Determining the extent of herbicide resistance in the rice growing regions of southern Australia

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Summary  Weed seed samples were collected from across the rice-growing regions of southern New South Wales (NSW). These samples were tested against a relevant range of commercial herbicides to establish benchmark levels of resistance for each weed and herbicide combination.

High levels of resistance to bensulfuron were detected in the three weed species tested. Approximately 50% of dirty Dora (Cyperus difformis L.), 40% of starfruit (Damasonium minus (R.Br.) Buchenau) and 38% of arrowhead (Sagittaria montevidensis Cham. & Schlechter) samples were resistant. All dirty Dora, arrowhead and starfruit samples were susceptible to all of the other herbicides tested. No barnyard grass samples were classed as resistant to any of the four herbicides tested.

A total of six barnyard grass species (barnyard grass (Echinochloa crus-galli (L.) P. Beauv.), awnless barnyard grass (E. colona (L.) Link), hairy millet (E. oryzoides (Ard.) Fritsch), prickly barnyard grass (E. microstachya (Wieg.) Rydb), Japanese millet (E. esculenta (A. Braun) H. Scholz) and channel millet (E. inundata P. W. Michael & Vickery)) were detected. Four species were collected during a first survey and five in the second. Three of the six species were present in both surveys.

In a number of the samples two different species were present. These species had slightly different rates of germination that resulted in them being at slightly different growth stages at the time of herbicide application. Where mixed populations occur in the field this is of major importance when dealing with any herbicides that have a narrow range of growth stages for effective application.

At present, herbicide resistance in weeds of rice appears to be confined to bensulfuron. However it remains important for farmers to implement appropriate herbicide resistance management in order to protect the viability of the herbicide options.

Keywords  Resistance, dirty Dora, arrowhead, starfruit, barnyard grass.

INTRODUCTION
Herbicide resistance is an ever increasing problem in Australia with Preston et al. (1999) documenting 22 species in which resistance has occurred, including three which are weeds of rice (Graham et al. 1996).

Two species of barnyard grass (barnyard grass and awnless barnyard grass) have developed resistance to propanil in the Americas (Valverde and Itoh 2001). Similarly populations of hairy millet have developed resistance to both molinate and thiobencarb in south-east Asia and North America (Valverde and Itoh 2001).

Resistance has been reported to bensulfuron which has been used extensively and almost exclusively on NSW rice crops for around ten years as the only form of aquatic weed control. Herbicide resistance is a major threat to the sustainability of Australia’s rice industry. Unlike terrestrial crops where the management options are numerous to overcome the problem of herbicide resistance, very few management tools are currently available to overcome this problem in the rice growing regions of southern Australia (Graham et al. 1996). Weed control in the rice industry has been dependent on only a few herbicides and significant levels of resistance are emerging to one of the major herbicides (bensulfuron).

A survey of farms conducted in early 1993 where rice crops had received three or more applications of bensulfuron found 94% of dirty Dora accessions, 40% of starfruit and 33% of arrowhead to be resistant to bensulfuron (Graham et al. 1996). Herbicide resistance testing became available to rice growers in 1994, with very limited uptake. Only 190 accessions have been received over 10 years of which 80% (153) were found to be resistant to bensulfuron. These accessions were biased towards situations where resistance was suspected and cannot be used as an industry benchmark.

The effectiveness of current strategies for managing resistance has not been quantified and thus the extent and levels of resistance need to be established to allow measurement of future performance. By determining the extent of resistance in various weed populations more strategic recommendations can be developed. This will enhance the life of current herbicides through better rotations; keep additional herbicide use to a minimum; and reduce the incidence of weed control failure due to resistance.

Resistant weed populations have the potential to: either significantly reduce the area suitable for rice growing; or increase the use of yield-damaging herbicides; and increase the possibility of MCPA drift damaging neighbouring crops e.g. grapes.
MATERIALS AND METHODS

The seed samples came from three sources: either collected in random surveys; randomly selected from Charles Sturt University’s resistance testing service; or from random samples from the Rice Growers Cooperative laboratories. All mills send a sample to the laboratory of every load of rice received. These samples were then sieved to collect any weed seeds present. Samples were obtained from the three major rice growing regions of NSW, Coleambally Irrigation Area (CIA), Murrumbidgee Irrigation Area (MIA) and Murray Valley Irrigation Districts (MVID).

