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ALLELOPATHIC POTENTIAL IN RICE TO CONTROL IMPORTANT RICE WEEDS IN THE RIVERINA

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PROJECT SUMMARY:

Currently, only two herbicides, Londax® (bensulfuron) and Taipan® (benzofenap) are available for the effective control of all four major broadleaf weeds infesting NSW rice paddocks. Prolonged and widespread use of these two herbicides in the rice growing regions increases the threat of herbicide resistance. The low likelihood of new herbicides in the foreseeable future increases the impact of herbicide resistance on the Australian rice industry.

Allelopathy, chemical interactions between plants, is an alternative control option. Weeds could be controlled by using crops which have been developed to exert their own weed control by releasing chemicals into the soil. These naturally occurring compounds could play a valuable role in an integrated weed management system, potentially reducing the amount of synthetic herbicides required for weed control.

In rice, the potential use of allelopathy in weed control has been explored by several researchers worldwide. Funding for work on allelopathic potential was provided by the Rice CRC as they recognised that the Australian weed community is very different and many of the weeds infesting rice paddocks are typically Australian problems not likely to be tackled by international research groups.

Twenty-seven rice cultivars were examined in the laboratory for their allelopathic potential against several currently important and potentially important rice weeds in Australia, namely barnyard grass (Echinochloa crus-galli), dirty dora (Cyperus difformis), lance-leaved water plantain (Alisma lanceolatum), starfruit (Damasonium minus), arrowhead (Sagittaria montevidensis) and S. graminea. Weed root growth inhibition ranged from 0.3 % to 93.6 % of the control depending on the cultivar and the weed species being tested. One weed was actually stimulated by Langi. Several rice varieties significantly inhibited root growth of more than one weed.

A field trial using starfruit as the test species was conducted to see if those cultivars which inhibited starfruit in the laboratory experiment also inhibited starfruit in the field and to determine whether allelopathy was an important factor in the resulting field performance. Twenty-four cultivars were used in a field trial based at the Yanco Agricultural Institute. Starfruit dry matter was measured as an indicator of weed inhibition. It was found that there was a correlation between laboratory and field results, and that allelopathy was an important contributor to field performance of a rice variety.

KEY WORDS**:** *Alisma*, *plantago-aquatica*, *alisma lanceolatum*, allelopathy, arrowhead, barnyard grass, bioassay, competition, C*yperus difformis*, *Damasonium minus*, dirty dora, *Echinochloa crus-galli*, equal compartment agar method (ECAM), field, *Oryza sativa*, rice, *Sagittaria graminea*, *Sagittaria montevidensis*, starfruit, water plantain

1. BACKGROUND TO THE PROJECT

Australia's high rice yields are jeopardised by the presence of weeds. Hassan *et al.* (1994) suggest that in upland rice, weeds can cause a 30-100% loss in yield. The species composition of weed communities in rice crops varies according to climate and the growing environment (Baltazar and De Datta 1992). Regardless of the weed composition, weeds compete with crops such as rice for resources, especially during establishment and early growth stages (Zimdahl 1980). Both *Echinochloa crus-galli* (barnyard grass) and *Cyperus difformis* (dirty dora) are among the top ten most important rice weeds (Smith 1983). According to Smith (1968), barnyard grass can affect rice yield at densities as low as 1-3 plants/m^2.

Though herbicides are the most cost effective and most widely used weed control method (Kim 1994), their use is becoming increasingly unpopular with the public. Community concern for the environment is rising and increased use of synthetic herbicides will not be welcomed. As the environmental consciousness of the public is heightened, environmental policies will provide for stricter limits on herbicide use. The limited number of chemicals available for weed control and the associated threat of herbicide resistance have also resulted in the notion of developing "natural" herbicides. Natural herbicide chemistries could offer new alternatives to those of the currently applied synthetic herbicides.

The development of natural herbicides could have a number of benefits. Pesticides from plants are more systemic and more easily biodegradable than synthetic ones (Rizvi and Rizvi 1992). Present-day synthetic herbicides have only 17 known molecular sites of action whereas plants produce a complex mix of chemicals whose active ingredients could have multiple sites of action (Duke 2002). These natural herbicide chemistries may exploit different sites of action which could minimise the threat of herbicide resistance, especially if multiple target plant sites were involved. Not only would they offer novel sites of action, but these natural compounds would show strong selection for target species (Duke and Lydon 1987). In this era of increased environmental consciousness, use of natural mechanisms such as allelopathy would be favoured over the increased application of synthetic herbicides. Narwal (1994) and Duke (1986) both note that 60% of the registered herbicides contain halogenated hydrocarbons which are a health risk. Duke (1998) suggests that micro-organisms that have coexisted with these natural compounds may have co-evolved and as such developed specific relationships with certain plants.

