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Can results from a laboratory bioassay be used as an indicator of field performance of rice cultivars with allelopathic potential against *Damasonium minus* (starfruit)?

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Abstract

Several weeds of rice in Australia have developed resistance to the main herbicide available for their control. Allelopathy is one phenomenon which could be incorporated into an integrated weed management system as a supplement or alternative to synthetic herbicides. Several rice cultivars were screened both in the laboratory and the field for allelopathic potential against a major rice weed, *Damasonium minus*. Results from the laboratory bioassay showed that there were significant differences between cultivars in their ability to inhibit *D. minus* root growth. *D. minus* root lengths ranged from 2.0 % (cv. Hungarian #1) to 32.6 % (cv. Rexmont) that of the control. In the field study, significant differences existed in the *D. minus* dry matter grown in association with different cultivars, ranging from 4.6 % (cv. Tono Brea) to 72.2 % (cv. Rexmont) of control. Comparison between laboratory and field results indicated a strong relationship between performance in the field and in the laboratory ($r^2 = 0.713$). Those cultivars ranked as allelopathic in the bioassay tended to have associated lower *D. minus* dry weight in the field. Eight of the top ten allelopathic cultivars in the bioassay were among the top ten suppressive cultivars in the field trial. This important finding indicates that at least some of the variation in field performance of cultivars may be predicted by their performance in bioassays.

Keywords: allelopathy, bioassay, *Damasonium minus*, equal compartment agar method, *Oryza sativa*

Introduction

Damasonium minus (R. Br. Buchenau) is, economically, one of the most important rice weeds in Australia. It belongs to the family Alismataceae which also encompasses several other important rice weeds, including dirty dora (*Cyperus difformis* L.), arrowhead (*Sagittaria montevidensis* Cham. & Schltdl.), *S. graminea*, water plantain (*Alisma plantago-aquatica*) and lance-leaved water plantain (*A. lanceolatum*). Only 2 herbicides are effective against this entire spectrum of rice weeds; bensulfuron (Londax®) and benzofenap (Taipan®). There is a high risk that their overuse will result in weed populations developing resistance to these herbicides. High levels of resistance to Londax® have already been reported in *C. difformis* (50 %), *D. minus* (40 %) and *S. montevidensis* (35 %) (Broster *et al.* 2001) and it is unlikely that new modes of action for rice weed control in Australia will become available (Pratley *et al.* 1998). The threat of increased herbicide resistance remains. Allelopathy is one phenomenon which could be incorporated into an integrated weed management system as a supplement or alternative to synthetic herbicides.

Although allelopathic potential in rice has been examined against several weeds and by several research groups (Fujii 1992; Dilday *et al*, 1994; Hassan *et al*, 1994; Olofsdotter *et al.* 1995; Marambe 1998), to date no research group has examined rice accessions for allelopathic effects against *D. minus*.

Both field and laboratory experiments are necessary to establish contributions from allelopathy and competition to the resulting combined interference in the field (Olofsdotter and Navarez 1995, 1996; Blum 1999; Inderjit and Weston 2000; Inderjit and Callaway 2003; Inderjit and Nilsen 2003). However, few papers have attempted to corroborate laboratory results via field experimentation. Already, there is an effective method available which eliminates competition in bioassay, the Equal Compartment Agar Method (ECAM), developed by Wu et al. (2000). The ECAM has been used successfully with several donor crops including wheat (Wu et al. 2000), rice (Seal et al. 2004) and barley (Bertholdsson 2005). There is ample light and water, and the agar medium contains no nutrients. Therefore, any observed effect on the seedling growth of the test species is due to chemicals being released by the donor seedlings into the water agar medium. Thus, potentially allelopathic cultivars can be identified for further experimentation to determine the contribution of allelopathy to plant interference in the field. Interactions with the environment are important dictators of allelopathic effects, and the expression of these allelopathic effects in the field is mediated by stress factors, soil characteristics and environmental conditions not tested in the laboratory bioassay.

Although root growth is generally a more sensitive parameter than shoot growth (Inderjit 1996) and is widely measured in bioassays, it is harder to assess root growth of established plants in the field and dry shoot mass was selected for measurement in the field trial.

As few papers have attempted to corroborate laboratory results via field experimentation, the objectives of this research were to identify the allelopathic potential in rice germplasm against the growth of *D. minus* in bioassay, observe the influence of these rice cultivars on weed growth in the field, and establish if a correlation existed between the performance of cultivars in bioassay and in the field.

