EVIDENCE FOR THE HERITABILITY OF MCPA- RESISTANCE IN GIANT BUTTERCUP (*RANUNCULUS ACRIS*)

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SUMMARY

Two experiments were conducted in a glasshouse at Lincoln. In experiment one, the MCPA-responses of seedlings of giant buttercup (*Ranunculus acris* L. subsp. *acris*) from three waste-place populations and a resistant dairy-farm population, were compared. The LD₅₀ values of the waste-place populations were 0.09, 0.28 and 0.36 kg ai/ha MCPA and 1.25 kg/ha for the dairy-farm population. In experiment two, seedlings from an MCPA-susceptible population were treated with the LD₅₀ dose MCPA. The LD₅₀ of the progeny of a polycross between the six least affected survivors was found to be, 0.68 kg ai/ha MCPA; 2.4 times greater than the LD₅₀ of the unselected controls. These results support the hypothesis that MCPA-resistance in giant buttercup is inherited.

Keywords: giant buttercup, *Ranunculus acris* L. MCPA resistance, herbicide resistance, inheritance

INTRODUCTION

Variation in the control of field populations of giant buttercup (*Ranunculus acris* L. subsp. *acris*) by MCPA (Popay *et al* 1984; Bourdôt and Hurrell 1990) lead to the suggestion that resistance to this herbicide may have developed in dairy pastures. Subsequent comparisons of the MCPA-responses of the seedling progeny of field populations from the South and North Islands, under uniform glasshouse conditions, confirmed that these populations varied genetically in their resistance to the herbicide (Bourdôt and Hurrell 1988; Bourdôt *et al* 1989a; Bourdôt *et al* 1990a). Correlations between the level of resistance to MCPA and degree of control obtained with this herbicide in dairy pastures, implicated resistance as the likely reason for poor control (Bourdôt *et al* 1990a, 1990b). Furthermore, the level of resistance in field populations is correlated with the level of historical exposure of the populations to MCPA, suggesting that the resistance has developed as a consequence of selection pressure exerted by the herbicide (Bourdôt *et al* 1990a, 1990b). The evidence for the latter is compelling and assumes that the resistance is inherited by the progeny of resistant individuals in a susceptible population.

The current contribution provides direct evidence, by way of a selection experiment, that resistance is inherited by the progeny of resistant plants. It also compares the MCPA-responses of roadside populations, unexposed to MCPA, with the response of a known resistant population from a dairy farm, providing further support for the correlation between historical exposure to MCPA and resistance to this herbicide.

MATERIALS AND METHODS

Experiment 1

Seeds of giant buttercup were collected from waste-place populations at Peel Forest (PF), Travis Swamp (TS) and Saltwater Creek (SC) in Canterbury in 1990, and from a known resistant population from the Silcock dairy farm in Takaka (S) in 1988. The waste-place populations had never been exposed to MCPA while population S had a long history of regular exposure (Bourdôt *et al* 1990b). After soaking the seeds overnight in 0.02% KNO₃ solution on 4 May 1990, they were dusted with a 1:1.6

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mixture of the fungicides benomyl (Benlate) and captan (Captan) and placed on moistened vermiculite germination beds at 20°C day/10°C night. On 5 June, one to two-leaf seedlings of each population were transplanted into plastic pots containing 2 litres of a sterilised bark-based potting mix.

There were 32 pots of populations PF, SC and S, and 12 pots of TS, with four seedlings per pot. The potted plants were placed in a glasshouse maintained at 20°C during the day and unheated at night. On 10 August, when they had produced five to eight leaves, plants were treated with a range of concentrations of the K salt of MCPA (IWD MCPA). For populations PF, SC and S, there were four pots of each of eight concentrations ranging from 0.198 kg ai/ha to 3.375 kg ai/ha, spaced according to a 1.5 multiplier. For population TS, there were two pots per concentration and the two highest rates were excluded. The MCPA was applied with a moving-belt sprayer delivering 200 litres/ha at 210 kPa through a single 8002E flat-fan Teejet hydraulic nozzle mounted 50 cm above the pots. The pots were arranged in the glasshouse in a randomised block design.

On 1 November 1990, 82 days after treatment, the number of deaths was determined as the sum of the number of obviously dead plants and the number deemed unlikely to survive (Bourdôt *et al* 1990a). The mortality data were analyzed using a generalised linear model (Dobson 1983) which fitted a probit curve to the graph of percent mortality vs log MCPA dose for each population. The model included terms which estimated block effects.

Experiment 2

Seeds collected in December 1988 from a MCPA-susceptible population of giant buttercup from the Jones farm in Takaka (J) and from the resistant S population, were germinated as in Experiment 1. On 23 May 1989, c. 200 cotyledonary seedlings of J and S were transplanted into small pots containing a 1:1 blend of peat and river sand, adjusted to pH 6, with nutrients added as slow-release capsules. Ridomil MZ 72 WP (80 g/kg metalaxyl + 640 g/kg mancozeb) was added to control any soil-borne fungi. The plants were placed in an unheated glasshouse. On 14 July 1989 when they had five to eight leaves, c. 180 plants of J and S were sprayed with the LD₅₀ MCPA estimated to be 0.55 kg ai/ha and 2.64 kg ai/ha MCPA for J and S respectively (Bourdôt and Hurrell 1988). The LD₅₀ was used to enable the most resistant individuals to be identified. Twenty plants of J and S were left as controls and all plants were returned to the glasshouse. The untreated plants were moved to larger pots on 4 August and on 31 August the nine plants of J and S least affected by the MCPA, were selected and transplanted to larger pots. On 26 September 1989 the six least affected of the nine selected plants of J and S, and 18 of the 20 controls of J and S, were transplanted into the field as closely spaced individuals in four spatially isolated groups to ensure crossing within (but not between) groups.

