WHITE CLOVER (*TRIFOLIUM REPENS*) RESISTANCE TO 2,4-D

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Initial screening of white clover biotypes collected from pastures and tested for susceptibility to 2,4-D showed significant differences between them. Subsequent tests showed that initial damage caused by 2,4-D was similar in both resistant and susceptible biotypes, but that the resistant biotypes then showed strong recovery. Differences in recovery rates would be even greater in a competitive pasture. Herbicide resistant biotypes may be useful as pasture components, and herbicide damage to regularly treated pastures may be less serious than it used to be, because of the development of clover resistance.

Keywords: phenoxy-herbicide resistance, white clover, *Trifolium repens*, pasture tolerance.

INTRODUCTION

The development of herbicide resistant biotypes of nodding thistle (*Carduus nutans*) and giant buttercup (*Ranunculus acris*) following repeated annual applications of MCPA or 2,4-D (Harrington *et al* 1988; Bourdôt and Hurrell 1988) suggests that biotypes of white clover (*Trifolium repens*) living in pastures regularly treated with phenoxy herbicides may also have developed resistance. Development of white clover resistance could be associated with weed resistance at some locations. This paper describes the collection and testing of white clover biotypes for resistance to 2,4-D.

METHODS

On 18 December 1989, stolon material of white clover was collected from pastures in the southern North Island (Table 1, A - R). Some of these pastures contained nodding thistles whose susceptibility to phenoxy herbicides was already known. Material was screened for tolerance to 2,4-D. Single nodes of each stolon were planted into soil mixture in polystyrene cups and grown in a glasshouse. Plants were watered regularly and treated with insecticides and fungicides as needed. The 18 biotypes were arranged in 12 randomised blocks. On 13 February 1990 all clover plants were placed in a laboratory spray booth and sprayed with 2,4-D amine at 1.5 kg/ha, in 370 litres water/ha, using a single nozzle (Teejet 730231). The plants were assessed on 6 March (21 days after spraying), using a 0 (dead) to 10 (most vigorous in the trial) scale, and re-assessed on 9 April (55 days after spraying), using a 0 (dead) to 5 (most vigorous) scale. On 14 February, stolon material of white clover collected from Takaka pastures (Table 1) containing susceptible (T) or resistant (S) giant buttercup was similarly planted and grown, and treated with 2,4-D amine on 30 April. The two biotypes were arranged in 10 randomised blocks. These plants were assessed on 7 June (38 days after spraying), on a 0 to 10 scale as before.

Five white clover biotypes (two plants which scored 9 in the first and 5 in the second of the above evaluations - 121A and 18B, and three from parent plants believed to be susceptible - 8J, 10E, 14E) were selected for further study and propagated by tissue culture. About 10 plants from each biotype were planted individually in 375 ml polystyrene cups on 10 January 1992 and a further 40 from each biotype planted on 21 February. All were grown in the glasshouse, watered regularly, and treated with fungicides and insecticides as needed. On 4 March, by which time each plant had covered the soil surface of its pot, all plants were lightly trimmed with scissors. On 13 March 10 plants of each biotype were treated with 0.5, 1, 2, 4 or 8 kg 2,4-D ester/ha (Dow Elanco Hi-ester 2,4-D, 72% ai) in the laboratory spray booth. A five replicate

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latin square factorial trial was set up in a shadehouse with each 'plot' comprising a pair of similarly treated pots of each biotype. On 1 April (19 days after spraying) each plant was assessed for injury symptoms on a 0 (no damage) to 10 (most damaged in the trial) scale. On 11 May (59 days after spraying), a further assessment was carried out of plant vigour on a 0 (dead) to 10 (most vigorous plant in the trial) scale. Ten untreated plants were also assessed.

RESULTS

In the screening trial, results varied considerably within each biotype, and there was no significant difference between the average score for each biotype. However, Table 1 shows that biotypes differed widely in the percentage of plants scoring 5 or less in the first assessment. Some white clover biotypes were clearly more resistant to 2,4-D than others. Of those plants which scored 5 or less, 42% were from sites with resistant weeds and 56% from sites with susceptible weeds. There were, however, some anomalous results.