Materials and Methods

Sterilisation and pre-germination of seeds

The seeds of rice and *D. minus* were surface sterilised in 2 % sodium hypochlorite (NaClO) for 15, and 5 minutes respectively. Both the rice and *D. minus* were rinsed 7 times with sterilised distilled water and transferred to petri dishes lined with Whatman #1 filter paper moistened with 7 mL of sterilised distilled water. The plates were then placed in a Precision Model 818 Low Temperature Incubator set at 27°C/22°C with a 14 h day/10 h night cycle.

Rice density experiment

Three rice cultivars, demonstrating varying allelopathic potential in previous research against *Sagittaria montevidensis* (Seal *et al.* 2004), were used as a guide to determine the ideal bioassay parameters to observe maximum differences between cultivars. Fortunately, these cultivars also demonstrated a range of allelopathic potential towards *D. minus* and could be used to establish an appropriate rice density for the bioassay. The Equal Compartment Agar Method (ECAM) bioassay developed by Wu *et al.* (2000), modified and utilised by Seal *et al.* (2004) in the *S. montevidensis* study, was used for the screening of rice accessions against *D. minus*. Beakers (250 mL) filled with 30 mL of 0.3 % nutrient-free water-agar were sown with 0, 3, 6, 9 or 12 pre-germinated cv. IET 1444, cv. Rexmont or cv. Woo Co Chin Yu (WCCY) seedlings. After 1 week of growth, 7 pre-germinated *D. minus* seedlings were added to the other half of the beaker. *D. minus* seedlings were removed from the system after 1 week of co-existence with the rice seedlings and their root length measurements were taken to the nearest 0.5 mm. *D. minus* without rice influence was used as the control treatment. All procedures prior to measurement were undertaken in a cross flow laminar flow cabinet to minimise bacterial and fungal contamination. Four replicates were arranged in a randomised complete block design in the aforementioned incubator.

Full screening of rice cultivars against D. minus

The experimental and incubation conditions used were identical to those described above. The initial density experiment with *D. minus* as the receiver plant showed that significant differences between the rice cultivars existed at 6 pre-germinated rice seedlings per beaker, so this density was used for the *D. minus* full screening experiment. *D. minus* without rice influence was used as the control treatment. Twenty-seven rice cultivars were screened in this bioassay (Table 1). All procedures prior to measurement were undertaken in a cross flow laminar flow cabinet to minimise bacterial and fungal contamination. Four replicates were arranged in a randomised complete block design in the aforementioned incubator. The experiment was repeated to verify the results, using a sub-sample consisting of half the original cultivars.

Field trial

This experiment was carried out during the 2000/2001 rice growing season at the Yanco Agricultural Institute in NSW. Each of 2 bays (4.5 m x 9.5 m) were sectioned in half to allow for 4 replicates of 25 squares (75 cm x 75 cm), arranged in a randomised complete block design. Each square was sown with 40-45 seeds of one of 23 rice cultivars or no rice in the case of the no-rice control. Pre-germinated rice was broadcast within a 10 cm radius of a fibreglass marker in order to identify squares during flooding. Bays were sprayed with alpha-cypermethrin (Dominex®) to control bloodworm. No other pesticides or fertilisers were used during the experiment. The study site located at Yanco Agricultural Institute has a history of establishing an almost monoculture-like infestation of either *D. minus* or *S. montevidensis*, depending on the season. The 2000/2001 growing season was dominated by a natural infestation of *D. minus*. The above-ground *D. minus* plant parts within a 50 cm x 50 cm quadrat were harvested 12 weeks after sowing. The *D. minus* dry weights were measured after a 72-hour drying period in an 80°C plant dehydrator.

Statistics

Data were subjected to an analysis of variance (ANOVA) using Genstat 7. Replicate means were converted to percent of control. Least significant differences (l.s.d.) for mean root length of *D. minus* were calculated to enable varietal comparisons. The data obtained from the full screening were skewed, presumably due to the zero *D. minus* root growth in some cases. Therefore, the percent control data were natural log transformed prior to analysis of variance. Field results were also skewed and so were natural log transformed. These transformed data satisfied the requirements for ANOVA. The correlation between the untransformed values for the field data and the laboratory bioassay data was examined. Coefficients of variation (CV) for replicates were also calculated using Genstat 7.

Results

Rice density experiments

The effects of rice density on *D. minus* root length are shown in Fig. 1. There were significant effects in both density (l.s.d. = 7.4, $p<0.001$) and in *D. minus* root growth between cultivars (l.s.d. = 5.7, $p<0.001$). Although there were significant differences between cultivars at 3 and 6 rice seedlings per beaker, the latter density was selected for the full screening. There is a possibility of hormesis, a slight stimulatory effect at very low concentrations (i.e. low densities) of allelochemicals (Rice 1984; Streibig 1988), which could complicate any interpretation at low levels such as 3 rice seedlings per beaker. Root length at the higher density (6 rice seedlings per beaker) ranged between 2 % (IET 1444) and 24 % (WCCY) of the control. At densities of 9 and 12 rice seedlings per beaker, there were no differences between cultivars, with all 3 resulting in less than 10 % root growth.

