing rice straw, or 3,4-dihydroxycinnamic acid, which has also been identified as an allelochemical. This is not necessarily a surprising result, considering that compounds produced by decomposing rice straw in the soil would not necessarily be the same as those found in water supporting growing rice plants.

We noted a trend for higher levels of 3-hydroxybenzoic acid, 4-hydroxy-benzoic acid, 4-hydroxyphenylacetic acid, 3-(4-hydroxyphenyl)propanoic acid, and 3-(3,4-dihydroxypheny1)propanoic acid in the allelopathic lines. All the compounds except 4-hydroxyphenylacetic acid have been confirmed by retention time and mass spectra compared with an authentic sample. The identification of 4-hydroxyphenylacetic acid is based on a very good match with the spectrum in the NIST90 computer library and the similarity of the mass spectrum to that of a known 2-hydroxyphenylacetic acid sample.

More control of ducksalad was found in the two allelopathic lines than in Rexmont. The number of ducksalad plants ± standard deviation was 13.8 ± 6.7 for Rexmont, 2.4 ± 2.7 for PI# 294400, and 5.0 ± 3.7 for PI# 277414. Ten no-rice controls were maintained along with the rice plants, however, and the number of ducksalad plants in the pots containing potting soil was 1.2 ± 3.1. We have observed that germination of ducksalad seed in potting soil is low, but we cannot explain why the ducksalad numbers were so low in the no-rice controls compared with the samples containing rice.

The distribution of the ducksalad numbers ranged from 1 to 27 pot^{-1} for Rexmont, 0-13 with one pot out of 14 having zero for PI# 294400, 0-10 with five pots out of 16 having zero for PI# 277414, and 0-9 with five pots out of 10 having zero for the no-rice controls.

We noticed that, when rice was grown in the water for 2 d, the water from the two allelopathic lines usually had a very distinct and strong odor of hydrogen sulfide. The chromatograms from these samples usually had a peak whose mass spectrum showed a very good match with S_8. The water containing the Rexmont samples had little or no odor.

We also noticed in our experiments, and Dilday noticed in both lab and field experiments in Stuttgart, that if ducksalad is controlled, it is because the ducksalad never germinates or doesn't elongate after it germinates. Because ducksalad germinates within 1 wk of flooding, the chemicals that are producing the allelopathic effect must be present at that time. Therefore, the chemicals are produced by very young rice plants before flooding or very shortly after flooding. Larger amounts or different types of allelochemicals may be produced by more mature plants, but they would not be responsible for the observed effect because weed control would have already been achieved by the young plants.

In the present experiment, almost all the samples were collected beginning 3 wk after flooding. Chemicals that were being produced at that time may be allelopathic, but they may have nothing to do with the observed effect if they weren't being produced at the earlier growth stage. This led to the next experiment using single plants and 50 g of soil.

50-g soil water transplant experiment. Samples were taken starting 4 d after emergence until 2 wk after flooding. Preliminary results on a limited number of samples show the presence of small amounts of some chemicals, but no differences between lines. No difference in the number of ducksalad plants growing in the remaining soil was observed.

5-g experiment. Because the allelopathic effect is apparently being caused by young rice plants, and because the chemicals causing the effect might be produced by microorganisms from chemicals exuded from the plants, we decided to grow rice in 5 g of soil in order to build the concentration of chemicals as high as possible to minimize the contribution from native compounds. We also decided to analyze the roots when the soil was analyzed rather than transplanting the rice to water. Because the soil was being extracted and all samples were collected before flooding, no determinations of ducksalad numbers could be made. The atmosphere inside the test tubes was very humid; condensation on the sides of the tubes was often present.

Table 6 shows the results from a set of samples. The compounds 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, tetradecanoic acid, 4-hydroxycinnamic acid, hexadecanoic acid, 9,12-octadecadienoic acid, 9-octadecanoic acid, octadecanoic acid, and dehydroabietic acid were confirmed by comparison with authentic samples on GC/MS. The spectrum for the compound identified as n-valeric acid gave a good fit with the spectrum for that compound in the

Table 6. Soil analysis results from a 5-g experiment, relative amounts.

Compound	Blank soil	Rexmont	PI# 312777
n-valeric acid[a]	9 B[b]	23 B	112 B
4-hydroxybenzaldehyde	1 B	3 B	11 A
4-hydroxybenzoic acid	7 B	10 B	43 A
4-hydroxy-3-methoxybenzoic acid	2 b	3 b	11 a
Tetradecanoic acid	11 B	21 B	43 A
4-hydroxycinnamic acid	0 B	4 B	26 A
Hexadecanoic acid	17	17	44
9,12-octadecadienoic acid	3	3	13
9-octadecanoic acid	24	27	71
Octadecanoic acid	19 B	31 B	115 A
Dehydroabietic acid	31	57	341
Scan 1720[c]	7	22	117

[a]Good match of the mass spectrum with that in the NIST90 computer library, but has not been confirmed with an authentic sample. [b]Numbers followed by different uppercase letters were significantly different at $P = 0.05$; numbers followed by different lowercase letters were not significant at $P = 0.05$ but were significant at $P = 0.1$. All others were not significant at $P = 0.1$. [c]Unknown compound that elutes at scan 1720 (21.5 min) and does not give a good match with any compound in the NIST90 computer library.

NIST90 computer library, thus indicating either correct identification of the compound or a compound with a similar structure. The compound labeled 1720 did not have any good fits in the library and is unlikely to be a compound in the library.

We noted a trend for each compound to be present in higher concentrations in the soil that grew PI# 312777 than in the soil growing Rexmont or in blank soil. We also noted that the mean concentrations of the compounds in Rexmont soil were higher than or equal to those from blank soil. For the 11 compounds, not including 4-hydroxycinnamic acid, the mean concentrations in soil growing PI# 312777 were 7.5 times higher than for the blank soil. The highest value was for scan 1720, which was 16.7 times higher for PI# 312777 than for the blank soil. The mean values for the Rexmont soil were 1.8 times higher than for the blank soil. The highest value was again for scan 1720, which was 3.1 times higher than for the blank soil. The mean values for hexadecanoic acid and 9,12-octadecadienoic acid were the same for the blank soils and Rexmont soil.

The concentrations in the roots were essentially the same for both PI# 312777 and Rexmont. If a given weight of roots were analyzed, the concentration in the roots on a 1-g basis might be the same for all plants, but plants having a larger root system would emit more chemicals into the soil. In this case, however, all the roots from each plant were ground, extracted, and analyzed. At this growth stage, we found little difference in root weight. The PI# 312777 roots were 37 ± 29 mg, with two plants having roots weighing 95 and 98 mg, and the Rexmont roots 23 ± 7 mg.

Comparison with field samples. In an experiment conducted at Stuttgart to differentiate between allelopathy and competition, PI# 312777 and PI# 475833 (Lemont) were grown in plots until they were approximately 15 cm tall. Glyphosate was applied to kill the rice plants and the plots were flooded. If the effect was caused by

Table 7. Analysis of Stuttgart soil after glyphosate was applied to rice at approximately 15 cm height, relative amounts.

Compound	Blank Soil	Lemont	PI# 312777
n-valeric acid[a]	74 B[b]	95 A	78 B
4-hydroxybenzaldehyde	15 b	16 b	23 a
Hexadecanoic acid	28	25	33
9,12-0ctadecadienoic acid	19 B	17 B	44 A
9-octadecanoic acid	24	28	31
Octadecanoic acid	57 b	64 b	109 a
Dehydroabietic acid	33	108	102
Scan 1720[c]	5	89	33

[a]Good match of the mass spectrum with that in the NIST90 computer library, but has not been confirmed with an authentic sample. [b]Numbers followed by different uppercase letters were significantly different at $P = 0.05$; numbers followed by different lowercase letters were not significant at $P = 0.05$ but were significant at $P = 0.1$. All others were not significant at $P = 0.1$. [c]Unknown compound that elutes at scan 1720 (21.5 min) and does not give a good match with any compound in the NIST90 computer library.

competition, little if any control of ducksalad should have been seen because the rice plants were no longer alive. If the effect was caused by allelochemicals that had been produced, the effect should have been seen if the chemicals were stable for 2–3 wk under flooded conditions. Prior to flooding, soil samples were taken from the top 2 cm from the above two treatments in addition to the no-rice controls. The samples were extracted and analyzed by GC/MS (Table 7). Six of the field samples were analyzed in duplicate. The average relative standard deviation for the 10 compounds in the six samples was 31%.

Although the glyphosate may have had an effect on the production of allelochemicals by the rice plant, the results of the soil analyses from the plots containing different rice lines differed. Plots containing allelopathic lines and those containing nonallelopathic lines showed a pronounced effect on the control of ducksalad, with the strongly allelopathic lines showing an almost complete control of ducksalad even 5 wk after flooding.

The 5-g study showed a trend of higher concentrations of 4-hydroxybenzoic acid, tetradecanoic acid, hexadecanoic acid, and 9-octadecanoic acid in soil growing PI# 312777 than in the no-rice controls. In the soils from the field study, the compounds were present, but no differences were found as a function of treatment. In the 5-g study, we noticed a trend toward higher levels of 4-hydroxybenzaldehyde, 9, 12-octadecadienoic acid, octadecanoic acid, dehydroabietic acid, and scan 1720 in the soil growing PI# 312777 than in the blank soil. This tendency held in the field samples, but the difference wasn't as pronounced. The results for blank soil and Lemont soil were similar for all compounds except dehydroabietic acid and scan 1720, for which higher concentrations were found in the Lemont soil. After the fields were flooded, control of ducksalad was stronger in the PI# 312777 plots and in some controls in the Lemont plots than in the no-rice controls.

Comparing these results with those of the 5-g experiment showed that humidity may influence the types and concentrations of compounds found. The greenhouse

samples were in a more humid environment, most of the time, than the field samples. As mentioned, care needed to be taken to make sure that the 5-g soil sample did not dry out, but a fine line exists between having the samples just moist and having them so wet that condensation formed on the tubes.

Conclusions

None of the solid-phase extraction systems works as well as an ethyl ether extraction to recover a broad range of acidic, slightly polar compounds from water.

Allelopathic and nonallelopathic rice was transplanted to deionized water 3-5 wk after flooding and left there for 48 h. The water from the allelopathic rice contained higher levels of 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-(4-hydroxypheny1)propanoic acid, 3-(3,4-dihydroxyphenyl)propanoic acid, and a compound tentatively identified as 4-hydroxyphenylacetic acid than did the water from the nonallelopathic rice. Ducksalad was controlled more in the soil containing allelopathic rice at the time of transplanting the rice to water than in the soil containing nonallelopathic rice.

When rice was grown in 5 g of soil and the soil was analyzed before flooding, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxycinnamic acid, 9,12-octadecadienoic acid, 9-octadecanoic acid, octadecanoic acid, tetradecanoic acid, hexadecanoic acid, dehydroabietic acid, a compound tentatively identified as valeric acid, and an unknown compound were all present in higher concentrations in the soil supporting the growth of PI# 312777. The soil from nonallelopathic rice Rexmont had concentrations for some compounds higher than those of the no-rice controls, but not as high as for PI# 312777. We found no differences in concentrations of 4-hydroxybenzoic acid, tetradecanoic acid, hexadecanoic acid, and 9-octadecanoic acid in surface soil samples taken before flooding from field plots that supported the growth of PI# 312777 up to a height of approximately 15 cm when compared with samples taken from plots growing Lemont or from no-rice control plots. The soil from the plots growing PI# 312777, however, did contain higher levels of 4-hydroxybenzaldehyde, 9,12-octadecadienoic acid, and octadecanoic acid when compared with soil from the Lemont or no-rice control plots.

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Notes

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CHAPTER 9

Adaptive autointoxication mechanisms in rice

C.-H.Chou

Crop debris has always been considered beneficial to succeeding crops. But evidence now indicates that crop residues can sometimes inhibit plant growth. In what is called adaptive autointoxication, an aspect of allelopathy, phytotoxins produced by a plant can limit the growth and/or population size of not only other plant species but also of its own species as well. For example, a 25% reduction in rice yield for a second crop in Taiwan was attributed primarily to the phytotoxins produced during the decomposition of rice residues left in the soil. Bioassays of phytotoxic substances obtained from the rice residues decomposing in the soil were done in laboratory and greenhouse experiments. The aqueous extracts of decomposing rice residues in the soil significantly reduced the growth of rice seedlings and other test plants. Major phytotoxic phenolics found included p-coumaric, o-hydroxyphenylacetic, vanillic, ferulic, and syringic acids. The phytotoxicity of these extracts persisted for up to 4 mo. N availability also appears to be affected. The amounts of NH_4^+-N and NO_3^--N in the soil were significantly higher in the first crop than in the second crop. Incorporating rice straw into the soil may decrease the availability of both forms of N. Autointoxication in rice appears to be a problem particularly in continuous monoculture ricefields and especially pronounced in fields with poor water drainage.

Introduction

A certain quantity of the unharvestable portion of a plant is left in the soil. This crop debris has always been thought to be beneficial to succeeding crops. But evidence now indicates that crop residues sometimes inhibit plant growth (Börner 1960, McCalla 1971, Patrick 1965, 1971). Residues of rye, timothy, tobacco, and barley that remained in the soil released great amounts of phytotoxic substances after 6–8 wk of decomposition (Toussoun et al 1968). The phytotoxins inhibited the respiration, germination, and growth of several different kinds of plants (Patrick and Koch 1958).

Many "sick" soils contain phytotoxins that produce a detrimental effect on some other plants or suppress the growth of the same plants from which the phytotoxins were derived (Börner 1971, Houng and Liu 1976, Kuwatsuka and Shindo 1973, Muller 1966, 1974, Rice 1984, Wang et al 1967). Wang and his associates studied the decline of sugarcane yield in Taiwan, and found that five growth inhibitors were present in the sugarcane soil. They indicated that these inhibitors were plant-originated and came

from the debris of sugarcane decomposed in soil. Similarly, farmers in Taiwan have always left a large amount of rice straw in the soil after harvesting. It was found that the rice residues submerged in soil could release phytotoxic substances during the decomposition period, especially under waterlogged conditions (Chou and Lin 1976, Chou and Chiou 1979, Chou et al 1981). A quantity of organic compounds produced during decomposition can be detrimental to the growth of rice seedlings, which usually makes the productivity of the second rice crop lower than that of the first crop (Chou and Lin 1976, Chou et al 1977). This intraspecific interaction is called autointoxication and it appears in many agricultural lands and in many crops (Rice 1984).

Chou et al (1977) indicated that the more rice residue left in paddy soil, the more phytotoxic phenolic compounds produced, along with a reduced amount of leachable nitrogen (N). Houng and Liu (1977), who studied the effect of rice straw and fertilizer on the growth and yield of rice, concluded that the application of N fertilizers during the two crop seasons was significantly different, with production being higher in the first crop season than in the second. Chou et al (1981) further confirmed that the influence of N application and rice straw decomposition in the soil may affect rice productivity and the chemical status of paddy soil.

Bioassay techniques

Three bioassay techniques were employed to determine the phytotoxicity of the extracts in the studies reported here. The sponge bioassay technique as described by Muller (1966) used lettuce seeds as the test material. The second bioassay technique—a modification of Muller (1966)—used presoaked rice seeds as the test material. In this bioassay, 10 rice seeds were planted on a 5 × 5-cm sheet of sponge and chromatographic paper moistened with test solutions, and placed in a petri dish (Chou and Young 1975). The petri dish was then sealed with a sheet of cellophane and allowed to incubate at 25 °C for 72 h. After incubation, lengths of the radicle and coleoptile were measured in millimeters.

The third bioassay was designed to determine the inhibitory effects of extracts on root initiation of mungbean hypocotyl. Five hypocotyl cuttings of mungbean seedlings were placed in a 50-mL test tube filled with 30 mL of test solution. The tube was then sealed with a sponge, covered with a piece of black paper, and incubated at 25 °C for 7 d in the light. The experiment was replicated three times. After incubation, the number of roots initiated from the hypocotyl cuttings was counted (Chou and Lin 1976).

Phytotoxicity of decomposing rice residues in soil

Effects on the growth of rice plants

To understand the effect of decomposing rice residues in the soil on the growth of rice plants, we conducted a series of experiments. The first experiment determined the phytotoxicity of aqueous extracts obtained from a 2-wk decomposition of a soil-straw mixture in which 3 kg of soil were mixed with 0, 25, 50, 75, or 100 g of straw. Extracts were bioassayed using presoaked rice seeds as the test material. Distilled water

Table 1. Effect of extracts on growth of rice seedlings[a].

