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An Assessment of the Extent of Serrated Tussock Resistance in the Rowsley Valley, Victoria.

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Summary
A 100 square km serrated tussock resistance survey in the Rowsley Valley of Victoria has confirmed that serrated tussock resistance is far more widespread than had been previously thought. Approximately 20% of surveyed sites surrounding a property previously confirmed with serrated tussock resistance to flupropanate had significant survival (resistance) to an application of label rate flupropanate compared to known susceptible serrated tussock plants treated at the same time. This suggests that resistance is not localised and should now be treated as a widespread issue. Any further Government investment that is deemed appropriate could be in the form of support for awareness and extension packages to affected land managers and/or ongoing research into new management techniques. This paper makes suggestions for how land managers can deal with the potential loss of flupropanate as a management tool and makes recommendations for how Governments and Industry could respond.

Keywords serrated tussock, *Nassella trichotoma*, flupropanate, resistance.

INTRODUCTION
Serrated tussock (*Nassella trichotoma* Trin. & Rupr. Barkworth) is a South American exotic unpalatable perennial grass that has been classified a Weed of National Significance in Australia due to its severe agricultural and environmental impacts (Thorp and Lynch 2000). It was first identified in Australia in 1936 and has since spread to occupy more than 2 million ha (Osmond *et al.* 2008) with an estimated potential distribution of 31 million ha (McLaren *et al*. 1998). It is costing Australia millions of dollars in lost agricultural production while also invading and replacing Australia's endangered native grasslands (McLaren *et al.* 1998). Despite years of research, there are still limited control options for managing weeds such as serrated tussock in Australia (Michalk *et al.* 1999). The only registered herbicides for control of serrated tussock in pastures are flupropanate, glyphosate and 2,2-DPA. The ability of organisms to develop resistance to a particular chemical control agent after constant exposure to that chemical over many generations is well documented in the scientific literature (Lebaron and Gressel 1982). Flupropanate is widely regarded as the most selective and effective herbicide for controlling serrated tussock while its residual action in the soil can prevent serrated tussock regrowing for three to five years (Campbell and Vere 1995). It is classified by as a Group J herbicide that inhibits plant lipid synthesis and is regarded as a relatively low risk herbicide for resistance (Croplife Australia 2008). Flupropanate is a soil active herbicide that can have a residual activity and can prevent serrated tussock from regrowing for three to five years (Campbell and Vere 1995).

Flupropanate resistance has been identified in a population of serrated tussock in Victoria with serrated tussock surviving application rates as high as 8 L ha^{-1}, which is four times the recommended rate used for controlling this species (Noble 2002). A national serrated tussock resistance survey was undertaken by the Victorian Department of Primary Industries during 2004 to determine the extent of resistance in Australia (McLaren *et al.* 2006) and resistance has now been confirmed at three sites in Australia (two in Victoria and one in NSW) (McLaren *et al.* 2008).

Durai (2008) conducted detailed serrated tussock population crossing studies of known flupropanate resistant and susceptible serrated tussock plants. His results show that resistance can come from both parents, strongly suggesting a genetic origin with 80-90% matching of seedling type to maternal parent type strongly indicating the involvement of a maternal component in the inheritance of flupropanate resistance, with a minor proportion of resistance heritable through pollen. He therefore hypothesised that the maternal cytoplasm of the female parent plays a significant role in the transmittance of flupropanate resistance. The minor transmission of resistance via pollen observed in all crosses suggested transmission also by a component in the pollen grains.

Another critical factor in serrated tussock resistance is understanding how serrated tussock reproduces. Durai (2008) showed that the majority (85 -90%) of serrated tussock flowers don't physically open (pollen is transferred within the closed flower) meaning that only 10-15% of serrated tussock flowers are available for pollen transfer. The implications of this are that a serrated tussock plant resistant to flupropanate will produce at least 85-90% resistant seeds as they will fertilise within the unopened flower. However, only a relatively small proportion (10-15%) of the flowers will send out resistant pollen to potentially spread flupropanate resistance great distances.

A critical issue for weed management authorities wishing to contain serrated tussock resistance to flupropanate is understanding the current extent of resistance infestations. If the resistant serrated tussock is confined within a very small area (ie. to a single property), then the serrated tussock resistance can be prioritised for management with Government assistance for direct control costs and compliance. If the serrated tussock resistance is widespread then management becomes more problematic and Government investment is likely to be directed towards extension and advice promoting integrated control.

