2,2-DPA resistance in giant Parramatta grass (*Sporobolus fertilis*)

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Summary *Sporobolus fertilis* seeds suspected of being resistant to 2,2-DPA (sodium 2,2-dichloropropionate) were collected near Grafton, NSW, Australia. A seedling Petri-dish assay showed that the 2,2-DPA concentration required to reduce shoot length by 50% in the resistant biotype was about 500 g a.i. kg^{-1}, five times greater than that for the sensitive biotype. Similarly, a pot-dose assessment in plants showed that the resistant population required 4 and 28 kg a.i. ha^{-1} for 50% reductions in shoot height and the proportion of live leaves per plant respectively, more than 2.5 and 14 times the recommended rate, whereas the sensitive plants were well controlled by the recommended rate. This shows that 2,2-DPA resistance has indeed developed in the field and will limit the use of 2,2-DPA for the control of *S. fertilis* in future.

Keywords *Sporobolus fertilis*, giant Parramatta grass, resistance, 2,2-DPA.

INTRODUCTION

Giant Parramatta grass, *Sporobolus fertilis* (Steud.) Clayton, is an aggressive perennial weed of pastures (Land Protection 2006) with a potential distribution of 23.7 million ha in Australia (D.A. McLaren pers. comm.). It is widely distributed along coastal areas of NSW and Queensland and out-competes native grass pastures (Betts and Officer 2001). It costs the Australian beef and dairy industries A$60m annually (Natural Resources and Mines 2001).

Current chemical control options include glyphosate, flupropanate and 2,2-DPA (Betts and Officer 2001). Both 2,2-DPA and flupropanate are Group J herbicides, which affect lipid metabolism and are thought to be at low risk of herbicide resistance (Avcare 2000). Flupropanate has some selectivity in native grasses, but is slow to act (3–6 months) and its residual activity excludes it from use with dairy cattle (Betts and Officer 2001). Also, the recent documentation of flupropanate resistance from a NSW population of *S. fertilis* limits its use (Ramasamy *et al*. 2007). Both glyphosate and 2,2-DPA are relatively non-selective and have no effect on lactating animals (Natural Resources and Mines 2001), but glyphosate is not effective on *S. fertilis*, leaving 2,2-DPA as the most viable option (Betts and Officer 2001). As resistance has been documented to flupropanate (Ramasamy *et al.* 2007), 2,2-DPA resistance might be predicted in *S. fertilis*. Support for this prediction was provided when *S. fertilis* plants at Grafton, NSW, were suspected of field resistance to 2,2-DPA. This study determined the degree of 2,2-DPA resistance in *S. fertilis* seeds collected from these plants at this property at Grafton, NSW.

MATERIALS AND METHODS

Seeds and plant material Seeds (hereafter called 'Resistant') were collected from *S. fertilis* plants suspected to be resistant to 2,2-DPA at a property near Grafton NSW in 2005. The plants had received applications of Propon® containing 740 g a.i. kg^{-1} 2,2-DPA as the sodium salt (Agricrop 2007) for an unknown number of times at 5–10 kg a.i. ha^{-1} for *S. fertilis* control. 'Sensitive' seeds were also collected, from *S. fertilis* plants that were known to be sensitive to 2,2-DPA, maintained in the NSW Department of Primary Industries.

Seedling assay Seeds were germinated in glass Petri dishes (90 mm diameter) with seed test paper (Whatman 182) and were assayed for resistance. Herbicide concentrations of 0, 1.85, 7.40, 29.6, 118.4, 473.6, 1894.4 and 7577.6 g a.i. kg^{-1} were prepared from Propon® and 5 mL of the solution added to each dish. Twenty-five firm seeds of each of the biotypes were placed in each dish and incubated at 22°C/15°C with a 12 h photoperiod for 15 days. After 15 days, shoot length was measured as an indicator of 2,2-DPA resistance, as in other resistance studies (Ramasamy *et al*. 2007). Experiments were arranged in a randomised design with four replicates per treatment.

Pot-dose study Pot-dose experiments were carried using plants eight months old, grown from Resistant and Sensitive seeds from NSW. Plants were treated

with 2,2-DPA as Propon® (740 g a.i. kg^{-1}) using a mechanical track sprayer in a spray cabinet with standard flat nozzles (SS10002) and a spray volume of 150 L ha^{-1} at 280 kPa. Herbicide doses were 0, 0.93, 1.85, 3.70, 7.40, 14.80 and 29.60 kg a.i. ha^{-1}, the maximum being four times the recommended field rate (Agricrop 2007), with three replicates per treatment. Four months after spraying, plants were assessed for resistance by recording the number of live and dead leaves and height per plant. All data were analysed using the statistical software Minitab (Version 13.20, Minitab 2004).

RESULTS

Both the seedling assay and the pot-dose study confirmed that *S. fertilis* seeds and plants tested showed resistance to 2,2-DPA. Shoot length declined with increase in herbicide concentration in both biotypes (Figure 1). There was a significant difference in shoot length between resistant and sensitive seedlings (ANOVA, F = 146.09, P <0.001) and with 2,2-DPA concentration (ANOVA, F = 243.3, P <0.001). The ED_{50} values for resistant and sensitive seedlings (extrapolated from 50% effect in Figure 1) were 500 and 95 g a.i. kg^{-1} respectively; thus the Resistant biotype was five times more tolerant than the Sensitive biotype.

