SESSION 8C

HERBICIDE RESISTANCE IN CROPS AND WEEDS: II

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THE USE OF GLUFOSINATE AS A SELECTIVE HERBICIDE ON GENETICALLY ENGINEERED RESISTANT TOBACCO PLANTS

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ABSTRACT

Glufosinate is a non-selective herbicide which acts by inhibiting plant glutamine synthetase. Recombinant DNA technology has been used to introduce and express into several plant species an enzyme that modifies the herbicide into a non-herbicidal form. Greenhouse tests and a subsequent field test demonstrated complete resistance of engineered tobacco plants to field dose applications of glufosinate.

INTRODUCTION

Recent progress in plant genetic engineering makes it possible to transfer and express new genes into plants. This technology has been applied with success to several crop plants such as tomato, potato, cotton and oilseed rape. Engineering resistance to total herbicides provides a new and attractive alternative for weed control in several crop plants. Progress towards engineering resistance has been obtained for glyphosate (Comai et al., 1985; Shah et al., 1986) and for the sulfonylurea and imidazolinone herbicides (Chaleff & Ray, 1984; Shaner & Anderson, 1985).

We present our results on engineering resistance to the non-selective herbicides glufosinate (Bayer et al., 1972) and bialaphos (Ogawa et al., 1973). Bialaphos is a tripeptide antibiotic produced by Streptomyces hygroscopicus. It consists of glufosinate, an analogue of L-glutamic acid and two L-alanine residues. Upon removal of these residues by peptidases, glufosinate is a potent inhibitor of glutamine synthetase. This enzyme plays a central role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants. It is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration. Inhibition of glutamine synthetase by glufosinate causes rapid accumulation of ammonia which leads to death of the plant cell. Glufosinate is chemically synthesised ('Basta', 200 g a.i./l) while bialaphos is produced by fermentation of S. hygroscopicus ('Herbiace', 330 g a.i./l).

Recently, a bialaphos resistance gene (bar) has been isolated from S. hygroscopicus, the bacterium that produces bialaphos. This gene encodes a glufosinate acetyltransferase (Thompson et al., 1987) which acetylates the free NH_2-group of glufosinate and thereby prevents its autotoxicity in S. hygroscopicus.

In this paper we report that a field test on transgenic plants expressing this gene proved their complete resistance to field dose applications of glufosinate.

MATERIALS AND METHODS

Transfer and expression of the bar gene in plants

Agrobacterium derived vectors were used to transfer a chimeric *bar* gene in tobacco, tomato and potato plants. The chimeric gene was expressed using a plant specific promoter. Transgenic plants containing this gene were regenerated from single cells using tissue culture techniques (De Block *et al.*, 1987).

Greenhouse tests

As a more sensitive indicator of glutamine synthetase inhibition, ammonia accumulation (De Block *et al.*, 1987) was measured in transgenic and non-transformed tobacco plants treated with glufosinate at 1600 and 4000 g a.i./ha, under greenhouse conditions.

Field experiment

In 1987, a field experiment was performed at the Tobacco Institute SEITA in Bergerac, France. Two transgenic tobacco lines (N78-107 and N78-108) were tested. They are both derived from *Nicotiana tabacum* cv Petit Havane SR1 which was used as the control tobacco line. Both transgenic lines contain a single copy of the herbicide resistance gene and hence segregate resistant and sensitive seedlings at a 3 to 1 ratio after selfing (F1). Seeds were germinated in the greenhouse and sensitive seedlings were eliminated by spraying with glufosinate at 1000 g a.i./ha. F1 progeny that expressed the resistance were transferred to the field on 10 June, 1987. Plots consisted of 5 rows, each of 20 plants. Tobacco seedlings were planted at 0.5 m x 0.3 m spacing giving a plot size of
2 m x 5.7 m. Treatments were arranged in randomised block design with 2 replicates.

Glufosinate was applied 20 days after planting at 1000, 2000 and 4000 g a.i./ha. Chemical treatments were made with an Oxford Precision knapsack sprayer at 2 kg/cm^2 pressure and at a volume of 500 l/ha. The crop was treated with a metalaxyl + maneb fungicide for mildew control. Flowering was prevented by removal of all flower heads, followed by treatment with a mixture of aliphatic alcohols to prevent lateral budding.

Crop resistance was assessed by measuring the length of the largest leaf 10 days after spraying.

RESULTS AND DISCUSSION

Greenhouse tests

The growth of transgenic tobacco plants was indistinguishable from non-transformed control plants. Glufosinate at 400 g a.i./ha killed control tobacco plants in 10 days. The 21 transgenic plants assayed were all resistant to the herbicide treatment at 4000 g a.i./ha. Two additional applications of the herbicide within a 4 week period did not affect growth of the plants. Treated plants flowered normally and set seed. Transgenic plants were also treated with 2640 and 6600 g a.i./ha bialaphos as the commercial formulation. They also proved resistant to these applications. The resistance was inherited in the F1 progeny of tobacco as a single dominant trait.

Ammonia accumulated in treated non-transformed control plants and increased 40-fold after 8 hours. Ammonia levels in transgenic plants did not significantly change over a 24 hour period after application of glufosinate. The levels were comparable to those present in untreated plants. This clearly showed that the glutamine synthetase of transgenic plants is not affected by the herbicide treatment.

Field experiment

TABLE 1

Effect of herbicide sprays on glufosinate resistant tobacco. Measurement of the length of the largest leaf (cm).

	Glufosinate (g a.i./ha)			
Tobacco line	0	1000	2000	4000
Control SR1	20.45	0*	0*	0*
N78-107	23.80	24.20	24.20	24.30
N78-108	20.00	23.10	24.05	23.65

* All plants destroyed by the herbicide

Table 1 shows that both transgenic tobacco lines were fully resistant to a post-emergence application of glufosinate. These plants did not show any symptoms of herbicidal activity even when glufosinate was applied at 4000 g a.i./ha. Normal field rates are 500 - 1500 g a.i./ha. There was no significant difference in plant growth between the different rates of glufosinate, nor between the two transformed lines. However, competition with weeds in the control treatment reduced growth in those plots. Enzymatic assays showed that glufosinate acetyltransferase was expressed at a level of 0.001% of total extracted protein in N78-108 and at 0.1% in N78-107 (De Block et al., 1987). Since both tobacco lines proved resistant to field dose applications, we conclude that the resistance gene is expressed in N78-107 at at least 100-fold above the level required for exhibiting complete resistance.

In conclusion, genetically engineered resistance to glufosinate has been confirmed under field conditions in transgenic tobacco. The resistance is due to the transfer and expression of a detoxifying enzyme in transgenic plants. The successful engineering of this detoxifying enzyme will be largely independent of the plant species used. It is expected that this will be of use in engineering herbicide resistance with other important crops such as sugar beet and oil seed rape.

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SELECTION FOR SULFONYLUREA HERBICIDE TOLERANCE IN OILSEED RAPE (*BRASSICA NAPUS*) USING MICROSPORE CULTURE

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ABSTRACT

Immature pollen grains (microspores) from the flower buds of spring oilseed rape R8311 were produced under controlled environment conditions and extracted in a modified B5 medium. Microspores were cultured *in vitro* using a modified NN medium to produce embryo growth (embryogenesis) after 30 days of culture. Embryos were then subject to a range of chlorsulfuron concentrations (0-100 ppb) for a further 30 days before being transferred to a herbicide-free regenerative medium and finally raised in a soil-based compost. Chlorsulfuron concentrations of > 1 ppb were sufficient to inhibit embryogenesis and reduce the capacity of embryos to develop both roots and shoots, however, plants could be successfully raised to maturity from the majority of herbicide treatments. Cytological studies showed herbicide treated and untreated plants to be genetically haploid. The potential value of the microspore culture technique as a method of selecting herbicide-tolerant crops is discussed.

INTRODUCTION

In Canada spring oilseed rape is the principal oilseed crop grown on some 2.8 million hectares. Presently, the choice of herbicides available for broad leaved weed control is quite restricted and this can limit the situations where the crop may be reliably grown. In an attempt to improve the availability of selective herbicides traditional methods of research have relied upon the screening of a multitude of chemicals. Alternatively, recent interest has focused on techniques which deliberately confer herbicide tolerance in a crop cultivar which is normally susceptible to that herbicide. Such herbicide tolerance is the consequence of an inheritable change in one or more than one plant gene. The techniques which have been used to confer herbicide tolerance in plants are numerous but they can be divided into three main categories: (1) classical plant breeding, (2) plant cell culture and (3) DNA recombination proceedures. These techniques have been described by Hughes (1983) and Chaleff (1985,1986) and the agronomomic value and limitations of herbicide-tolerant oilseed rape cultivars by Marshall (1987).

In this paper we report the development of the *in vitro* culture of immature pollen grains (microspores) in oilseed rape (*Brassica napus*) for

the purpose of selecting and raising embryos which show tolerance to the sulfonylurea herbicide chlorsulfuron.

MATERIALS AND METHODS

Plant culture

Spring oilseed rape plants, R8311 (ex Ringot, France) were raised singly in 15 cm diameter pots containing a soil-based compost. Plants were grown in a growth room with a day/night temperature at 20/15 °C, 70 % relative humidity and a 16 h photoperiod (300-400 $\mu Em^{-1}s^{-1}$). Plants were watered as required and given a weekly application of liquid fertilizer (N:P:K 20:20:20). After bolting, flower buds (2-5 mm in length) were selected from the main axis for microspore culture.

Microspore culture

The proceedures used were based on earlier studies by Chuong & Beversdorf (1985). All operations were carried out in a laminar flow hood and the growth media filter sterilised. Flower buds were surface sterilised by immersion into 2% sodium hypochlorite solution for 20 minutes and rinsed three times in sterile double distilled water. After removing a sepal from each bud the stage of bud development was assessed by examining the ratio of the length of the flower petal to the anther. Only buds whose petal length was between one quarter and one half of the sepal length were used for culture.

Six buds were placed into a small glass homogenizer containing 2 ml of B5 media containing 13 % sucrose (Gamborg et al. 1976). After grinding down the buds with a plunger, the homogenate was filtered through nylon cloth (40 µm pore size) and washed into a centrifuge tube with a further 7.5 ml of B5 media. The microspores were then centrifuged at slow speed (500 rpm) and the pellet resuspended with fresh media. This cleaning proceedure was repeated a further three times. Finally the pellet was resuspended in 7.5 ml of NN media (Nitsch & Nitsch 1967) containing 13 % sucrose, 30 mg l^{-1} glutathione, 800 mg l^{-1} glutamine, 100 mg l^{-1} serine, 0.5 mg l^{-1} naphthalene acetic acid and 0.05 mg l^{-1} benzyl adenine. The concentration of microspores in the suspension was checked using a haemocytometer in order that the final concentration of suspension placed in each of three petri plates (60 x 15 mm) was approximately 200,000 (per 2.5 ml). Plates were incubated in the dark at 32°C for 3-4 days, then transferred to 25 °C for a further 21 days. Finally, the plates were illuminated by fluorescent light at 25 °C and the number of embryos recorded at 25-30 days of culture. Embryos complete with root and shoot buds were selected for uniformity of size and placed on the appropriate selective media (described below).