Most importantly, a natural herbicide may more readily circumvent the issue of herbicide resistance, which poses a threat to the multi-million dollar crop industry. Although Narwal (1999) states that allelochemicals have potential in weed control as they "are free from the problems associated with present herbicides" (p. 227), caution must still be practised when developing a natural herbicide. Putnam and Duke (1978) suggest that weeds would develop resistance to the natural allelochemicals just as they would to synthetic herbicides. Despite the risk, allelopathy could be a feasible supplement or alternative to synthetic herbicides for rice weed control. Allelopathy or other natural suppression mechanisms could play a valuable role in an integrated weed management system, potentially reducing the amount of synthetic herbicide required for weed control.

It has been suggested that the focus of weed management should be the maintenance of weeds below the economic threshold rather than the total elimination of weeds (Kim 1994).

Allelopathic capability could give a crop the competitive edge it needs to survive the critical period during germination and establishment when competition is at a maximum (Kim 1994). Allelopathy can be exploited for weed control in many ways. Besides the use of allelopathic mulches or maintaining a cropping sequence that allows allelopathic residues to remain in the paddock, there is the option of obtaining crop varieties that have been bred to possess both allelopathic capability and good agronomic features (Putnam and Duke 1974, Rice 1995). The latter option has yet to be realised, but much research has focussed on this potential outcome. Although it will not solve all the problems associated with weed control, allelopathy could play a valuable role as one component of an integrated weed management system and prove to be a useful tool in crop production.

The discovery of allelopathic potential in rice is attributed to Dr. Robert Dilday in Stuttgart, Arkansas who screened over 10 000 rice accessions during 1988 and 1989. Around 3.5% of the lines screened demonstrated a degree of weed control suggesting that allelopathic potential does exist within the species (Dilday *et al.* 1994). Accessions were considered allelopathic if they resulted in a 10 cm or greater weed-free radius around the rice plant. Proportional weed control, compared to a non-allelopathic line, was also considered.

Since Dilday's initial work on rice allelopathy (Dilday *et al.* 1989, 1991), many papers have been written which support the finding that there are distinct differences between rice varieties in their allelopathic ability. Researchers such as Olofsdotter *et al.* (1995, Olofsdotter and Navarez 1996) in the Philippines, Hassan *et al.* (1994) from Egypt, Marambe (1998) in Sri Lanka and Fujii (1992) of Japan have also looked at allelopathic potential in rice. Chemicals released from rice plants have been implicated in inhibition of germination and growth of weed species such as barnyard grass, dirty dora, duck salad, red stem and *Trianthema portulacastrum*. Although Dilday's results are promising for weed control in US rice paddocks, the Australian weed community is very different. It is not known whether crops allelopathic towards US rice weeds would be effective against Australian weeds. Therefore, research on the main broadleaf weeds infesting rice paddocks in NSW was required to determine the potential contribution of rice allelopathy towards control of Australian rice weeds.

Previously, a PhD project was undertaken which focussed on allelopathic potential in rice to control arrowhead in NSW rice crops. The following report is based on results from one-year postdoctoral funding which was granted to examine additional important weeds in rice-based farming systems. The full detailed results from project 3205 have already been published (Seal *et al.* 2004 a, 2004 b, 2004 c), are under review (Seal *et al.* [pending]) or are due to be submitted (Seal et al [in prep]).

2. OBJECTIVES

Previously, 28 rice cultivars were screened for allelopathic potential against arrowhead. A one-year continuation of the project aims to:

- screen a number of rice accessions from different countries for allelopathic capability against additional weeds infesting Australian rice crops
- determine if any rice varieties have high allelopathic potential against more than one weed

- determine if those cultivars that performed well in the bioassay result in actual weed control in a field situation

- compare those cultivars found to have effects on more than one weed

3. INTRODUCTION

Due to Australia's dominant practice of aerially sowing into flooded bays, germination and establishment of barnyard grass is largely suppressed. However, such aquatic conditions are ideal for other major weeds of rice which can decrease the crop yield and quality. Currently, only two herbicides, Londax® (bensulfuron) and Taipan® (benzofenap) are available for the effective control of all four major broadleaf weeds infesting NSW rice paddocks. Prolonged and widespread use of these two herbicides in the rice growing regions increases the threat of herbicide resistance. The extent of herbicide resistance in NSW rice crops has been evaluated by Broster *et al.* (2001). Dirty dora, starfruit and arrowhead all had high levels of Londax® resistance, with, 50%, 40% and 35% resistance in weed accessions, respectively. Because Australia represents a small economic market, little incentive exists for the large international chemical companies to develop new herbicides targeted to Australian rice weeds. The low likelihood for new herbicides in the foreseeable future increases the impact of herbicide resistance on the Australian rice industry. An alternative control option is allelopathy, a natural mechanism which occurs widely in natural plant communities (Bell and Koeppe 1972).