Condition	Amount of straw mixed (g)	Radicle growth		Coleoptile growth	
		Length (mm)	Control (%)	Length (mm)	Control (%)
Aerobic	0	30.3	79.1	13.3	101.8
	25	24.3	63.4[c]	14.6	111.7
	50	18.0	47.0[c]	14.8	113.4
	75	22.3	58.1[c]	13.6	104.2
	100	14.8	38.7[c]	11.6	89.1
Anaerobic	0	26.0	68.0[b]	13.4	102.0
	25	24.4	63.7[c]	15.8	121.0
	50	15.6	40.7[c]	12.0	92.0
	75	11.5	30.1[c]	13.2	101.0
	100	10.4	27.2[c]	14.2	109.0

[a]Extracts were obtained from soil (3 kg) mixed with different amounts of straw under aerobic and anaerobic decomposition conditions for 2 wk. Distilled water was used as a control. [b,c]Statistical significance at the 5% (b) and 1% level (c) by using analysis of variance.
Source: Chou and Lin 1976.

served as a control. The data revealed that the radicle was significantly suppressed by a straw amendment as low as 50 g, and that the dry weight decreased significantly with increasing amounts of straw applied; however, coleoptile growth was not inhibited (Table 1) (Chou and Lin 1976).

The second experiment was designed so that a soil-straw mixture (3 kg:200 g) was saturated with water and allowed to decompose for 1, 2, and 4 wk under greenhouse conditions. Soil alone was treated in the same manner and served as a control. At the end of each decomposition time, five 3-wk-old rice seedlings were planted in a pot containing rice residues. One month after transplanting, the rice plants were harvested for examination and measurement. It was found that the control rice seedlings were normal and usually more than 66 cm tall. But the rice seedlings grown in the decomposing material were less than 36 cm tall, and the roots were shorter and dark brown. The degree of inhibition of rice growth did not vary with the length of decomposition time (Chou and Lin 1976).

Phytotoxicity of aqueous extracts in the soil

Two bioassays were performed to determine the phytotoxicity of extracts obtained from the decomposition of rice residues and of soil alone against lettuce growth. In the first experiment, extracts were prepared from the mixture of dry straw-root-soil (100:100:3,000, g:g:g), which was left to decompose for 1, 2, and 4 wk. It was found that after 1 wk the extract from the soil without rice residues showed no phytotoxicity; however, the extracts from the soil with decaying rice residues revealed more than 70% inhibition (Fig. 1A). After 1 wk of decomposition following the first extraction as mentioned, the straw-root soil was returned to the same pots each time for a period of further decomposition. Meanwhile, an adequate amount of distilled water was added to the mixture before decomposition took place. The aqueous extracts of

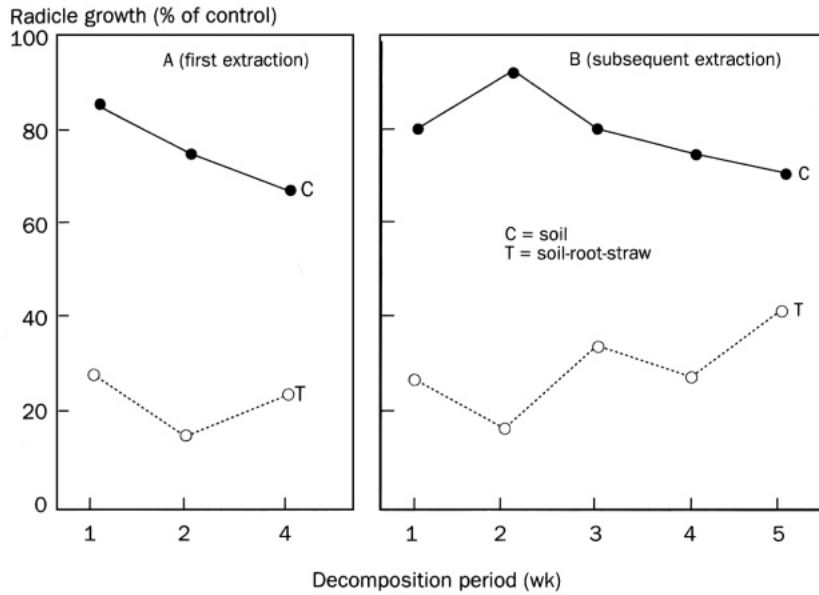

Fig. 1. Phytotoxic effect of extracts from decomposition of rice residues in the soil on radicle growth of lettuce. (A) Extracts were obtained from soil alone and from soil-root-straw after the indicated period of decomposition; (B) subsequent extractions were made each week from the soil and soil-root-straw samples, and the residues were returned to the same pot each time for a period of further decomposition. (Adapted from Chou and Lin 1976.)

each treatment were obtained for each time period by using the same technique. That extraction was designated as subsequent extraction. Naturally, the extract of the first extraction was different from the first extract of the subsequent extraction. Therefore, the extracts made from the same pot at 1-wk intervals also showed significant inhibition, and subsequent extracts in the following 4 wk continued to show inhibition (Fig. 1B) (Chou and Lin 1976).

In the second experiment, extracts were obtained from a decaying mixture of straw-soil (200 g:3 kg) by using the same preparation process. The phytotoxicity of the extracts from the decaying rice straw was significantly higher than that from the soil alone (Fig. 2A). The toxicity was obviously high in the straw-soil extract, but gradually decreased with increased decomposition time and subsequent extractions. Nevertheless, the phytotoxicity (above 40%) still persisted in extracts after 16 wk of decomposition (Fig. 2B) (Chou and Lin 1976).

Effects of aqueous extracts on root initiation of mungbean hypocotyl cuttings

It was evident that the rice roots growing in the decomposing rice residues were much poorer than those in the control. We therefore attempted to determine whether rice-

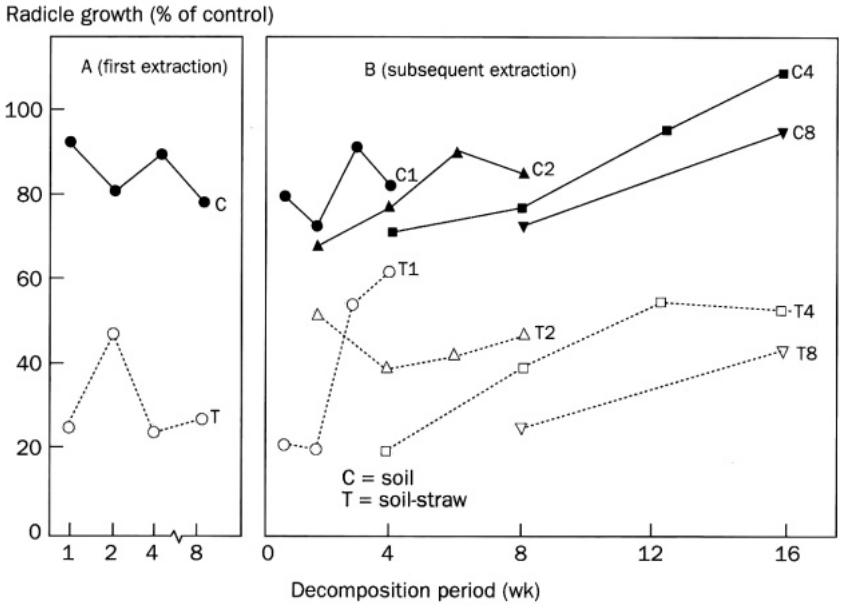

Fig. 2. Phytotoxic effect of extracts from decomposition of rice residues in the soil on radicle growth of lettuce. (A) Extracts were obtained from soil alone and from soil-straw after the indicated period of decomposition; (B) subsequent extractions were made from the soil and soil-straw samples. From the C1 and T1 sets of pots, extractions began at the end of the first week; from the C2 and T2 at the end of the second week; from the C4 and T4 at the end of the fourth week; from the C8 and T8 at the end of the eighth week. After each extraction, the residues were returned to the same pot for a period of further decomposition. (Adapted from Chou and Lin 1976.)

straw-soil extracts affected adventitious root initiation from the hypocotyl cuttings of mungbeans. The results showed that root initiation of mungbeans was greatly retarded by extracts from decomposing residues, but proceeded normally in control soil extracts. In fact, extracts from soil without rice residue stimulated root initiation when compared with the use of distilled water. The degree of inhibition decreased with increased decomposition time (Fig. 3), and the retarded hypocotyl cuttings became dark brown and quite fragile (Chou and Lin 1976).

Allelopathic activity in relation to environmental stresses

Dynamics of Eh (oxidation-reduction potential) in paddy soil

The results of an Eh measurement in paddy fields of Nankang showed that the Eh ranged from -100 mV to +300 mV during the first (spring) rice crop season and from -200 mV to +l00 mV during the second (summer) crop season (Fig. 4A). In the pot experiment, the soil ranged from -100 mV to +400 mV in the treatment with soil alone, which served as a control, but ranged from -300 mV to + 100 mV in the treat-

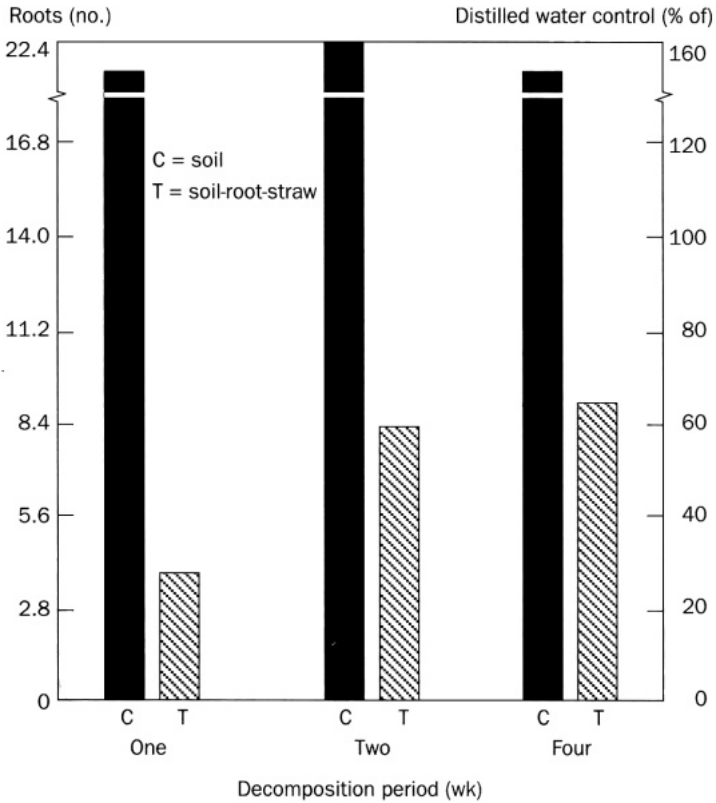

Fig. 3. inhibition of root initiation of hypocotyl cuttings of mungbeans affected by extracts of soil alone (C) and soil-residue (T) at different decomposition periods. (Adapted from Chou and Lin 1976.)

ment of soil mixed with rice residues (SR) during the first crop season of 1977 (Fig. 4B). The Eh was down to the reduced state at the tillering stage of 30–45 d after transplanting rice seedlings into the soil, and at the panicle stage of 80–90 d after transplanting (Fig. 4B). The difference in the Eh was significant when the N fertilizers ammonium sulfate and potassium nitrate were applied onto the field or to plots during the tillering and panicle stage in 1977 (Figs. 4A and B) (Chou and Chiou 1979).

These results clearly indicated that the soil Eh was significantly low, reaching the reduced state in the early growing period of the second crop season, and was particularly high in the soil mixed with rice residues left in the paddy field. A similar pattern was found in the pot experiment when the soil was mixed with rice residues. During the early stage of the second crop season, the surface soil temperature was almost always above 30 °C, which expedited the decomposition of rice residues in the soil.

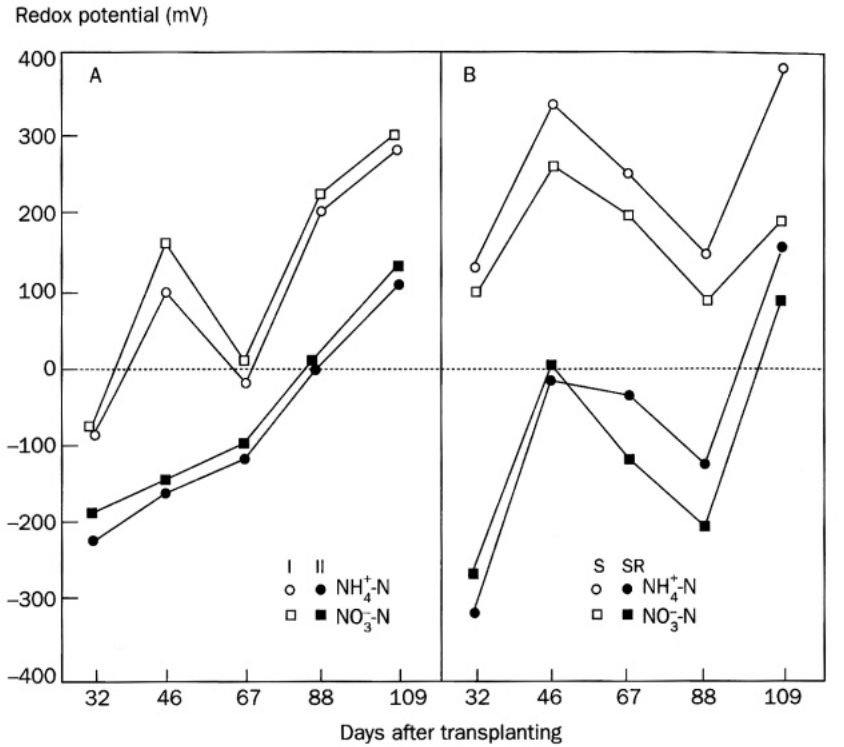

Fig. 4. Effects of different culture treatments on the redox potential of soils in the field (A) and in pots (B) at five sampling times during the first crop season (I) and second crop season (II) of 1977. The soil received separate applications of ammonium sulfate (NH_4^+-N) and potassium nitrate (NO_3^--N) as topdressings in both the field and pot experiments. Pots were filled with either 10 kg of soil alone (S) or soil mixed with 100 g of chopped rice straw (SR). The mixture was allowed to decompose for 3 wk before the rice seedlings were transplanted. Each point on the figure was obtained from the mean of at least five replications. Data for the other figures were treated in the same manner. (Adapted from Chou and Chiou 1979.)

Dynamics of extractable nitrogen as affected by incorporating rice residues in soil

In pot experiments, water-extractable NH_4^+-N and NO_3^--N were determined. The NH_4^+-N content was generally higher in the soil alone (S) than in the soil-residue mixture, except in the period 40–70 d after transplanting. This finding mostly agreed with findings in field soil. The NO_3^--N content was about one-tenth the NH_4^+-N content and was significantly higher in the S soil than in the SR soil, except during the last 40–70 d after transplanting. This result clearly indicated that the available nitrate-N would rapidly decrease when the soil was incorporated with rice residues. The results also suggest that the nitrate-N could be denitrified or easily leached out, resulting in its fast disappearance.

We concluded that the amounts of NH_4^+-N and NO_3^--N were significantly higher in the second crop than in the first crop, and that the quantity of NH_4^+-N was about 10 times higher than that of NO_3^--N. Both forms of soil N were significantly affected by both N dressing and rice residue incorporation. Additionally, rice straw incorporated into the soil may decrease both forms of available N (Chou and Chiou 1979).

Effects of culture treatments on soil-leachable cations

It was thought that under various culture treatments the amount of available minerals in the soil might be affected and that this would consequently influence rice growth. Under the same experimental treatments (i.e., with rice residues and without straw), the cations present in the aqueous leachates of soil—namely Na^+, K^+, Zn^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, and Mn^{2+}—were determined. Chou and Chiou (1979) found that the concentrations of Cu^{2+}, Zn^{2+}, and Fe^{2+} were lower than 0.1 ppm (1 g soil basis), those of K^+ and Mn^{2+} were lower than 6 ppm, and those of Ca^{2+}, Na^+, and Mg^{2+} were above 10 ppm. The Mg^{2+} content was much higher than 30 ppm. Compared with the results of N dressing treatments, the amounts of listed cations, except Fe^{2+} and K^+, were significantly higher in the NH_4^+-N dressing than in the NO_3^--N dressing in the first crop of 1977. In addition, the amounts of all cations were higher in the former than in the latter in the second crop season of 1976.

