Heritability of flupropanate resistance in *Nassella trichotoma*

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Summary Extensive use of flupropanate over the last 20 years has selected for resistance in *Nassella trichotoma* (serrated tussock) across Australia. To determine its genetic basis, reciprocal crosses were made between resistant and sensitive parents. In the F_1 generation, both sensitive and resistant plants produced seedlings true to type when mated with the same type and 7–10% of the opposite type when mated with the opposite type. The heritability of flupropanate resistance from both the parents suggests a genetic origin. The 80–90% matching of seedling type to maternal parent type strongly indicates mostly a maternal component in the inheritance, with a minor proportion through pollen.

Keywords *Nassella trichotoma*, heritability, resistance, flupropanate, maternal inheritance.

INTRODUCTION

Herbicide resistance is an evolutionary phenomenon in which the gene frequencies within a plant population change as a result of selection pressure by chemicals, due to mutation, breeding system and gene migration (Slatkin 1987). The breeding system of a species determines the genotypic frequencies of its perpetuating population in each generation. Most weed species are highly self-pollinated and the spread of resistance is therefore reduced in autogamous plants (Jasieniuk *et al*. 1996).

Flupropanate is a Group J herbicide that is a selective herbicide for the control of serrated tussock and other unpalatable weedy grasses (Campbell and Vere 1995). It is considered to present a low risk for resistance, but the extensive usage of flupropanate has resulted in flupropanate resistance in serrated tussock, with populations surviving application rates as high as 8 L ha^{-1}, four times the recommended rate (Noble *et al*. 2005). A national mail survey conducted by McLaren *et al*. (2006) for flupropanate resistance identified nine properties that had serrated tussock suspected of flupropanate resistance, four of which were confirmed by seed tests (McLaren *et al*. 2008) suggesting establishment of resistant plants in the field. A local survey conducted in Victoria showed that resistant plants were found up to 3.5 km from the original site of discovery (Ramasamy *et al*. 2008), which suggests a likely movement of genes or simultaneous natural mutations. Understanding the inheritance of flupropanate resistance would help to predict the likelihood of resistance spreading in the future. This study therefore investigated the inheritance of flupropanate resistance in controlled and natural pollinations between resistant and sensitive serrated tussock in pot trials.

MATERIALS AND METHODS

Origin of parent plants Resistant serrated tussock parent plants were originally collected from Diggers Rest (North-west of Melbourne, Victoria) in 2001 and type was earlier confirmed in seed and pot trials (Noble *et al*. 2005). Sensitive serrated tussock plants were collected from Victoria University, St Albans Campus, Victoria, and type confirmed in earlier pot trials. All resistant and sensitive plants used in breeding experiments were cloned from these plants. Tillers were potted into 12 cm diameter pots with standard potting mix and left to establish. Plants were irrigated and fertilised, as needed, during growth to maturity in a glasshouse at 15–25°C and natural daylight.

Manual crossing (2004) Five resistant and five sensitive plants were transferred into separate glasshouses with the same environmental conditions to minimise contamination with foreign pollen. Manual breeding was performed during Oct–Dec 2004. All crosses are described as female × male parent (♀ × ♂); S is used to designate sensitive and R to designate resistant parent plants.

Panicles selected for crossing were supported by bamboo sticks and covered by brown paper bags on the previous evening to avoid contamination by foreign pollen. The florets were very small (1–2 mm) with a bifid feathery stigma. Emasculation followed by pollination was performed early in the morning in florets that had anthers extruded but not dehisced. Pollen grains were collected from the donor parents and dusted on the stigmatic surface of emasculated floret using fine needles. All pollinating tools were

sterilised in 70% ethanol. The crossed florets were tagged and protected with paper bags. The mature seeds were harvested inside the bags and stored at ambient temperature for 3 months to overcome dormancy before testing.

Selfing Three resistant and three sensitive plants were selfed by covering the panicle with a paper bag prior to anthesis and leaving it undisturbed until the seeds matured. Seeds were collected and stored as before. Three resistant and three sensitive plants were also grown in a separate glasshouse in close proximity to allow natural pollination. During anthesis, the panicles were manually shaken every morning to supplement the pollination. Once flowering was complete, the resistant and sensitive plants were separated to avoid seed contamination. Mature seeds were harvested and stored as before.