Selection for chlorsulfuron tolerance

Chlorsulfuron was prepared immediately prior to plating embryos in the regeneration medium to minimise the risk of herbicide metabolism or hydrolysis. Technical grade chlorsulfuron (95.0 %) was prepared by dissolving 10.52 mg in 1 ml of acetone then further dissolving the stock solution in 5 mM phosphate buffer (pH 7.0) to the desired final concentration. The chlorsulfuron solutions were filter sterilized and added to slightly cooled autoclaved B5 media containing 2.0 % sucrose and 0.8 % purified agar (Difco). Twenty petri plates (90 x 15 mm) of each

treatment, including controls were prepared each containing six embryos. Treatments corresponded to 100, 50, 10, 5 and 1 ppb chlorsulfuron (experiment 1) and 10, 5, and 1 ppb (experiment 2) together with controls. The experiments were arranged in a randomised block design within a growth room maintained at 25 ^{o}C with a 16 h photoperiod provided by fluorescent lights. After 30 days, the number of embryos with roots and or shoots was recorded prior to the next transfer to non-selective regenerative media.

Plant regeneration

After one month on selective media, embryos showing signs of regeneration were transferred to non-selective regeneration media to promote further root and shoot development. MS media (Gamborg et al. 1976) with 2.0 % sucrose, 5 mg l-1 napthalene acetic acid and 0.5 mg l-1 benzyl adenine was used for this purpose. Plants which were successfully regenerated were subcultured as required or transplanted into peat pellets and maintained in a mist propagation unit until they were suitable for transfer into a growth cabinet. Healthy plants were then raised in pots containing a soil-based compost.

Chromosome counts were carried out to determine the ploidy levels of the regenerated plants. Root tips were fixed for one hour in 2-3 ml of distilled water containing 3 drops of monobromonaphthalene (Darlington & LaCour 1975). The roots were stored in 3:1 v/v ethanol and acetic acid for 24 hours before hydrolysis and staining with modified carbol fuchin. The chromosome complement of haploid plants were doubled as required for future study using a colchicine (0.05 %) root soak for 6 hours (Winkle & Kimber 1976).

RESULTS AND DISCUSSION

The success of the microspore technique was assessed by examining the numbers of healthy embryos which were raised per anther in experiments 1 and 2 prior to the chlorsulfuron-selection stage (Table 1). Microspore culture of oilseed rape produced embryos at success rates which varied with the donor plant. Such variation in the degree of embryogenesis has been previously documented and is known to involve the genotype-environment interaction (Dunwell 1985). While variability in the degree of embryogenesis is to be acknowledged under the conditions reported herein, these and subsequent experiments carried in our laboratory generated an average of 3.5 embryos/anther. The effectiveness of this technique is presently sufficiently good to generate material for herbicide selection experimentation.

TABLE 1 Embryo production from microspore-derived cultures of oilseed rape (R8311) after 30 days in vitro

Plant donor	Total number of healthy embryos/anther	
	Experiment 1	Experiment 2
1	0	0
2	0	0.03
3	7.0	0
4	7.1	0
5	0	1.01
6	0	14.70
7	4.4	0.64
8	3.0	0.59
9	-	0.49
10	-	15.30
MEAN	2.7	3.3

The degree of success attained from microspore-derived embryos following a 30 day period on a selection medium is presented in Tables 2 and 3.

TABLE 2 The regenerative ability and condition of microspore-derived oilseed rape embryos (R8311) after 30 days in vitro (experiment 1)

Chlorsulfuron selection treatment (ppb)	Embryo number recorded and condition					
	Dead	Necrotic		Healthy/Green			Total
		shoot	root	shoot	root	both	
Control	55	-	1	16	1	47	120
1	61	-	33	5	12	3	114
5	78	-	18	4	9	-	109
10	50	-	64	-	-	-	114
50	56	-	54	1	9	-	120
100	69	-	41	-	11	-	121

TABLE 3 The regenerative ability and condition of microspore-derived embryos of oilseed rape (R8311) after 30 days *in vitro* (experiment 2)

Chlorsulfuron selection treatment (ppb)	Embryo number recorded and condition						
	Dead	Necrotic		Healthy/Green			Total
		shoot	root	shoot	root	both	
Control	-	-	-	20	-	100	120
1	-	-	-	17	-	97	114
5	30	41	-	7	23	19	120
10	66	46	-	-	8	-	120

The regeneration percentage for controls was almost twice as great in experiment 2, 100 % (Table 3) compared to experiment 1 (Table 2). This can be explained partly by examining the morphology of early embryo development. In experiment 1 the embryos were mainly globular in shape whereas in experiment 2 the embryos were torpedo shaped, green with a well defined axis. The latter type of embryos grew consistently depending upon the selection medium producing callus, roots and shoots within a few days of transfer from the liquid NN medium. In these particular experiments it was found that most of the embryos used in the selection experiments originated from plants with one or two flowers open and from buds with the petal length one half the length of the anther. This is likely to coincide with the stage of uninucleate microspore development as identified by Keller *et al*. (1975).

The effect of different concentrations of chlorsulfuron on the growth and development of the microspore embryos was investigated. The frequency of embryogenesis was not influenced consistently by chlorsulfuron. In experiment 1 only 15.0 % of the green embryos produced roots and shoots on 1 ppb chlorsulfuron and at higher concentrations regeneration was completely inhibited. In experiment 2, 85 % of the embryos produced roots and shoots on 1 ppb (Table 3). This may be due to differences in the stage of embryo development at the commencement of the herbicide selection as previously described. The sensitivity of the embryos of oilseed rape *in vitro* to such low concentrations of chlorsulfuron are in agreement with studies carried out in callus cultures of tobacco (*Nicotiana tabacum*) by Chaleff & Ray (1984).

Cytological examination of the plants which grew beyond the regeneration media stage into vegetative potted plants showed that they were all haploid. This is perhaps surprising since the degree of genetical variation which can be produced in microspore culture often results in

irregularity of the ploidy level due to spontaneous chromosome doubling (Keller et al. 1975). The ability to generate and select haploid plants which show tolerance in vitro to chlorsulfuron has several research implications. Haploid genotypes are particularly suitable in future breeding work where the new trait, herbicide tolerance is to be introduced into existing varieties. First, by virtue of the haploid genotype there are no problems associated with the expression of the herbicide tolerance trait when it is controlled by a recessive gene. Subsequently, homozygous diploid plants can be produced rapidly for breeding studies using a colchicine treatment. Furthermore, the mechanism of inheritance of the chlorsulfuron tolerance trait can be determined using diploid material as previously described by Chaleff & Ray, (1984) and the biochemical basis for chlorsulfuron selectivity assessed by measuring its effects on the inhibition of the enzyme acetolactate synthase (Ray, 1985).

In conclusion, the use of microspore culture as an alternative to suspension culture or callus culture could prove a useful alternative technique where herbicide tolerance is to be selected in vitro. In oilseed rape, a species which has not been successfully regenerated from suspension culture and is rather difficult to regenerate from callus, microspore culture is a prolific alternative approach which has the advantage of generating haploid plants. Further studies are in progress to examine the degree of chlorsulfuron tolerance in the R8311-derived plants and the biochemical basis for selectivity. In future, microspore culture might be examined as a method of selecting herbicide tolerance in other herbicide families outwith the sulfonylureas and be extended to other crop species.

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HERBICIDE RESISTANCE IN BLACK-GRASS (ALOPECURUS MYOSUROIDES)

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ABSTRACT

Thirty-eight stocks of black-grass (Alopecurus myosuroides) were tested for resistance to chlorotoluron (= chlortoluron). The most resistant samples came from five farms at Peldon, Essex. One Peldon population was tested for cross-resistance to isoproturon, metoxuron, pendimethalin, trifluralin, terbutryn, simazine, chlorsulfuron, tri-allate, ethofumesate, propyzamide, imazamethabenz, diclofop-methyl, fluazifop-butyl, tralkoxydim and SMY1500 (4-amino-6-tert-butyl-3-ethylthio-1,2,4-triazin-5(4H)-one). A stock susceptible to chlorotoluron (Rothamsted) was used as a standard. The Peldon stock showed a degree of cross-resistance to all herbicides except propyzamide, ethofumesate and trifluralin. Two Peldon soils were used in a pot experiment which demonstrated that both resistance and soil adsorption of herbicides can reduce herbicide activity at Peldon. The two factors had an approximately equal and additive effect in reducing chlorotoluron performance. In Petri-dishes, pendimethalin had a greater effect on early shoot growth of seedlings from the Rothamsted than from the Peldon populations. The possibility of using a Petri-dish test for the detection of resistance is discussed.

INTRODUCTION

Black-grass (Alopecurus myosuroides) showing a high degree of resistance to chlorotoluron (new BSI common name for chlortoluron) was first detected in the UK, at Peldon in Essex, in 1984 (Moss & Cussans 1985). The degree of resistance was less than commonly occurs with triazine resistance but the chlorotoluron-resistant black-grass also showed a degree of cross-resistance to several other herbicides - isoproturon, methabenzthiazuron, pendimethalin, terbutryn, diclofop-methyl and chlorsulfuron (Moss & Cussans 1987). In nutrient culture experiments the concentration of chlorotoluron required to control Peldon black-grass was ten times greater than that required to control a standard stock (Moss & Cussans 1987). Studies on the biochemical mechanisms of resistance are now in progress (Kemp & Caseley 1987). This paper describes further studies of resistance on the following topics :-

- the distribution of resistant black-grass, especially in Essex
- the detection of cross-resistance to other herbicides at doses recommended for application in the field
- the relative importance of resistance and herbicide adsorption by soil in causing poor herbicide performance in the fields at Peldon
- the development of a petri dish test for detecting resistance using seeds.

MATERIALS AND METHODS

Sources of black-grass seeds

All samples were collected in 1986. The two seed stocks used in all experiments were from the following locations :-

Rothamsted (Hertfordshire) : a winter wheat crop in the 'no weedkillers' section of Broadbalk field which has never received herbicide in its 140 year history. This was used as a standard reference stock in all experiments.

Peldon A1 (Essex) : a winter wheat field where resistance to chlorotoluron was first identified in 1984 (Moss & Cussans 1985).

In addition, thirty-six other seed stocks collected from winter wheat fields where chlorotoluron or isoproturon had been applied regularly for at least the previous twelve years were tested for resistance to chlorotoluron. Letters refer to different farms and numbers differentiate fields on the same farm (Table 1). These stocks were from the following locations :-

Sixteen samples from fields on five farms within 4 km of Peldon village. Peldon A & B were the two farms where chlorotoluron-resistant black-grass was detected in 1984 and 1985 (Moss & Cussans 1985, 1987). Peldon A1, A2 and B1 are the fields referred to as 'Lower 16 acres', 'Twitch' and 'Melondowns' by Orson (1987).