TABLE 1: Percentage of plants scoring 5 or lower in screening trial, March 1990. Scoring scale: 0 = dead, 10 = very vigorous.

Biotype	Source	%	Biotype	Source	%
E ²	Ohiti	83	J ¹	College Rd	50
H	Hickey Rd	83	M ²	Mason Ridge	50
D	Linton	75	O ¹	Glenalvon	50
K ¹	Argyll Rd	67			
A	Opapa	58	G ²	Awanui	45
C ¹	Tauhara	58	T ²	Jones	40
F ¹	Te Onepu	58	N ¹	Raukawa	33
I ¹	Waikareao	58	Q ¹	Kia Ora	25
L ²	Matapiro	58	B ¹	Argyll Rd	25
P ²	Ohutu	58			
R	Poukawa	58			
S ¹	Silcock	55			

^{1,2} Plants collected from pastures where nodding thistles or giant buttercup were resistant¹, or susceptible² to 2,4-D or MCPA (Bourdôt and Hurrell 1988; Harrington 1989).

At the first assessment of 2,4-D rates and clover biotypes, at 19 days after spraying, one biotype (18B) had an average score of 3.5, significantly lower than the average score of 4.1 for the others. There was no significant interaction between herbicide rate and biotype, and the effect of 2,4-D rates on average scores, across all biotypes, are shown in Table 2. Damage scores increased with herbicide rate in all biotypes.

TABLE 2: Average damage scores (0 = no damage, 10 = worst damage) at different rates of 2,4-D ester, across all clover biotypes, 19 days after spraying.

Rate (kg/ha)	0.5	1.0	2.0	4.0	8.0	SEM (0.05)
Score	1.3	2.6	4.1	5.3	6.8	0.12

By the time of the second assessment, 59 days after spraying, biotypes differed in their recovery rates (Table 3), and herbicide rate by biotype interactions were significant. Biotype 121A was less affected by 2,4-D than the others. Biotypes 10E

and 14E were more susceptible than the others, and 8J and 18B were intermediate.

TABLE 3: Average vigour scores (0 = dead, 10 = very vigorous) for clover biotypes 2 months after spraying.

Biotype	Rate of 2, 4-D ester (kg ai/ha)					Mean
	0.5	1.0	2.0	4.0	8.0	
121A	7.1	7.1	5.7	5.5	4.8	6.0
18B	4.1	3.3	3.2	3.3	3.1	3.4
8J	6.9	6.2	5.7	4.1	3.6	5.3
10E	5.9	4.7	3.2	2.6	1.2	3.5
14E	4.8	3.6	2.5	1.9	0.9	2.7
			SEM (0.05)			0.19

DISCUSSION

White clover, in monoculture, was relatively hard to kill with 2, 4-D. Even susceptible cultivars, although badly damaged by 8 kg/ha, were not completely killed within 2 months. Biotypes selected for apparent tolerance to 2,4-D were much more resistant than other cultivars. In a competitive pasture, damage due to 2,4-D would probably be very much worse, and recovery after spraying would be much slower. Both susceptible and resistant biotypes showed similar initial damage to 2,4-D. Resistant and susceptible biotypes of nodding thistle and giant buttercup also show similar initial effects of phenoxy-herbicide application (K. Harrington and G. Bourdôt, pers. comm.)

Results suggest some relationship between herbicide tolerance in clovers and in weed species in the same paddock. Differences between white clover and weed species in seed bank size, seed dormancy, plant emergence and growth patterns may affect development of herbicide resistance.

Reduced plant and animal productivity caused by MCPA or 2,4-D application to pasture have been measured in the past (Meeklah 1958; Lewis 1956) and have been attributed to reduced pasture quality and lower availability of fixed nitrogen. These effects may have become smaller over the years on pastures regularly treated with herbicides if the clover component of the sward has developed herbicide resistance. Herbicide-resistant cultivars of white clover could be usefully sown in pastures likely to be regularly sprayed. Both 2,4-D and MCPA are very similar in chemical structure and nodding thistle biotypes resistant to one are usually resistant to the other. Although testing is required, 2,4-D resistant white clover is probably also resistant to MCPA.

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