Natural crossing (2005) The manual crossing (2004) produced only limited seeds and so natural crossing was used to obtain a greater number of seeds. Ten resistant and ten sensitive plants were placed inside a rectangular cage (95 × 145 × 95 cm) covered by white muslin. To circulate pollen grains, a fan was fixed under the roof of the cage facing downwards; an automatic timer operated the fan for 8 h, from 6 a.m. to 2 p.m., and plants were shaken manually every morning. Once flowering was complete, resistant and susceptible plants were moved to two separate benches in the same glasshouse to prevent seed contamination. The seeds of both were harvested and stored as before.

Seedling assay for F_1 identification Firm seeds were tested for resistance, in three replicate 9 cm diameter glass Petri dishes with 10 seeds each. Two control samples, of known resistant and sensitive seeds, were also included (Noble *et al*. 2005). F_1 seeds were placed on Whatman 182 seed test paper moistened with 5 mL of 40 mg L^{-1} (30 mg a.i. L^{-1}) flupropanate as Taskforce®. Shoot length (mm) was measured after 14 days at 25°C to distinguish the sensitive from the resistant biotypes (Noble *et al*. 2005). Seedlings were categorised as sensitive if the shoot length was <10 mm, whereas resistant seedling shoots were up to 60 mm long. It was assumed that seeds from manual crosses were true hybrids. Seedlings were recovered and transplanted into trays with a standard seedling raising mix and grown at 25°C with a 12 h photoperiod of 270 μmoles m^{-2} s^{-1} light provided by halide lamps for 3–4 months. Surviving seedlings were transferred to 6 cm diameter pots with potting mix to produce F_2 seeds.

RESULTS

Manual crosses (2004) Plants in all treatments produced seeds. Flowering depended on the prevailing atmospheric weather condition; only 5–10% of the florets bloomed on sunny days and none on cloudy days. The small size of florets and thin pedicels made crossing difficult and about 30% of unopened florets were lost during the emasculation and tagging process. All seeds tested germinated and resistance ranged from 0–100% (Table 1). All selfed seedlings behaved true to their parents, in that all sensitive plants produced only sensitive seedlings and vice versa. Naturally pollinated sensitive plants produced 10% of resistant seedlings and resistant plants produced 17% of sensitive seedlings. Manually crossed plants showed similar results to naturally pollinated plants, although the seed yield was less. Both sensitive and resistant plants produced seedlings true to type when mated with the same type. Sensitive plants produced 10% of resistant seedlings and resistant plants produced 7% of sensitive seedlings.

Only resistant seedlings survived transplantation to pots, but they did not flower in 2005 or 2006 and so it was impossible to raise an F_2 generation within the period of study. Sensitive seedlings deteriorated and died in trays within 2 weeks.

Natural crosses (2005) The number of seeds tested per plant varied six-fold because plants varied in their seed production. Three of each biotype produced chaffy ill-filled seeds that were excluded from seed testing. Seed germination varied from 99–100%. All selfed seedlings behaved like the parents. Reciprocal crosses produced reciprocal results, in that crosses with the sensitive female parent produced 15% (7.5–21.3%) resistant seedlings and crosses with the resistant female parent produced 84% (81.2–88.9%) resistant seedlings (Table 2). The results produced in this experiment were similar to the manual crosses. Sensitive seedlings in both the crosses died within 2 weeks of germination. All the surviving resistant seedlings in both the crosses were transplanted into pots to raise an F_2 generation, but none flowered in 2007 or 2008, and so it was not possible to study the F_2 generation within the period of the study.

DISCUSSION

The heritability of flupropanate resistance from both parents strongly suggests a genetic origin. The 80–90% matching of seedling type to maternal parent type strongly indicates a maternal component in inheritance, with a minor proportion of resistance heritable through pollen. It is therefore hypothesised that the maternal cytoplasm of the female parent plays

Table 1. Resistance to flupropanate (30 mg a.i. L^{-1}) in seedlings from manual crosses, selfing and natural pollination in 2004. All crosses were female × male, R = resistant, S = sensitive. Data are counts.