Allelopathy is the positive or negative impact of any biochemical interactions between plants including algae and micro-organisms (Rice 1984). Studies have been done on the exploitation of allelopathy via the use of allelopathic mulches and the application of cover crops (Olofsdotter *et al.* 1995). The development of crops with the capability to exert their own weed control via allelopathy is another option which has been gaining attention. These naturally occurring allelochemicals could play a valuable role in an integrated weed management system, potentially reducing the amount of synthetic herbicides required for weed control.

In rice, the potential use of allelopathy in weed control has been explored by several researchers worldwide on the germination and growth of weeds such as red stem, duck salad, barnyard grass, dirty dora and *Trianthema portulacastrum* (Dilday *et al.* 1994, Olofsdotter *et al.* 1995, Olofsdotter and Navarez 1996, Hassan *et al.* 1994, Marambe 1998 and Fujii 1992). However, the Australian weed community is very different and many of the weeds infesting rice paddocks are typically Australian problems. It is not known whether crops allelopathic towards US rice weeds would be effective against Australian weeds.

As such, this study focussed on screening rice germplasm for allelopathic potential against several currently important and potentially important rice weeds in Australia, namely barnyard grass (*Echinochloa crus-galli*), dirty dora (*Cyperus difformis*), lance-leaved water plantain (*Alisma lanceolatum*), starfruit (*Damasonium minus*), arrowhead (*Sagittaria montevidensis*) and *S. graminea*.

4. METHODOLOGY

The fine details of the following screening (laboratory) work can be found in Seal *et al.* (2004 a, in prep).

4.1 ECAM Bioassay

The Equal Compartment Agar Method (ECAM) developed by Wu *et al.* (2000) and modified by Seal *et al.* (2004 a) was used for the screening of rice accessions. Ideal bioassay parameters required to observe maximum differences between varieties were established for arrowhead, barnyard grass, lance-leaved water plantain, *Sagittaria graminea* and starfruit. The initial rice density experiments showed that significant differences between the rice cultivars existed at the following rice densities per beaker: 3 rice for *S. graminea*, 5 rice for *A. lanceolatum* and 6 rice for arrowhead, starfruit and barnyard grass. As such, these densities were used for the full screening of 27 rice cultivars, with different countries of origin, maturity and stage of improvement, for allelopathic potential. However, this experiment was not undertaken for dirty dora, as rice density had no effect on dirty dora root growth. Weeds without rice influence were used as the control treatments.

4.2 Field Trial

The method used to conduct this trial has already been outlined in Seal *et al.* (pending). This experiment was carried out during the 2000/2001 rice growing season at the Yanco Agricultural Institute in NSW. Briefly, two flooded rice bays were sectioned and sown with the seeds of 24 cultivars or no rice in the case of the no-rice control. Bays were sprayed with alpha-cypermethrin (Dominex®) to control bloodworm. No other pesticides were used during the experiment, so any observed weed control was due to plant interference (both allelopathic and competitive effects) between the rice and the starfruit. The study site located at Yanco Agricultural Institute was dominated by a natural infestation of starfruit. Starfruit dry weights were used as an indicator of the impact of rice on starfruit growth.

5. RESULTS

The results of the screening work, and greenhouse and field trials have already been published in Seal *et al.* (2004 a), are under review (Seal *et al.* [pending]) or are due to be submitted (Seal *et al.* [in prep]).

5.1 ECAM Bioassay

As mentioned, rice seedling densities used in all bioassay ranged from 3 to 6 seedlings per beaker. In all cases, there were no significant differences between cultivars at rice densities of 9 seedlings per beaker and above. Dirty dora, however, did not respond to increasing rice densities. Even using 25 rice seedlings per beaker of a reported allelopathic cultivar (Dular) towards dirty dora (Hassan *et al.* 1994) did not impact root growth.