This paper reports on an assessment of the extent of serrated tussock resistance occurring within a 100 square km region surrounding a known serrated tussock resistance site located in the Rowsley Valley of Victoria. This project aims at helping Government scope their future response to the serrated tussock resistance issue.

MATERIALS AND METHODS

Field component – serrated tussock sampling

A known serrated tussock population resistant to flupropanate occurs on a property in the Rowsley Valley of Victoria (37º41' 144º21') (McLaren *et al.* 2008). To assess whether serrated tussock within the general vicinity of this "resistant property" were also resistant to flupropanate, serrated tussock samples (a serrated tussock tiller with roots attached) were collected from within a 5 km radius (100 square kms) of this property during May 2008. The 100 square kms were gridded and assigned numbers (1-100). For a 2 km radius (16 square kms) surrounding the known serrated tussock resistant property, roadside and paddock collections of serrated tussock samples were made within each square km. For the additional 84 sq km away from the 16 kms selected around the affected property, 50% of the gridded sites were selected using a random number generator (42 sites x 10 serrated tussock plants = 420 plants). A further 2 sites were sampled on advice from local landowners. An additional 40 individual serrated tussock plants were collected from St Albans Victoria, (37º45' 144º47') during May 2008 that was previously known to have serrated tussock susceptible to flupropanate (Figure 2.). In total, 640 individual serrated tussock plants were assessed for flupropante resistance.

At each sampled location, 10 individual serrated tussock plant samples (tiller and roots) were collected and placed into labelled plastic bag recording date collected, location name and latitude/longitude. Samples were returned to DPI Frankston and each sample location was potted into two 15cm pots using standard potting mix (5 individual serrated tussock samples per pot). After potting, the serrated tussock plants had their leaves trimmed to aid in recovery after transplanting and were then grown for 3 months in a greenhouse at an average temperature of 20°C, watered on alternate days and were randomised fortnightly until plants were growing actively.

Application of flupropanate

The sampled serrated tussock plants were sprayed with Taskforce® (745 g a.i./L flupropanate) using a mechanical track sprayer in a spray cabinet with a standard flat nozzle (SS11002), to deliver a spray volume of 150 L/ha at 280 kPa at the recommended field rate (1.49 kg a.i./ha). Known flupropanate sensitive serrated tussock plants collected from St Albans were included in the experiment as controls. Assessment of flupropanate resistance was based on a visible injury scale of 0=healthy to 9=dead for each individual serrated tussock plant sampled. Assessments of flupropanate impacts to the surveyed serrated tussock samples were made at 89, 120, 173, 212 and 262 days after treatment (DAT).

RESULTS

Statistical Analysis

The sites plus untreated controls were analysed as a two replicate fully randomised one-way analysis of variance, with each pot being a unit of analysis (Payne 2006). The mean damage scores of each survey site, and of the untreated control, were compared to the St Albans treated control using 95% and 99% hypothesis tests using one-sided Dunnett's simultaneous comparisons (Miller 1981). These tests allow comparisons of many treatments with a control, while maintaining the nominal significance level.

The statistical analysis of flupropanate impact on the sixty serrated tussock sites surveyed from the Rowsley Valley 89 days after treatment are shown in Figures 1 and 2. Eleven out of the sixty surveyed sites (18%) were not significantly affected by the flupropanate treatment (99% probability) suggesting they were truly resistant to flupropanate. Similarly, an additional eight sites (12%) were not significantly affected by the flupropanate treatment (95% probability) suggesting they were very likely to be resistant to flupropanate. Thus, almost 30% of the sites surveyed in the Rowsley Valley were comparatively unaffected by the recommended (2 lt/ha) flupropanate treatment compared to the known flupropanate susceptible serrated tussock plants collected from St. Albans (Sites 61 and 62). Serrated tussock plants sampled from collection sites 22 and 54 died before application of the flupropanate treatment suggesting that they may have been already sprayed with herbicide at the time of collection.

