

Field survey of flupropanate-resistant *Nassella trichotoma* in Victoria

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Summary: A population of *Nassella trichotoma* (serrated tussock) in Victoria was confirmed as resistant to flupropanate (sodium 2,2,3,3-tetrafluoropropionate) at a property north-west of Melbourne in 2002. To determine the spread of flupropanate resistance, *N. trichotoma* plants were collected randomly from 87 sites in paddocks and on roadsides within 5 km of the resistant plants in 2003. The plants were grown in the glasshouse until maturity and assessed for flupropanate resistance by a pot-dose method. Plants resistant to flupropanate were found up to 3.5 km from the site where resistant plants were initially identified. Seeds from these plants showed 0-100% resistance, with sensitive plants often having a low ($\leq 5\%$) of resistant seed. These results suggest movement of flupropanate resistance through seeds or pollen and shows that its spread had occurred within one year of detection. This research emphasizes the importance of integrated weed management and the need to rotate herbicide groups to avoid the risk of resistance.

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

INTRODUCTION

Serrated tussock (*Nassella trichotoma* (Nees) Hack. ex Arechav) is a perennial noxious weed of South American origin and is a Weed of National Significance in Australia (Thorp and Lynch 2000). It invades native grasslands and has huge impacts on agricultural production and the environment. The potential distribution of serrated tussock, based on current infestations, has been estimated at 32 million ha (McLaren *et al.* 1998). Serrated tussock costs \$5m pa to the Victorian (Nicholson *et al.* 1997) and \$40.3m pa to the New South Wales (Jones and Vere 1998) economies. Serrated tussock also costs \$12.9m pa to the Australian Wool Industry (Cook and King Pty Ltd 1988).

Flupropanate, glyphosate and 2,2-DPA are the only three herbicides registered for serrated tussock control. Flupropanate (sodium 2,2,3,3,-

tetrafluoropropionate) is considered the most effective herbicide because of its selectivity (Campbell and Gilmour 1979). In addition, the long residual action of flupropanate in soil inhibits the germination and growth of seedlings (Campbell *et al.* 1979). Flupropanate belongs to the Group J herbicides, which act on lipid synthesis and were thought to be at low risk of herbicide resistance (Avcare 2000).

In 2001, a population of serrated tussock from Diggers Rest, Victoria was suspected of being resistant to flupropanate. Dose-response studies and seedling trials confirmed flupropanate resistance to at least four times the recommended dose (Noble *et al.* 2005). The mechanism of resistance is not known, nor is its heredity. The spread of flupropanate resistance from the original site by seed or pollen transport would pose dangers for eradication of the resistant population. Early detection of its spread and extent would help to develop management strategies to combat the risk of resistance.

The current study was undertaken to determine the extent of the spread of resistant serrated tussock into the surroundings area from the site of documented flupropanate resistance.

MATERIALS AND METHODS

A field survey was carried out in Diggers Rest, Victoria, within a 5 km radius of the spot where flupropanate resistance was suspected in 2001 and confirmed in 2002 (Noble *et al.* 2005). Serrated tussock plants were collected randomly from 87 sites in paddocks and on roadsides in Diggers Rest between July and November 2003. Plants were potted in 10 cm diameter pots with standard potting mix and grown on in a glasshouse with natural lighting and heating at DPI Frankston. After 8 months, groups of tillers were separated from each plant, potted into 10 cm pots with standard potting mix and left to establish for 4 months.

Plant tests: Three replicate pots of tillers of uniform size (girth and height) from each original serrated tussock plant were selected for the

experiment. Serrated tussock plants from five of the sites (Sites 69, 72, 73, 76, and 77) were not tested due to insufficient plant material. The remaining 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 (SS10002), to deliver a spray volume of 150 L/ha at 280 kPa at the recommended field rate (1.49 kg a.i./ha). Control plants were sprayed with water only. Leachates from the treated plants were collected and replaced into the pots. Known resistant and sensitive serrated tussock plants, originally collected from Diggers Rest and St Albans respectively, were included in the experiment as controls. Plants were grown for 4 months in a greenhouse at 21-25°C with 12 h light/dark respectively, watered on alternate days and randomised fortnightly. Assessment of flupropanate resistance was based on a visible injury scale of 0=healthy to 10=dead, as followed in other resistance studies (Falk *et al.* 2005; Preston 2006).

Seed assay: Seeds were collected from each plant established in the glasshouse in 2004-5. Plants were housed within one glasshouse and free pollination was allowed. Seeds were germinated and seedlings grown in glass Petri dishes (90 mm diameter) with seed test paper (Whatman 182). A concentration of 40 mg/l (30 mg a.i./l) (equivalent to 8 l/ha, 4 times the recommended rate) was prepared from Taskforce and 5 ml of the solution added to each Petri dish. Twenty-five firm seeds from each plant were placed in each Petri 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 flupropanate resistance and seedlings were classified as resistant or sensitive (Ramasamy *et al.* 2007). Experiments were arranged in a randomized design with four replicates per treatment.

RESULTS

Plant tests: Plants from nine (10%) sites from up to 3.5 km from the original site survived the recommended field rate of flupropanate (Fig. 1). In three sites, separate plants from the same site behaved differently, some plants dying and others surviving. Plants from six of the sites (Sites 7, 12, 25, 30, 39, and 83) showed varied results within the replicates of one plant, with one or two of the three replicates dying and the other one or two surviving. Sensitive biotypes were killed by the normal field dose of flupropanate. All sensitive plants exhibited the expected browning symptoms after 2 months, including sensitive control plants from St Albans. None of the resistant control plants from Diggers Rest was affected by flupropanate.