In the case of starfruit and arrowhead, the per cent control data were skewed so the data were natural log transformed and then statistically analysed. For *A. lanceolatum*, *S. graminea* and barnyard grass, statistical analysis was performed using the per cent control data. Comparisons between the cultivars were made on the basis of per cent control data. When the full screening experiments were repeated using a sub sample consisting of approximately half of the original cultivars, the correlation co-efficient between the two data sets for each weed was over 0.95 in all cases.

Significant differences existed between the rice cultivars in their ability to suppress root growth of each of the weeds studied. Arrowhead root lengths ranged from 0.3 % (Takanenishiki) to 71.3 % (Basmati) that of the control and A. *lanceolatum* root lengths ranged from 2.6 % (Ratna) to 93.6 % (Rexmont). Barnyard grass root lengths ranged from 20.8 % (Italpatna) to 107.9 % (Langi) and *S. graminea* root lengths ranged from 1.5 % (Amaroo) to 82.1 % (Langi) that of the control. Starfruit root lengths ranged from 2.0 % (Hungarian #1) to 32.6 % (Rexmont) that of the control.

Correlation analysis between weed root inhibition due to various cultivars was examined among various combinations of all five weeds for which the full screening experiment was undertaken. All correlations were significant, although they varied depending on the weeds being compared. The highest correlation occurred between lance-leaved water plantain and *S. graminea* (r = 0.93) whereas the lowest correlation occurred when comparing barnyard grass with arrowhead (r = 0.58). A list of the most allelopathic and least allelopathic cultivars can be found in Table 1.

Table 1. Inhibition of weed root growth by different rice cultivars as determined by the ECAM bioassay of 27 varieties

Mean Weed Root Inhibition (% of Control) *

Most Allelopathic Cultivars		Least Allelopathic Cultivars	
Giza 176	92.5	Toro	57.7
Amaroo	92.4	TN-1	54.7
Takanenishiki	92.3	UPR 82-1-	54.7
Ratna	91.5	BG 34/8	52.1
Italpatna	91.3	Woo Co Chin Yu	45.8
Hungarian #1	90.5	Rexmont	31.4
Jarrah	90.5	Langi	27.4

*mean root inhibition was calculated using data from all five tested weed species

All allelopathic cultivars inhibited weed root growth by more than 90%. The data presented in Table 1 includes results from barnyard grass which is not a member of the Alismataceae. If only Alismataceae weeds are considered, all allelopathic cultivars inhibited weed root growth by more than 94 % (values not shown). Amaroo and Jarrah, two varieties which are widely cultivated in the Riverina, appear to have high allelopathic potential. Langi, which is also a commonly used variety, does not show much promise for future weed control.

5.2 Field Trial

Significant differences existed in the starfruit dry matter grown in association with different cultivars. Dry weight ranged from 4.6 % (Tono Brea) to 72.2 % (Rexmont) of control.

Twenty-three cultivars which were used both in the laboratory and in the field trial were used for the comparison. A correlation between the raw untransformed starfruit root growth laboratory data (mm) and the starfruit dry weight field data (g) resulted in an r^2 value of 0.700 (correlation coefficient is 0.837). Eight of the most allelopathic cultivars in the bioassay were in the top ten highest ranked allelopathic cultivars in the field. Seven of the ten least allelopathic cultivars in the bioassay were among the top ten least-allelopathic cultivars in the field.

Table 2. Reduction in Starfuit Dry Matter Grown in Association with Different Rice Cultivars

Starfruit Dry Weight (% of Control)			
Most Interfering Cultivars*		Least Interfering Cultivars	
Tono Brea	95.4	Palmyra	60.6
Hungarian #1	93.7	Pelde	60.2
Kingmen T. C. M.	93.1	CI Selection 63	60.0
Giza 176	86.4	Kyeema	59.9
Amaroo	85.6	Woo Co Chin Yu	58.7
Takanenishiki	81.6	BG 34/8	44.8
IET 1444	80.7	Rexmont	27.8

* the word ‘interfering’ has been used to reflect that field observations are the result of both competitive and allelopathic interactions

6. DISCUSSION

The role of allelopathy in plant-plant interference is often discounted, with attributions of influence of one plant on another being competition. It is necessary therefore to undertake both laboratory and field experiments to corroborate the contribution of allelopathy to plant interference. However, few papers have attempted to validate laboratory results via field experimentation. Therefore, this project, has attempted to draw correlations between results from both laboratory and field trials.