A map of the survey region showing sites and results of the serrated tussock flupropanate survey is shown in figure 2. Two of the sites showing resistance (sites 44 and 45) come from the original property identified with resistance (figure 2). It can be seen that the resistance is quite widespread and is not isolated to a single property. Several "resistant" sites occur along Reids Rd (Sites 21, 20, 19, 23) and close to Glenmore Rd (Sites 34, 37, 53, 33, 25) (Figure 2). By the last assessment (day 262 DAT) 14 of the 60 flupropanate treated serrated tussock survey sites still had alive serrated tussock plants (Figure 3). The majority (11 out of 14) of these corresponded to those sites identified as resistant locations from assessment 1 (89 DAT).

DISCUSSION

This 100 square km serrated tussock resistance survey of the Rowsley Valley of Victoria has confirmed that serrated tussock resistance is far more widespread than had been previously thought. Approximately 20% of surveyed sites had significant survival to label rate flupropanate application (resistance) compared to known susceptible serrated tussock treated at the same time.

The Victorian Serrated tussock Working Party in collaboration with the Victorian Department of Primary Industries and affected local Governments have been driving a concerted serrated tussock compliance program with the ambitious aim of preventing all serrated tussock in Victoria from seeding. This program has been highly successful and it has been estimated that of the 130,000 ha of serrated tussock identified in Victoria in 1998, 45,000 ha are now under long term control (Osmond *et al.* 2008). The herbicide flupropanate, has been widely regarded as the best chemical control option for serrated tussock management as it has some selectivity (Campbell *et al*. 1979) and its residual action can prevent serrated tussock germinations for several years after application (Campbell and Vere 1995). The potential loss

of flupropanate as a control tool for serrated tussock due to resistance would severely limit control options for land managers and potentially set back the many serrated tussock management gains made by the Victorian Serrated Tussock Working Party.
The likely spread of serrated tussock flupropanate resistance will depend upon the nature of the inheritance, the breeding system and the amount of gene flow. The maternal nature of the inheritance, coupled with the high proportion of self-pollination (Harding 1983) and cleistogamy, has probably resulted in rapid establishment of resistant seeds among the field populations (Durai 2008). Coupled with a low persistent transmission of resistance by pollen up to many kilometres away, this suggests the likelihood that flupropanate resistance has already escaped from the original site to surrounding areas (Durai 2008).

Living with flupropanate resisitant serrated tussock:

1. **Rotate your herbicides.** There are currently three herbicides - flupropanate, glyphosate and 2,2-DPA registered for control of serrated tussock in pastures. If using chemical control, land managers should alternate the use of these herbicides from year to year. Both flupropanate and 2,2-DPA are Group J herbicides (Croplife Australia 2008). Land managers should be aware that *Sporobolus fertilis* plants resistant to flupropanate have also shown some resistance to 2,2-DPA (Ramasamy *et al*. 2008). Serrated tussock plants not dieing 6-12 months after flupropanate application (depending on rainfall) should be targeted for spot spraying with glyphosate or physical removal.
2. **Reduce serrated tussock population levels.** To reduce the likelihood of developing resistance, land managers should attempt to keep serrated tussock populations as low as possible. Fewer serrated tussock individuals will mean fewer chances of selecting resistant individuals. Increasing beneficial plant competition is a key factor in managing serrated tussock. Practicing good agronomy by using competitive pasture species with appropriate use of fertiliser, grazing management, disease management and weed control is critical. Mechanical control through chipping and cultivation are excellent ways of controlling serrated tussock and minimising resistance. Land managers should also consider crop/pasture rotations to help minimise resistance where appropriate.
3. **Stop serrated tussock seeding.** The key to serrated tussock management is reducing the seedbank. If land managers can prevent seed set for several years and there is little recruitment from surrounding properties, then the serrated tussock seedbank will decrease through time. In some situations slashing, burning or spray topping serrated tussock can be useful tools to reduce seeding. Using combinations of grazing to reduce the height of beneficial grasses and chemical wipers to apply herbicide selectively to serrated tussock is also a very useful tool. Development of new seed drill technology for rocky terrain (Rock-hopper – AgriCon Pty Ltd) is also providing more options for rehabilitation of what was previously non-arable land.
4. **Change land use:** In some difficult situations it may be better to change land use from pastures to Agroforestry (Campbell and Nicol, 1999) or to cropping (Osmond *et al.* 2008) or in some situations, if there is good competition, simply locking land up and removing grazing can be enough to provide enough competition to reduce serrated tussock dominance. In some difficult non-arable, situations it has been better to promote re-vegetation and competition using tea-tree to smother dense serrated tussock populations (Osmond *et al.* 2008).
5. **Importance of Integrated Control:** This survey has identified several new serrated tussock populations potentially resistant to flupropanate in Victoria. There is a real risk that flupropanate will become less effective if land mangers don't quickly change the way they are using it. The consequences are more herbicide usage, greater serrated tussock dominance, greater herbicide pollution, increased environmental damage and reduced profits for farmers. Land mangers need to consider mechanical control, cropping rotations, pasture rehabilitation and grazing management to reduce the likelihood of resistance. A common theme with herbicide resistance is that weeds will quickly adapt through natural selection if they are constantly exposed to the same management technique (Warwick 1991). Land managers need to confuse the weed by applying a range of different weed