Full screening of rice cultivars against D. minus

The results from the full screening of rice cultivars against *D. minus* are shown in Figs. 2a and b. The per cent control data were skewed (Fig. 2a) so the data were natural log transformed and then statistically analysed (Fig. 2b). Significant differences existed between cultivars in their abilities to inhibit star fruit root growth (l.s.d. = 0.5, $p<0.001$). *D. minus* root lengths ranged from 2.0 % (Hungarian #1) to 32.6 % (Rexmont) that of the control. When the experiment was repeated using a sub sample consisting of half of the original cultivars, the co-efficient of determination between the two data sets was 0.92 ($r = 0.96$, $p < 0.001$). The results of the correlation analysis are shown in Fig. 3. The coefficient of variation for the replicates is 2.5 %.

Field trial

The results from the field trial are shown in Fig. 4. The per cent control data were skewed (Fig. 4a) so the data were natural log transformed and then statistically analysed (Fig. 4b). Significant differences existed in the *D. minus* dry matter grown in association with different cultivars (l.s.d. = 1. 8, $p< 0.05$). Dry weight ranged from 4.6 % (cv. Tono Brea) to 72.2 % (cv. Rexmont) of control. In Fig. 4b, all cultivars resulting in *D. minus* dry weights lower than cv. Taichung Native 1 are significantly different from the no-rice control plot. The coefficient of variation for the replicates is 22.9 %.

Comparison between laboratory and field results

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 *D. minus* root growth laboratory data (mm) and the *D. minus* dry weight field data (g) resulted in an r^2 value of 0.70 (correlation coefficient is 0.84). Fig. 5 shows the correlation. Eight of the most allelopathic cultivars in the bioassay were in the top ten highest ranked suppressive cultivars in the field. Seven of the ten least allelopathic cultivars in the bioassay were among the top ten least-suppressive cultivars in the field.

Discussion

Artificial laboratory conditions such as those in the ECAM bioassay do not attempt to mimic field conditions. These controlled, replicated tests serve to separate allelopathy from competition and test the current allelopathic theories about synergy and chemical interactions. In the field, it is impossible to separate the effects of allelopathy and competition. The role of allelopathy in plant-plant interference is often discounted, with sceptics attributing any influence of one plant on another to competition. It is necessary therefore to undertake both laboratory and field experiments to demonstrate the contribution of allelopathy to plant interference.

Few studies have considered the relative importance of allelopathy in plant interference. However, greenhouse and field trials by Nilsson (1994) show that both allelopathy (via leaf leachates) and competitive ability of *Empetrum hermaphroditum* influenced the overall interference in Scots pine (*Pinus slyvestris*) growth. The author suggests that only when there is a reduced allelopathic effect can the Scots pine negate the effects of competition. It follows that the effect of competition is conditional and may depend on the presence of an allelopathic effect. Therefore, allelopathy via a donor plant could hinder the defences of a target plant and render it more susceptible to competition.

In the bioassay studies, it was found that a range of allelopathic potential exists in the rice germplasm tested. *D. minus* root growth inhibition by rice seedlings ranged from 67 % (cv. Rexmont) to 97 % (cv. Tono Brea 439). Of the 28 screened cultivars, four had been selected from the literature as non-allelopathic controls against other weeds. In the bioassay, cv. Rexmont was found to be the least allelopathic cultivar, although it still suppressed *D. minus* root growth by more than 60 %. In the field, cv. Rexmont was also the least suppressive cultivar, suppressing *D. minus* growth by only 27 %, relative to the control as measured using dry matter production. These results are similar to those of Dilday *et al.* (1994) who found that this cultivar was non-allelopathic towards ducksalad (*Heteranthera limosa*) in the field and as such, was considered as the non-allelopathic control in their trials. However, two cultivars which had been chosen for their apparent 'non-allelopathic' effects were strongly suppressive in this study. The cultivar Giza 176, which had no allelopathic effect on dirty dora or barnyard grass (Hassan *et al.* 1994), was among the top five most suppressive cultivars in both laboratory and field studies. Cultivar Palmyra, which is non-allelopathic towards ducksalad (Dilday *et al.* 1994) ranked among the ten most allelopathic cultivars against *D. minus* in this study. These reported non-allelopathic cultivars suppressed *D. minus* root growth by 96.6 % and 95.6 % respectively in the bioassay. In the field cv. Giza 176 ranked as fourth most suppressive,, inhibiting *D.*

minus dry matter by 86 %. Cv. Palmyra, however, was not among the top 10 suppressive cultivars in the field trial where it was intermediate to low in such activity. The cultivar's competitive attributes may have confounded the allelopathy effects. The fourth cultivar, Aus 196, which was found to be non-allelopathic against barnyard grass and dirty dora (Hassan *et al.* 1994), had intermediate suppressive activity both in the bioassay and the field.