Fourteen samples from fields within an area of Essex bounded by Maldon, the A12 road, Colchester and Mersea Island. These are identified by the name of the nearest village or town.

Five other fields in East Anglia, identified by county.

One sample from Faringdon, Oxfordshire, where a low level of resistance to chlorotoluron was first detected in 1982 (Moss & Cussans 1985).

Screening of seed stocks for resistance to chlorotoluron

The experiment comprised a randomised block design with five replicates. For each seed stock there was one treated and one unsprayed pot per replicate. Ten pre-germinated seeds were sown in sandy loam soil in each 8.75 cm diameter pot. After emergence, seedlings were thinned to leave six plants/pot. Chlorotoluron (2.25 kg a.i./ha) was applied at the two-leaf stage in 322 litres water/ha at 210 kPa through a single 'Spraying Systems' 8001 'Tee-jet' nozzle on a laboratory sprayer. Pots were placed in a glasshouse and watered as necessary. Because few symptoms were visible 2½ weeks later, all previously sprayed pots were treated with an additional 1.75 kg a.i. chlorotoluron/ha. Foliage fresh weight was recorded 4½ weeks after initial spraying.

Tests for cross-resistance at field recommended rates of sixteen herbicides

Rothamsted and Peldon A1 seeds (300/container) were incorporated into the top 5cm of a sandy loam soil (5.6% o.m. pH 6.8) in separate plastic containers (27 x 18 x 10 cm deep) with drainage holes. The experiment comprised a randomised block design with five replicates. Herbicide doses are given in Table 2. Apart from chlorsulfuron, the rates used were those currently recommended for the control of black-grass in the field.

Herbicides were applied in the same manner as the first experiment. Pre-emergence herbicides were applied on 7 October 1986 immediately after sowing and then all containers were buried to the rim in a field. Post-emergence treatments were applied on 21 November 1986 when black-grass had 2 - 2½ leaves per plant. Containers were returned to the field immediately after spraying. There were three untreated containers per seed stock in each replicate. Foliage fresh weight was recorded on 25 February 1987.

Relative influence of resistance and herbicide adsorption by soil on the activity of chlorotoluron

The two soils used in this experiment were collected from the surfaces of minimally cultivated and ploughed plots of an ADAS cultivation experiment at Peldon. Rothamsted or Peldon A1 seeds were sown in pots containing either the minimum cultivated ('adsorptive') or ploughed ('less-adsorptive') soil. The four factorial combinations of soil and seed stocks were used. Herbicide adsorptive capacity of the soils was determined and expressed as Kd values for chlorotoluron (Moss & Cotterill 1985).

The experiment consisted of a randomised block design with three replicates. Eight pre-germinated seeds were sown in each 7.5 cm pot. Emerging seedlings were thinned to leave six plants per pot. Ten rates of chlorotoluron, in the range 0.25 - 28.0 kg a.i./ha, were applied at the two-leaf stage in the same manner as the first experiment. There were three untreated pots per replicate for each soil/seed combination. Pots were placed in a glasshouse and foliage fresh weights were recorded 4 weeks after spraying. Dose response curves were fitted using the Rothamsted Maximum Likelihood Programme and ED_{50} values calculated (Ross 1980). Log ED_{50} values were analysed by analysis of variance. ED_{50} is the estimated herbicide dose required to reduce foliage fresh weight to 50% of untreated plants.

Petri-dish test for detection of resistance

A petri dish experiment was conducted to study germination and early seedling development of Rothamsted and Peldon A1 stocks when exposed to pendimethalin. Twenty-five seeds were placed in each 9 cm Petri-dish containing three Whatman No.4 and 1 glass fibre filter papers. There were two dishes per treatment. Seven ml of 1 mg/l pendimethalin solution were added to each treated dish. Distilled water was added to control dishes. Dishes were placed in polythene bags in a controlled environment cabinet (18 ^{o}C 14 h day, 12^{o}C 10 h night). After two weeks the lengths of the radicle and primary shoot were recorded for each germinated seed. The number of shoots touching the lid of each Petri-dish, 12 mm above the seeds, was also counted.

RESULTS

Screening of stocks for resistance

There were large differences in response to chlorotoluron treatment (Table 1). The Rothamsted standard was well controlled (87%). For ease of comparison, the stocks can be placed arbitrarily into three categories :-

'Resistant' : 6 stocks where control was between 0 and 24%
'Intermediate' : 9 stocks where control was 25 - 75%
'Susceptible' : 23 stocks where control was 76 - 100%

All the samples from fields on the two Peldon farms (A & B), where

TABLE 1

Effect of chlorotoluron (4.0 kg/ha) applied post-emergence on 38 black-grass seed stocks. Stocks listed in order of insensitivity to chlorotoluron. Underlined stocks are those used in all other experiments.

		% reduction in foliage fresh weight			% reduction in foliage fresh weight
1.	Peldon A3	-4	20.	Essex	84
2.	Peldon B2	6	21.	Peldon H2	84
3.	Peldon A1	12	22.	Great Totham	84
4.	Peldon B3	15	23.	Layer Marney A	85
5.	Peldon C	18	24.	Suffolk	85
6.	Peldon A4	21	25.	Lincoln A	86
7.	Peldon A5	27	26.	Tolleshunt Major	86
8.	Peldon B1	32	27.	Witham A1	86
9.	Peldon D	45	28.	Rothamsted	87
10.	Peldon E1	54	29.	Fingringhoe A1	88
11.	Peldon E2	57	30.	Layer Marney B	88
12.	Tiptree	60	31.	Cambridge	89
13.	Peldon B4	61	32.	Fingringhoe A2	90
14.	Peldon A2	63	33.	Tolleshunt D'Arcy	92
15.	Faringdon	73	34.	Witham A2	93
16.	Peldon F	77	35.	Maldon	94
17.	Peldon G	79	36.	Goldhanger	94
18.	Peldon H1	80	37.	Kelvedon	95
19.	Salcott	82	38.	Lincoln B	98
				S.E. ±	5.5

resistance to chlorotoluron had been detected previously, were in the resistant or intermediate category, none were susceptible. Samples from three other farms (Peldon C, D & E) within 4 km of Peldon were also in the intermediate or resistant category. However, four other stocks from Peldon were in the susceptible category. The five stocks collected from elsewhere in East Anglia were susceptible to chlorotoluron. Of the 15 stocks in the resistant and intermediate categories only two, Tiptree and Faringdon, were collected from the area beyond 4 km of Peldon. The Tiptree stock was collected from a field about 11 km west of Peldon.

Tests for cross-resistance to field rates of herbicides

All herbicides except for trifluralin, ethofumesate and propyzamide, were more effective on the Rothamsted than on the Peldon stock (Table 2). Fluazifop-butyl gave good control of both stocks, but Rothamsted was controlled significantly better ($p \leq 0.05$) than the Peldon stock. Ethofumesate and propyzamide completely killed both stocks. Post-emergence applications of chlorotoluron and isoproturon were more effective than pre-emergence applications on both stocks.

Influence of resistance and herbicide adsorption on chlorotoluron activity

The adsorptive capacipies of the two soils, expressed as Kd values for chlorotoluron were: ploughed = 4.7; minimum cultivated = 11.1.

TABLE 2

Effect of herbicides on two black-grass seed stocks grown in containers in the field. Arcsin transformed data in parentheses.

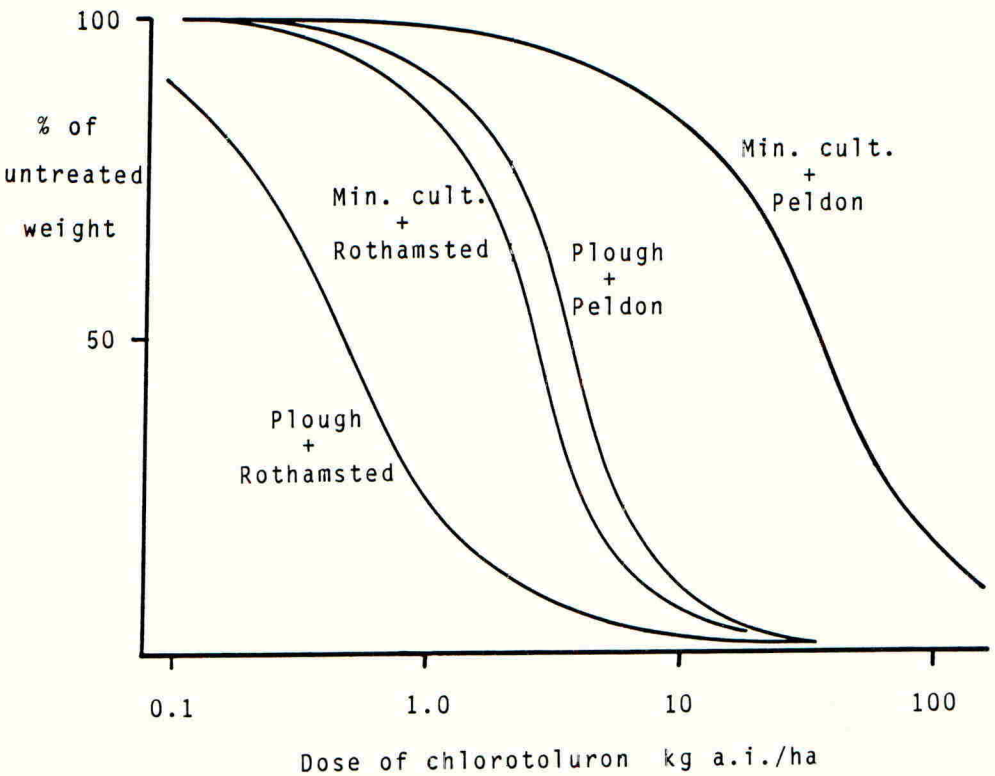
	Rate of application (kg a.i./ha)	% reduction in foliage fresh weight			
		Rothamsted		Peldon A1	
Pre-emergence treatments					
chlorotoluron	3.50	84	(66.5)	15	(22.2)
isoproturon	2.50	66	(54.5)	30	(33.3)
terbutryn	2.80	81	(64.8)	36	(37.0)
simazine	1.15	73	(59.0)	53	(46.7)
pendimethalin	1.98	95	(77.7)	43	(40.6)
trifluralin	1.20	87	(69.0)	86	(68.4)
tri-allate granules	2.25	84	(66.7)	46	(42.7)
ethofumesate	1.40	99	(83.8)	100	(88.9)
chlorsulfuron	0.03	56	(48.5)	35	(36.0)
Post-emergence treatments					
chlorotoluron	3.50	98	(82.1)	30	(32.7)
isoproturon	2.50	98	(82.3)	62	(52.1)
metoxuron	4.38	98	(82.9)	50	(45.0)
propyzamide	0.70	99	(86.6)	100	(87.7)
imazamethabenz	0.50	76	(61.1)	8	(16.2)
SMY1500	1.75	99	(84.5)	68	(56.0)
diclofop-methyl	1.14	99	(86.6)	55	(47.9)
fluazifop-butyl	0.25*	99	(83.8)	93	(74.3)
tralkoxydim	0.20*	99	(86.6)	75	(59.9)
		S.E. ±	(1.97)		

* = a non-ionic wetter ('Agral') used with these treatments

There were substantial differences in the response to chlorotoluron (Fig 1). High doses of herbicide were required to control Peldon (resistant) black-grass growing in minimum cultivated (adsorptive) soil (ED_{50} = 32.50 kg a.i./ha). In contrast, control of Rothamsted (susceptible) black-grass growing in ploughed (less adsorptive) soil was achieved at much lower rates of herbicide (ED_{50} = 0.46 kg a.i./ha). The other two soil/seed combinations gave intermediate dose response curves (ED_{50} Min. cult./Rothamsted = 2.56 kg a.i./ha; Plough/Peldon = 3.56 kg a.i./ha). Analysis of the log ED_{50} values showed that there was no significant interaction between soil and seed source. The relative influence of adsorption and resistance was determined from the ratios of ED_{50} values, which is the same as the difference between two log values. The mean value for effect of resistance (0.996, detransformed = 9.89) was similar to that for adsorption (cultivation) effect (0.853, detransformed = 7.129). This indicates that in this experiment resistance and soil adsorption had an approximately equal influence on chlorotoluron performance.