Seeds were collected from the four groups in February 1990 and the MCPA-responses of the seedling progenies were compared using the methods described for Experiment 1. There were eight replicate pots of four plant and eight concentrations of MCPA, giving 256 plants per progeny group. The MCPA treatments were applied and assessed simultaneously with those for Experiment 1, and the effects were analyzed by the same method.

Grey mould (*Botrytis cinerea*) caused deaths in both experiments and was controlled with 1 g/litre Rovral WP (250 g/litre iprodione) and 2.5 g/litre Euparen (500 g/kg dichlofluanid).

RESULTS

Experiment 1

The percent mortality was highly variable, particularly for populations TS and PF resulting in a poor fit to the model (Fig. 1a). This is attributable to the random deaths caused by the grey mould infections. Nevertheless, the Canterbury roadside populations, TS, PF and SC, had significantly lower LD₅₀ values than the Takaka dairy farm population (S). The roadside populations did not vary significantly among themselves in response to MCPA (Table 1, Fig. 1a).

Experiment 2

The seedling progeny of the six J plants selected as the most tolerant to MCPA (J-selected), were 2.4 times more resistant to MCPA than the progeny of the random selection of J plants (J-unselected) according to their LD_{50} values (Table 1, Fig. 1b). By contrast, there was no evidence in either the dose response curves or the LD_{50} values, that the progeny of the six S plants selected as the most tolerant to MCPA (S-selected), were more resistant to MCPA than the progeny of the random selection of S plants (S-unselected). For clarity, the dose response curve for the latter has been omitted from Fig. 1b.

Fig. 1: Dose-response curves for mortality of the giant buttercup populations in (a) Experiment 1; Travis Swamp [TS] (Δ), Peel Forest [PF] ($\circ$), Saltwater Creek [SC] ($\square$), Silcock [S] ($\blacktriangle$) and (b) Experiment 2; Jones-unselected ($\bullet$), Jones-selected ($\circ$) and Silcock-unselected ($\blacktriangle$).

TABLE 1: LD₅₀ values (kg ai/ha MCPA) derived from the dose-response curves from Experiments 1 and 2, shown in Figures 1a and 1b respectively.

Population	Log ₁₀ LD ₅₀	LD ₅₀
Experiment 1		
Travis Swamp (TS)	-1.059	0.09
Peel Forest (PF)	-0.552	0.28
Saltwater Creek (SC)	-0.446	0.36
Silcock (S)	0.097	1.25
LSD (5%) [PF, SC, S]	0.250	
LSD (1%) [TS vs others]	0.818	
Experiment 2		
Jones-unselected	-0.548	0.28
Jones-selected	-0.169	0.68
Silcock-unselected	0.309	2.04
Silcock-selected	0.292	1.96
LSD (5%)	0.149	

DISCUSSION

The purpose of this study was to test further the hypothesis that the resistance to MCPA in giant buttercup (Bourdôt *et al* 1990b), has developed as a result of selection pressure exerted by repeated exposure to the herbicide. In other weed species there is little evidence that resistance to phenoxy herbicides has developed as a result of their repeated use (Bourdôt *et al* 1990b). Support for this hypothesis can come from two types of data. If it could be shown that successive generations of a sprayed weed population required increasing doses of the herbicide to achieve control, then this would constitute very good evidence. Unfortunately data of this type (apart from anecdotal) are never available because difficulties with control inevitably precede investigations. The alternative is to look for a correlation between resistance and historical spraying. This approach has been followed for giant buttercup. In all cases to date, when the progeny of field populations previously exposed and never exposed to MCPA, have been compared, LD₅₀ values for MCPA have been statistically higher for previously exposed populations (Bourdôt and Hurrell 1988; Bourdôt *et al* 1989a; Bourdôt *et al* 1989b; Bourdôt *et al* 1990a, 1990b). The results of Experiment 1 in the present study again support the hypothesis since the three roadside populations from Canterbury formed a group having LD₅₀ values for MCPA from 3 to 14 fold lower than the values for the frequently exposed dairy-pasture population.

For herbicide resistance to develop in a susceptible weed population; i.e. for the proportion of resistant individuals to increase in successive generations; the resistance must be inherited by the progeny of the resistant individuals. That this occurs in giant buttercup is confirmed by Experiment 2 since the progeny of the polycross between the six survivors least affected by MCPA in the susceptible J population, were 2.4 fold more resistant than unselected controls. Such a rapid increase in resistance in one generation is unlikely in the field even with an effective kill rate of 97% as simulated in Experiment 2. This is because plants arising annually from susceptible soil-borne seed will "dilute" the resistance level in the plant population (Gressel and Segel 1982). The absence of a detectable increase in resistance in the already "resistant" S population may imply that an upper limit in resistance has been reached in S. Alternatively this result could be explained by a failure to select the most resistant plants for the polycross, or it may be that a shift occurred which was too small to detect in this experiment. The latter seems unlikely given the logarithmic nature of resistance increase (Gressel and Segel 1982).

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