Cultivars selected for their apparent allelopathic properties, or lack thereof, in respect of a particular weed did not perform as expected when grown in conjunction with other weed species. Results from this study concur with the findings by others who suggest that allelopathic potential is species specific (Dilday *et al.* 1991; Hassan *et al.* 1994; Olofsdotter and Navarez 1996; Dilday *et al.* 1998; Hassan *et al.* 1998; Olofsdotter *et al.* 1999; Chung *et al.* 2001, Seal *et al.* 2004). It is therefore unlikely that any one cultivar will be allelopathic to all weed species although there may be scope where weeds belong to the same family.

In the current bioassay, the effects on the receiver plant are due to allelopathy via chemicals exuded into the agar by rice roots. Because allelopathy and competition cannot be separated in the field, those cultivars which performed well in the field can only be described as having "high weed suppressing ability" rather than being highly allelopathic.

When the bioassay results are compared with results from the field trial, there is a significant relationship ($r = 0.837$, $p < 0.001$) between performance in the field and in the laboratory (Fig. 5). Those cultivars ranked as allelopathic in the bioassay tended to produce lower *D. minus* dry weight in the field. Eight of the top 10 allelopathic cultivars (which excludes any cultivars not common between the 2 experiments) in the bioassay were among the top 10 suppressive cultivars in the field trial.

Although a significant correlation was obtained, and a positive correlation with actual yield results is promising, no claims are made of cause and effect. Determination of any sole cause of the observed weed suppression would require controlling all variables but the one being tested, and this is difficult to do in the field for allelopathy.

To date, few reports indicate that laboratory results could serve as a suitable indicator of allelopathic potential or of the contribution that allelopathy can make towards field performance. Olofsdotter *et al.* (1999) screened 111 rice cultivars for allelopathic effects in both the laboratory and the field. This work showed that inhibition of barnyard grass root growth in bioassay by rice cultivars was somewhat indicative of their suppressing performance in the field. In this case, comparisons were drawn between the barnyard grass dry weight from the field trial taken eight weeks after seeding and barnyard grass root growth in the laboratory experiment. There was a linear relationship between the laboratory and field results with correlations ranging from 0.41 to 0.65 over the three field seasons. This is an important finding which indicates that at least some of the variation in field performance of cultivars can be predicted by their performance in bioassays.

Inderjit and Callaway (2003) indicated that allelopathy in plant communities cannot be predicted by laboratory studies alone, regardless of how detailed the studies. This is due to the myriad other factors which have impact in a field situation. However, such essential laboratory studies can identify key cultivars which possess outstanding allelopathic potential. Such cultivars may be selected as ideal candidates for field evaluation and further chemical analysis and mechanism studies. Performance in the field is imperative in authenticating the allelopathic impact of any cultivar.

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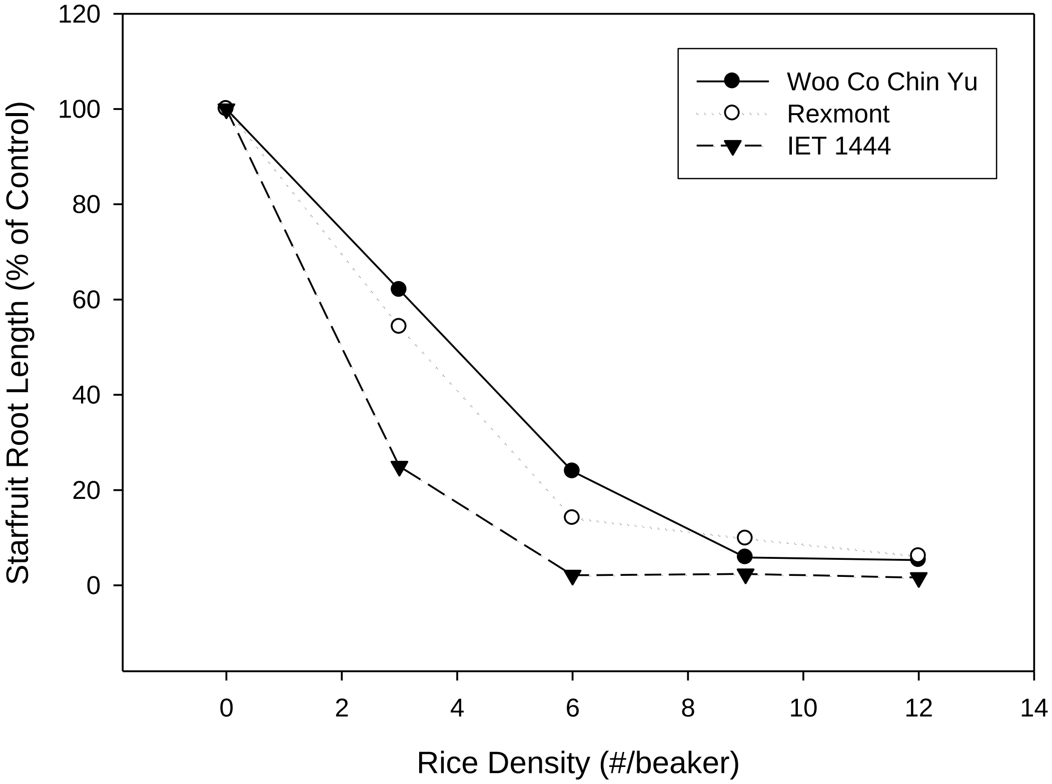