FIGURE 1

Dose response curves for the effect of chlorotoluron on foliage fresh weight of Rothamsted and Peldon black-grass growing in soil removed from ploughed or minimum cultivated land.



Petri-dish tests for determining resistance There was a big difference between the two stocks in early shoot development in the presence of pendimethalin. Shoots emerging from Rothamsted seeds (mean length = 2.3 mm) were much shorter than those emerging from Peldon seeds (mean = 18.2 mm). The assessment of numbers of shoots reaching the Petri-dish lid demonstrated this difference even more clearly. The numbers of primary shoots touching the Petri-dish lid as a percentage of control dishes was 0% for Rothamsted and 76% for Peldon seeds. Root length was reduced substantially by about 95% in both stocks, but pendimethalin had no effect on germination. In control dishes, there was no difference in mean shoot length between the two stocks (Rothamsted = 44.2 mm, Peldon = 44.3 mm).

DISCUSSION

The results showed that resistance or partial resistance to chlorotoluron occurs on at least five farms in the Peldon area of Essex. There were big differences in degree of sensitivity between samples collected within the Peldon area. Black-grass samples from 80 fields have

been tested for resistance to chlorotoluron in this, and previous, experiments (Moss & Cussans 1985, 1987). With two exceptions, the samples collected more than 4 km from Peldon have been susceptible to chlorotoluron. It is of interest that partial resistance was found on a field near Tiptree, 11 km from Peldon. More intensive sampling in this area was conducted in summer 1987. Partial resistance at Faringdon was first detected in 1982 (Moss & Cussans 1985) but is still marginal.

The experiment in containers showed that a degree of cross-resistance to many different herbicides occurs. Resistance was sufficient to cause substantial reductions in herbicide efficacy at normal field rates. The herbicides used came from several different chemical groups and had varied modes of action. While cross-resistance to herbicides with a similar mode of action commonly occurs with triazine resistant weeds (Fuerst et al. 1986) it is surprising that black-grass from Peldon shows resistance to herbicides with several different modes of action. However different degrees of resistance occur within the same chemical group. For example, Peldon black-grass was partially resistant to pendimethalin whereas trifluralin (also a dinitro-aniline) was equally effective on both stocks. The excellent level of control achieved by propyzamide and ethofumesate could have masked small differences in sensitivity to these herbicides by the two seed stocks. Further experiments at a range of doses will be needed to confirm that both stocks respond identically to these herbicides.

The use of field containers permits the evaluation of herbicides under semi-field conditions. Most of the herbicide treatments controlled Rothamsted black-grass to the extent expected from our knowledge of their relative efficiency as black-grass herbicides. The activity of soil-acting herbicides may benefit from the fine soil tilth in the containers. However, the lack of crop competition may impair the apparent activity of some herbicides.

The minimum tillage system used on some farms at Peldon has been shown to result in the development of an adsorptive surface layer which can reduce the activity of many soil-acting herbicides. The difference in adsorptive capacity of soil would be expected to result in differences in herbicide activity (Moss 1984, 1985). Thus, poor herbicide performance in the field could be attributed to cultural factors rather than to resistance. The pot experiment using soils from Peldon showed that adsorption and resistance are both capable of substantially reducing the activity of chlorotoluron. Resistance and adsorption had an approximately equal and additive effect on herbicide activity. It is important, therefore, that both factors are considered during the appraisal of results from field experiments at Peldon (Orson 1987). The relative importance of adsorption and resistance is likely to vary between fields depending on several factors. Firstly, the adsorptive capacity of the surface soil is likely to vary considerably between fields. Secondly, the degree of resistance is known to vary over relatively short distances. Thirdly, the herbicide used will influence the relative importance of these two factors depending on the degree of resistance to that herbicide and any foliar activity. These results highlight the difficulty in detecting resistance solely on the basis of field observations.

Differences in resistance to pendimethalin between seed stocks could be detected in a Petri-dish test by differences in shoot length after 2 weeks. The Petri-dish test may be useful as a rapid screening technique for testing seed stocks for resistance. However, resistance may occur elsewhere without

the extent of cross resistance we have found. In these cases, resistance to other herbicides is unlikely to be detected using pendimethalin. Further evaluation of the Petri-dish test is in progress.

Practical conclusions

There is no evidence that the resistance problem at Peldon is widespread in other parts of the UK. However, resistance may be developing, or may already occur elsewhere but has yet to be detected. Herbicide resistance in black-grass has the potential to become a major problem because of the degree of cross-resistance we have found. Although the limited evidence indicates that some herbicides are effective on the Peldon black-grass (ethofumesate, propyzamide) none of the cereal herbicides we tested gave adequate control of Peldon black-grass.

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FIELD TRIALS ON THE EFFICACY OF HERBICIDES ON RESISTANT BLACK-GRASS (ALOPECURUS MYOSUROIDES) IN DIFFERENT CULTIVATION REGIMES.

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ABSTRACT

The results of field trials on Alopecurus myosuroides, which is resistant to some herbicides, are discussed. Ploughing, when compared with shallow cultivations, reduced the population of the weed and led to increased control with herbicides. This indicates that the problem of control on the sites tested was due to adsorption of soil-applied herbicides on to organic matter and burnt straw residues as well as to herbicide resistance. Resistance was confirmed by testing the seed collected from the trials on a sandy loam soil where adsorption of soil applied herbicides was low.

INTRODUCTION

Alopecurus myosuroides (black-grass) is a major weed of winter cereals in England (Elliot et al, 1979). A significant area of the winter cereal crop is sprayed (Sly, 1986) with a range of effective herbicides in order to control this weed (Baldwin, 1979).

Control with soil applied herbicides can be affected by adsorption onto organic matter and burnt straw residues (Moss, 1984). Such problems are a common feature in systems where continuous autumn sown crops, established after minimal tillage, are grown on very heavy clay soils.

In 1984, as a result of an advisory inquiry, samples of A. myosuroides seed were collected from Peldon Hall Farm, near Colchester, Essex and sent to the Weed Research Organization, Oxford. Tests showed that the seed was resistant to chlorotoluron (Moss & Cussans, 1985) and subsequently to other herbicides (Moss & Cussans, 1987). A herbicide evaluation trial was carried out in the crop year 1984/85 in the field where the problem was first identified (Clarke, 1987). Tests on other seed stocks reveal that other farms in the Peldon area also have A. myosuroides which are resistant to herbicides (Moss, 1987).

The cropping system at Peldon Hall is continuous autumn sown wheat established by minimal tillage on a marine silty clay loam. Soil analysis showed that the adsorption of soil applied herbicides was very high. It was decided that two tillage experiments would be carried out to investigate the efficacy of herbicides in the systems of shallow cultivation, ploughing every year and 'rotational' ploughing ie ploughing once every four or five years. The objective of these experiments was to establish, in the field, whether and to what level the problems of control could be attributed to either herbicide adsorption or herbicide resistance.

Additionally, field trials were carried out at Peldon Hall to evaluate the efficacy of tank-mixtures based on isoproturon for the control of herbicide resistant A. myosuroides where the crops were established by shallow cultivation techniques.

Seed from trials on A. myosuroides were collected from the Peldon trials and from other trial sites in eastern England and sown on a sandy loam soil in Cambridge. The resistance of the resulting plants was evaluated by the application of two rates of chlorotoluron.

MATERIALS AND METHODS

All the trials were sprayed with a modified Van de Weij sprayer with an Oxford Precision Sprayer boom, fitted with Lurmark F02-80 fan nozzles at a pressure of 2.1 to 2.5 bar. A volume equivalent to 200 l/ha was used. The trials at Peldon Hall and Brickhouse were on the winter wheat cultivar Virtue and received commercial treatments of fertiliser, fungicides and insecticides.

Cultivation trials

Site selection was difficult because of the patchy nature of the weed. Sites were selected at Peldon Hall in the field named Twitch and on Brickhouse, the neighbouring farm, in a field named Melondowns. Seeds collected from these sites were tested and were described as being intermediate in their resistance to herbicides (Moss, 1987). The patchy nature of the weed not only affected site selection but trial methodology.

Large main plots, measuring 24 m by 18 m, were either ploughed to a depth of 25 cm or shallow cultivated to 5 cm at Peldon Hall or 10 cm at Brickhouse, in late August to early September. The three main tillage treatments were laid out in a randomised block design with four replicates. Six herbicide treatments were applied to sub-plots each measuring 12 m by 6 m. Representative members of the major herbicide groups which are effective on A. myosuroides were chosen for evaluation (Table 1).

TABLE 1

Herbicides used in the cultivation trials - rates and formulations

Treatment	Herbicide	Rate (a.i./ha)	Formulations
1	pendimethalin	2.0 kg	330 g a.i./l
2	terbutryn	2.8 kg	500 g a.i./l
3	diclofop-methyl	1.14 kg	380 g a.i./l
4	chlorotoluron	3.5 kg	500 g a.i./l
5	isoproturon	2.5 kg	500 g a.i./l
6	chlorsulfuron/ metsulfuron-methyl	20.0 g	20% w/w

Untreated areas were established in each of the sub-plots by covering at random, with polythene, two quadrats of 1 m x 1 m at the time of spraying. Assessments were carried out in the following July on the basis of head numbers. The A. myosuroides was then removed from the untreated areas.

At Peldon Hall, the experiment was carried on into a second year with the untreated areas being placed in a different position within the sub-

plots. The same herbicide treatments were applied to the same sub-plots for the second year. The rotational ploughing plots were established by returning the designated main plots to shallow cultivations. Site details are given in Table 2.

TABLE 2

Site details for the cultivation trials.