These data indicated that different N fertilizer applications definitely influenced the availability of leachable cations in the soil. Furthermore, comparing the data of cation amounts in two crop seasons, the concentrations of four cations—Cu^{2+}, Fe^{2+}, Mn^{2+}, and K^+—were shown to be higher in the first crop season, whereas those of other cations—Zn^{2+}, Ca^{2+}, Mg^{2+}, and Na^+—were higher in the second. In some paddy fields of Taiwan, Zn^{2+}, Fe^{2+}, Ca^{2+}, and Na^+ were significantly higher in the S soil than in the SR soil. However, regardless of the fertilizer treatment, K^+ and Mg^{2+} were more concentrated in the SR soil. It is obvious that introducing rice straw into the soil increases the amount of some cations and decreases that of others (Chou and Chiou 1979).

Effects of culture treatments on the growth and yield of rice plants

The tiller number was significantly higher in the NH_4^+-N dressing treatment than in the NO_3^--N treatment, as observed by rice plants growing in the field in the second crop season of 1976 (Fig. 5A). In the pot experiment, the tiller number was usually lower in the SR soil than in the S soil (Fig. 5B).

Table 2 shows the yield components of rice plants affected by different culture treatments. These yield components were higher in the NH_4^+-N treatment than in the NO_3^--N treatment. In the NH_4^+-N dressing, these components were nonsignificantly different between the S and the SR soil, but were significantly different between them in the NO_3^--N dressing. These data suggest that the suppressive effect of rice productivity can be antagonized by NH_4^+-N fertilizer, which agrees with our previous findings (Chou et al 1977) and those of Chandrasekaran and Yoshida (1973).

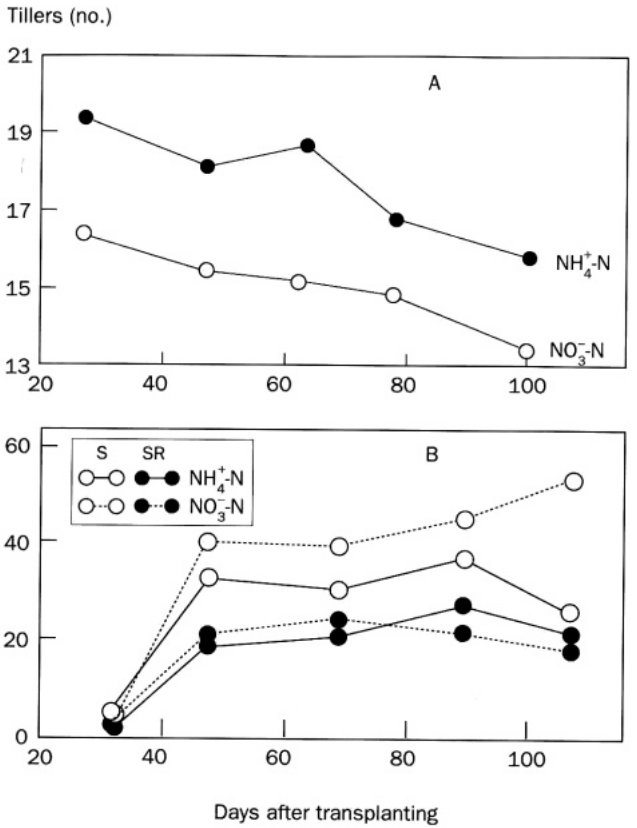

Fig. 5. Effects of N fertilizer treatments on the tillering number of rice plants growing in the field (A) in the second season of 1976 and in pots (B) during the first crop season of 1977. (Adapted from Chou and Chiou 1979.)

In conclusion, both the vegetative and reproductive growth of rice plants were significantly affected by culture treatments, showing that the NH_4^+-N dressing had a beneficial effect on rice growth and exhibited an antagonistic interaction with rice residues decomposed in soils.

Effect of temperature and fertilizer on soil phytotoxicity

Phytotoxicity revealed from the decomposing rice residues in the soil was thought to be affected by temperature and fertilizer dressing (Lin and Chang 1976, Houng and Liu 1977). A series of experiments were conducted with soil and a soil-straw mixture in pots and placed under various temperatures from 15 °C to 35 °C, with 5 °C intervals. Aqueous extracts from soil and soil-straw were bioassayed.

Table 2. Yield components of rice plants in pot soils.

Yield component ($hill^{-1}$)	Soil treatment					
	NH_4^+-N			NO_3^--N		
	Content (ppm)		Decrease	Content (ppm)		Decrease
	S[a]	SR	(%)	S	SR	(%)
Panicles (no.)	38.8	32.3	17.0	26.3	19.5	26 *
Total panicle weight (g)	64.3	58.2	10.0	52.3	42.3	20
Ripening rate (%)	90.9	89.1	1.0	97.7	92.7	5
Testing weight (g 100 $seed^{-1}$)	27.0	27.2	0.5	28.0	26.0	7
Grain weight (g)	61.9	55.2	11.0	50.3	40.1	21*
Yield (g m^{-2})	1,546.3	1,381.0	11.0	1,256.4	1,003.6	(%)

[a]The pot was filled with 10 kg of soil alone (S) and the soil was mixed with 100 g of soil-residue mixture (SR); nitrogen fertilizers were then applied. *Statistical significance at the 5% level.
Source: Chou and Chiou 1979.

In the lettuce bioassay, the phytotoxicity of the soil-straw extracts ranged from 75% to 100% inhibition, whereas that of the control soil extracts was below 30% (Fig. 6A). In the rice bioassay, the soil-straw extract was also more phytotoxic than the control soil extract, but less inhibition was usually shown than that exhibited by the lettuce bioassay. Phytotoxicity, as measured by growth inhibition, was highest at 20–25 °C and decreased at both lower and higher temperatures (Fig. 6B).

Although the lettuce bioassay showed that soil phytotoxicity persisted for the duration of the experiment, phytotoxicity on rice seedling growth had virtually disappeared after 6 wk of anaerobic incubation of the soil-straw mixtures. The decrease in soil phytotoxicity on rice over time was more noticeable at temperatures above 30 °C. It appears that at such temperatures the decomposition of rice straw in the soil would be enhanced, thus resulting in phytotoxicity in the soil of shorter duration (Chou et al 1981).

Phytotoxins produced during decomposition of rice residues in the soil

Identity of phytotoxins affected by culture treatments

We have previously reported seven phytotoxins—vanillic, *p*-hydroxybenzoic, (*cis* and *trans) p*-coumaric, syringic, *o*-hydroxyphenylacetic, and ferulic acids—present in decomposing rice residues in paddy soil (Chou and Lin 1976, Chou et al 1977). The dynamics of these compounds as affected by fertilizer treatments was further demonstrated.

In the first crop season, only ferulic and syringic acids were found in lower amounts in the NH_4^+-N dressing treatment than in the NO_3^--N treatment, whereas in the second crop season other compounds were also found in lower amounts in the NH_4^+-N

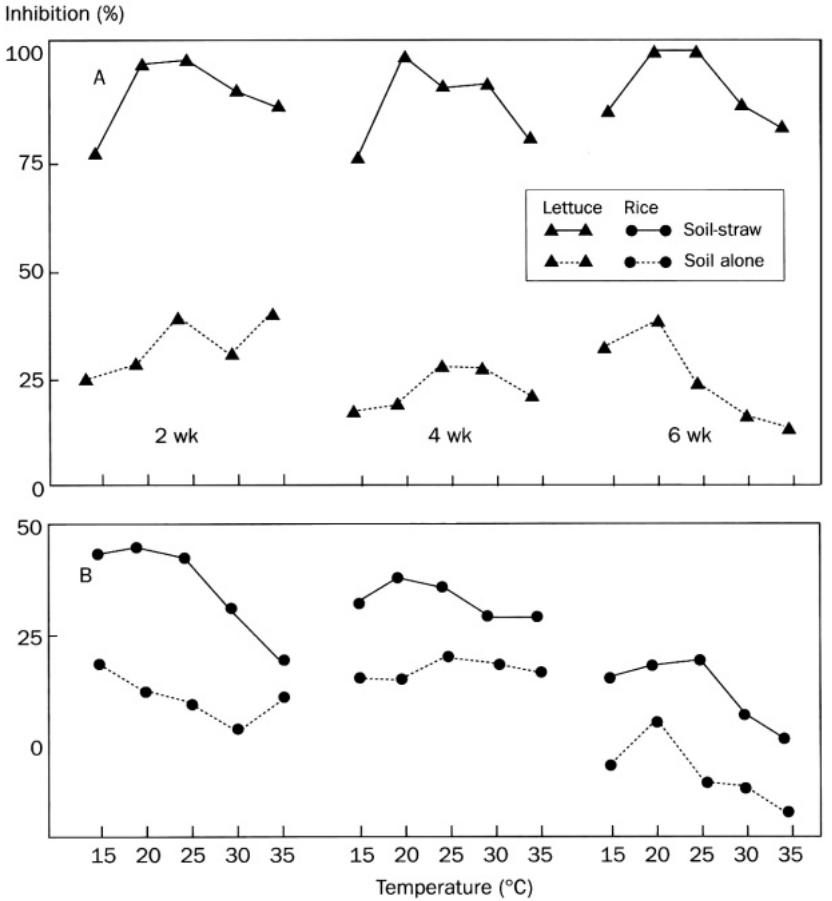

Fig. 6. Effect of temperature on phytotoxicity produced during rice straw decomposition in the soil for 2, 4, and 6 wk. Phytotoxicity is expressed as inhibition (%) of growth of lettuce (A) or rice (B) radicles in various soil extracts compared with distilled water controls. (Adapted from Chou et al 1981.)

treatment (Chou and Chiou 1979). When the quantities of these compounds were compared between two crop seasons, we found that the amount of four compounds—ferulic, *p*-hydroxybenzoic, *cis*-*p*-coumaric, and *o*-hydroxyphenylacetic acids—was significantly higher in the first crop season than in the second (Chou and Chiou 1979).

An analysis of these phenolic acids in pot experiments showed higher concentrations of phytotoxic phenolics in the soil-residue mixture than in the soil-alone control. The ratio of soil alone to soil-residue mixture in terms of the quantitative comparison of phytotoxins was generally lower than 1, which was more significant in the NO_3^--N dressing treatment. Phytotoxic phenolics were found in significantly higher

amounts in the soil-residue mixture, indicating that rice straw is a major source of these plant phenolics.

Effects of temperature and fertilizer on the quantity of phytotoxin production

Except for *o*-hydroxyphenylacetic acid, the other six compounds are ubiquitously distributed in agricultural soils of Taiwan (Wang et al 1967). But the quantity of these compounds was often variable in different soils and under different culture treatments (Chou and Lin 1976, Chou et al 1977, Chou and Chiou 1979). The amount varied with the temperature and duration of incubation (Fig. 7). The concentration of the compounds isolated tended to be higher in the extracts from treated soil incubated at 20–30 °C, and lower in the extracts from soils incubated at either 15° or 35 °C. This pattern was most evident in the contents of ferulic, syringic, vanillic, and *p*-hydroxybenzoic acids obtained from the alcohol extraction. No decrease in the level of phytotoxin accumulation in the treated soil was apparent after 6 wk of incubation.

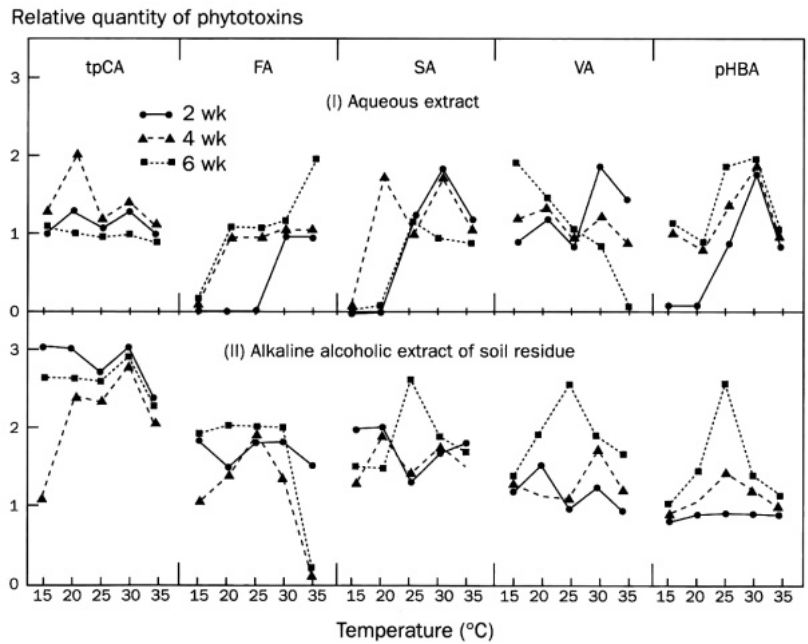

Fig. 7. Relative quantity (arbitrary scale) of phytotoxic phenolics produced during the decomposition of rice straw in the soil. The phytotoxins were obtained by (I) ether extract of aqueous extract and (II) alkaline alcoholic extract of the residue of each incubated soil-straw mixture. Abbreviations are tpCA = *trans-p*-coumaric acid, FA = ferulic acid, SA = syringic acid, VA = vanillic acid, and pHBA = *p*-hydroxybenzoic acid. (Adapted from Chou et al 1981.)

Discussion

The autointoxication mechanism of rice is thought to be influenced by environmental factors, such as temperature, light, soil texture, nutrient availability, and the degree of water drainage. Some of these effects have been discussed earlier. In addition, under poor water drainage, the quantity of phytotoxic substances may be significantly higher than under normal conditions. Koeppe et al (1976) demonstrated that a higher concentration of chlorogenic acid and its isomers was found in the extracts of sunflower plants grown under phosphate deficiency compared with phosphate-sufficient plants. This result could mean that the survival of plants growing in nutrient-poor soil may depend on the production of phytotoxic substances, thereby excluding other plants without competing with them for available nutrients. Therefore, autointoxication or allelopathy (Muller 1970, 1974) could play a significant role in the important initial stages of plant development, such as seed germination, radicle growth, tillering, panicle initiation, and other processes. This autointoxication or allelopathy may help us understand the formation of a dominant species and the productivity of crop plants. Nevertheless, the relation of phytotoxins to rice productivity is only one aspect; important factors mentioned previously should also be considered for the cause of lowered rice yield in the second crop season.

Patrick and Mikkelsen (1971) indicated that redox potential (Eh) in the soil is brought about by the use of the available oxygen by soil microorganisms and by the production of reduced metabolites. The SR soil with ammonium sulfate might reduce Eh; however, the Eh was slightly oxidizing in soil to which potassium nitrate was added. These findings agree with those of Patrick and Mikkelsen (1971), who found that nitrate and manganese dioxide are reduced at a fairly high potential (above 200 mV). In addition, they also pointed out that most of the chemical changes that occurred in flooded soils were associated with microbial metabolism. Wu et al (1976) concluded that, at the maximum tillering stage, the lower the redox potential in the soil, the more sulfate reducers and denitrifiers on the surface of the roots. Among those reducers, *Pseudomonas putida* was found to be the most common, but none of those organisms was found with normally growing rice roots. At this stage, the concentration of phytotoxic substances in the soil was also high, especially *p*-hydroxybenzoic acid. Our results agreed with their findings, and we found more than seven potentially phytotoxic substances. These phytotoxins were predominant in fields with poor water drainage, particularly in the area where rice residues had been previously incorporated into the soil. A large-scale experiment was conducted by improving water drainage in the paddy field of the Kaohsiung Agricultural Improvement District in 1976 (Wu et al 1975). The results of this experiment show that rice yield has increased by at least 30% since that time.

The two crop seasons have quite different temperatures. During the early growing period of the second crop season, the surface soil temperature surpasses 30 °C, thus expediting the decomposition of rice residues left in the soil, and resulting in the release of greater quantities of organic substances. In this situation, phytotoxins could accumulate and naturally would have a detrimental effect on rice growth. In addition,

phytotoxins could also affect the population of N-fixing microorganisms or nitrifying bacteria in the soil of paddy fields. This effect of phytotoxins on microbes has not been evaluated in this study. Such effects have been demonstrated by Rice and his coworkers (Rice 1984) however. The high accumulation of phytotoxins at 20–25 °C during the 6–9-wk interval of incubation could have a severe effect on crop production. At the early stages of the second crop season, the daytime temperature is usually above 30 °C, which could expedite the decomposition of rice stubble remaining in the soil, resulting in the release of large amounts of phytotoxins. As the crop season progresses, the daytime temperature gradually drops below 15 °C, which would slow the decomposition of rice residues. Because phytotoxicity in paddy soil under natural conditions during the second crop season has been observed to persist for more than 4 mo (Chou and Lin 1976), both tillering and panicle initiation of rice plants could be significantly retarded, resulting in a reduction in rice yield (Chou et al 1977).