120
100
80
60
40
20
0
Starfruit Root Length (% of Control)
0
2
4
6
8
10
12
14
Rice Density (#/beaker)
Woo Co Chin Yu
Rexmont
IET 1444

Fig. 1

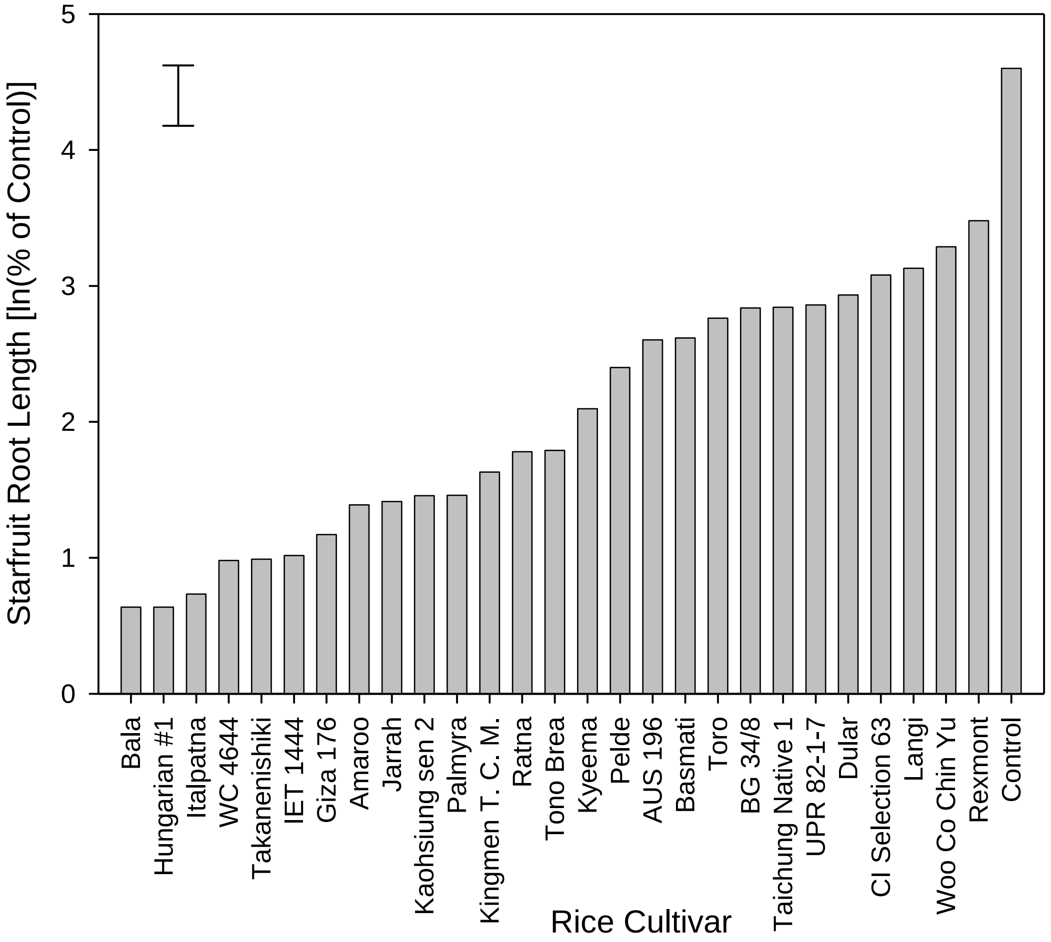

Starfruit Root Length [ln(% of Control)]
0
1
2
3
4
5
Bala
Hungarian #1
Italpatna
WC 4644
Takanenishiki
IET 1444
Giza 176
Amaroo
Jarrah
Kaohsiung sen 2
Palmyra
Kingmen T. C. M.
Ratna
Tono Brea
Kyeema
Pelde
AUS 196
Basmati
Toro
BG 34/8
Taichung Native 1
UPR 82-1-7
Dular
CI Selection 63
Langi
Woo Co Chin Yu
Rexmont
Control
Rice Cultivar