	Peldon Hall (Twitch Field)		Brickhouse (Melondowns Field)
	1985/86	1986/87	1985/86
Application 1:			
Date	14/10/85	1/10/86	18/10/85
Crop G.S.	0	0	0
Weed G.S.	0	0	0
Treatment No.	1,2	1,2,6	1,2
Application 2:			
Date	30/12/85	3/1/87	11/12/85
Crop G.S.	12	23(1)	12
Weed G.S.	11-12	22-23(1)	11-12
Treatment No.	4,5,6	3,4,5	4,5,6
Application 3:			
Date	11/3/86	-	11/3/86
Crop G.S.	12-13	-	12-13
Weed G.S.	12	-	12
Treatment No.	3	-	3

(1) In the ploughed plots the crop G.S. = 21-22 and weed G.S. = 21

The soil surface was dry at the time of the pre-emergence applications. However, the farmers did achieve seedbeds which were well within product recommendations regarding clod size. The application of diclofop-methyl is not recommended for the control of A. myosuroides which is beyond the four leaf/one tiller stage.

Evaluation of isoproturon tank-mixes

Two trials were laid down in the winter of 1986/87, one at Peldon Hall and one at Brickhouse. Both trials were superimposed onto commercial crops of winter wheat established after shallow cultivations. The Peldon Hall trial was laid down in the field named lower 16 acres, where resistance was first identified (Moss & Cussans, 1985) and was described as resistant by Moss (1987). The trial at Brickhouse was on a site in the field named Melondowns, where the resistance was described as intermediate. The treatments are listed in Table 4 and were chosen on the basis of the most recent pot and container results from Long Ashton Research Station (Moss, 1987). Both trials were sprayed on 9 January, when the crop growth stage on both sites was three tillers and the growth stage of A. myosuroides was up to three tillers. The repeat application of PP 604 was carried out on 15 April. A. myosuroides was assessed in July on the basis of head

numbers. Again, it should be noted that the application of diclofop-methyl alone is not recommended for the control of A. myosuroides beyond the four leaf/one tiller stage. Similarly chlorsulfuron/metsulfuron-methyl is recommended for application in the autumn but not later than 25 December.

Testing of A. myosuroides seed from Peldon and other sites in eastern England for resistance to chlorotoluron

Seed was collected in mid to late July from the Peldon trials and a number of other ADAS trial sites and stored in open trays until planting. The seed was drilled in single rows, eight metres in length in a sandy loam soil at Anstey Hall, Cambridge, where the level of herbicide adsorption was low. The seed rate was approximately one seed every 0.5 cm. The single row plots were laid out in randomised blocks with four replicates. In the autumn of 1985, there were 10 stocks tested and in the autumn of 1986, there were 14 stocks. Seed from the stock bed at the Weed Research Organization, known to be susceptible to chlorotoluron, was included in both year's trials.

The seed was planted in a dry soil on 28 October in 1985 and on 16 October in 1986. Chlorotoluron was applied at 1.75 kg a.i./ha and 3.5 kg a.i./ha immediately after planting in strips two metres wide across the rows. The site was then protected from birds and mammals with a net.

The trials were assessed the following May by hand harvesting the total green matter of A. myosuroides from a one metre length/treatment of each row. The green material was weighed and then dried in an oven to obtain dry weights.

RESULTS

The statistical information provided in the tables is for P=0.05.

Cultivation trials

The results are presented in Table 3. The adsorption levels (Kd(chlorotoluron)) were very high in the shallow cultivation plots. The control of A. myosuroides was higher where the soil was ploughed than where it was shallow cultivated. Ploughing significantly reduced the number of heads of the weed in the untreated areas, which confirms the findings of Moss (1978). Ploughing reduced populations more at Peldon Hall where the shallow cultivations were carried out at a depth of 5 cm compared to 10 cm at Brickhouse.

Evaluation of isoproturon tank-mixes

The level of control was very poor for most treatments (Table 4). The Kd(chlorotoluron) at the Peldon Hall site was extremely high. It should be noted that the rate of SMY 1500 applied at Peldon Hall was less than intended.

Testing of A. myosuroides seed from Peldon and other sites in eastern England for resistance to chlorotoluron

The results of the test on seed collected in July 1985 and tested over the winter 1985/86 are not published. All the stocks tested were completely controlled by the 1.75 kg a.i./ha rate of chlorotoluron with the exception of the two stocks collected from Peldon. The stock from the original field, where resistance was first identified (lower 16 acre), collected from plants that received isoproturon during the autumn of 1984, was not controlled by the full rate of 3.5 kg a.i./ha chlorotoluron. A

seed stock collected from the same field but from an area that did not receive any herbicide in the 1984/85 cropping season was more susceptible. These stocks were re-tested the following year and the results are given in Table 5.

TABLE 3

Kd(chlorotoluron) values of the soil (Moss, 1984), head/m^2 of A. myosuroides in the untreated areas and percentage control of heads of A. myosuroides within main treatments, in the cultivation trials

	Kd	(heads /m^2)	pendim- ethalin	terbut- ryn	diclofop -methyl	chlorot oluron	isopro turon	chlors- ulfuron
Brickhouse, 1985/86					SED = 13.8	d.f. = 49		
Shallow cult.	15	(336)	54	71	44	-4	59	72
Plough	8	(180)	84	80	58	63	90	87
Peldon Hall, 1985/86:					SED = 15.5	d.f. = 50		
Shallow cult.	18	(915)	57	33	16	41	82	87
Plough	5	(107)	74	79	31	81	94	93
Peldon Hall, 1986/87:					SED = 22.4	d.f. = 45		
Shallow cult.	11	(3667)	-24	-12	-27	-6	24	23
Rotation pl.	4.2	(1394)	16	38	0	28	43	59
Plough	3.7	(1280)	29	48	23	45	68	78

Broad-leaved weed competition reduced the fresh weight of the untreated A. myosuroides in the 1986/87 trial. This occurred evenly over the site. The results in Table 5 confirm the findings of Moss (1987). The seeds collected from the cultivation trials in 1986 (Brickhouse, Melondowns and Peldon Hall, Twitch) were less resistant than those from the fields where the problem was first identified (Peldon Hall, lower 16 acre). In addition, the findings of the previous year that the stock of seed from Peldon Hall, lower 16 acre, collected from plants sprayed with isoproturon was more resistant than the seed collected from plants not receiving herbicide, were confirmed. A similar trend also occurred with the different seed stocks collected from Melondowns and Twitch. Stocks collected from other trial sites throughout the eastern area of the country were more susceptible but the control of the stock from Chittering requires further investigation.

DISCUSSION

The results, along with those of Moss (1987) indicate that the poor control of A. myosuroides on certain fields in the Peldon area was due to two factors. High adsorption of soil applied herbicides was partially responsible but even when ploughing was carried out, the results were not completely satisfactory. However, the adsorption was not always reduced to a level where soil applied herbicides would work very effectively (Eagle, 1987). Tests on the seed grown in soil favourable for control confirm that herbicide resistance was also a cause for the poor control. However, the first year of the cultivation trial did indicate that with favourable conditions and ploughing, satisfactory control of A. myosuroides, deemed to

be intermediate in resistance, could be achieved with single applications of isoproturon and chlorsulfuron/metsulfuron-methyl. Applications were made to larger A. myosuroides in 1986/87 and this may partially explain the results obtained in that year, although it should be noted that poor control of the weed was commonly achieved commercially on many farms in the eastern area of England.

TABLE 4

Herbicides, Kd (chloroturon) (Moss, 1984), rate of use and percentage control of heads of A. myosuroides in the evaluation of isoproturon tank-mixes trials.

Herbicide	Rate a.i./ha	Peldon Hall 16 acre Kd = 23	Brickhouse Melondowns Kd = 9
		SED=11.4 d.f.=28	SED=8.4 d.f.=18
isoproturon	2.5 kg	21	13
diclofop-methyl	1.14 kg	24	14
PP 604(1)	0.2 kg	52	16
followed by PP 604(1)	0.35 kg		
chlorsulfuron/metsulfuron -methyl	20.0 g	28	18
isoproturon+diclofop-methyl	2.5+1.14 kg	40	20
isoproturon+chlosulfuron/ metsulfuron-methyl	2.5 kg +20.0 g	28	49
isoproturon+PP 604(1)	2.5+0.2 kg	78	61
followed by PP 604(1)	0.35 kg		
SMY 1500(2)	1.75 kg	15	52
No of heads/m2 (untreated)		(1801)	(2404)

(1) All treatments of PP 604 (tralkoxydim, formulation FD 4026) were applied with the addition of 0.1% non-ionic wetter (Agral) in the spray solution
(2) 4-amino-6-tert-butyl-3-ethylthio-1,2,4-triazin-5(4H)-one in formulation UK 220. 1.4 kg a.i./ha applied at Peldon Hall.

Ploughing not only increased the efficacy of the herbicides tested but also reduced the untreated population of A. myosuroides. On these soil types, farmers are reluctant to plough due to difficulties in achieving a consolidated and fine seedbed and an even establishment of crop plants. However at Peldon, the alternative of minimum tillage will almost certainly mean more herbicide treatments and the tests on the seed indicate that each application of a herbicide may result in the surviving plants becoming more resistant. This aspect of the problem requires further investigation as the implications are of great practical significance.

TABLE 5

Foliage weight(g)/metre of row of A. myosuroides when harvested in May 1987, untreated compared to 1.75 kg a.i./ha or 3.5 kg a.i./ha chlorotoluron.

Location and year of seed collection	Untreated	1.75 kg a.i./ha chlorotoluron	3.5 kg a.i./ha chlorotoluron
		SED = 44 d.f. = 78	
Peldon Hall(16 acre) 1985(1)	150	141	141
Peldon Hall(16 acre) 1985	193	386	348
Peldon Hall(Twitch) 1986(1)	162	284	182
Peldon Hall(Twitch) 1986	157	234	244
Brickhouse(Melondowns) 1986(1)	134	153	25
Brickhouse(Melondowns) 1986	208	208	168
Wragby, Lincolnshire 1986	212	107	3
Marshland St James, Norfolk 1986	140	10	7
Woburn, Bedfordshire 1986	103	2	1
Weed Research Organization 1985	248	8	0
Henham, Essex 1986	89	19	1
Thrapston, Northants 1986	216	45	2
Chittering, Cambridgeshire 1986	160	70	0
Odell, Bedfordshire 1986	119	15	6

(1) Seed was collected from plants not treated with herbicides. All other seed stocks were collected from plants treated with 2.5 kg a.i./ha isoproturon.

The trials confirmed the field container experiments described by Moss (1987). There appeared to be cross resistance to different groups of herbicides. The most promising herbicide combination of those tested in the field appeared to be PP 604 when tank-mixed with isoproturon. However, chlorsulfuron seemed to work more effectively in the cultivation trials at Peldon than in the field container experiments. This may be because this herbicide appears to be unaffected by adsorption onto soil organic matter and burnt straw residues and in ADAS trials, it has always worked more effectively in the relatively dry climate of eastern England in comparison with the west of the country, where the container experiments were carried out.