Chou et al (1981) also indicated that the amount of the phenolics obtained from alcohol extracts of the incubated soil mixtures followed the same increasing order of magnitude as those shown by the phytotoxicity tests: soil + fertilizer < soil + straw < soil + straw + fertilizer. Only negligible amounts of phytotoxins were found in the control soil, without straw or fertilizer. There was no definite pattern of accumulation or disappearance of the individual phenolics that could be related to temperature differences. Ferulic acid, however, was absent in the absence of straw, syringic acid decomposed rapidly regardless of the sequence of temperature change, *cis-p*-coumaric acid was produced slowly, and both forms of *p*-coumaric acid were quite persistent.

Furthermore, the concentrations of cations—namely, K^+, Ca^{2+}, and Mn^{2+}—were higher in the first crop season, whereas those of Na^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} were higher in the second crop season in Nankang paddy soil regardless of the N fertilizer application. Most of our findings agree with those of Patrick and Mikkelsen (1971), who found that the rapid decrease in Eh after flooding is a characteristic of soil that is low in reducible iron and manganese. When the pot soil was mixed with rice straw, the amount of K^+ was significantly higher than in soil alone, but the amount of Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} was on average significantly lower in the soil in terms of the ratio of soil alone to soil-residue mixture. In several poorly drained areas in Taiwan, such as Changhwa, Taitung, and Pintung, Zn deficiency is particularly noticeable during the second crop season (Chou and Chiou 1979).

Growth and yield of rice plants were affected by soil treatments, and root development was retarded by phytotoxins present in the soil-residue mixture (Chou 1978, unpublished data), although this suppression could be overcome by applying NH_4^+-N fertilizer. Chandrasekaran and Yoshida (1973) concluded that ammonium sulfate effectively eliminated the injury. Phytotoxic organic acids can be detoxified by ammonium ions, thus leading to well-developed root systems. Soil phytotoxicity might also be reduced by incorporating humic substances (Wang et al 1971). Wang and Li (1977) found that protocatechuic acid, one of the phytotoxins related to *trans-p*-coumaric acid, could be polymerized into humic acid by using clay minerals as heterogeneous

catalysts. If this is the case, incorporating humic acid into the soil may accelerate soil detoxification, and possibly increase rice productivity. The polymerized phytotoxins fixed into a humic complex might be depolymerized under some conditions, resulting in free phenolic compounds that will produce an immediate phytotoxic effect on nearby susceptible plants.

Harborne (1977) indicated that aquatic plants may produce shikimic acid as an adaptation to avoid accumulating toxic intermediates such as ethanol. If this is the case for rice plants, a substantial quantity of shikimic acid could accumulate in paddy soil. Shikimic acid is a key precursor for synthesizing phytotoxic phenolics, such as *p*-hydroxybenzoic, p-coumaric, vanillic, and ferulic acids, as well as for synthesizing plant growth stimulators, such as indole 3-acetic acid (Kefeli 1971). Shikimic acid might favor forming growth inhibitors instead of stimulators when paddy soil is under poor water drainage and unfavorable temperature conditions. This possibility needs to be examined further in field experiments.

In conclusion, although rice residues decomposed in the soil produced a substantial amount of phytotoxic phenolics and other organic acids, which suppressed the growth and productivity of rice plants, the inhibition was not fully expressed. The findings of limited suppression of rice plants by these compounds supported the idea of an adaptive autointoxication mechanism proposed by Whittaker and Feeny (1971).

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CHAPTER 10

Allelopathic strategies for weed management in the rice-wheat rotation in northwestern India

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Within the last 25–30 yr, rice cultivation in northwestern India has grown in importance where irrigation is ample during the summer rainy season. Rotating the summer rice crop with a winter wheat crop has become very popular because of the high yield potential of both crops. Unfortunately, both crops are heavily infested with weeds, forcing farmers to use large amounts of herbicides. The heavy use of herbicides in the rice-wheat rotation has contributed to several problems in northwestern India: (1) a change in natural weed flora, (2) development of herbicide-resistant weeds, (3) human health hazards, and (4) contamination of groundwater resources. Allelopathic strategies may provide an alternative to overcome the serious ecological problems associated with herbicide use. Field, pot culture, and laboratory studies show that introducing weed-smothering crops into the rotation can suppress weed populations considerably in current and succeeding crops. For example, early summer (April-June) fodder crops such as sorghum, pearl millet, and maize drastically reduced the weed population and its biomass. The suppressive effects of pearl millet also persisted as much as 45 d into the next sorghum crop. Including such summer fodder crops before the rice crop in the rice-wheat rotation may provide enough weed control in the rice crop to reduce considerably the need for herbicides. In addition, substituting winter fodder crops of oat and berseem (*Trifolium alexandrinum*) in place of wheat also controlled weeds during the winter season. Additional allelopathic studies are needed before a satisfactory weed management strategy can be put in place for the rice-wheat rotation.

Introduction

In the past two decades, with the expansion of irrigation facilities in northwestern India (the states of Punjab, Haryana, and Uttar Pradesh), area under rice has increased substantially. In this region, rice is grown during the summer rainy season; as a result, weeds are a major problem. The rice crop is harvested from late October to mid-November; by this time, the sowing of most winter crops, except wheat, is over. The rice-wheat rotation is popular with farmers in irrigated areas because it is high-yielding. As occurs with rice, weeds are also a major problem in wheat. Therefore, most herbicides in these states are used in the rice-wheat rotation. This gave rise to various problems: (1) the natural weed flora changed to grass species because of herbicidal control of broadleaf weeds, (2) *Phalaris minor* developed resistance to its recom-

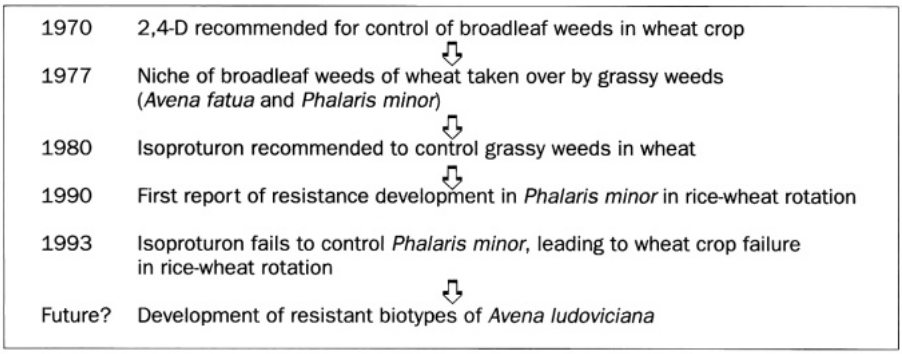

Fig. 1. Development of herbicide resistance in grassy weeds in the rice-wheat rotation in India.

mended herbicide isoproturon (Malik and Singh 1995) (Fig. 1), (3) human health hazards appeared during herbicide spraying, and (4) groundwater became contaminated. An alternative for overcoming these problems is to use allelopathic strategies for weed management for sustainable agriculture. These strategies include (1) using weed-smothering crops, (2) using crop residues for weed control, (3) using phytotoxins from plants or microbes as herbicides, and (4) using synthetic derivatives of natural products as herbicides. Because these weed management strategies do not cause the problems associated with herbicides, they could lead to more sustainable agriculture.

During the past 10 years, there has been an acute shortage of labor at harvest of rice and wheat; hence, harvest of these crops is often combined. In areas with cropping intensity of 300% or higher, only 7–10d are available between the harvest of the previous crop and the sowing of the next crop. Farmers therefore burn the rice and wheat straw to prepare a seedbed for the next crop. Putnam and DeFrank (1983) reported a high potential of crop residues for weed control, so we incorporated rice and wheat crop residues to explore their potential for weed control under Indian conditions. Weed-smothering crops suppress weeds through interference, that is, competition and allelopathy. Narwal (1984) mentioned several weed-smothering crops for the summer season (sorghum, sudan grass, pearl millet, hemp, soybean, cowpea, and alfalfa) and winter season (barley, rye, oat, buckwheat, sweet clover, rapeseed). This paper presents the results of our research on the use of crop residues and weed-smothering crops for weed management.

Materials and methods

Field, pot, and laboratory bioassay studies were conducted at the Haryana Agricultural University in Hisar, India (296° 10' N latitude, 75° 40 E longitude, and 215 m above sea level), in two separate sets (each consisting of several experiments) on summer and winter crops.

Summer crops

In northern India, the summer season extends from April to October, including the early summer (April-June) and the main rainy season (July-September). During this period, the availability of photosynthetically active radiation (PAR) and soil moisture, particularly during the rainy season, is maximum. This, coupled with high temperatures (mean 30–35 °C), provides ideal conditions for weed growth. Our research, consisting of both field and laboratory studies, was conducted to determine (1) the effects of wheat residue management on the germination and growth of weeds and (2) the smothering effect of crops on associated weed species (Sarmah 1992).

Field study. The treatments consisted of three wheat (WH 283) straw management practices (removed, burned, and soil incorporated) and six fodder crops—sorghum (*Sorghum bicolor*) JS 20, pearl millet (*Pennisetum glaucum*) HHB 60, maize (*Zea mays*) Ageti 76, clusterbean (*Cyamopsis tetragonoloba*) HG 75, cowpea (*Vigna unguiculata*) HFC 42-1, and fallow. The treatments were replicated four times in a split-plot design, with straw management practices in the main plot and test crops in the subplot. After the wheat crop was harvested with a combine harvester, the field was divided into three equal strips of 60 × 6 m each. In the first strip, wheat straw was removed completely, in the second it was burned, and in the third it was incorporated into the soil with a disc plow. Thereafter, the field was irrigated and each strip was plowed separately three times with a disc harrow. Each strip was further subdivided into six plots of gross and net sizes of 6 × 3 m and 5 × 2 m, respectively. Crop seeds were drilled with a hand plow in rows 30 cm apart according to recommended seed rates. The crops were sown in the last week of May and harvested in the second week of August; they were grown with recommended cultural practices. Observations on the population of broadleaf, grassy, and total weeds and dry matter m^{-2} were recorded from 15 d after sowing (DAS) at fortnightly intervals until harvest. In both years, all the crops were harvested for fodder at 70 DAS.

Residual effect. For the observation trial, the residual effect of the straw management and fodder crops treatments was studied on the weed population in the succeeding sorghum crop and in undisturbed plots. Three treatments were sown with sorghum and one was left unplowed and designated as an undisturbed plot. The experimental field was irrigated and then plowed twice with a disc harrow; sorghum was then sown 4 d after the harvest of the previous fodder crop. Just after the sorghum was sown and before seedling emergence, the experimental plots were marked according to the layout of the original trial. The total weed population was recorded at 15 and 45 d after the harvest of the previous fodder crop in undisturbed plots and at 15 and 75 DAS in the sorghum crop.

Bioassays: Wheat straw. The treatments consisted of three aqueous extract concentrations (control, 5%, and 10%) of wheat straw and five summer weed species—*Trianthema portulacastrum,* pigweed (*Amaranthus* spp.), sunberry (*Physalis minima),* barnyard grass (*Echinochloa crus-galli*), and crowfoot grass (*Dactyloctenium aegyptium*). The wheat straw samples were collected at the wheat harvest, cleaned, dried, and ground in a Wiley mill. An aqueous extract of 10% (w/v) was prepared by

soaking 100 g of dried ground samples in 1,000 mL of distilled water at room temperature (30 °C) for 24 h. The extract was filtered through muslin cloth and Whatman No. 1 filter paper and then centrifuged at 3,000 rpm for 20 min. A 5% extract was prepared by diluting the 10% solution. Fifty seeds of the five test weed species were placed in each sterilized petri dish lined with two Whatman No. 1 filter papers. The treatments were replicated four times in a completely randomized design. For each treatment, 5 mL of extract or distilled water as a control were added per petri dish on the first day. Thereafter, extract or distilled water was applied as needed. Petri dishes were incubated at 29 ± 2 °C and germination (%) was recorded at 48, 144, and 240 h after sowing. Root and shoot length and dry weight of 20 randomly selected seedlings per petri dish were recorded at 240 h.

Bioassays: Wheat field soil. The treatments consisted of three aqueous extract concentrations (control, 25%, and 50%) of wheat field soil and five weed species as in the wheat straw bioassay. The soil samples (0-30 cm depth) taken from the experimental field at the harvest of the wheat crop were thoroughly mixed and undecomposed materials were removed by sieving. For aqueous extracts, a 50% solution (w/v) was prepared by adding 500 g of soil to 1,000 mL of distilled water. This was kept for 24 h and occasionally stirred. Then the slurry was filtered through Whatman No. 1 filter paper and the filtrate was centrifuged at 3,000 rpm for 30 min. The supernatant was collected and a part of the extract was further diluted with water to get a 25% concentration. Thereafter, this bioassay followed the method of the wheat straw extract bioassay using the same test weed species.

Pearl millet accessions

The maximum smothering effect of pearl millet on weeds compared with other summer crops (Narwal and Sarmah 1996) encouraged us to screen pearl millet genotypes for weed suppression following the pattern of earlier studies with cucumber (Putnam and Duke 1974), oat (Fay and Duke 1997), and sunflower (Leather 1983). We conducted a field trial to determine the smothering capability of 13 accessions of pearl millet (*Pennisetum typhoides*) on weeds. Because the field was highly infested with weeds, we conducted the study with a natural weed seed bank in the field. At the pearl millet harvest at 100 DAS, we recorded the weed population and dry weight. The two most dominant weed species (*Trianthema portulacastrum* and *Amaranthus spinosa*) were recorded separately. Other species, such as *Cyperus rotundus* and *Physalis minima,* were present in some plots.

Winter crops

Pot study. This study was done in pots in an open-air net house to determine (1) the suppression effect of crops on weeds and (2) the influence of soil incorporation of rice straw on weeds (Tamak 1991). The treatments consisted of three rice residue managements (control soil—without straw or stubble, soil + stubble, and soil + stubble + straw) and five test crops—wheat (*Triticum aestivum*) HD 2329, oat (*Avena sativa*) OS6, berseem (Egyptian clover) (*Trifolium alexandrinum*) Muscavi, lentil (*Lens*

esculenta) L 9-12, and fallow. The treatments were replicated 11 times in a completely randomized design. The experiment was conducted in soil brought from a rice field in the paddy growing belt, Pabnawa village, Kaithal District, Haryana. The rice straw, stubble, and soil (upper 15-cm layer) were collected after the harvest of the summer rice crop in November 1990. The soil was sieved through a 2-mm sieve to remove crop and weed residues. It was filled in pots (30 cm height, 30 cm diam) at 5 kg of soil pot^{-1}. As in the treatments, rice stubble and stubble + straw were mixed in the top 15 cm of soil in the pots, in the same proportion as found in the field, that is, 1.1 g of stubble (2.2 t ha^{-1}) and 2.6 g of stubble + straw kg^{-1} soil (5.2 t ha^{-1}). Presowing irrigation was applied and the next day 5 seeds pot^{-1} of all the test crops were sown. The crops were raised with recommended cultural practices except for weed control.

Bioassay study. The treatments consisted of five aqueous extract concentrations (control-distilled water, stubble 5% and 10%, and stubble + straw 5% and 10%) of rice residues and four winter weeds — *Phalaris minor; Avena ludoviciana, Chenopodium album,* and *Convolvulus arvensis.* A part of the rice crop residues (straw and stubble) used for the pot culture was also used for the bioassay. The residues were washed with water to remove adhered soil particles and dried in an oven at 70 °C. The samples were ground in a Wiley Mill so that they could pass through a 0.2-mm sieve. To prepare aqueous extracts of stubble of 5% and 10% concentration, 50 and 100 g of ground stubble were soaked in 1,000 mL of distilled water; likewise, straw + stubble extracts of 5% and 10% concentration were prepared. Some 50 g (35 g of straw + 15 g of stubble) and 100 g (70 g of straw + 30 g of stubble) of the above plant materials were soaked in 1,000 mL of water. After 24 h of soaking at room temperature (22 °C), the extracts were filtered through Whatman No. 1 filter paper and then centrifuged at 3,000 rpm for 20 min to remove all solid plant material. The bioassay then followed the method of the wheat straw extract bioassay using the above test weed species.

Results and discussion

Summer crops

Field study. In both years, the straw management treatments stimulated weed seed germination, whereas growing the forage crops decreased the weed population. The residual effect of the straw management practices and crops also persisted into the following crop season. The higher weed population in 1991 than in 1990 was due to rainfall immediately after crop emergence, which favored a quicker germination of weed seeds (Narwal and Sarmah 1996).