Fig. 2

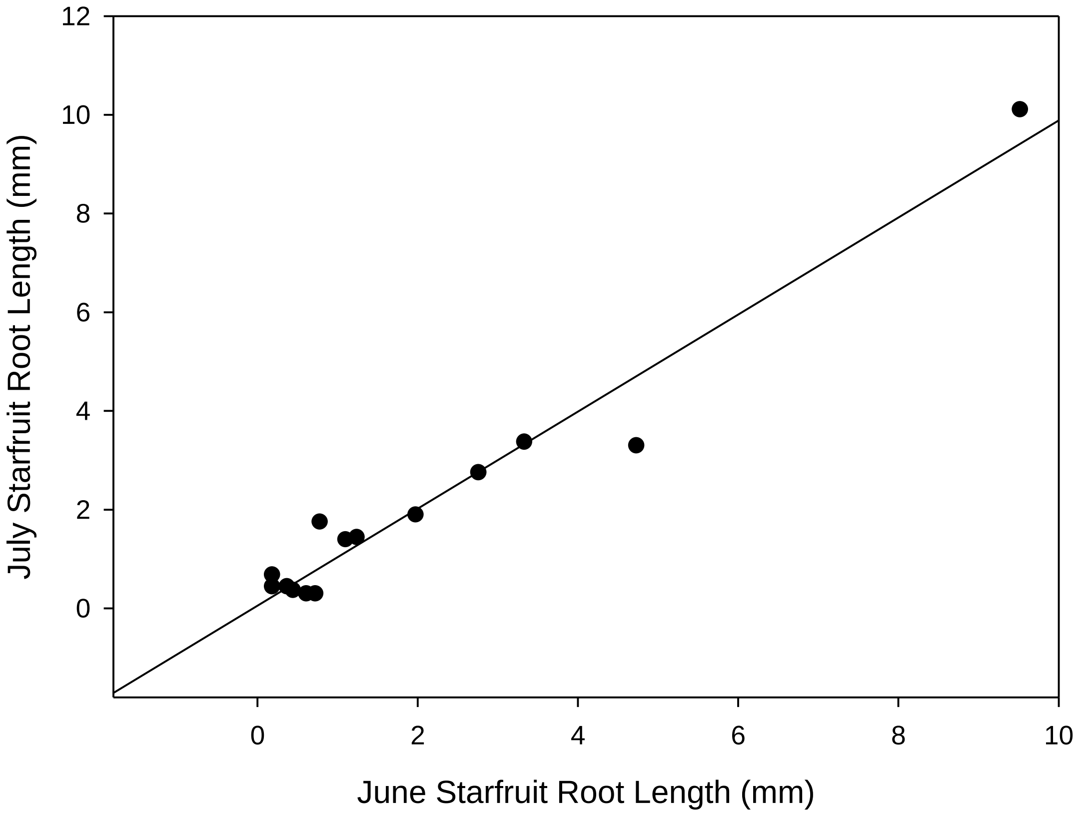
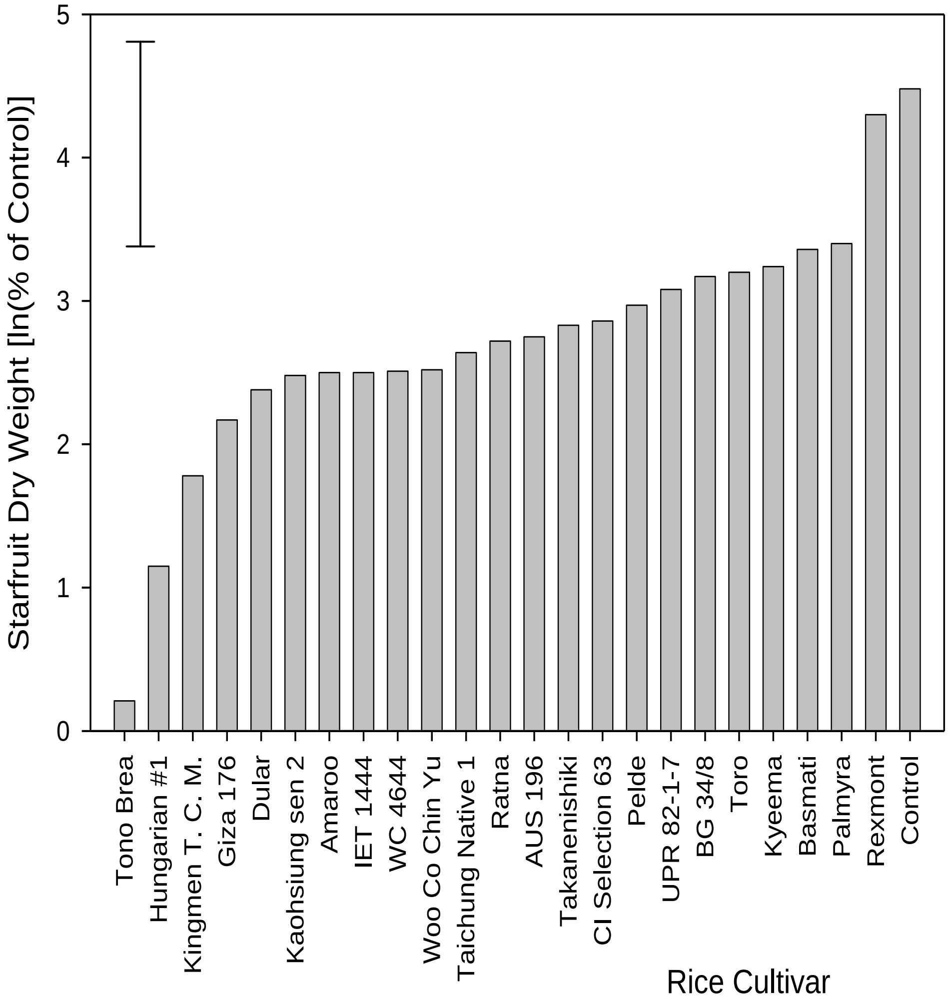