The stocks of A. myosuroides, which are described as resistant, have great economic significance to the farms on which they occur. The reasons for the occurrence of resistance on these farms are not understood and consequently, the potential for the problem to arise on other farms cannot be quantified. The cause of resistance therefore requires urgent attention as well as the monitoring of populations of the weed in the main arable areas of England. Tests on other trial sites in eastern England indicate that the problem is at present isolated. In addition, further trials work is required to try to identify economic control measures.

ACKNOWLEDGEMENTS

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SYNERGISTIC EFFECTS OF 1-AMINOBENZOTRIAZOLE ON THE PHYTOTOXICITY OF CHLOROTOLURON AND ISOPROTURON IN A RESISTANT POPULATION OF BLACK-GRASS (ALOPECURUS MYOSUROIDES)

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ABSTRACT

The phytotoxicity of chlorotoluron and isoproturon in resistant and susceptible populations of black-grass (Alopecurus myosuroides) has been investigated by treatment of hydroponically grown seedlings with the herbicides incorporated in the liquid medium. Seedlings from the resistant population survived concentrations of chlorotoluron more than ten times the maximum sustained by the susceptible population. The difference between the responses of the two populations was smaller for isoproturon.

Incorporation in the nutrient solution of the P450 mixed-function oxidase inhibitor 1-aminobenzotriazole (ABT) synergised herbicide activity against the resistant population but had little effect upon phytotoxicity in the susceptible population. These effects suggest that degradation and detoxification of chlorotoluron and isoproturon may be more rapid in the resistant population compared with the susceptible population.

INTRODUCTION

Resistance to chlorotoluron and isoproturon has been found in populations of black-grass where these herbicides have been used intensively in winter cereals for over ten years (Moss & Cussans 1985, 1987; Moss 1987). Two to three-fold increases in ED_{80} values were generally found, but a population at Peldon in Essex showed exceptionally high resistance to chlorotoluron (16-fold increase) compared to a population from a site at Rothamsted Experimental Station in which herbicides have not been used in its 140 year history. The present work compares the responses of hydroponically grown seedlings from these two populations to incorporations of chlorctoluron and isoproturon in the liquid medium, and investigates the influence of simultaneous incorporations of 1-aminobenzotriazole (ABT) on these responses.

MATERIALS AND METHODS

Samples of black-grass seed (20g) collected from the Peldon and Rothamsted populations were pre-germinated on moist filter paper by incubating for 8 days at 17.5'C in the dark with daily 3 hour light periods (Atlas "Grow-lux" 6 x 8W in a Gallenkamp cooled incubator). Newly germinated seeds which were at a uniform stage of development (20% of total) were selected for growing on in liquid nutrient ($Ca(NO_3)_2$ 0.75mM, KNO_3 2.5mM, KH_2PO_4 0.5mM, $MgSO_4$ 0.75mM, $NaNO_3$ 1mM, Ferric EDTA 9.22μM, H_3BO_3 9.22μM, $CuSO_4$ 0.16μM, KCl 14.1μM, $MnSO_4$ 3.6μM, $NH_4Mo_7O_{24}$ 0.106μM, $ZnSO_4$ 0.77 μM). Groups of ten Peldon plus ten Rothamsted seedlings per 500ml of liquid nutrient were grown in a constant environment (16 h. day, 20°C, Photosynthetically active radiation within waveband 400-700 nm. (PAR) 325μM m^{-2} sec^{-1}, r.h. 75%; 8hr. night 16°C, r.h. 82%).

After 10 days, when the seedlings had developed 1-2 unfolded leaves (growth stage 11-12; Tottman and Broad 1987), chlorotoluron was incorporated into the liquid medium at nine concentrations across the range 0.01-5 mg/l. Similar treatments were prepared using isoproturon. Control plants were maintained in herbicide-free nutrient. Three replicates of all these herbicide treatments were also prepared and given additional treatments of ABT at concentrations of 5, 7.5 and 10 mg/l respectively.

Nutrient solutions plus herbicide and ABT adjuvants were changed every five days. Damage to plants was assessed by measuring fresh weight of foliage three weeks after the commencement of herbicide treatment.

RESULTS

The effects of increasing concentrations of (i) chlorotoluron and (ii) isoproturon in the liquid medium upon fresh weight of foliage in the Peldon and Rothamsted populations of black-grass are illustrated in Fig 1. Chlorotoluron at concentrations > 0.05 mg/l are phytotoxic to the Rothamsted population, but the Peldon population suffers equivalent damage only at concentrations > 0.5 mg/l (Fig. 1a and 1b). In the case of isoproturon the difference between the two populations is smaller. Concentrations > 0.05 mg/l are again phytotoxic to the Rothamsted population, and comparable damage is inflicted in the Peldon population by concentrations > 0.2 mg/l (Fig. 1c and 1d).

The enhanced growth observed in seedlings of the Peldon population exposed to subtoxic concentrations of herbicide is attributable to a loss of competition from the Rothamsted plants which are killed by these concentrations. The effect is eliminated when the two populations are grown in isolation, but herbicide phytotoxicity is unaffected.

The influence of ABT on chlorotoluron and isoproturon phytotoxicity in both Peldon and Rothamsted black-grass populations are compared in Fig. 1. Treatment with ABT alone reduced the fresh weight of foliage by 10-30%. This was accompanied by a reduction in leaf length and some necrosis at the leaf tips. Increasing concentrations of ABT progessively reduces tolerance to chlorotoluron in the Peldon population (Fig. 1a). ABT at 5 mg/l substantially suppresses the tolerance, and 10 mg/l almost eradicates it. The development of chlorosis and later necrosis associated with chlorotoluron is now comparable to that in the Rothamsted population. Likewise the less pronounced tolerance to isoproturon in the Peldon population is removed by 5mg/l ABT, though applications of higher concentrations have little further effect (Fig. 1c). These synergistic effects on chlorotoluron and isoproturon phytotoxicity are not as apparent in the Rothamsted population (Fig. 1b and 1d).

DISCUSSION

Chlorotoluron is degraded and detoxified in plant tissues and soils via ring-methyl oxidation, N-demethylation and conjugation of the corresponding metabolites (Gross et al., 1979; Cole & Owen, 1987a). Analogous pathways of degradation have been demonstrated for isoproturon in soil (Mudd et al., 1983). The selective properties of these herbicides have been attributed to their rapid degradation and detoxification by the crop plants (Ryan et al., 1981; McIntosh et al., 1981). In wheat and barley, the major route of chlorotoluron degradation is via ring-methyl oxidation reactions (Gross et al., 1979; Ryan et al., 1981). This is particularly apparent in those

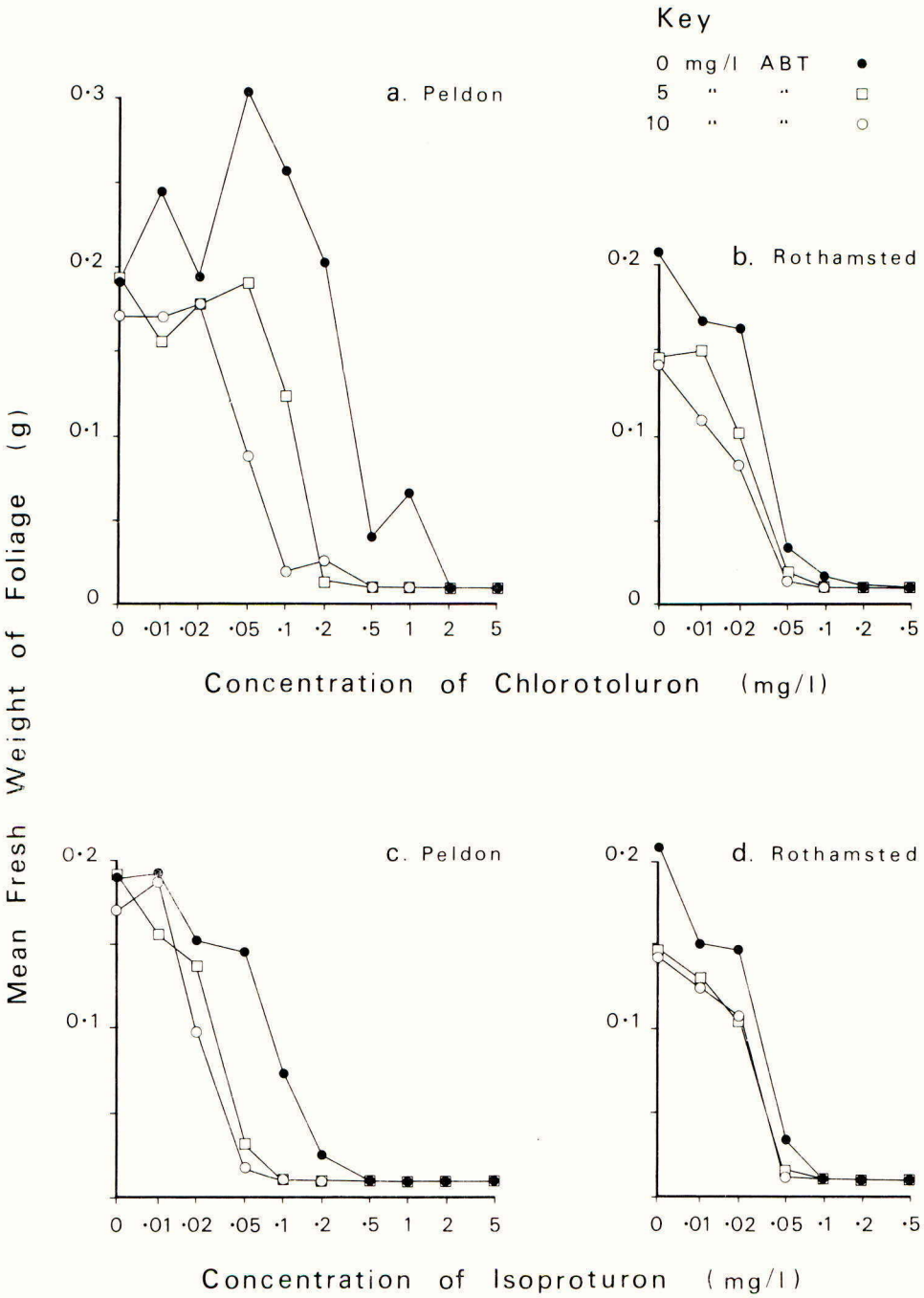

Figure 1. Synergistic effects of 1-aminobenzotriazole (ABT) on phytotoxicity of chlorotoluron and isoproturon in Peldon and Rothamsted populations of black-grass (Alopecurus myosuroides).

cereal varieties which are most tolerant to chlorotoluron (Ryan & Owen 1983), but in resistant cotton (Gossypium hirsutum) N-demethylation is the predominant pathway (Ryan et al., 1981; Cole & Owen, 1987a).