All species had seed collected through the surveys, although for barnyard grass this only occurred in the second project. Dirty Dora and barnyard grass were the only species collected from the samples from Rice Growers laboratories while some starfruit samples were provided from the resistance testing service seed bank at CSU.

Circular plastic tubs (11 cm diameter) were filled to a depth of 3 cm with a red clay loam and placed in a glasshouse with the temperature maintained at 20–30°C. The tubs were watered to field capacity and sown with either 0.1 g of seed or 25 seeds for the larger seeded species. The tubs were then watered to maintain field capacity until germination was nearly complete at which stage they were flooded to a depth of 2–3 cm. The tubs were fertilised with urea at a rate of 50 kg N ha⁻¹ when the plants were at the one leaf growth stage.

Slight modifications to this procedure were necessary for starfruit and barnyard grass. For starfruit, three to four days after sowing, 110 mL of a 150 µg mL⁻¹ ethephon solution was added to enhance germination. The tubs containing barnyard grass were not flooded but were maintained at around field capacity for the entire experiment. All treatments were replicated three times and the tubs were arranged in a randomised block design.

For the four weed species tested, a total of seven herbicides were used; bensulfuron, thiobencarb, benzofenap, MCPA, propanil, clomazone and molinate (Table 1).

Four rates of herbicide were applied, 0, 0.5, 1 and 2 times the recommended field rate. The herbicides were applied using one of two methods. MCPA and propanil were applied in a spray cabinet using a Tee-Jet nozzle moving at 6.6 km h⁻¹ applying 83.8 L ha⁻¹ total spray volume at a pressure of 300 kPa.

The remaining herbicides were applied at the recommended rate by injecting 10 mL of the appropriate dilution into the flood water of each of the tubs. For the 0.5 and 2 times recommended rate treatments 5 mL and 20 mL, respectively, of the same dilution were injected into water in the tubs. Herbicides were applied at the appropriate growth stages as per label recommendations.

Twenty-eight days after herbicide application the plants were scored for percentage survival and compared to the controls. The control was rated 10 and each treatment within that replication was then compared to it. Data analysis was conducted using two-way analysis of variance with scores expressed as proportions of their untreated controls.

RESULTS AND DISCUSSION

For all herbicides except MCPA, samples were classified as resistant if the mean for the three herbicide rates used (0.5 recommended rate (0.5R), recommended rate (1R) or 2 times recommended rate (2R)) had a score of greater than two or a score higher than the least significant difference (LSD) if that was greater than two. For the samples sprayed with MCPA the threshold used was 2.5 or the LSD as the MCPA was applied to the plants at a later growth stage.

Dirty Dora  Seventy seed accessions were screened to the five herbicides recommended for dirty Dora. All samples were well controlled by the application of benzofenap and thiobencarb.

Three samples were poorly controlled by propanil at 0.5R, but at 2R all three samples were well controlled (LSD = 1.6, P <0.001). All but one sample was well controlled by the application of MCPA (LSD = 1.8, P <0.001).

Upon retesting these four samples were controlled by the appropriate herbicide showing that other factors may be involved in the poor control experienced. These samples may have been poorly controlled simply as a result of insufficient herbicide being absorbed by the plant for it to be effective because of the characteristics of the plant itself. Variability in the efficacy of dirty Dora control by MCPA is an annual occurrence in the rice industry. The two herbicides with poor control are the only two where the method of application is by spraying and not adding a herbicide solution to the flooded tubs.

Of the 67 samples screened to bensulfuron, 31 were considered resistant as they had scores of greater than 2.8 for the mean of the three herbicide rates used (LSD = 2.802, P <0.001) (Figure 1).

Starfruit  None of the 35 samples of starfruit were classified as resistant to either benzofenap or MCPA. When treated with bensulfuron, samples with a mean score of 2.5 or greater were classified as resistant (LSD = 2.471, P <0.001). Fifteen accessions were classified as resistant (Figure 1) and of these seven
were from the survey and the other eight from the resistance testing service. There was no difference in the level of resistance for the two regions (CIA and MVID) from which samples were obtained in the random survey.