12
10
8
6
4
2
0
July Starfruit Root Length (mm)
0
2
4
6
8
10
June Starfruit Root Length (mm)

Fig. 3



5
4
3
2
1
0
Starfruit Dry Weight [ln(% of Control)]
Tono Brea
Hungarian #1
Kingmen T. C. M.
Giza 176
Dular
Kaohsiung sen 2
Amaroo
IET 1444
WC 4644
Woo Co Chin Yu
Taichung Native 1
Ratna
AUS 196
Takanenishiki
CI Selection 63
Pelde
UPR 82-1-7
BG 34/8
Toro
Kyeema
Basmati
Palmyra
Rexmont
Control
Rice Cultivar

Fig. 4

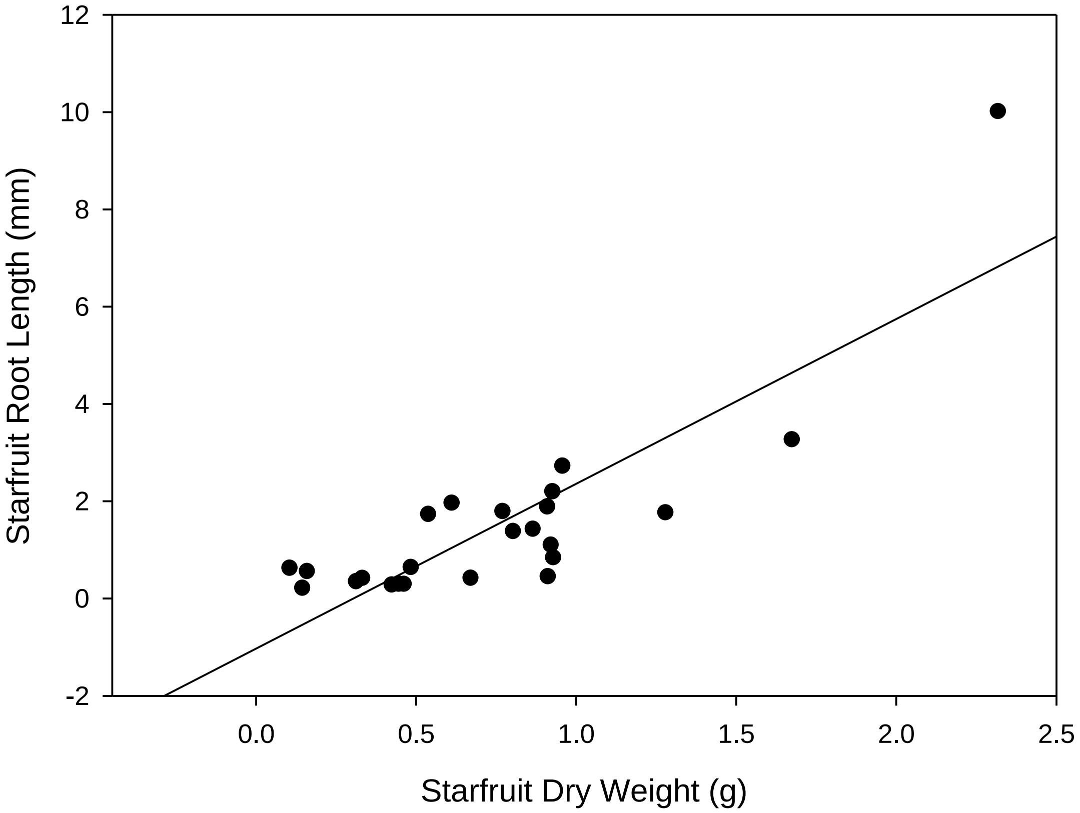

Starfruit Root Length (mm)
12
10
8
6
4
2
0
-2
0.0
0.5
1.0
1.5
2.0
2.5
Starfruit Dry Weight (g)

Fig. 5

Table 1: Cultivar and pedigree information for 27 rice cultivars selected for analysis

Cultivar	Experiment	Origin	Type	Pedigree
Amaroo	B and F	Australia	japonica	Calrose/M7
Aus 196	B and F	Bangladesh	japonica	Unavailable
Bala	B	India	japonica	N22/Taichung Native 1
Basmati	B and F	Pakistan	indica	Unavailable
BG 34/8	B and F	Sri Lanka	japonica	IR8-246///PP/MAS/H501
CI Selection-63	B and F	Cuba	indica	No info from IRRI
Dular	B and F	Bangladesh	japonica	Dumai/Larkochi
Giza 176	B and F	Egypt	japonica	Calrose 76/Giza 172//GZ 242
Hungarian #1	B and F	Hungary	japonica	Unknown
IET 1444	B and F	India	indica	TN-1/CO29
Italpatna	B	Italy	japonica	Unavailable
Jarrah	B	Australia	japonica	M7*2/Somewake
Kaonsiung shen 2	B and F	Taiwan	japonica	TN-1/Pa Mi Fen
Kingmen T. C. M.	B and F	China	japonica	Unavailable
Kyeema	B and F	Australia	japonica	Pelde//Della/Kulu
Langi	B	Australia	japonica	Kulu/CI9187//M7/3/Pelde
Palmyra	B and F	USA	japonica	Caloro/Blue Rose
Pelde	B and F	Australia	indica	Century Patna 231/Calrose//Bluebelle
Ratna	B and F	India	japonica	TKM6/IR8