Weed density. The results of both years showed that the fodder crops significantly influenced the weed population throughout the growing season (Table 1). The crops had a variable effect on the density of broadleaf and grassy weeds. In general, the suppression effect was greater on the broadleaf weeds than on the grassy weeds.

Broadleaf weeds. Among the broadleaf weeds, *Trianthema portulacastrum* is a major weed. It constitutes up to 70% of the population of broadleaf weeds and up to 50–60% of the total weeds. Weed population data for the fodder crops harvest (70 DAS) showed that ail crops suppressed weeds more than the fallow, but varied in

Table 1. Effect of forage crops on weed density (mean of two years).

Crop	*Trianthema portulacastrum*	Weed population (no. m^{-2})		
		Broadleaf (% inhibition versus fallow control)	Grassy	Total
Sorghum	55.3	59.8	87.3	71.1
Pearl millet	82.9	85.3	91.8	87.4
Maize	76.6	76.9	80.9	79.4
Clusterbean	78.3	64.9	65.5	73.9
Cowpea	56.3	63.2	54.2	64.0

their degree of suppression (Table 1). Pearl millet was the most smothering crop and suppressed the population of *T. portulacastrum* 80.8–85.8% and broadleaf weeds 79.3–91.2% compared with the fallow. Broadleaf weed suppression followed this order: pearl millet > maize > clusterbean > cowpea > sorghum.

Grassy weeds. Like the broadleaf weeds, the fodder crops differed in their suppression effect on grassy weeds. Pearl millet caused maximum suppression (83.7–100%) in grassy weeds, followed by sorghum (78.2–96.4%). The cereal fodders exerted greater smothering than the legumes.

Total weeds. Pearl millet proved to be the most smothering crop and suppressed the weed population 80.7–94.0% compared with the fallow. The higher weed suppression of pearl millet may be due to its faster growth rate. It competed well with weeds and its root exudates may contain certain allelochemicals that might have inhibited weed seed germination and seedling growth. Narwal et al (1992) also reported a smothering ability of pearl millet genotypes in weed suppression. Sunlight interception studies with the test crop canopies showed that more PAR (photosynthetically active radiation) reaches the soil surface in pearl millet than in maize, clusterbean, and cowpea, and even then pearl millet smothers weeds (Sarmah 1992). This indicates the role of pearl millet allelopathy in weed suppression.

The cereal fodders caused more weed suppression than the legumes because of taller plants, which offered competition for light, soil moisture, nutrients, and space. Allelochemicals in root exudates may also inhibit germination and early seedling growth of weeds.

Weed dry matter. The straw treatments also increased dry matter (DM) accumulation in weeds (Table 2). Straw burning and soil incorporation increased DM production by 7.3–20.7% and 27.1–28.7%, respectively, compared with straw removal. In bioassays, the wheat straw and soil extracts also increased seedling growth (shoot and root length and DM) of all the test weed species.

All the crops, throughout their growth, significantly reduced the dry weight of weeds. As it was for weed density, pearl millet was the most efficient crop in reducing weed DM accumulation, followed closely by maize. Sorghum significantly reduced weed DM versus clusterbean and at 45 and 60 DAS versus cowpea. Both legumes performed identically. The reduction in weed dry weight compared with that of the

Table 2. Inhibitory effect of forage crops x straw treatment interactions at 70 days after sowing on weed dry weight (mean of two years).

Crops	Straw removed	Straw burned	Straw incorporated	Mean
		(% inhibition versus fallow control)		
Sorghum	64.5	82.9	81.7	75.7
Pearl millet	98.6	97.8	96.0	96.9
Maize	95.9	93.4	92.3	93.4
Clusterbean	88.0	60.7	72.4	73.7
Cowpea	86.4	80.6	72.8	79.0
Mean	86.7	83.1	83.0	

Table 3. Residual suppression effect of preceding fodder crops and straw treatment on weed density in the succeeding sorghum crop.

Treatments in previous crop	Sorghum		Fallow/undisturbed plot	
	15 DAS[a]	70 DAS	15 DAH	70 DAH
		(% inhibition versus fallow control)		
Sorghum	66.0	0.5	25.8	60.1
Pearl millet	86.6	22.6	54.0	67.6
Maize	27.4	26.7	38.6	43.3
Clusterbean	65.4	16.3	6.8	32.9
Cowpea	23.5	4.8	13.4	39.0

[a] DAS = days after sowing, DAH = days after harvest of preceding crop.

control (fallow) at 70 DAS for pearl millet, maize, sorghum, cowpea, and clusterbean was 96.9%, 93.4%, 75.7%, 79.0%, and 73.7% (Table 2), respectively. The cereal fodders caused a greater reduction in DM than the legume fodders. Among the cereals, pearl millet produced a maximum reduction of 88.8–96.1 % in DM production of weeds.

Residual suppression effect. In undisturbed plots and in sorghum, the residual effect of straw management treatments and the preceding fodder crops persisted up to 70 d after harvest of the fodder crops (Table 3). The straw management treatments increased the weed population, similar to the results of the original experiment.

In both years, the residual effect of the preceding forage crops clearly reduced the weed population throughout the crop season compared with the fallow plots. The residual suppression effect of the legumes (cowpea and clusterbean) on weeds was much less when compared with the cereal fodders. Pearl millet most effectively reduced the weed population through the next crop season. Maize followed pearl millet, whereas sorghum, clusterbean, and cowpea had similar suppression effects. Perhaps allelochemicals exuded as root exudates in soil from previous fodder crops continued to suppress the weeds, as did the preceding crops. Pearl millet caused the maxi-

mum suppression (54–67.6%) compared with the fallow, possibly because of the release of some phytotoxins from the decomposing stubble.

The weed population in undisturbed plots followed a similar trend to that of the sorghum crop. The maximum weed population was recorded in the fallow plots and the minimum in plots vacated by pearl millet. Maize followed pearl millet in residual suppression effects. The suppression effect persisted up to 45 d after harvest of the previous fodder crop.

This shows that the residual suppression of the preceding crops will keep the weed population under check during the early growth stages of the succeeding crop. If the next crop is sown within 7–10 d after harvest of the previous crop (as practiced in the multiple cropping system in Asia), then the weed density in the subsequent crop may remain under check (below the economic threshold level) during the first 35–40 d of crop growth, that is, during the critical phase. During this period, the next crop will develop enough canopy to suppress or smother the weeds. Currently, to reduce the problems associated with herbicides, weed scientists do not recommend using herbicides until the weed population reaches the economic threshold. Therefore, including smothering crops with residual effects, such as pearl millet, in crop rotations may provide weed management in the subsequent rice crop without the use of herbicides.

Pearl millet accessions

All the pearl millet genotypes reduced the weed population of *Trianthema portulacastrum, Amaranthus* spp., and total weeds. Nine genotypes significantly smothered the total weed population. Of these, HHB 68 ranked first in weed suppression (72.3%), closely followed by 88004A × 833-2 (71.1%), HHB 60 (57.5%), and 863A × HTP 88/33 (57.2%) (Table 4). Other genotypes suppressing 50% of the weed population were 88006A × 90/4-5 (54.6%) and 843A × HTP 88/47 (53.5%). Four genotypes—81A × HC-4, 88006A x 833-2, 861A × 77/273, and HHB 67—suppressed

Table 4. Suppression effect of pearl millet accessions on population of weeds m^{-2}.

Accession	Total weeds (suppression of weed population (%) versus fallow control)	*Trianthema portulacastrum*
HHB 68	72.3	73.0
88004A x 833-2	71.1	72.6
HHB 60	57.5	57.3
863A x HTP 88/33	57.2	42.4
88006A x 90/4-5	54.6	55.7
843A x HTP 88/47	53.5	46.2
81A x HC-4	38.5	41.1
88006A x 833-2	36.7	26.9
861A x 77/273	34.2	38.5
HHB 67	32.6	74.8
843A x 77/371	22.4	53.8
HHB 50	18.0	52.4

weeds > 30%. The weed population of *T. portulacastrum* varied greatly with different genotypes of pearl millet. Genotype HHB 67 suppressed its population by 74.8%, followed closely by HHB 68 (73.0%) and 88004A × 833.2 (72.6%). Smothering of this weed species by other genotypes—HHB 60 (57.3%), 8806A × 90/4-5 (55.7%), 843A × 77/371 (53.8%), and HHB 50 (52.4%)—was quite satisfactory. Pearl millet cultivars HHB 50, HHB 60, 81A × HC-4, 843A × HTP 88/33, and 843A x HTP 88/47 significantly suppressed the *Amaranthus* population. Shetty and Rao (1981) also reported that pearl millet suppressed the growth of weeds much more strongly than did groundnut.

All pearl millet genotypes significantly reduced weed DM. Variety HHB 67 and genotype 88004A × 833-2 caused maximum suppression of 87.7% and 85%, respectively, versus the control. The pearl millet genotypes did not significantly differ in their plant populations, plant height, and fodder yields. Therefore, differences in weed populations in different genotypes indicate variability in the suppression effect of the genotypes.

Winter crops

Pot study. Soil incorporation of rice residues suppressed the plant density of weeds, although test crops exhibited inhibition or stimulation and decreased weed biomass.

Broadleaf weeds: Weed density. Soil incorporation of rice stubble or stubble + straw decreased weed density (Table 5). The suppression of weeds was greater in earlier stages—15 DAS—and decreased in later stages (45 DAS). Perhaps the phytotoxicity of the decomposing rice residues decreased 15 d after soil incorporation.

The test crops had a variable effect on weed density. Wheat and berseem decreased the weed population, whereas oat and lentil increased it. Perhaps the root exudates of later crops stimulated weed germination. Berseem was the most smothering crop for the broadleaf weeds and caused 52.9% suppression at 30 d, versus 16.8% for wheat at 45 d. Oat suppressed the weed population slightly more than did lentil.

Table 5. Effect of rice residues and test crops on weed population plot^{-1}.

Treatment	Broadleaf weeds[a]			Grassy weeds[a]		
	15	30	45	15	30	45
	(% inhibition (–)/stimulation (+) versus fallow control)					
Soil-incorporated rice residues						
Stubble	–11.7	–5.5	–7.9	–26.5	+4.1	+9.5
Stubble + straw	–12.3	–7.9	–9.0	–41.2	+1.1	+8.0
Test crops						
Wheat	–2.9	–2.4	–16.8	–5.6	+39.8	+77.1
Oat	+4.7	+33.9	+16.8	–6.7	+32.1	+88.5
Berseem	–3.7	–52.9	–39.3	–5.6	+59.0	+57.3
Lentil	+4.0	+25.3	+14.8	–7.8	+9.0	+34.4

[a]At 15, 30, and 45 days after sowing.

Table 6. Effect of rice residues and test crops on weed biomass pot[1].

Treatment	Broadleaf weeds[a]				Grassy weeds[a]			
	60	90	120	135	60	90	120	135
	(% inhibition (–)/stimulation (+) versus fallow control)							
Soil-incorporated rice residues								
Stubble	–49.4	–61.4	–43.0	–33.4	+60.0	+126.7	+286.3	+12.6
Stubble + straw	–46.9	–58.6	–39.8	–32.3	+40.0	+68.3	+214.7	+10.0
Test crops								
Wheat	–3.5	–15.2	–44.2	–34.9	0.0	–41.5	–46.5	–24.7
Oat	–35.7	–57.3	–75.4	–66.7	0.0	–47.7	–62.3	–31.2
Berseem	–53.3	–79.0	–87.5	–83.0	–50.0	–78.5	–90.0	–43.7
Lentil	–26.1	–7.6	–44.8	–50.2	–16.7	–78.0	–84.0	–37.6

[a]At 60, 90, 120, and 135 days after sowing.

Table 7. Inhibitory effect of rice residues and test crops on biomass yield of broadleaf weeds at 135 days after sowing.

Crops	Rice residue treatments			
	Control	Stubble	Stubble + straw	Mean
	(inhibition (%) versus fallow control)			
Wheat	30.3	56.0	15.8	34.9
Oat	65.4	78.5	59.7	66.7
Berseem	79.3	88.9	86.8	83.0
Lentil	46.5	54.3	51.0	50.2
Mean	55.4	69.4	53.3	

Broadleaf weeds: Weed dry matter. In contrast to the plant population of weeds, both soil-incorporated rice residues and test crops considerably decreased the dry biomass of weeds (Table 6). Soil-incorporated rice stubble caused a greater suppression in weed DM than stubble + straw. The inhibitory effects of decomposing crop residues on the growth of many weed species is known (Guenzi and McCalla 1966, Guenzi et al 1967, Leather 1983, Purvis et al 1985, Putnam and DeFrank 1983, Putnam and Duke 1978). Kuwatsuka and Shindo (1973) isolated 13 phenolic acids in rice straw and its decayed products. The suppression effect increased up to 90 DAS and decreased afterwards. The toxicity of phenolic acids decreased with progress in decomposition (Guenzi et al 1967). The smothering effect of crops on weed DM followed this order: berseem > oat > lentil > wheat (Table 7). The degree of suppression increased up to 120 DAS in crops and was maximum in berseem (87.5%), oat (75.4%), lentil (50.2%), and wheat (44.2%) compared with fallow at 120 DAS. Altieri and Doll (1978), Leather (1983), Chou (1987), and Putnam (1988) have also reported suppression effects of several crops on numerous weeds.

Grassy weeds: Weed density. The effect of rice residues and test crops on grassy weeds was entirely different from that on broadleaf weeds (Table 5). The soil-incorporated rice stubble or stubble + straw suppressed the weed population only up to 15 DAS. The inhibitory effect was greater in stubble + straw incorporation than in stubble alone, but both had an identical stimulatory effect. All the test crops suppressed weed density up to 15 DAS, but increased weed density afterwards until 45 DAS. Oat and wheat caused a maximum increase in weed populations.

Grassy weeds: Weed dry matter. Both soil-incorporated rice stubble or stubble + straw increased the DM of grassy weeds and the stubble proved superior to stubble + straw up to 120 DAS (Table 6). All the test crops inhibited grassy weeds and the smothering effect of crops at 120 DAS followed this order: berseem (90.0%) > lentil (84.0%) > oat (62.3%) > wheat (46.5%).

Bioassay study: Broadleaf weeds. The aqueous extracts of both rice stubble and stubble + straw at two concentrations (5% and 10%) inhibited the germination and seedling growth of *Convolvulus arvensis* (Table 8). The stubble + straw extract, regardless of concentration, was more inhibitory than the stubble extract. This indicates that the straw contains more inhibitory allelochemicals than the stubble. The inhibition in germination and seedling growth was concentration-dependent, that is, higher concentrations (10%) were more inhibitory than lower concentrations (5%). The extracts had a similar inhibitory effect on shoot length and dry weight, but were more inhibitory to root elongation than to dry weight.

The *Chenopodium album* seeds did not germinate because of dormancy.

Bioassay study: Grassy weeds. The aqueous extracts of rice residues were more inhibitory to grassy weeds than to broadleaf weeds.

Table 8. Inhibitory effect of aqueous extracts of rice residues on seed germination and seedling growth of weed species at 10 days after sowing.

Extract	Concentration (%)	Germination (%)	Shoot		Root	
			Length	Dry weight	Length	Dry weight
			(inhibition (%) versus water control)			
Convolvulus arvensis						
Stubble	5	6.3	28.3	34.4	1.4	0.7
	10	12.5	58.3	60.2	52.6	42.0
Stubble + straw	5	9.4	37.2	37.5	25.7	17.3
	10	17.7	66.1	64.1	61.8	46.0
Avena ludoviciana						
Stubble	5	22.6	7.0	8.7	32.3	21.8
	10	38.9	29.8	30.5	60.5	43.3
Stubble + straw	5	31.0	20.3	15.4	43.3	31.5
	10	46.4	49.8	37.5	68.9	53.9
Phalaris minor						
Stubble	5	15.4	22.7	20.1	14.2	11.4
	10	30.8	62.5	60.2	40.8	35.0
Stubble + straw	5	21.8	14.4	12.7	10.1	8.3
	10	37.2	100.0	100.0	100.0	100.0

Avena ludoviciana. Both extracts and their concentrations inhibited germination and seedling growth of *A. ludoviciana* and the inhibition was concentration-dependent (Table 8). The stubble extract had an identical inhibitory effect on shoot length and dry matter, whereas stubble + straw extract was more inhibitory to shoot DM than to its length. Stubble and stubble + straw extracts, however, caused more inhibition in root DM than in root elongation.

Phalaris minor: Like *A. ludoviciana* and *C. arvensis,* both the extracts and their two concentrations inhibited germination and seedling growth of *P. minor* and the inhibition was concentration-dependent (Table 8). The extracts were more inhibitory to DM and elongation of shoots than to those of roots. The stubble + straw extract at 10% caused 100% inhibition of seedling growth.