The P450 mixed function oxidase inhibitor ABT is synergistic to chlorotoluron and isoproturon phytotoxicity in wheat (Cabanne et al., 1985; Gaillardon et al., 1985), and in cell suspension cultures of cotton and maize (Zea mays) (Cole & Owen, 1987b). Both oxidation of alkyl groups and N-demethylation are inhibited, but the extent to which each is affected appears to be dependent upon the molecular structure of the herbicide and the plant species. The stunting effects associated with applications of ABT might similarly be associated with inhibition of cytochrome P450 dependent oxidation and demethylation in gibberellin and sterol biosynthesis (Buchenauer et al., 1984; Burden et al., 1987; Hedden & Graebe, 1985).

These findings together with the results of the present work suggest that resistance to chlorotoluron and isoproturon in the Peldon black-grass population may be attributable to rapid herbicide degradation and detoxification. Resistance based upon such enhanced enzymic activity might explain cross resistance in the Peldon population to other herbicides of varied modes of action including terbutryne, diclofop-methyl, chlorsulfuron and pendimethalin (Moss & Cussans, 1987; Moss, 1987).

Rotations and mixtures of herbicides probably have limited potential for controlling cross resistance of this nature, and increased applications of herbicide are neither economical nor environmentally desirable. ABT cannot be used in practice since synergy also occurs in herbicide-treated cereals. However, oxidase inhibitors can be species specific (Gressel & Shaaltiel, 1987). Other synergists of the type therefore may provide an effective means of overcoming resistance associated with rapid herbicide degradation.

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FURTHER INVESTIGATIONS INTO THE RESISTANCE OF CHICKWEED (STELLARIA MEDIA) TO MECOPROP

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ABSTRACT

The experiments described in this paper have confirmed the presence of mecoprop-resistant chickweed (Stellaria media) in a large area to the north of Bath (Avon). There was at least a six-fold difference in sensitivity between the resistant and susceptible plants. Comparison of eighteen stocks showed that the distribution of resistance was irregular, as resistant plants were collected from fields adjacent to those containing susceptible ones. There were also indications that plants from some fields were intermediate in sensitivity. Plants resistant to foliage applications of mecoprop were also less sensitive to soil applications of this herbicide, indicating that effects on herbicide uptake and translocation are unlikely to be the cause of the resistance. In an experiment to test for cross-resistance, both mecoprop-resistant and susceptible seed stocks were equally sensitive to the growth regulator herbicides, dicamba, benazolin and fluroxypyr.

INTRODUCTION

The occurrence of herbicide resistant weeds has become increasingly common in recent years, frequently as a result of repeated use of the same or closely related herbicides. The most serious and widespread problem, worldwide, is resistance to the triazine herbicides (LeBaron & Gressel 1982), but in the UK, although present on some horticultural holdings, triazine resistance is of minor importance. Of greater potential concern in England, is the occurrence of resistance to chlorotoluron and a number of other unrelated herbicides in black-grass (Alopecurus myosuroides) in Essex (Moss 1987, Moss & Cussans 1987). Annual meadow grass resistant to paraquat has also been identified in England (Putwain 1982).

Resistance to growth regulator herbicides has only rarely been reported (LeBaron & Gressel 1982), despite their extensive use for over forty years. However, preliminary experiments reported in 1985 indicated that chickweed (Stellaria media) from two fields near Bath (Avon) was resistant to the normal field rates of the phenoxy-alkanoic acid herbicide, mecoprop (Lutman & Lovegrove 1985). It was not clear how this resistance had arisen, as the farms concerned did not have a long history of the use of this herbicide. Nor was it clear whether the two fields concerned were isolated examples, or were part of a much more widespread problem. The earlier experiments had indicated that there was some cross-resistance to other phenoxy-alkanoic acid herbicides but field observations had suggested that the growth regulatory herbicide, fluroxypyr might still be effective.

Thus, there was a need to confirm the occurrence of resistance, to delineate the extent of resistance in the Bath area, to identify those herbicides that would be effective against the mecoprop-resistant plants and to understand the mechanisms of resistance involved. The five

experiments described in this paper attempted to provide this information.

MATERIALS AND METHODS

General

In all the experiments described, five chickweed plants were grown from seed in a standard potting compost in 9 cm pots, on capillary matting in an unheated glasshouse, during summer 1986. The foliar treatments of mecoprop and the other herbicides were all applied with a laboratory pot sprayer fitted with a 'Spraying Systems' 80015 'TeeJet' nozzle delivering 300 l/ha at a pressure of 207 kPa. The soil drench treatments in Experiments 1 and 2 were applied with a syringe, each pot receiving 10ml of herbicide solution, the dose being calculated on the basis of the surface area of the pot.

Experimental Design and Analysis

All five experiments were of a randomised block design with three replicates. The herbicide treatments were applied at a range of doses so that dose response curves could be calculated. The Rothamsted Maximum Likelihood Programme (MLP), constrained to an asymptote of zero, was used to fit logistic curves to the fresh and dry weight data. In general, the fresh weights gave more consistent results than the dry weights, as the dry weights of dead and live plants differed less than their fresh weights. From these curves the doses required to reduce plant weights by 50%, compared to the weight of the untreated controls, were calculated (ED_{50}). In Experiments 4 and 5 parallel curve analysis (Ross 1978) was used to group stocks of chickweed, according to their susceptibility.

Foliage and soil applications of mecoprop (Experiments 1 and 2)

The chickweed plants in these two experiments were treated with mecoprop (K salt, 570 g a.e./l a.c.) 4 - 5 weeks after sowing on 14 May (Expt 1) or 23 June (Expt 2), when the plants were well established and beginning to flower. Visual assessments of effects were recorded approximately two weeks after treatment and the fresh and dry weights of the plants measured in the following week. Five stocks of chickweed were studied in both experiments. These were from the Weed Research Organisation, Oxford (WRO), Long Ashton Research Station, Bristol (LARS), May & Baker's Ongar Research Station (ONG), the first resistant field identified near Bath (BAO) and the second resistant field (BAN), also near Bath.

In Experiment 1 plants from the two Bath stocks were sprayed with a range of doses of mecoprop from 1.4 to 7.0 kg a.e./ha and those from the three others were treated with doses from 0.2 to 2.6 kg a.e./ha. The soil drench treatments were eight doses of mecoprop equivalent to: 0.25 - 5.5 kg a.e./ha for WRO, ONG, and LARS and 2.5 - 13.0 kg a.e./ha for the two Bath stocks. The soil drench treatments in Experiment 2 were eight doses of mecoprop from 2.0 to 16.0 kg a.e./ha, applied to all five stocks. In both experiments the growth of the treated plants was compared with untreated controls. The fresh and dry weight data were used to generate the dose response curves.

Performance of other herbicides (Experiment 3)

The activities of benazolin (K salt, 300 g a.e./l a.c.), dicamba (Na salt, 240 g a.e./l a.c.) and fluroxypyr (ester, 200 g a.i./l e.c.) against the same five stocks of chickweed (WRO, LARS, ONG, BAO, BAN) were assessed in the third experiment. As in Experiments 1 and 2, the plants were sprayed 4 - 5 weeks after sowing and were harvested 2 -3 weeks after

spraying. Fresh and dry weights of foliage were recorded. Visual symptoms of damage were also noted. Four doses of each herbicide, 0.1 - 0.8 kg a.e./ha for dicamba and benazolin and 0.06 - 0.24 kg a.i./ha for fluroxypyr were compared on all five seed stocks. The weights of the treated plants and the untreated controls were used to produce the dose response curves and the ED_{50}s.

Extent of resistance (Experiments 4 and 5)

In these two trials plants grown from seed collected from chickweed plants from up to eighteen fields from the Bath area (Fig. 1) were tested for their sensitivity to mecoprop. A known susceptible seed stock (WRO) was included for comparison. The plants were sprayed on 30 July (Expt 4) or 11 Sept (Expt 5) and were harvested 19 or 12 days after treatment, respectively. Eight doses of mecoprop (K salt, 570 g a.e/l a.c., 1.4 - 11.4 kg a.e./ha) were applied in Experiment 4 to the nine stocks tested and six doses (0.8 - 7.8 kg a.e./ha) to the nineteen stocks treated in Experiment 5. Untreated control plants were also included. As in the previous experiments response curves from the fresh and dry weight data of the different stocks were produced.

RESULTS

Foliage and soil applications of mecoprop

In the first experiment, the dose response curves based on the fresh weight data, for the foliar applications of mecoprop, on the three non-Bath seed stocks, were similar, giving ED_{50}s between 0.74 and 1.13 kg a.e./ha (Table 1). The Bath plants were much less sensitive with ED_{50}s of around 8.0 kg a.e./ha. Because of the degree of resistance shown by the Bath plants, even the highest dose tested achieved only a modest degree of control. There was at least a seven-fold difference in sensitivity between the Bath plants and the others. All the plants exhibited an epinastic response to the mecoprop within 24h of treatment, especially at the higher doses, the leaves curled and the stems and petioles became twisted. The symptoms slowly disappeared from the Bath plants but not from the others.

TABLE 1

The effect of foliage and soil applications of mecoprop on the growth of five stocks of chickweed. Data = $\log_{10}$ dose (kg a.e./ha) required to reduce fresh weights by 50%, compared to the growth of the unsprayed controls (ED_{50})

Stock	Experiment 1						Experiment 2		
	Foliage Application			Soil Application			Soil Application		
	Log ED_{50}	(ED_{50})	S.E. of mean	Log ED_{50}	(ED_{50})	S.E. of mean	Log ED_{50}	(ED_{50})	S.E. of mean
WRO	0.066	(1.16)*	0.079	0.904	(8.01)	0.169	0.784	(6.08)	0.079
LARS	-0.128	(0.74)	0.064	0.767	(5.86)	0.115	0.305	(2.02)	0.157
ONG	0.053	(1.13)	0.065	0.856	(7.17)	0.129	0.629	(4.26)	0.066
BAO	0.910	(8.13)	0.045	1.446	(27.9)	0.190	1.965	(92.3)	0.458
BAN	0.892	(7.80)	0.039	1.674	(47.2)	0.207	1.830	(67.6)	-

* detransformed doses

The activity of mecoprop applied to the soil was much poorer than that following the foliar treatments. Despite the use of higher doses, none of the treatments achieved a very high degree of control. The data were also considerably more variable. The calculated ED_{50}s from the fresh weight data, for the WRO, LARS and Ongar seed stocks were between 5.9 and 8.0 kg a.e./ha, whilst those of the two Bath stocks, which were almost unaffected by the mecoprop, were 28 and 47 kg a.e./ha (Table 1). The results from the soil applications of the second experiment were similar to those of the first. The ED_{50}s of the three non-Bath stocks were between 2 and 6 kg a.e./ha, whilst those of the Bath stocks were even higher than in the first experiment, 68 and 92 kg a.e./ha. These very high ED_{50}s again reflect the lack of effect from all the doses of mecoprop tested. Clearly, in both trials, the Bath stocks were less susceptible to mecoprop applied through the soil than the others.

