The extent of herbicide resistance in rice crops of southern New South Wales

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Abstract
Herbicide resistant weeds are a major problem in the cropping regions of Australia. The rice industry is no exception since weed control in the rice industry has been dependent on only a few herbicides.A random survey in 1999 reported significant levels of resistance to one of the major herbicides (bensulfuron or Londax®) across the rice growing regions of southern New South Wales. The survey was repeated in both 2016 and 2017 to assess current herbicide resistance levels. Resistance was confined to bensulfuron. Since the previous survey in 1999, bensulfuron resistance had increased from 46% of dirty dora populations to 70% in 2017 and for arrowhead from 38% to 61%. The extent of resistance in starfruit at 50% was similar to that reported for the 1999 survey. While there was no resistance confirmed to other herbicides or species, three accessions (two with dirty dora and benzofenap; one with barnyard grass and propanil) had survival levels above the resistance thresholds used, these populations require further testing to confirm resistance status.

Key Words
Herbicide resistance, rice, bensulfuron

Introduction
Weed control in the rice industry has been dependent on very few herbicides and significant levels of resistance have been reported to one of the major herbicides (bensulfuron or Londax®) (Pratley *et al.* 2000). Approximately 50% of *Cyperus difformis* (dirty dora), 40% of *Damasonium minus* (starfruit) and 35% of *Sagittaria montevidensis* (arrowhead) accessions were found to be resistant to this herbicide in a 1999 survey. Four of the seven basic herbicide programs for aerial sowing of rice use bensulfuron as the main herbicide for aquatic weed control. Where this herbicide is unable to be used due to resistance, the other three herbicide programs use benzofenap for control of aquatic weeds (Troldahl *et al.* 2016). This significant level of resistance to bensulfuron has placed increased pressure on benzofenap (Taipan®) in respect of resistance evolution.

The 1999 survey found no resistance to any other herbicide used in rice crops at that time (Broster *et al.* 2004). However resistance has been found in weeds of rice crops in 45 other species across 27 other countries and to 10 different herbicide groups (Heap 2015). The resistant weeds include seven different species of *Echinochloa*, several of which have been found in the rice growing regions of NSW (Pratley *et al.* 2008). While no resistant populations of *Echinochloa* spp. have been reported in Australian rice fields, populations of *E. colona* resistant to atrazine or glyphosate have been reported in other Australian cropping regions (Heap 2015).

With continued herbicide use, the likelihood of weed populations evolving resistance increases. Therefore there is a need for continued surveillance in this area to maintain or increase the awareness of herbicide resistance in the rice industry, at both the grower and advisory level, as a means of monitoring and managing the problem.

Methods
Random field surveys
Two random field surveys were conducted, one in February / March 2016 and the second in March 2017. Across the two surveys a total of 122 paddocks were visited. Paddocks were selected by driving through the rice growing regions and at specific intervals stopping at the nearest rice paddock and collecting samples. Two people sampled the paddocks with one walking through the middle of the bays and one sampling nearer to the edges and banks. Weed species collected included *Echinochloa crus-galli* (barnyard grass), *Echinochloa colona* (awnless barnyard grass), *Diplachne fusca* (Silver top), *Cyperus difformis* (dirty dora), *Cyperus eragrostis* (umbrella sedge), *Damasonium minus* (starfruit), *Sagittaria montevidensis* (arrowhead),

Alisma plantago-aquatica (water plantain) and *Ammannia multiflora* (jerry jerry). If no weeds were found in the selected paddock this event was still recorded.

Plant establishment

Plants were sown into plastic tubs, with no drain holes, filled to a depth of 3-4 cm of 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 0.1g of seed or 0.2 g of seed for the larger seeded species. When germination was nearly complete the tubs were flooded to a depth of 2–3 cm. Treatments were replicated three times. Samples were not tested to all herbicides or all replicates if seed availability or germination levels were insufficient. Barnyard grass samples were either thinned to 25 plants (2016 survey) or pre-germinated in petri dishes with 12 seedlings transplanted per tub (2017 survey).

Resistance screening

For the weed species tested, ten herbicides were used: cyhalofop-butyl (Barnstorm®), profoxydim (Aura®), bensulfuron (Londax®), propanil (Stam®), benzofenap (Taipan®), MCPA, thiobencarb (Saturn®), molinate (Ordram®), paraquat (Gramoxone®) and clomazone (Magister®). Table 1 shows which herbicides were applied to each weed species along with rate applied (g a.i./ha) and application timing.

Herbicides were applied using one of two methods. Cyhalofop, profoxydim, MCPA, paraquat and propanil were applied in a laboratory spray cabinet using a twin nozzle boom with XR11001 nozzles travelling at 5 km per hour applying 85 L of water per hectare at a pressure of 250 kPa. The remaining herbicides (bensulfuron, benzofenap, molinate, thiobencarb and clomazone) were applied by injecting 10 mL of an appropriate dilution into each of the flooded tubs to obtain the recommended rate per hectare. Herbicides were applied when the plants were at the appropriate growth stages as per label recommendations (Table 1).