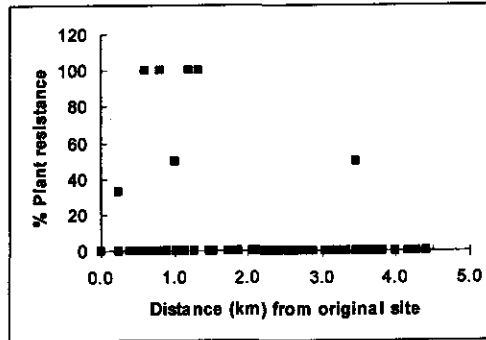


Fig. 1. Variation in resistance of plants collected at different distances from the original flupropanate-resistant plants.

Seed tests: Resistant seedlings were produced from seeds collected from plants up to 3.5 km from the original site (Fig. 2). Seeds from three plants within 0.6 km from the same paddock as the original site produced seeds with 100% resistance, but only 33% of sensitive plants produced only sensitive seedlings. Most (45%) of the remainder produced seeds with $\leq 5\%$ resistance, but four produced up to one-third resistant seed (Fig. 3). Resistant plants also did not all produce only resistant seed. There was only a weak relationship between plant and seed resistance (Fig. 4). Seeds from control plants behaved as expected, with 100% matching to the parent plants.

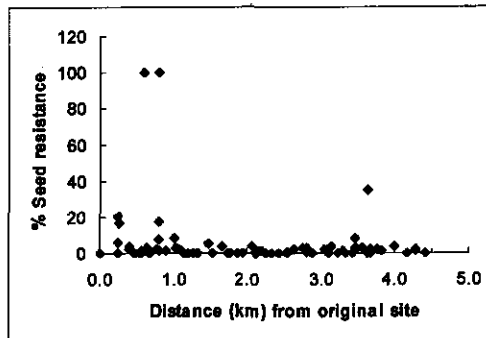


Fig. 2. Variation in resistance of seedlings from seeds of plants collected at different distances from the original flupropanate-resistant plants.

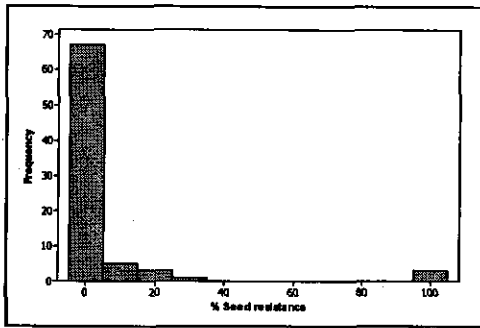


Fig. 3. Histogram showing frequency of resistance in seedlings from seeds of plants collected at different distances from the original flupropanate-resistant plants.

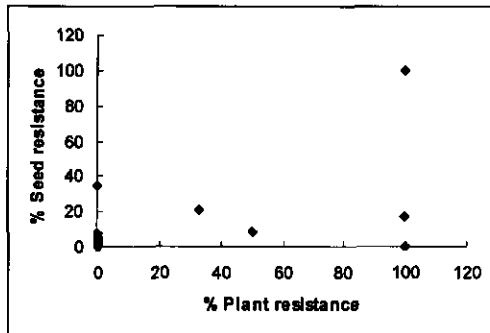


Fig. 4. Relationship between resistance of glasshouse-grown plants and seeds collected from them.

DISCUSSION

This flupropanate resistance survey suggests the movement and establishment of resistance as far as 3.5 km from the location where resistance was first identified in 2001 (Noble *et al.* 2005). This suggests that eradication may already be too late to prevent the spread of resistance to other locations. Nine property managers across Australia suspected flupropanate resistance in serrated tussock in a mail survey (McLaren *et al.* 2006) and three of those sites have now been confirmed as flupropanate-resistant, surviving 13.5 kg a.i./ha (D.A. McLaren, pers. comm.). It is now appropriate to assume that resistance has already spread around the properties identified in that survey too and to take appropriate remedial action.

The occurrence of resistant plants close to the original resistance site strongly suggests the flow of resistance genes via seed or pollen. Farm machinery and livestock were probably responsible for seed movement within the paddock and even along the roadsides, but seeds of serrated tussock can travel as far as 16 km in the direction of the prevailing wind

(Healy 1945). Pollen grains carrying resistance genes could also travel many kilometres, e.g. pollen of genetically modified creeping Bent Grass (*Agrostis stolonifera*) was recorded up to 55 km away within 3 h (Van de Water *et al.* 2007).

The high proportion of sensitive plants with even a low percentage of resistant seeds suggests that resistance genes exist in the population collected around the site where resistance was first confirmed. Breeding was not controlled in the glasshouse housing all the plants and so a mixture of self- and cross-pollination would have occurred (Taylor 1987). Controlled breeding experiments in serrated tussock (unpublished data) have shown that flupropanate resistance is governed by both nuclear and cytoplasmic genes, with pollen able to transmit resistance to progeny of sensitive plants.

Contradictory results from testing plants collected at the same site can be explained by a mixture of resistant and sensitive plants at that site. It is more difficult to explain the contradictory results from testing groups of tillers from what appeared to be the same plant in the field. Re-testing of these samples is currently in progress and may bring clarification.

The findings of this study reinforce the need to practice integrated weed management to control serrated tussock. The implications of serrated tussock herbicide resistance are its increased dominance as a weed, increased costs for land managers, more herbicide usage and higher environmental pollution as a consequence.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support provided by the Australian Commonwealth Government through the Natural Heritage Trust and CRC for Australian weed management.

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