management techniques. This survey reinforces the need to practice integrated weed management to control serrated tussock.

Recommendations

1. Produce a serrated tussock flupropanate resistance brochure highlighting what land managers should be looking for if they suspect resistance on their property. This brochure will also highlight what actions should be undertaken by land managers to reduce the impact of serrated tussock flupropanate resistance.
2. Conduct an extension program within the Rowsley valley district to inform land managers of the serrated tussock resistance issue.
3. Provide land managers within the affected region with a copy of the Serrated Tussock Best Practice Management manual that documents a range of integrated serrated tussock management options.
4. Contact and inform local Shires, Councils, Parks and herbicide contractors about the flupropanate resistance issue.
5. Prioritise identified flupropanate resistance serrated tussock locations for spot treatments using glyphosate.
6. Conduct a state-wide serrated tussock resistance survey.
7. Where deemed appropriate, support for awareness and extension packages to affected land managers and/or ongoing research into new management techniques including the use of classical/inundative biological control of the serrated tussock seed-bank using soil borne pathogens.

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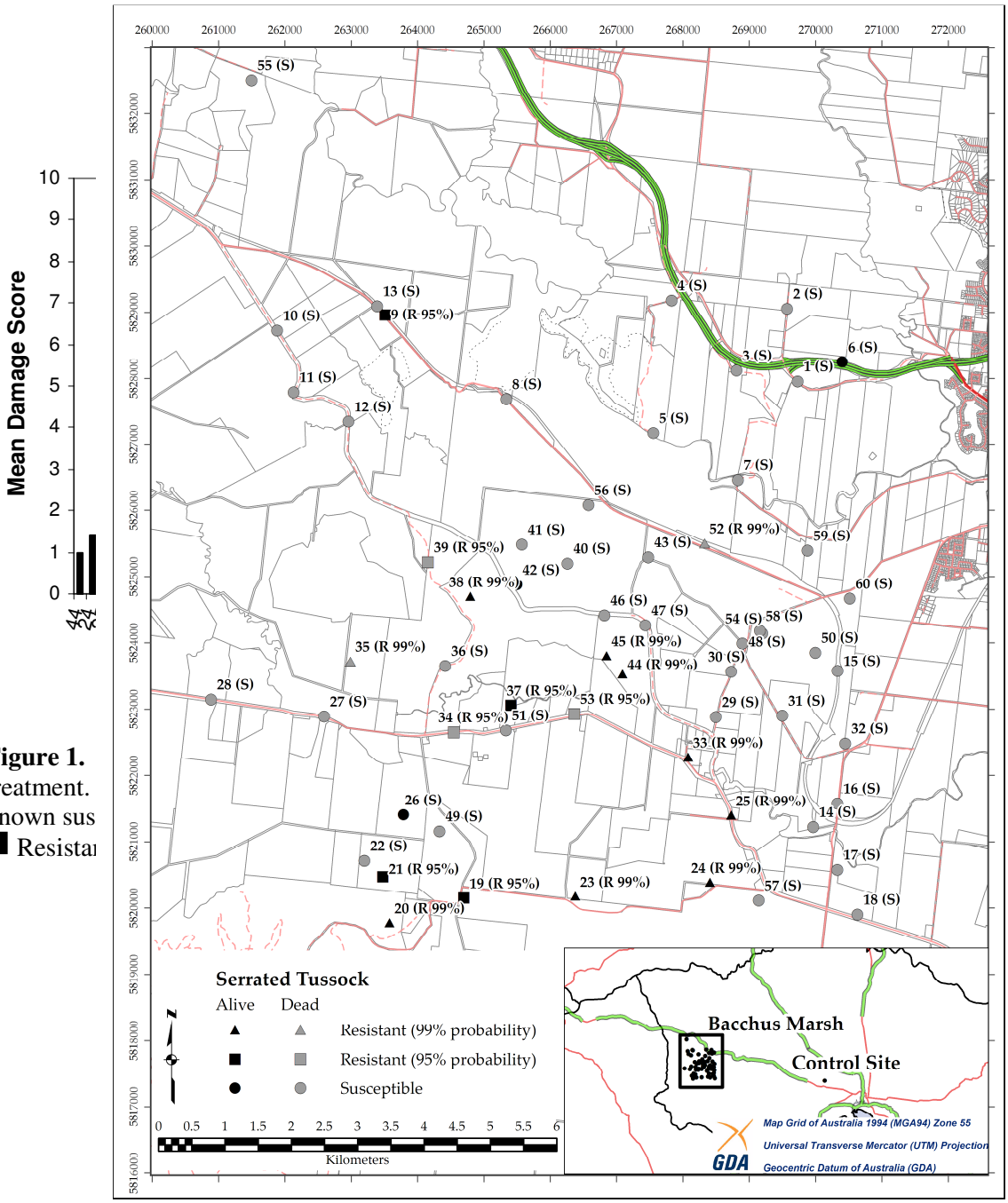