Prospects of nonchemical weed management in the rice-wheat rotation

Herbicide resistance has started developing in biotypes of the major weed *Phalaris minor* of the rice-wheat rotation. Therefore, in the future, more weeds may develop such tolerance, and herbicide-based weed control may not be sustainable. Thus, the use of allelopathic strategies seems to be the only way to manage weeds for the sustainability of the rice-wheat rotation for farmers of the grain bowl of northwestern India. Our preliminary studies with summer and winter crops have amply demonstrated the great potential of smothering crops to control weeds without herbicides in this rotation. This goal might be achieved if more detailed studies are undertaken to exploit the untapped allelopathic potential of these crops for weed management.

Pearl millet

In northwestern India, there is a fallow period of about 80 d between the wheat harvest in mid-April and transplanting of the rice crop in the first half of July. This period is most productive and favorable for crop growth because of the clear sky (maximum PAR), high temperatures, and freedom from pests. But it is a scarce period for green fodder because of the maturity and drying of winter fodders. Therefore, farmers in irrigated areas grow fodder crops of maize, sorghum, pearl millet, cowpea, and clusterbean in a small part of their land. Among these fodder crops, pearl millet has the fastest growth rate and produces the maximum biomass per day. According to the results of our study, pearl millet could be grown during this fallow period (mid-April to the end of June) to smother the summer weeds, as well as to control weeds in the subsequent rice crop through its residual suppression effect. Likewise, double cropping of rice-barley reduced weed occurrence in the rice crop by 30% compared with a single crop of rice. This was attributed to the allelopathic effect of barley residues (Choi et al 1995). To improve soil fertility and fodder quality, a mixture of pearl millet + clusterbean can be grown. To further improve the residual suppression effect, pearl millet accessions can be screened for this character as is being done in rice.

Rice residues and winter crops

To reduce the infestation and growth of weeds in ricefields, Egyptian clover (*Trifolium alexandrinum*) and oats (*Avena sativa*) can be grown. Egyptian clover effectively controls the weeds, through its frequent cutting for fodder, which prevents seed setting in weeds. Therefore, growing of Egyptian clover after rice for 2–3 yr may significantly reduce weed populations in the field. Bioassays have shown that a 10% aqueous extract of stubble + straw completely checked the seedling growth of *Phalaris minor* (Table 8). This needs further investigation.

Based on our studies, we suggest new 3-yr rice-wheat rotations for weed management without the use of herbicides (Fig. 2). These rotations are based on weed-smothering crops for summer (pearl millet) and winter (oat, Egyptian clover) seasons. To solve the problems of weed infestations in individual crops of rice and wheat, separate rotations for both crops have been suggested. We hope that more research on this will help to develop new alternate rotations.

Conclusions and future lines of research

These studies have shown that allelopathic strategies including the use of smothering crops could provide weed control in a rice-wheat rotation. Although these crops do not provide complete weed control, they can manage weed populations at the economic threshold. This may either eliminate or minimize the use of herbicides and thus overcome the major problems associated with herbicides. The order of weed suppression in summer crops was pearl millet > maize > sorghum > clusterbean > cowpea; the order in winter crops was berseem > oat > lentil > wheat. Studies with accessions of pearl millet showed that this crop exhibited greater variability in its weed-smothering ability.

Rotation 1

Pearl millet	Rice	Wheat	Pearl millet	Rice	Wheat	Pearl millet

Rotation 2

Pearl millet	Rice	Oats	Pearl millet	Rice	Berseem	Fallow

Rotation 3

Pearl millet	Rice	Berseem	Fallow	Rice	Oats	Pearl millet

Fig. 2. Rotations based on allelopathic strategies for weed management in the rice-wheat rotation (pearl millet, oats, and berseem are fodder crops).

Further studies are urgently needed to overcome the problem of herbicide resistance and to develop allelopathic strategies for nonchemical weed management for the sustainability of the rice-wheat rotation. These studies should:

1. Screen smothering crops for their weed-smothering potential.
2. Screen varieties in promising crops for smothering potential.
3. Develop crop rotations with allelopathic crops having the potential to control weeds.

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CHAPTER 11

Allelopathic effect of *Lantana camara* on rice and associated weeds under the midhill conditions of Himachal Pradesh, India

G.L. Bansal

Lantana camara L., one of the world's 10 worst weeds and introduced in India during the early part of the nineteenth century, has become a major problem in the state of Himachal Pradesh. The fast growth of the weed has been responsible for substantial economic losses in the state's agricultural, animal husbandry, and forest industries. However detrimental the weed itself is to agriculture, it has been found that aqueous extracts from *L. camara* twigs inhibit, to various degrees, germination and seedling growth in rice-associated weeds, such as *Echinochloa colonum, Digitaria sanguinalis, Panicum psilopodium,* and *Commelina benghalensis.* This inhibitory effect was substantially relieved when activated charcoal was added to the soil medium in laboratory tests, which indicates that allelopathy is most likely involved. In four of five rice varieties tested, these extracts did not affect germination. *L. camara* twigs incorporated into the soil under field conditions significantly increased chlorophyll content in transplanted rice and subsequently grain yield, while at the same time significantly reducing the abundance of two weeds, *P. psilopodium* and *C. benghalensis.* It would appear that *L. camara* has some useful allelopathic effects that warrant more study.

Introduction

Mineral nutrients and organic matter are an integral part of agriculture. Because soil nutrients are depleted in crop production, they need to be replenished by exogenous sources such as fertilizers. Modern agriculture depends heavily on chemical fertilizers. But rising fertilizer prices and their limited availability and the low purchasing power of marginal and small farmers have demonstrated the importance of using organic wastes as a source of nutrients. This is true for most of the developing world but even more so in heavily populated countries such as India (Tandon 1995).

India has many organic materials that can be used to complement fertilizers. Farmers can effectively convert crop residues and undesirable plants (weeds) rich in nitrogen into organic energy by incorporating them into the soil under proper management practices (Bansal 1987).

Evidence shows that incorporating some organic wastes as green manure during the process of decomposition releases certain substances that can inhibit the germination and development of various weed species (Guenzi and McCalla 1962). Thus, incorporating these organic wastes may reduce land preparation and the competitive ability of weeds to such an extent that crop yield can be increased considerably. In addition, for any new technology to be popular and practical, it must be economically sound.

Wild sage (*Lantana camara* L. var. Aculeata), a plant introduced in India around 1810 (Rao et al 1971) for ornamental purposes, has spread alarmingly in Himachal Pradesh besides in other parts of India. This obnoxious weed poses a serious problem for flora and fauna because of its toxic substance Lantadene A (Sharma et al 1981), and it contains certain allelopathic compounds (Jain et al 1989). The green twigs of *Lantana* on a dry weight basis are reported to contain more than 2.0% N and 1.5% K, but they are poor in P (Bhardwaj and Kanwar 1991). We therefore conducted research to (1) check the allelopathic effect of *Lantana* extracts on rice and associated weeds, and (2) study and compare the effect of decomposition of *Lantana* in different intervals on rice growth and yield, and on associated weeds.

Materials and methods

Two experiments were conducted to meet the objectives:

Experiment I. For allelopathic studies, a 10% aqueous extract of *Lantana* twigs (shoots with leaves) was prepared by soaking 100 g of fresh matter in 1,000 cc of water for 24 h. A part of this extract was diluted to 1% and 5% with water. One hundred seeds each of three weeds—*Echinochloa colonum, Panicum psilopodium,* and *Digitaria sanguinali*—were tested for their germination (in three replications) on filter paper lined in petri dishes (10 cm diam). The effect of these aqueous extracts was also studied in the presence of activated charcoal (500 mg petri dish^{-1}) to see whether charcoal can modify the effect of extracts. The seedling growth of 10 germinated seeds in each case was recorded after 1 wk (Table 1). The germination of four rice varieties—Himalaya-1, Himalaya-2, Himdhan, and China-988—was also tested with these extracts (Table 2).

Experiment II. To study the effect of cornposting of *Lantana,* fresh *Lantana* twigs were put into pits (4 x 4 m) for 3, 2, and 1 mo before starting the decomposition experiment. The decomposed and fresh *Lantana* was incorporated into the field at the time of puddling at 10 t ha^{-1}. The experiment consisted of 13 treatments—incorporation of fresh *Lantana* twigs decomposed for 1, 2, and 3 mo and three levels of nitrogen (0, 45, and 90 kg ha^{-1}), with one absolute control—and was conducted at the experimental farm of the Department of Agronomy from the end of June to October 1986. The plot size was 6 x 4 m and each treatment was replicated three times in a randomized block design. Variety Himalaya-1 was used. Observations were recorded on plant height, number of tillers, chlorophyll content (at maximum tillering), and grain, straw, and weed dry weight.

Table 1. Effect of Lantana twig extract on germination and seedling growth of some weeds, with and without charcoal.

Extract (%)	Weed species								
	E. colonumn			*P. psilopodium*			*D. sanguinalis*		
	Seed germination	Seedling growth (cm)		Seed germination	Seedling growth (cm)		Seedling germination	Seed growth (cm)	
		Root	Shoot		Root	Shoot		Root	Shoot
0 (control)	64.7	2.97	5.37	65.33	1.76	3.12	58.6	1.35	2.45
1	57.3 (88.6)[a]	1.90 (63.9)	5.08 (94.48)	56.6 (86.59)	0.97 (55.09)	2.47 (79.04)	56.0 (95.2)	1.30 (96.2)	2.36 (96.28)
1+charcoal	60.6 (94.6)	1.98 (66.52)	6.02 (111.97)	63.3 (96.84)	1.48 (84.06)	3.24 (103.68)	59.3 (100.81)	1.32 (97.68)	3.16 (128.92)
5	54.6 (84.3)	1.74 (58.46)	5.04 (93.74)	42.0 (64.26)	0.97 (55.09)	2.25 (72.0)	45.3 (77.01)	1.09 (80.66)	1.78 (72.62)
5+charcoal	58.0 (89.6)	1.79 (60.14)	5.15 (95.79)	50.0 (76.5)	1.50 (85.2)	3.02 (96.64)	56.6 (96.22)	1.14 (84.36)	2.57 (104.85)
10	46.0 (71.8)	1.76 (59.13)	5.07 (94.30)	39.3 (60.4)	0.96 (54.52)	2.14 (68.48)	36.0 (61.4)	0.94 (69.56)	1.17 (47.73)
10+charcoal	53.3 (83.2)	1.79 (60.14)	5.26 (97.83)	45.3 (69.30)	0.96 (54.52)	2.36 (75.52)	44.6 (75.82)	0.96 (71.04)	1.53 (62.42)
CD at 0.05%	1.5	0.23	NS	4.6	0.21	0.18	1.9	NS	0.23

[a] Numbers in parentheses indicate the percentage value of control. NS = nonsignificant.

Table 2. Effect of aqueous extracts of plant parts of *Lantana camara* var. Aculeata twigs on germination (%) of some rice varieties.

Extract (%)	Rice variety			
	Himalaya-1	Himalaya-2	Himdhan	China-988
0 (control)	97	87	87	99
1	98	92	67	94
5	98	92	65	96
10	91	100	65	97
CD at 5%	NS[a]	NS	8.5	NS

[a] NS = nonsignificant.

Results

Allelopathic effect on weeds

Lantana extract suppressed seed germination in all the associated weeds and the suppressive effect increased with an increase in percentage of the extract. The effect was most pronounced in *P. psilopodium*, followed by *E. colonum* and *D. sanguinalis* (Table 1). The activated charcoal significantly alleviated the suppressive effect of the extract of higher concentration, with the alleviation being more pronounced in *D. sanguinalis,*

Table 3. Effect of incorporating *Lantana camara* var. Aculeata on rice growth and yield.

Treatments	Plant height (cm)	Tillers (no.)	Effective tillers plant^{-1}	Chloro-phyll (mg g^{-1})	Grain yield (Q ha^{-1})[a]	Straw weight (Q ha^{-1})	Weed dry weight (Q ha^{-1})
3 mo composted *Lantana*	48.0	6.7	1.4	1.1	29.7	51.5	3.2
+ 45 kg ha^{-1} N	52.2	10.3	1.5	1.3	30.3	61.8	5.2
+ 90 kg ha^{-1} N	56.7	12.2	1.5	1.5	30.6	65.1	3.4
2 mo composted *Lantana*	47.1	6.6	1.5	1.4	27.1	58.0	3.7
+ 45 kg ha^{-1} N	49.0	10.9	1.5	1.2	29.9	62.8	1.8
+ 90 kg ha^{-1} N	55.2	10.3	1.4	1.2	29.5	61.5	2.2
1 mo cornposted *Lantana*	48.3	6.3	1.5	1.5	30.0	61.7	2.5
+ 45 kg ha^{-1} N	51.3	8.1	1.5	1.5	30.2	47.6	1.9
+ 90 kg ha^{-1} N	52.2	9.1	1.6	1.4	33.8	68.6	3.2
Fresh *Lantana*	44.0	12.6	1.4	1.7	25.5	68.5	2.5
+ 45 kg ha^{-1} N	45.4	15.2	1.7	2.0	38.9	74.8	3.1
+ 90 kg ha^{-1} N	46.4	12.0	1.5	1.8	35.8	67.6	3.5
Absolute control	44.8	5.6	1.5	1.0	22.1	51.4	2.1
CD at 0.05%	6.0	2.5	NS[b]	0.3	2.1	NS	NS

[a]Q = quintal, 1 quintal = 100 kg. [b]NS = nonsignificant.

followed by *E. colonum* and *P. psilopodium.* For seedling growth, root elongation was affected more than shoot elongation in all the weeds, with the suppressive effect being most pronounced in *P. psilopodium,* followed by *D. sanguinalis* and *E. colonum* (Table 1). But the activated charcoal did not significantly reduce the suppressive effect on root growth in any of these weeds. Shoot growth was significantly suppressed at a higher concentration in *D. sanguinalis,* followed by *P. psilopodium.* Interestingly, although the charcoal reduced the suppression of shoot growth at a higher concentration in these two weeds, the extracts at the lowest concentration (1%) significantly promoted shoot growth in *D. sanguinalis.*

Allelopathic effect on rice varieties

The effect of *Lantana* extracts remained nonsignificant for seed germination in all the rice varieties, even with the increase in extract concentration, except for Himdhan, which was sensitive to *Lantana* (Table 2).

Allelopathic effect of *Lantana* composting on rice and associated weeds

Plant height of rice was not affected significantly by *Lantana* compost or nitrogen application (Table 3). Numbers of rice tillers were highest with fresh *Lantana* treatments but were not significantly different among the compost treatments. Applying N increased tillering, and this was most pronounced in *Lantana* composted for 3 mo, followed by 2 and 1 mo. The treatment effects were nonsignificant for effective tillers plant^{-1}. Chlorophyll content remained unaffected in *Lantana* composted for 3 mo, but increased with a reduction in composting interval, and was the highest in fresh *Lantana* with 45 kg ha^{-1} of N.

Table 4. Number of weed species 0.25 m^{-2} as affected by different treatments at harvest.

Treatments	*Cyprus rotundus*	*Echinochloa colonum*	*Panicum psilopodium*	*Commelina benghalensis*
3 mo composted *Lantana*	10.0	8.3	23.2	20.1
+ 45 kg ha^{-1} N	5.6	10.0	21.7	27.0
+ 90 kg ha^{-1} N	1.3	6.6	26.0	23.0
2 mo composted *Lantana*	9.3	8.0	24.7	24.3
+ 45 kg ha^{-1} N	4.3	6.6	23.0	24.0
+ 90 kg ha^{-1} N	3.3	8.0	26.3	31.7
1 mo composted *Lantana*	7.0	9.3	27.7	22.7
+ 45 kg ha^{-1} N	4.3	10.3	26.3	20.7
+ 90 kg ha^{-1} N	3.0	9.6	22.3	22.0
Fresh *Lantana*	4.6	3.6	22.7	27.3
+ 45 kg ha^{-1} N	5.0	9.3	24.7	25.0
+ 90 kg ha^{-1} N	7.0	6.3	22.7	30.3
Absolute control	12.3	13.0	39.3	39.7
CD at 0.05%	NS[a]	NS	3.5	3.9

[a]NS = nonsignificant.

Incorporating *Lantana* significantly increased grain yield in all the treatments, but the effect was most remarkable in fresh *Lantana* with 45 kg ha^{-1} of N, followed by fresh *Lantana* with 90 kg ha^{-1} of N (Table 4). Applying N, however, invariably increased straw, but the effects remained statistically nonsignificant. Weed dry weight did not show any consistent trend and the treatment effects were again nonsignificant.