Rexmont	B and F	USA	indica	Newrex/Bellemont
Taichung Native 1	B and F	Taiwan	japonica	Tie-cha-oo-chien/Tsai-yuan-chung
Takanenishiki	B and F	Japan	japonica	Unavailable
Tono Brea 439	B and F	Dominica	indica	Unavailable
Toro	B and F	USA	indica	Bluebonnet/C4-II-1-8
UPR 82-1-7	B and F	India	indica	IR 20/IR 24
WC 4644	B and F	Philippines	japonica	Tx*2/TN-1
Woo Co Chin Yu	B and F	Taiwan	japonica	Unavailable

B represents those cultivars included in the laboratory bioassay
F represents those cultivars included in the field trial

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Fig. 1 Effect of rice density and cultivar on *D. minus* root growth in the ECAM bioassay [l.s.d. (cultivar) = 5.7, $p < 0.001$, l.s.d. (concentration) = 7.4, $p < 0.001$, l.s.d. (interactions) = 12.7, $p < 0.01$].

Fig. 2 Effect of different rice cultivars on *D. minus* root growth in the ECAM bioassay using natural log transformed data (l.s.d. = 0.55, $p < 0.001$).

Fig. 3 Correlation of *D. minus* root lengths as affected by different rice cultivars during June and July bioassays ($r^2 = 0.92$, $p < 0.001$).

Fig. 4 Effect of rice cultivar on *D. minus* dry matter production in a field trial at the Yanco Agricultural Institute in the 2000/2001 rice growing season using natural log transformed data (l.s.d. = 1.8, $p < 0.05$).

Fig. 5 Correlation between *D. minus* root length in the ECAM bioassay (laboratory) and *D. minus* dry weight from the field trial ($r^2 = 0.70$, $p < 0.001$).

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Table 1: Cultivar and pedigree information for 27 rice cultivars selected for analysis