Figure 1. treatment. known sus ■ Resista

Figure 2. Map of serrated tussock resistance survey locations and resistance probabilities after treatment.

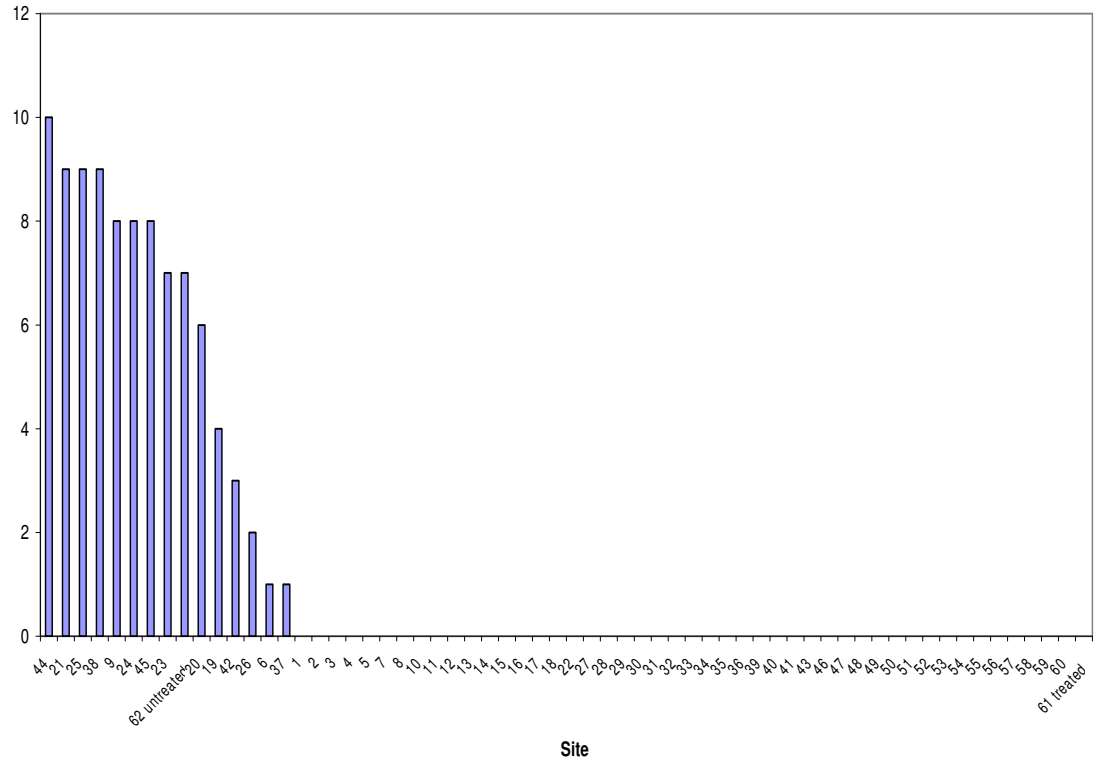

Figure 3. Number of surviving serrated tussock plants 262 DAT. (10 = all surviving).