Incorporating *Lantana* reduced the number of weeds in all the species, but the effect was significant only for *P. psilopodium* and *Commelina benghalensis.* No consistent trend in weed count could be recorded with the application of nitrogen.

Discussion

The results in this paper clearly demonstrate the suppressive effect of *Lantana* extract in rice on weed germination and weed seedling growth. This suppressive effect was significantly reduced with activated charcoal. *Lantana* extract did not affect rice germination. The suppressive effect of *Lantana* on weeds may be caused by allelopathy. *Lantana* has also been reported to be allelopathic against milkweed vine *(Morrenia odorata* Lindl.), velvetleaf *(Abutilon theophrasti* Medik), and fern *[Cyclossus dentatus* (Forsk) Ching]. Jain et al (1989) reported that *Lantana* is allelopathic because of the presence of phenolic compounds. Allelochemicals from *Lantana camara* L. var. Aculeata may, however, be different and need to be identified.

Incorporating composted *Lantana* to increase rice yield is not new (Chakor and Sharma 1989). Bhardwaj and Kanwar (1991) also reported that *Lantana* decomposes faster than wheat straw, but not as fast as sesbania *[Sesbania cannabina* (Retz.) Pers. (syn. *S. aculeata* Pers.)], without any significantly inhibitory effect on rice. The most significant finding of this investigation, however, is that fresh *Lantana* not only saved 45 kg ha^{-1} of N (besides extra labor and energy used for decomposing) but also enhanced tillering and chlorophyll content in rice besides generating higher yield. It

would appear that *Lantana* has some useful allelopathic compounds or hormonal substances that increased the number of effective tillers and chlorophyll content (and thus photosynthesis) in rice. In fact. when incorporated at 10 t ha^{-1} in rice, *Lantana* adds 80 kg ha^{-1} of N. Apart from enhancing yield, *Lantana* also reduced the proportion of two weeds (*D. sanguinalis* and *P. psilopodium*) significantly, perhaps also because of allelopathy.

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CHAPTER 12

Potential of allelopathy for weed management in wet-seeded rice cultivation in Sri Lanka

B. Marambe

Experiments conducted in Egypt and in the United States have shown that several rice varieties collected from Sri Lanka are allelopathic to major weeds. Despite the potential importance of allelopathy in agriculture, evidence is lacking on rice allelopathy in Sri Lanka, where wet-seeded cultivation is the major practice of peasant farmers. Experiments have been carried out using four rice varieties—BG1165-2, BG 34-11, LD 183-3, and LD 183-7—to analyze their allelopathic potential in controlling *Echinochloa crus-galli* (L.) Beauv. Knowledge of rice allelopathy and its allelochemical functions offers several attractive possibilities for cropping practices in Sri Lanka.

Introduction

Low rice productivity and environmental degradation have been major problems for the past decade in paddy cultivation in Asia. Maintaining high yields of rice, Sri Lanka's staple food crop, has become more difficult as population growth and expansion have reduced land area and increased the demand for rice (Naylor 1994). Weeds are an important constraint to increased rice production. Moody (1994) reported that appropriate weed management strategies are urgently needed to maintain the yield stability of rice and reduce production costs.

In recent years, because of the use of large quantities of herbicides and their potential hazards, such as residual effects, contamination of food chains and groundwater, and other consequences, scientists have been looking for alternative ways to manage weeds to enhance crop production (Marambe and Sangakkara 1996). Allelopathy is one of the emerging techniques identified and studied as an effective and environmentally friendly method. This paper presents the current status of weed control in rice cultivation in Sri Lanka and focuses on the prospects for using allelopathy as a weed management strategy in wet-seeded rice cultivation.

Weed constraints to rice production

A significant portion of the agricultural land in developing countries in the tropics is heavily infested with weeds (Akobundu 1992), and controlling weeds is a big challenge to Asian farmers. During the past 15 years, wet seeding has replaced transplanting as the dominant method of rice establishment in Sri Lanka, mainly because of

Table 1. Estimated potential losses in rice production in South Asia from diseases, animals, and weeds (1988-90).

Country	Yield (kg ha^{-1})	Potential crop losses (%)			Attainable yield (kg ha^{-1})
		Diseases	Animals	Weeds	
Bangladesh	2,550	12–17	50–55	50–55	5,930
Bhutan	1,660	30–35	45–50	50–55	4,190
India	2,630	35–40	50–55	55–60	6,600
Myanmar	2,910	27–32	45–50	55–60	6,670
Nepal	2,310	30–35	45–50	50–55	5,850
Pakistan	2,290	25–30	45–50	55–60	6,440
Sri Lanka	3,010	27–32	35–40	60–65	6,430

Source: Oerke 1995.

Table 2. The major weeds in wet-seeded lowland rice cultivation in Sri Lanka.

Botanical name	Family	Life cycle
Echinochloa crus-galli (L.) Beauv.	Poaceae	Annual
Ischaemum rugosum Salisb.	Poaceae	Annual
Isachne globosa (Thunberg) Kuntze.	Poaceae	Perennial
Leptochloa chinensis (L.) Nees.	Poaceae	Annual
Cyperus iria L.	Cyperaceae	Annual
C. difformis L.	Cyperaceae	Annual
Fimbristylis miliacea (L.) Vahl.	Cyperaceae	Annual
F. dichotama (L.) Vahl.	Cyperaceae	Annual
Monochoria vaginalis (Burm. f.) Presl.	Pontederiaceae	Perennial
Sphenoclea zeylanica Gaertn.	Sphenocleaceae	Annual
Ludwigia octovalvis (Jacq.) Raven.	Onagraceae	Annual

increasing labor costs. This shift has been accompanied by an increase in weed problems (Amarasinghe 1986, Moody 1994).

The estimated loss of rice production in South Asian countries when crop protection measures are not practiced clearly indicates that losses caused by weeds are much higher than those caused by diseases or animals (Oerke 1995, Table 1). Quantitative losses from weed interference depend on rice variety, agronomic practices, type of rice culture, and water management (Moody 1991). In addition, the replacement of traditional rice varieties—which were tall, vigorously growing, and of long duration—with short-stature modern cultivars has significantly increased weed infestation (Amarasinghe 1986). The frequent wetting and drying cycles experienced in most of the ricefields in Sri Lanka as a result of intermittent rainfall or rotational water use have also favored prolific weed growth.

Weerakoon and Gunawardena (1983) identified more than 134 species belonging to 32 plant families as weeds in ricefields of Sri Lanka. A more comprehensive study carried out by Chandrasena (1988) indicated that weed diversity is the highest in the family Poaceae, with at least 70 species, and next highest in *Cyperaceae,* with more than 55 species. Only 11 weed species, however, have been identified as troublesome (Table 2).

Weed control strategies

The use of herbicides has become the most common practice in controlling weeds in wet-seeded rice fields. Naylor (1994) reported that herbicides are used in 90–100% of the wet-seeded rice fields in Sri Lanka. Although alternative and integrated weed management techniques have been introduced, most farmers still rely on herbicides because of a lack of labor, the wet-seeding method of crop establishment, and prolific weed growth resulting from poor land preparation techniques and poor water management. Several negative effects of chemical weed control, however, have been recorded in the recent past, and these side effects were usually attributed to the incorrect use of certain herbicides. Shifts in weed flora, with a lower species diversity, accompanied by the appearance of resistant species or biotypes, have also been observed repeatedly. In addition, damage to highly sensitive rotational crops caused by carryover from herbicides with high soil persistence has been recorded. During weed management in other field crops, the detection of traces of certain herbicides (such as atrazine and diuron) in ground- and surface water has produced animated discussions on the impact of herbicides on the environment (Bulcke 1996).

The cost of weed control under Sri Lankan conditions increased three- to four-fold from 1982 to 1995, indicating that, despite the large-scale application of herbicides, weed control in ricefields has been less than optimum because of increased costs. In addition, the country is now experiencing repercussions from the continuous use of herbicides for weed control in rice. Several incidents of the poor control of *Echinochloa crus-galli* have been reported. An increasing number of reports on resistance of this weed to propanil, a photosystem II inhibitor, in rice have also been mentioned in countries such as Greece (Giannopolitis and Vassiliou 1990) and the United States (Hoagland et al 1997), as well as Sri Lanka (Marambe et al 1997).

Recent and future legal measures will have a significant impact on crop production and weed control. The adjectives “integrated” or “sustainable” are now commonly associated with agriculture, crop production, and weed control. Many new developments are aimed at reducing the use of or dependence on chemicals to control weeds. Appropriate crop management techniques, such as the use of allelopathic rice in weed control, should therefore be investigated for the long-term productivity of continuous rice cropping.

Rice allelopathy in weed management

For weed management strategies, the allelopathic capacity of crops to suppress weeds has immediate utility. Most work, however, has focused on the effects of weed allelopathy on crop productivity and information on the reverse is scarce. A few studies have used allelopathic effects as a practical means of directly controlling weeds (Liang et al 1983). Crops such as cucumber (Putnam and Duke 1974), soybean and sunflower (Masantini et al 1977, Kim 1995), barley (Putnam and DeFrank 1983), and sorghum (Kim 1995) are allelopathic to weeds.

The allelopathic capacity of the rice plant has been identified recently (Dilday et al 1990, 1994, this volume, Chapter 2, Fujii 1992, Smith 1992, Lin et al 1993, Hassan et al 1994, this volume, Chapter 3, Olofsdotter and Navarez 1995, 1996) and other studies are under way to use the crop's allelopathic potential to control weeds. Information on rice allelopathy indicates its significant potential as an environmentally friendly way to control weeds in ricefields.

Despite the significance of allelopathy in the agricultural sector, there is no documented evidence on allelopathic effects of rice in Sri Lanka (L. Amarasinghe, personal communication; Gunasena 1992). Studies conducted in Egypt (Hassan et al 1996) and the United States (Dilday et al 1996), however, used several rice varieties obtained from Sri Lanka that were allelopathic to weeds (Table 3). Hassan et al (1996) reported that three Sri Lankan rice varieties suppress the growth of *Echinochloa crus-galli,* and one variety is allelopathic to *Cyperus difformis.* These two weeds are identified as troublesome in wet-seeded rice cultivation in Sri Lanka (Table 2). In the Philippines, Olofsdotter and Navarez (1996) reported that 19 rice cultivars were allelopathic to *E. crus-galli* by inhibition of root elongation. Hassan et al (1996) also reported that all allelopathic rice cultivars demonstrated allelopathic activities after the 3-leaf stage. Dilday et al (1996) found that more than 400 accessions from the germplasm collection of the USDA Rice Research Institute in Arkansas, including an accession from Sri Lanka (Table 3), have shown allelopathic activity. Fujii (1992) reported that 24 rice varieties were allelopathic. Ten rice cultivars from Japan have also been demonstrated as having allelopathic effects on *Monochoria vaginalis* and *C. difformis* (Shibayama and Matsuo 1996).

Moody (1995) reported that weed populations could be managed with phytotoxic crop residues. Straw of allelopathic rice varieties has inhibited the growth of *Heteranthera limosa* (Dilday et al 1990) and *Cyperus iria* (Lin et al 1993). In Sri Lanka, farmers use rice straw as an organic amendment to ricefields. It is also well known that rice straw releases phenolic acids that can act as allelopathic agents. Significant weed control effects using rice straw, however, have not been reported under Sri Lankan conditions, probably because of high temperatures, which result in the rapid decomposition of the material, as explained by Putnam (1985).

Several rice varieties are currently under investigation in Sri Lanka for their allelopathic potential, such as BG1165-2, BG 34-1l, LD 183-3, and LD 183-7 (Table 3), at the Faculty of Agriculture, University of Peradeniya, in collaboration with the

Table 3. Reports on allelopathic activity of Sri Lankan rice varieties.

Variety	Target weed	Control (%)
BG 1165-2	*Echinochloa crus-galli*	60-80
LD 183-3	*E. crus-galli*	70-80
LD 183-7	*E. crus-galli*	60-70
LD 180-10 B	*Cyperus difformis*	40-60
BG 34-11	*Ammannia coccinea*	70

Rice Research and Development Institute of Sri Lanka. These studies are evaluating the allelopathic potential of these cultivars in controlling *E. crus-galli,* the major weed in wet-seeded rice cultivation in Sri Lanka. The experiments use relay seeding, as described by Navarez and Olofsdotter (1996). Based on the results of the laboratory experiments, field trials will be conducted to analyze the allelopathic activity of the selected rice varieties using different weed species as target plants.

Obtaining evidence for rice allelopathy

Growing interest in rice allelopathy suggests that rice has potential as an allelochemical donor to natural ecosystems. Knowledge of rice allelochemical functions offers several possibilities for agricultural practices. Most important to the future of allelopathy as a science is an appraisal of its methods, because the greatest difficulty in identifying allelopathic effects of plant sources is to assay them precisely.

Laboratory bioassays are an important part of allelopathic research. Many bioassays, however, show little or no correspondence to field interactions (Inderjit and Dakshini 1995) because of their dissimilarity to field conditions. Stowe (1979) determined that bioassays using plant extracts, foliar washes, and decomposing litter inhibited germination and growth even when the plants were not suspected of allelopathy. Bell (1974), Reynolds (1975), and Marambe and Sangakkara (1996) reported that the pH or osmotic potential of plant extracts could result in positive effects in allelopathy bioassays. A detailed account of laboratory assays in allelopathy and their implications is given by Inderjit and Dakshini (1995).

Potential of rice allelopathy for weed management in Sri Lanka

Extensive evidence indicates that allelopathy may contribute to vegetation patterns in natural ecosystems. This phenomenon is now recognized as an important ecological factor in plant-plant interactions. A considerable amount of allelopathy research was published in the mid-1980s (Rice 1984, Putnam 1985, Putnam and Tang 1986, Waller 1987). Recently, allelopathy has been considered within a broader scope of research involving sustainable agriculture that is known to be low-input and resource-conserving. In many parts of the world, allelopathy has been studied for use in weed control (Elliot and Cheng 1987, Rizvi and Rizvi 1992), low-input agriculture (Gliessman 1987), intercropping systems, and nutrient recycling (Rizvi and Rizvi 1992).

Agricultural systems could achieve sustainability if methods adopted for this goal are ecologically sound, resource-conserving, and not too costly. Good crop husbandry accounts for more than 50% of the weed control on farmland. In this respect, allelopathic interactions are considered crucial for manipulating ecosystems. Ecologists have often demonstrated that allelopathy influences vegetation patterns. The exploitation of this technology in weed management has been attempted through screening for allelopathic types in crop germplasm collections. Because of the magnitude of weed problems in Sri Lankan rice farming, and the implications of the heavy use of herbicides, knowledge of rice allelopathy and allelochemical functions offers several possibilities for improving cropping practices in the country.

Olofsdotter and Navarez (1995) reported that research to achieve the goal of a weed-suppressing rice cultivar should be multidisciplinary, and that the process is not an easy task. The proven success of breeding programs for pest- and disease-resistant crops, however, suggests a similar potential to capitalize on rice allelopathy. Thus, developing cultivars with allelopathic potential would probably be the best and most environmentally friendly technique to overcome the problems caused by weed infestations in rice production in Sri Lanka, as well as in other countries.

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Notes

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CHAPTER 13

Allelopathic effects of gooseweed extracts on growth of weed seedlings

C. Premasthira and S. Zungsontiporn

Because gooseweed (*Sphenoclea zeylanica* Gaertn.) has been reported to have allelopathic potential, we studied the effects of gooseweed extracts on weed growth in the laboratory. The seeds of various grasses, broadleaf weed species, and rice were treated with three concentrations of gooseweed extracts, equivalent to 0.1, 1.0, and 5.0 g of fresh weight. To determine the growth of tested plants as affected by the gooseweed extract solutions, the lengths of the longest root and shoot of the weed seedlings were measured 7 d after treatment. The tested plants were differentially inhibited by a given concentration of gooseweed extract. Seedlings of the following species were more significantly different on growth inhibition than those of the untreated controls at the lowest concentration of extract solution: *Leptochloa chinensis* (L.) Nees, *Chloris barbata* Sw., *Dactyloctenium aegyptium* (L.) B.P., *Pennisetum pedicellatum* Trin., *Pennisetum setosum* L.C. Rich., *Hygrophilla erecta* Hochr., *Mimosa invisa* var. inermis Adelb., *Hyptis suaveolens* Poit., and *Scirpus articulatus* L. When the concentration of extract solution was increased, growth of the weed seedlings decreased; however, growth of the rice seedlings was less inhibited than that of the weeds.