Twenty-eight days after herbicide application the tubs were visually assessed based on percentage survival. For the aquatic species (dirty dora, umbrella sedge, arrowhead, starfruit and water plantain), with smaller seeds and seedlings and therefore high germination levels, seedling numbers were estimated before and after herbicide application. For the barnyard grass and silver top, plant numbers were counted before and after spraying to determine survival percentage

Table 1: Herbicide rates used in the resistance screening and growth stage (leaf number) for application

Herbicide	Group	Rate (g a.i. / ha)	Barnyard grass	Awnless barnyard grass	Dirty dora	Umbrella sedge	Starfruit	Arrowhead	Water plantain	Silver grass
cyhalofop	A (fop)	285	2-4 lf	2-4 lf						2-4 lf
profoxydim	A (dim)	75	2-4 lf	2-4 lf						
bensulfuron	B (SU)	51			0-3 lf	0-3 lf	0-3 lf	0-3 lf		
propanil	C	4080	2-3 lf	2-3 lf						
benzofenap	H	600			0 lf	0 lf	0 lf	0 lf	0 lf	
MCPA	I	675			>5 lf					
thiobencarb	J	3000	0-3 lf	0-3 lf	0-2 l					
molinate	J	3600	2-4 lf	2-4 lf						2-4 lf
paraquat	L	400	2-4 lf	2-4 lf						
clomazone	Q	288	2-4 lf	2-4 lf						2-4 lf

Accessions were classed as Resistant, Developing Resistance or Susceptible using standard thresholds. A survival percentage of 20% or greater were classed as Resistant, between 10% and 20% as Developing Resistance while a survival percentage of less than 10% was classed as Susceptible. Data analysis was only undertaken for the resistance screening for the barnyard grass to compare those accessions with levels of survival below the thresholds for Developing Resistance.

Results

Bensulfuron resistance levels

The level of resistance to bensulfuron had increased since the last survey, with 70% of the dirty dora populations resistant compared with 46% reported in the previous survey. Fifty percent of starfruit accessions were resistant, the same level found in the 1999 survey while the percentage of resistant arrowhead populations had increased from 38% in 1999 to 61% in this survey (Table 2).

Table 2: Percentage of accessions resistant to bensulfuron in the 1999 and current surveys

Herbicide	Populations tested	Populations resistant	% resistant	% resistant 1999 survey
Dirty dora	70	49	70	46
Starfruit	26	13	50	50
Arrowhead	23	14	61	38

Other herbicides

Two dirty dora populations were classed as resistant to benzofenap and one barnyard grass accession classed as developing resistance to propanil. These populations will need to be retested at several rates to confirm resistance, as resistance to these herbicide in these species has not been reported previously in Australian rice fields.

No accessions from any species were classified as resistant to any of the other tested herbicides (see Table 1 for species and herbicide combinations). However, some populations did have plants that survived the herbicide application but the level of survival was below the threshold for resistance. Despite the significant number of accessions with surviving plants, no accession had surviving plants in all replicates for any herbicide.

Surviving plants were recorded for barnyard grass accessions screened to cyhalofop, molinate, propanil and clomazone. Several of these accessions had survival percentages significantly higher than the susceptible accessions and the other survey accessions (propanil - $P<0.001$, lsd = 2.95%; molinate $P<0.001$, lsd = 2.95%) and clomazone $P<0.001$, lsd = 2.16%) but still below the 10% survival threshold for classification as developing resistance. The presence of some barnyard grass accessions with a low number (below resistance thresholds) of plants surviving herbicide application was also apparent in a previous survey (Broster 2014). Additionally, although the resistance status was assessed using visual scores rather than survival percentages, two other previous resistance surveys for barnyard grass have also reported differential responses to the herbicides, i.e. not all accessions are completely controlled (Pratley *et al.* 2000; Pratley and Broster 2004).

Two accessions of dirty dora also had plants that survived the application of MCPA. Variable responses to MCPA have been observed in a previous resistance survey (Pratley *et al.* 2000) and in the field. The physical structure of the dirty dora plant, erect with shiny leaves can mean insufficient herbicide remains on the plant from a spray application. Additionally this survey also reported that some accessions had plants that survived benzofenap application, although on re-testing these accessions were shown to be susceptible (Pratley *et al.* 2000)

Conclusion

This project found that the rate of herbicide resistance to bensulfuron has increased in the two of the three major aquatic weed species (dirty dora and arrowhead) since the last major resistance survey conducted in 1999. The rate of increase was much lower than that experienced by other sulfonylurea herbicides in dryland crops over a similar time period. The findings from the 1999 survey provided an awareness of the problem that, due to its smaller size relative to dryland cropping, the rice industry has been able to better address than more extensive cropping systems. Additionally, the smaller rice crop area in the mid to late 2000s resulted in wider rotations and therefore fewer herbicide applications over this time.

The presence of significant levels of bensulfuron resistance in association with an awareness of the impact herbicide resistance can have on management, production and ultimately profitability has been an influence in management change. Previously, about 90% of rice in Australia was aerially sown (Graham *et al.* 1996). However, since the discovery of bensulfuron resistance in the aquatic weeds, the proportion of ground sown rice has now increased to a level where only 26% of rice was aerially sown (R. Ford personal communication, 2018). This change in sowing method has resulted in a change in weed spectrum as ground (dry) sowing favours dryland species (e.g., barnyard grass) over the aquatic species favoured by aerial sowing.

That no barnyard grass accessions were found to be resistant to the higher risk herbicides, cyhalofop and profoxydim herbicides despite the change to sowing systems that favour this weed is most likely a combination of; awareness of herbicide resistance, the low rice crop area in recent years and the existence of alternative summer crops that use different herbicides for barnyard grass control.

The findings from this research show that herbicide resistance, while present, is limited to a single herbicide. However, the ineffectiveness of this herbicide has already resulted in management change and associated increased selection pressure on alternative herbicides. To maintain the long term effectiveness of the current herbicides there needs to be an increase in alternative, non-herbicidal control options, such as harvest weed seed control and allelopathy, for weed control.

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