In the pot-dose study, a herbicide effect (leaf death) was first observed with doses greater than 1.85 kg a.i. ha^{-1} after two months of treatment in the Sensitive biotype. At four months, the proportion of green leaves in Sensitive plants decreased with increase in 2,2-DPA concentration (Figure 2). By contrast, Resistant plants were unaffected by as much as 15 kg a.i. ha^{-1}. There were significant differences in the proportion of live: dead leaves with plant biotype (ANOVA, F = 291.40, P <0.001) and 2,2-DPA concentration (ANOVA, F = 41.43, P <0.001). The ED_{50} values required to reduce the proportion of live leaves by 50% in the Resistant and Sensitive biotypes were 28 and 1.9 kg a.i. ha^{-1} respectively (Figure 2), indicating that the resistant biotype was 14 times more tolerant than the sensitive biotype.

A decrease in height was also observed with increase in 2,2-DPA concentration (Figure 3). At four months, there were significant differences in height with plant biotype (ANOVA, F = 141.19, P <0.001) and 2,2-DPA concentration (ANOVA, F = 46.43, P <0.001). The ED_{50} values required to reduce the height by 50% in the Resistant and Sensitive biotypes were 4 and 1.5 kg a.i. ha^{-1} respectively, suggesting that the Resistant biotype was 2.5 times more tolerant than the Sensitive biotype.

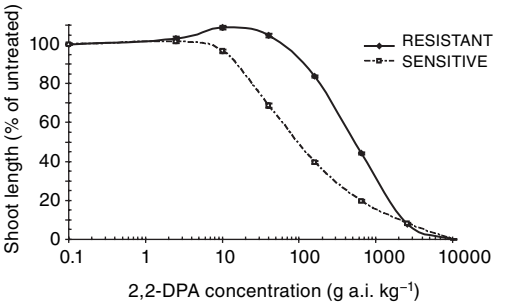

Figure 1. Effect of 2,2-DPA on shoot growth of *Sporobolus fertilis* seedlings at 15 days after treatment. Vertical bars represent 2x standard error of the mean.

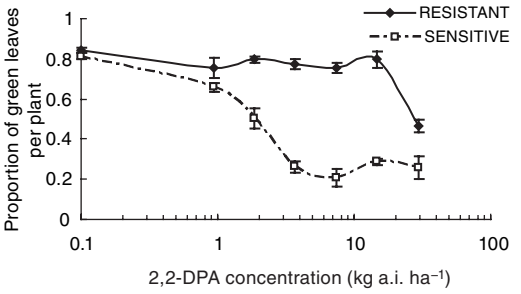

Figure 2. Effect of 2,2-DPA on proportion of green leaves in *Sporobolus fertilis* potted plants with increase in concentration. Vertical bars represent 2x standard error of the mean.

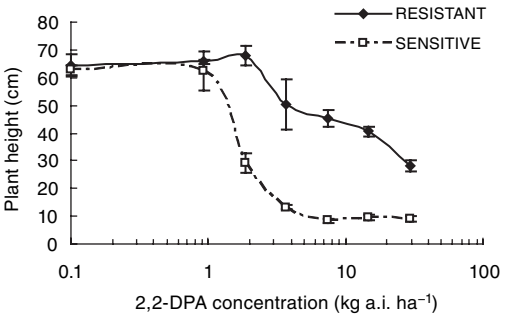

Figure 3. Effect of 2,2-DPA on the height of *Sporobolus fertilis* with increase in concentration. Vertical bars represent 2x standard error of the mean.

DISCUSSION

This study has confirmed that 2,2-DPA resistance has evolved in *S. fertilis*. Although 2,2-DPA is classified in Group J, thought to be at low risk of developing herbicide resistance, this report suggests that the intensive use of this and other herbicides with similar modes of action can select for resistance (Heap 2005). Coupled with the previous report of resistance of *S. fertilis* to flupropanate, another Group J herbicide (Ramasamy *et al*. (2007), this demonstrates that, even with low-risk herbicide groups, herbicide resistance can and does evolve in the field to more than one herbicide (Heap 2005).

The large difference in herbicide effects between Resistant and Sensitive biotypes is consistent with resistance detection and classification (Heap 2005). Resistant *S. fertilis* plants did not die even at 14 times the field recommended rate and resistant seedlings also showed 5–6 times more resistance than the sensitive biotype. Similarly, Perez and Kogan (2003) confirmed that a population of *Lolium multiflorum* resistant to glyphosate showed an ED_{50} of five to six times (seedlings) and two to four times (mature plants) the dose of sensitive plants. The greater effect on leaf death than plant height is consistent with the browning seen in the field after herbicide application. There was a discrepancy between estimates of resistance from the leaf and height parameters (14 and 2.5 times respectively); the resistance estimate from the live:dead leaves ratio is more accurate, since total plant height rather than height increment was measured.

With its enormous seed production (150,000 seeds m^{-2}) and high seed viability (90–100%) (Natural Resources and Mines 2001), *S. fertilis* can rapidly build up the soil seed bank. Seeds of *S. fertilis* are easily dispersed as contaminants in pasture seeds and hay, through irrigation channels, and on livestock and farm machinery (Natural Resources and Mines 2001). The migration of seeds from resistant plants provides a source for spread and establishment of resistance genes in the population, although the seed bank is long-lived (10 years, Natural Resources and Mines 2001) and this is likely to delay the development of widespread resistance (Jasieniuk *et al.* 1996). Pollen grains from resistant plants may also act as a source of resistance genes, but so far there is no information on the heritability of the resistance.

This report of 2,2-DPA resistance in *S. fertilis* is of great concern to the pastoral farmers in South-east Australia. Research should be undertaken to estimate the extent of resistance and to raise awareness of it, as for flupropanate resistance in *N. trichotoma* (McLaren *et al*. 2006). This evolution of resistance restricts the use of 2,2-DPA and so leaves farmers with reduced control options. Hence growers need to employ integrated management strategies to avoid the risk of resistance to this herbicide in future and to combat it when it arises.

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