Introduction

Gooseweed (*Sphenoclea zeylanica*) is a herbaceous annual weed, belonging to the Sphenocleaceae family. A native species in South Africa, it is now distributed worldwide in tropical and subtropical regions. Gooseweed is a serious weed of rice in the Caribbean area, India, Pakistan, and Southeast Asia, and is a weed in rice in 17 countries (Holm et al 1977). In Thailand, gooseweed is a common weed in paddy rice, especially in the Central Plain. Gooseweed is also a serious weed in transplanted rice and is fourth among the 10 most common weed species in Thailand's Central Plain. It is found in about 25% of fields (Chootummatat et al 1994). Gooseweed is always a dominant species of the weed community in paddy rice. The weed may be highly competitive for nutrients, light, water, and carbon dioxide, and it may release toxic substances that suppress growth of associated weeds.

Many reports attest that weeds and crops can produce substances that suppress growth of associated weeds and crops (Sanchez et al 1973). Phytotoxic substances of plants have a different degree of inhibition on various plant species (Drost and Doll 1980). Preliminary research reported that gooseweed contained a substance that

strongly inhibited rice seedling growth (Premasthira and Zungsontiporn 1985). To investigate the effect of gooseweed extract on the growth of some weeds, our group conducted this experiment in 1985.

Materials and methods

Gooseweeds at the flowering stage, which contain a high concentration of plant growth-inhibiting substances, were collected as plant samples. A total of 750 g of fresh plant samples were homogenized 5 times (w/v) with cold methanol by using a Nippon-Seiki blender. Extract solutions of 0.1, 1.0, and 5.0 g in equivalent fresh weight were poured into 30 x 120-mm glass vials containing 1.5 g of cellulose powder. These vials were kept in a vacuum oven for 24 h to evaporate the methanol. After drying, 4 mL of distilled water were added to each vial. Six uniformly germinated seeds of 12 grass weed species, 11 broadleaf weed species, two sedge species, and rice were placed in each vial. The vials were covered with vinyl film and placed in a growth chamber (30 °C at 3,500 lux). The longest root and second leaf sheath of grass weeds and the longest root and shoot of broadleaf weeds were measured 7 d after seeding.

Results and discussion

The length of the longest root and second leaf sheath of the grass weed seedlings was measured 7 d after seeding. The results showed that each weed species was differentially inhibited by the gooseweed extract solution. Growth of the weed seedlings decreased when the concentration of the extract solution increased.

Paddy grass weed seedlings

The effects of a gooseweed extract solution on the growth of paddy grass weed seedlings showed that the root length ofjungle rice [*Echinochloa colona* (L.) Link], wrinkle duck-beak (*Ischaemum rugosum* Salisb.), red sprangletop [*Leptochloa chinensis* (L.) Nees], and rice (*Oryza sativa* L.) seedlings was shorter than that of the untreated controls at the lowest concentration of extract solution. Only the root length of red sprangletop, however, was significantly shorter. The root length of barnyardgrass [*E. crus-galli* (L.) Beauv.] was significantly longer at the same concentration (Fig.1A). The root length of these weed seedlings was significantly shorter when the extract solution concentration was increased to 1 and 5 g equivalent fresh weight.

The leaf sheath length of these weed seedlings was not significantly shorter than that of the untreated seedlings at the lowest concentration, but it was significantly shorter at the higher concentration (Fig. 1B). At the same concentration, the root length of weed seedlings was more inhibited than the leaf sheath length. Among the paddy grass weed seedlings, red sprangletop was the most sensitive to the extract solution of gooseweed.

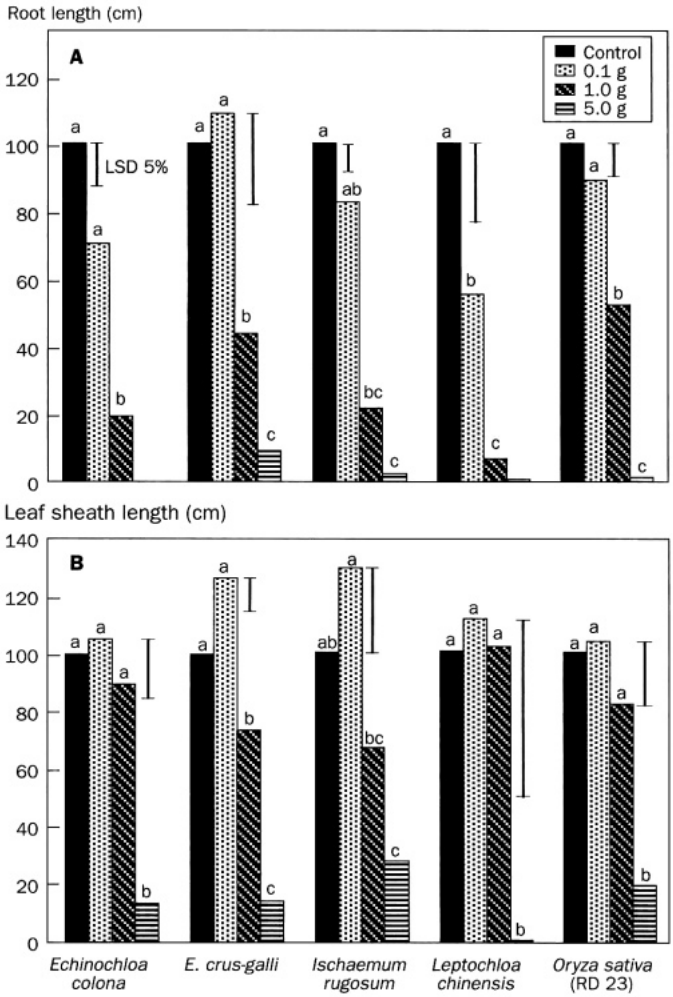

Fig. 1. Effects of methanol extracts of gooseweed *(Sphenoclea zeylanica)* on root (A) and leaf sheath (B) growth of paddy grass weed seedlings.

Upland grass weed seedlings

Gooseweed extract affected the growth of upland grass weed seedlings. The root length of *Brachiaria reptans* (L.) Gard. & Hubb., *Chloris barbata* Sw., *Dactyloctenium aegyptium* (L.) B.P., *Pennisetum pedicellatum* Trin., and *Pennisetum setosum* L.C. Rich. was significantly shorter than that of the untreated control, but the root length of *Cenchrus echinatus* L. and *Pennisetum polystachyon* (L.) Schult. was significantly longer at the lowest concentration of extract solution. When the extract concentration was increased, the root length of all tested weeds was significantly shorter (Fig. 2A).

The extract solution of gooseweed showed different effects on the leaf sheath length of these upland grass weed seedlings. The leaf sheath length of *Brachiaria reptans, Pennisetum pedicellatum,* and *P. setosum* was significantly longer than that of the untreated controls. Only the leaf sheath length of *Dactyloctenium aegyptium* and *P. polystachyon* was significantly shorter at the lowest concentration of extract solution. The leaf sheath length of these weed seedlings was significantly shorter at the higher concentration of extract solution (Fig. 2B). *Chloris barbata* and *D. aegyptium* were the most sensitive of the upland grass weed species to gooseweed extracts.

The longest root and shoot of the broadleaf weed seedlings were measured 7 d after seeding. The results showed that each weed species was differentially inhibited by the gooseweed extract solution. The growth of weed seedlings decreased when the concentration of the extract solution increased.

Paddy broadleaf weed seedlings

Gooseweed extract at the lowest concentration affected the growth of paddy broadleaf weed seedlings. The root length of *Aeschynomene indica* L. was longer than that of the untreated control, whereas *Corchorus olitorious* L., *Eclipta prostrata* L., *Hygrophilla erecta* Hochr., *Phaseolus lathyroides* L., *Cyperus procerus* Rottb., and *Scirpus articulatus* L. had a shorter root length. Only the root length of *H. erecta* and *S. articulatus* was significantly shorter. The root length of these weed seedlings was significantly shorter at a higher extract concentration (Fig. 3A).

The shoots of these paddy broadleaf weeds had differential sensitivity to gooseweed extract solution. At the lowest concentration of extract solution, the shoot length of *Phaseolus lathyroides, Cyperus procerus,* and *S. articulatus* was longer than that of the untreated controls. Although the shoot length of the other weeds was shorter, only *A. indica* and *Corchorus olitorious* showed a significantly shorter shoot length (Fig. 3B). The shoot length of these weeds was less inhibited than the root length at the same concentration of extract solution. Among the paddy broadleaf and cyperus weeds, *H. erecta* was more sensitive to gooseweed extract than the other species.

Upland broadleaf weed seedlings

The root length of *Celoasia argentea* L., *Hyptis suaveolens* Poit., *Mimosa pigra* L., *Mimosa invisa* var. inermis Adelb., and *Mimosa invisa* Mart. was inhibited by gooseweed extract. Only the root length of *H. suaveolens, Mimosa invisa* var. inermis

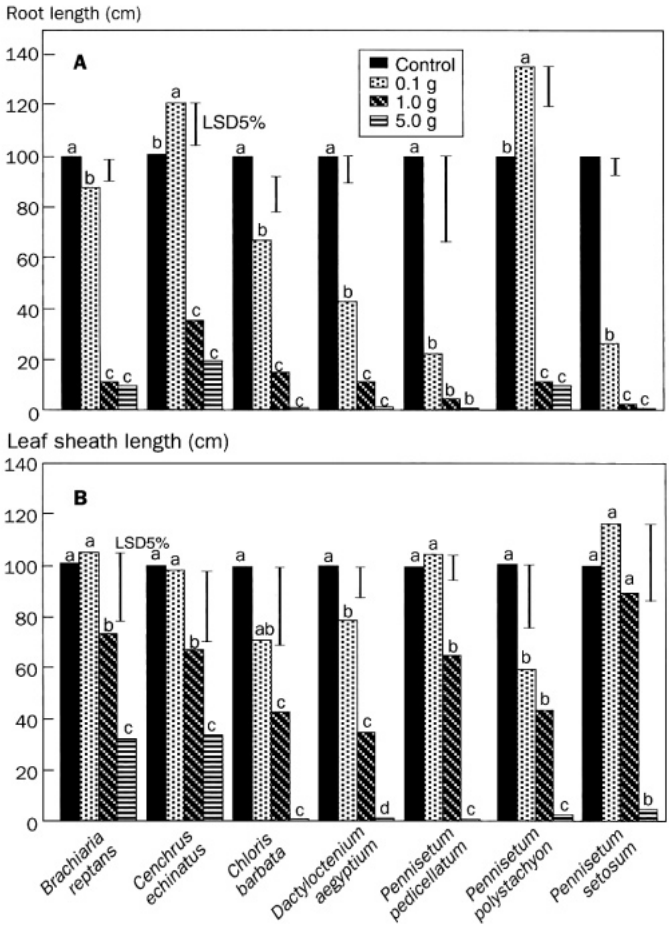

Fig. 2. Effects of methanol extracts of gooseweed *(Sphenoclea zeylanica)* on root (A) and leaf sheath (B) growth of upland grass weed seedlings.

Adelb., and *Mimosa invisa* Mart. was significantly shorter than that of the untreated controls. The root length of *Aeschynomene americana* was significantly longer (Fig. 4A).

The shoots of these weeds were more insensitive to gooseweed extract than their roots. At the lowest extract concentration, the shoot length of these weeds was not significantly different from that of the untreated controls, except for *A. americana,* whose shoot length was significantly shorter.

All tested upland broadleaf weeds were inhibited by the gooseweed extract.

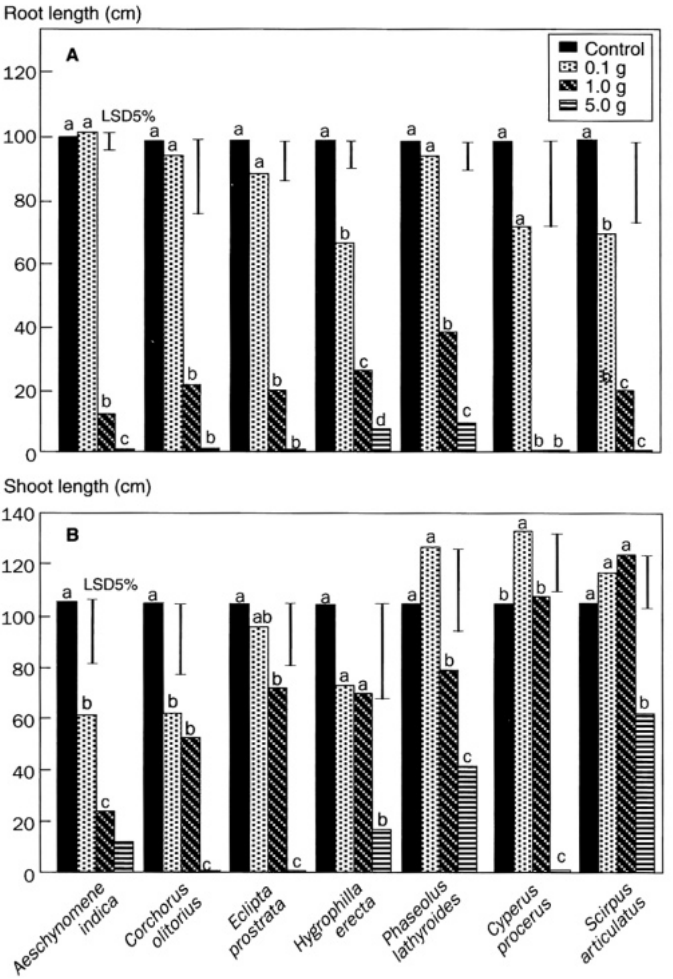

Fig. 3. Effects of methanol extracts of gooseweed (*Sphenoclea zeylanica*) on root (A) and shoot (B) growth of paddy broadleaf weed seedlings.

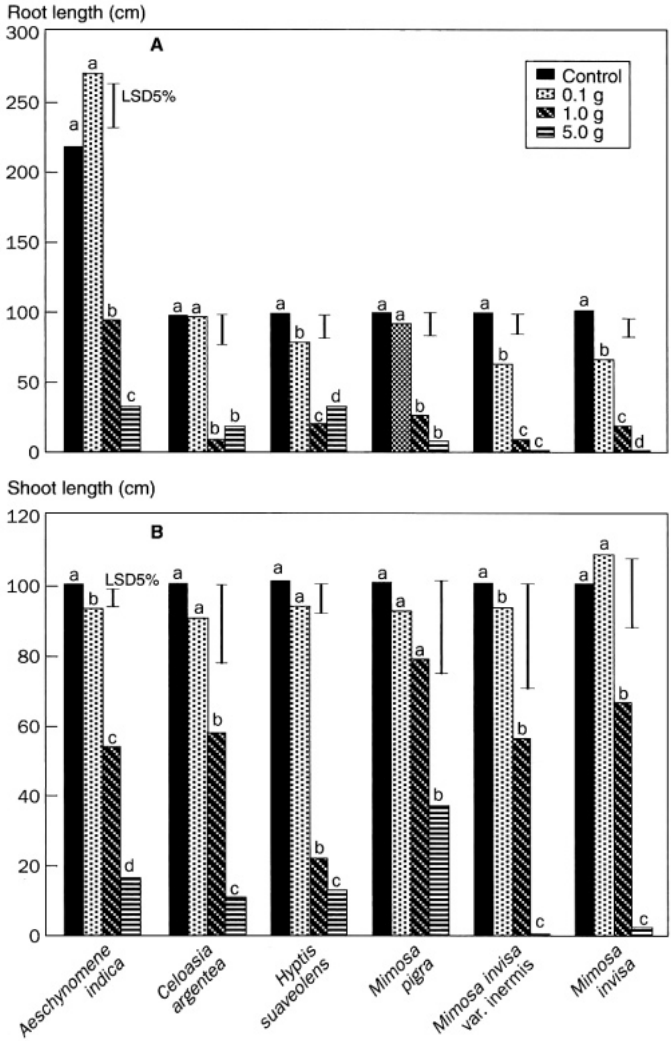

Fig. 4. Effects of methanol extracts of gooseweed (*Sphenoclea zeylanica*) on root (A) and shoot (B) growth of upland broadleaf weed seedlings.

Conclusions

Some substances in gooseweed are allelopathic and can inhibit the growth of some weed seedlings. The extracted substances showed both stimulatory and inhibitory effects on weed seedling growth, depending on species and extract concentration. Normally, gooseweed is incorporated into the soil before rice is planted, and thus substances may leach out and suppress weed growth in the paddy field. In the future, gooseweed might play an important role in weed control in paddy, particularly in transplanted rice. Good management of a cropping system is necessary for the efficient use of allelopathic weeds in weed control. The identification of these active substances and their behavior in the soil, however, should be determined.

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