It was determined via the initial laboratory bioassay on arrowhead (Seal *et al.* 2004a) that those rice cultivars selected from the literature for their reported allelopathic effects do not necessarily behave the same way against other weeds. Several research groups have reported this apparent species specificity of allelopathy (Dilday *et al.,* 1991; Hassan *et al.,* 1994; Olofsdotter and Navarez, 1996; Chung *et al.,* 2001).

Although it seems unlikely that a specific variety will be able to inhibit the growth of all weed species, it is possible that there are cultivars which can be allelopathic towards more than one weed in a family. In this study, 6 of the 7 most allelopathic cultivars, as determined via calculation of the mean root inhibition using data from all five tested weed species, were consistently in the top ten allelopathic cultivars when each weed was screening separately. This is also true for barnyard grass, which is not a member of the Alismataceae.

Root length inhibition due to all 27 rice cultivars were compared between weeds. There were significant correlations between the weeds. *A. lanceolatum* and *S. graminea* had the highest correlation co-efficient (0.93) while barnyard grass and arrowhead was the least co-related overall (0.58). The lowest correlation between members of the Alismataceae occurred between starfruit and lance-leaved water plantain (0.74). A few of the lowest correlations between weeds occurred when comparing barnyard grass against members of the Alismataceae. From this observation, it becomes apparent that there could be cultivars which possess multi-weed effects within a family.

To determine if such allelopathic effects on weed root inhibition in the laboratory play an important role in plant interference in the field, one weed was selected for a field trial. Comparisons could then be made between cultivar performance against this weed, starfruit, from the laboratory bioassay and the field trial. There was a significant correlation between performance in the field trial and in the bioassay (correlation co-efficient 0.837). Those cultivars which ranked as highly allelopathic in the bioassay tended to perform well in the field. This finding suggests that allelopathy is an important contributing factor to observed plant interference in the field. It also suggests that laboratory results could be suitable for establishing likely field performance, which few researchers have examined. However, Olofsdotter *et al.* (1999) did attempt to draw comparisons between laboratory and field results. Their results also suggest that bioassay results are somewhat indicative of their field performance (correlation co-efficient = 0.41-0.65, depending on the season). These are exciting results which indicate that the allelopathic potential observed in laboratory bioassays can be realised in a field situation, and that allelopathy could play a substantial role in weed management.

7. IMPLICATIONS AND RECOMMENDATIONS

Although such research does not imply that the use of allelopathic mulches or natural compounds released in the root exudates can be substituted for synthetic herbicides, it does establish the potential contribution of allelopathy to weed management systems. Research based in the laboratory and in the greenhouse is essential in the progressive demonstration of the presence, effect and feasibility of using allelopathy as a tool for weed management in the field. Prior to the development of rice cultivars capable of exerting some degree of weed control via allelopathic root exudates, research needs to be done on both the chemical and genetic basis to rice allelopathy. These are necessary stages in reaching the long-term goal of breeding cultivars with high allelopathic potential and good agronomic characteristics.

8. A DESCRIPTION OF THE PROJECT INTELLECTUAL PROPERTY AND OF ANY COMMERCIALLY SIGNIFICANT DEVELOPMENTS ARISING FROM THE PROJECT.

None at this stage.

9. RECOMMENDATIONS

As several cultivars have been identified which significantly inhibit the growth of more than one weed, pedigrees could be examined to establish any direct genetic similarities. Future work could focus on chemical analysis of rice root exudates from these cultivars using both GC/MS/MS methods established during previous work and searching for additional chemicals. If the chemical(s) responsible for the observed allelopathic effect can be identified, it will be possible to determine whether there is a genetic basis to rice allelopathy.

The chemistry of allelopathy remains as the Achilles' heel of allelopathy research. Characterisation of the chemicals responsible is an essential step in the development of a natural herbicide. Recently, more than 22 compounds have been identified in the root exudates of allelopathic and non-allelopathic rice seedlings and 15 have been quantified (Seal *et al.* 2004 b). The real challenge is to determine which compounds play a key role in the observed allelopathic effect (Seal *et al.* 2004 c). None of the 22 previously identified compounds in the rice root exudates appears to be directly responsible for the observed allelopathic effect.

Allelochemicals can be utilised in a number of ways. New herbicide chemistries can be identified and synthetically produced or commercially useful rice varieties with good agronomic characteristics can be selected as part of an integrated weed management system. As allelopathic effect is expressed largely during germination and establishment of crop plants when competition with weeds is at a maximum, adoption of allelopathic cultivars would reduce the need for pre-emergence herbicides.

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