**Arrowhead**  Sixty-nine samples of arrowhead were screened to three herbicides. None were classified as resistant to either benzofenap or MCPA. For bensulfuron 26 samples were considered resistant as they had scores of greater than 3.6 for the mean of the three herbicide rates (LSD = 3.596, P < 0.001) (Figure 1).

There was a difference in the level of resistance present in the two regions surveyed. Twenty-two of the 39 samples from the CIA were resistant (56%) while only six out of 24 samples tested from the MIA were resistant (25%).

![Figure 1](image_url)  Mean range of scores for dirty Dora, starfruit and arrowhead after treatment with bensulfuron at 0.5R, 1R and 2R. Numbers denote proportion of LSD for each species.

### Barnyard grass
A total of six different species were found in the two studies (barnyard grass, awnless barnyard grass, hairy millet, prickly barnyard grass, Japanese millet and channel millet). Four species were found in the first (Pratley et al. 2000). The majority of the samples in both surveys were barnyard grass either as a pure sample or with another species present.

In the second study five barnyard grass species were detected (Pratley and Broster 2002). Two of the five species (Japanese millet and channel millet) identified from this survey were not detected in the previous survey while one found in the previous survey (prickly barnyard grass) was not detected in this project. The three most common species from the previous survey, barnyard grass, hairy millet and awnless barnyard grass, were also the most common species in this survey.

A wide range of phenotypes was present within barnyard grass, with awned and awnless forms detected as well as large panicled forms. With such within species variability possible mis-identifications between hairy millet and the large panicle form of barnyard grass are possible. With there being a zero tolerance for hairy millet in rice seed crops this potential for mis-identification may have serious financial outcomes for growers.

From the two studies 191 samples of barnyard grass were tested to four herbicides. No samples were classed as resistant to propanil, molinate or clomazone.

Of all the different herbicides evaluated in this trial resistance was only detected to bensulfuron (Table 1). All three weed species in this study, normally controlled by bensulfuron, exhibited levels of resistance of greater than 37%. This herbicide has been the one of choice for many rice farmers as it is easy to use and has a wide spectrum of weed control.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Herbicides used in the resistance screening and benchmark levels of resistance for the four species surveyed.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>Dirty Dora</td>
</tr>
<tr>
<td>Herbicide</td>
<td>g a.i. ha⁻¹</td>
</tr>
<tr>
<td>Bensulfuron</td>
<td>51</td>
</tr>
<tr>
<td>Thiobencarb</td>
<td>4000</td>
</tr>
<tr>
<td>Benzofenap</td>
<td>600</td>
</tr>
<tr>
<td>MCPA</td>
<td>675</td>
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<tr>
<td>Propanil</td>
<td>5760</td>
</tr>
<tr>
<td>Clomazone</td>
<td>300</td>
</tr>
<tr>
<td>Molinate</td>
<td>3225</td>
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</table>
The high level of resistance to bensulfuron in samples of these three major weeds is of major concern for the rice industry. No other herbicide has the same wide spectrum of weed control, with the same window of application or lack of constraints on application. Resistance to this herbicide will result in other less effective, harder to use herbicides being used to control these weeds with a decrease in the profitability of the rice industry as a whole.

Fortunately, none of the barnyard grass samples were found to be resistant to any of the tested herbicides. Herbicide resistance in barnyard grass has been reported overseas (Valverde and Itoh 2001). In Arkansas some barnyard grass populations have been shown to tolerate over 20 times the normal recommended rate of propanil. These fields had been sown to rice for greater than 20 consecutive years. A survey of farmers in this area determined that a simple crop rotation greatly reduced the probability of resistance (Kendig and Fishel 1995). This could be the reason why barnyard grass resistance has not yet been detected in Australia as no fields have been sown to rice with such intensity.

This benchmarking study provides appropriate background against which to generate extension programs.

ACKNOWLEDGMENTS
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REFERENCES