	Manually crossed				Selfed		Naturally pollinated	
Treatment	S × S	S × R	R × S	R × R	S	R	S × R	R × S
No. of resistant seedlings	0	3	28	30	0	30	3	25
No. of sensitive seedlings	30	27	2	0	30	0	27	5
% resistant	0	10	93	100	0	100	10	83

Table 2. Resistance to flupropanate (30 mg a.i. L^{-1}) in seedlings from natural crosses and selfed plants in 2005. All crosses were female × male, R = resistant, S = sensitive. Data are counts.

		Seeds	Germinated progeny					Seeds	Germinated progeny		
Cross	Plant	tested	R	S	% R	Cross	Plant	tested	R	S	% R
S × R	A	109	19	88	17.7	R × S	A	22	18	4	81.8
	B	134	13	121	9.7		B	145	129	16	88.9
	C	145	31	114	21.3		C	143	119	24	83.2
	D	135	22	113	16.2		D	128	104	24	81.2
	E	26	3	23	11.5		E	121	102	19	84.2
	F	122	21	101	17.5		F	120	100	20	83.3
	G	146	11	135	7.5		G	23	19	4	82.6
	Total	817	120	695	14.7		Total	702	591	111	84.1
S_{selfed}		30	0	30	0	R_{selfed}		30	30	0	100

a significant role in the transmittance of flupropanate resistance. The minor transmission of resistance via pollen in all crosses suggests transmission also by a component in the pollen grains.

The inheritance of flupropanate resistance in serrated tussock is markedly similar to the strong maternal inheritance of resistance in the Group C herbicides (e.g. the azines). Inheritance was 90% associated with the female parent, but with a small percentage of the resistance associated with the male parent, especially with triazine in *Echinochloa crus-galli* (Gawronski 1985). The source of maternal resistance is normally the chloroplast and the source of resistance in pollen is normally the plastid (Darmency and Gasquez 1981). The strong non-Mendelian maternal inheritance here suggests a cytoplasmic origin, most likely in the chloroplast or mitochondrion, especially if the pollen grains carry plastids. Plastids have been recorded in mature pollen grains in grasses (*Lolium perenne* mature pollen grains contain 550–820 amyloplasts) (Pacini *et al*. 1992). The minor variation in the proportion of the opposite type in progeny of both crosses suggests a small percentage of transmission via plastids in pollen grains (Singh 1990).

The spread of resistance depends upon 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, probably results in rapid establishment of resistant seeds among the field population. Personal observations during the study revealed that 65% of the florets in a panicle were chasmogamous and 35% cleistogamous, but only 10–15% of the florets bloomed and only on sunny days. The proportion of cleistogamous and chasmogamous florets is highly correlated with environmental parameters, including soil moisture and humidity, before and after flowering (Taylor 1987). In field conditions, less than 1% of the florets opened in inflorescences, suggesting self-pollination as the dominant mode of reproduction in serrated tussock (Harding 1983). This would accelerate the establishment of resistance in a population from an initial mutation.

The migration of pollen grains and seeds carrying resistance will accelerate the rate of spread across field populations. Serrated tussock seeds travelled up to 15 km (Healy 1945) from the point of origin, with a high possibility of seed dispersal even further through machinery and livestock. Pollen grains of grasses can travel many kilometres in optimal weather conditions. Van de Water *et al*. (2007) recorded a maximum gene flow distance of 21 km through pollen grains in genetically modified bent grass (*Agrostis stonifera*). How far pollen can travel and still be viable depends on climate and proximity of receptive plants.

High maternal inheritance, coupled with high self-pollination, has probably resulted already in the build-up of a flupropanate-resistant seed bank in the field population and flupropanate resistance has already escaped from the original sites to surrounding areas. Adopting integrated weed management techniques to manage serrated tussock infestations would reduce the development of new resistant populations; otherwise the spread of resistance is inevitable.

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