Performance of other herbicides

Dicamba

The sensitivity of the five stocks of chickweed to dicamba was similar. All the plants showed epinastic symptoms. The ED_{50}s, based on the dose response curves of the fresh weights, were all between 0.28 and 0.43 kg a.e./ha, the least sensitive were the plants from WRO and Ongar (Table 2).

Benazolin

The performance of benazolin was not greatly affected by the origins of the plants, as curled leaves and twisted stems were observed on all five stocks. All five stocks were controlled by benazolin, the ED_{50}s being between 0.30 and 1.11 kg a.e./ha (Table 2). Again, the WRO and Ongar plants were slightly less susceptible than the others.

TABLE 2

The sensitivity of five stocks of chickweed to dicamba, benazolin and fluroxypyr. Data = $\log_{10}$ dose (kg/ha) required to reduce foliage fresh weight by 50%, compared to the growth of the unsprayed controls (ED_{50}).

Stock	Dicamba		Benazolin		Fluroxypyr	
	Log ED_{50} (ED_{50})	S.E. of mean	Log ED_{50} (ED_{50})	S.E. of mean	Log ED_{50} (ED_{50})	S.E. of mean
WRO	-0.370(0.43)*	0.081	-0.212(0.61)	0.097	-0.950(0.112)	0.123
LARS	-0.550(0.28)	0.100	-0.322(0.48)	0.167		
ONG	-0.352(0.44)	0.084	0.044(1.11)	0.380	-1.079(0.083)	0.088
BAO	-0.552(0.28)	0.033	-0.323(0.48)	0.119	-1.382(0.041)	0.235
BAN	-0.520(0.30)	0.096	-0.521(0.30)	0.056	-1.331(0.047)	0.125

* detransformed dose

Fluroxypyr

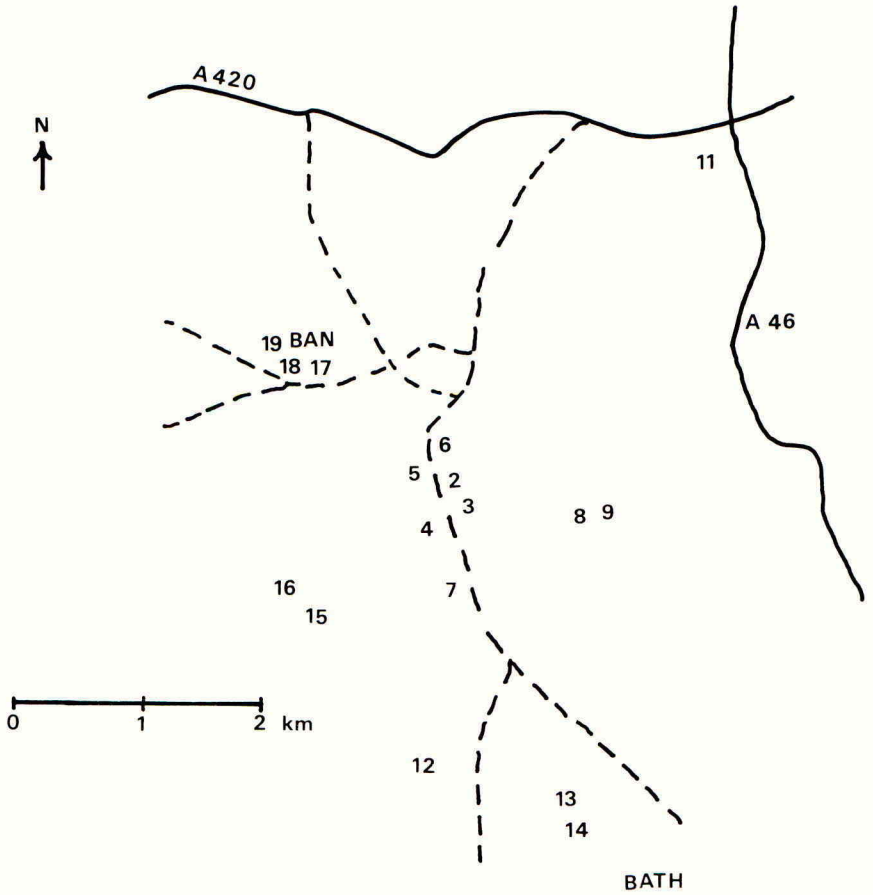
Plants from the four stocks tested rapidly became twisted following the application of fluroxypyr. The ED_{50}s indicated that the two non-Bath stocks were marginally more tolerant than the mecoprop-resistant Bath stocks (Table 2) but these apparent differences were not supported by the statistical analysis.

Extent of resistance

The first test of resistance to include seven of the additional seed stocks from near Bath showed that some were as resistant as the original stocks, whilst others were not. There was a very high degree of control, from even the lowest dose of mecoprop, of the plants from WRO and those from fields 2,3 and 5, near Bath (Fig. 1 and Table 3). As a consequence of this, the fit of the dose response curve was poor and the MLP programme did not provide standard errors for the calculated ED_{50} value. In contrast, fields 6 and 7 appeared as resistant as the BAO standard, with ED_{50}s of 4.2 and 3.3 kg a.e./ha. Parallel curve analysis confirmed that stocks 2, 3 and 5 were as sensitive as WRO and 6, 7, and 8 were as tolerant as BAO.

FIGURE 1

The distribution of fields sampled for mecoprop-resistant chickweed in an area to the north of Bath.



These two groups were different from each other and from stock 9, which was intermediate. The overall level of control in this trial was higher than that achieved in Experiment 1.

TABLE 3

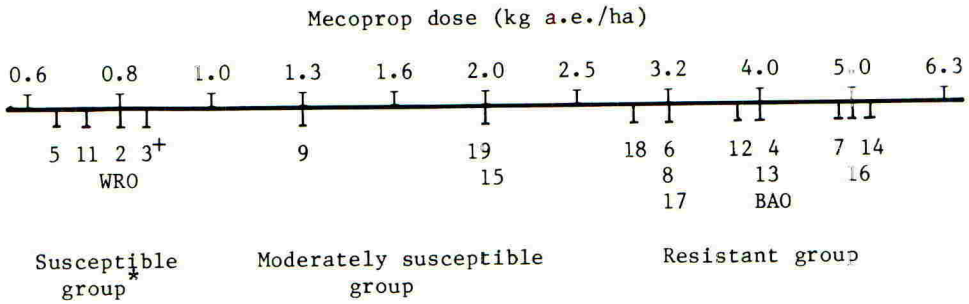
Relative sensitivity of nine stocks of chickweed to foliage applications of mecoprop (Experiment 4). Data = $\log_{10}$ dose (kg a.e./ha) required to reduce foliage fresh weights by 50%, compared to the growth of the unsprayed controls (ED_{50}).

Stock	Log ED_{50} (ED_{50})	S.E. of mean	Stock	Log ED_{50} (ED_{50})	S.E. of mean
WRO	-1.458(0.035)*	-+	6	0.619(4.16)	0.061
BAO	0.597(3.95)	0.034	7	0.515(3.27)	-
2	-1.547(0.028)	-	8	0.274(1.88)	-
3	-1.169(0.068)	-	9	-0.045(0.90)	0.206
5	-3.451(0.0004)	-			

* detransformed values
\+ because of the inadequacy of the dose range (all doses severely damaged the plants), no standard errors are available

FIGURE 2

Relative sensitivities of nineteen stocks of chickweed to foliage applications of mecoprop (Experiment 5). Data = detransformed $\log_{10}$ dose (kg a.e./ha) required to reduce foliage fresh weights by 50% compared to the growth of the unsprayed controls (ED_{50}).



* Groupings produced by parallel curve analysis
\+ see Figure 1 for the location of the fields containing the nineteen seed stocks

In the final experiment a range of responses to mecoprop were identified in ninteen stocks of chickweed, including the standards 'WRO' and 'BAO'. The parallel curve analysis of the fresh weight data identified a susceptible group (WRO,2,3,5,11) with an ED_{50} of approximately 0.7 kg a.e./ha, and a resistant group (BAO,4,6,7,8,12,13,14,16,17) with an average

ED_{50} of aproximately 4.0 kg a.e./ha (Fig. 2). Stock 18 was not quite as resistant as the main resistant group, and plants from fields 9, 15 and 19 were clearly intermediate in response.

DISCUSSION

The results of our experiments confirmed the presence of chickweed resistant to mecoprop in an area of at least 30km^2 to the north of Bath (Fig. 1). The full extent of the affected area is still not fully defined as the chickweed plants from some fields distant from the centre of the area were still resistant. However, plants from the most remote site (11) were susceptible. Earlier experiments (Lutman & Lovegrove 1985) included only one 'susceptible' stock, from WRO, so it could be argued that this was ultra-sensitive. However, the first experiment clearly showed that three different standard stocks (WRO, LARS, ONG) were all equally sensitive. The ED_{50} values for the trials indicated that there was at least a six-fold difference in tolerance between the resistant and susceptible stocks.

The variability in the level and distribution of resistance in the final experiment was unexpected. The plants from fields 2, 3 and 5, which were susceptible to mecoprop, were adjacent to the original resistant field (BAO) and were very close to fields 4 and 6, which contained resistant plants. There were also indications in this experiment and in Experiment 4 that some stocks were intermediate in response, particularly stock 9. Most of the seeds used in the experiments were produced by a small number of plants collected from each of the fields. So it is not clear whether this apparent variation between adjacent fields was due to differences in susceptibility within fields that had not been identified because of the small numbers of plants collected, or to real differences between them. Further, more intensive, sampling of the fields is needed.

Previous experiments have shown that mecoprop-resistant plants were also resistant to the related phenoxy-alkanoic acid herbicides MCPA and dichlorprop (Lutman & Lovegrove 1985). In the experiments reported here there was no indication that mecoprop resistance also extended to the growth regulator herbicides benazolin, dicamba and fluroxypyr. Thus, the mecoprop cross-resistance appears to be restricted to closely related herbicides, unlike the extensive cross-resistance identified by Moss & Cussans (1987) for chlorotoluron-resistant black-grass. As there is a lack of resistance to other herbicides, a number of alternative products containing effective post-emergence herbicides are available to the farmer.

There are a number of possible explanations for the resistance of the chickweed plants. The uptake or translocation of the herbicide may be affected, or the plants may vary in their ability to metabolise mecoprop. As plants resistant to foliar applications of mecoprop were also shown to be less sensitive to soil applications of this herbicide it seems unlikely that the primary cause of resistance is related to lowered uptake or translocation. Poor performance from phenoxy-alkanoic acid herbicides on some weed species (e.g. MCPA on cleavers, Galium aparine) seems to be due to the ability of the weed to detoxify the herbicide. Thus it is possible that metabolic differences may account for the resistance shown in these experiments. Some support for this theory is provided by the response of the treated plants to mecoprop. Epinastic symptoms were produced by all stocks but these symptoms subsequently disappeared from the plants that were resistant. Detailed biochemical studies are planned to identify the basis of this resistance.

It is frequently stated that herbicide resistance develops because of the repeated use of the same herbicide. This may be true for the development of triazine-resistant weeds in maize monocultures but appears not to be true for this mecoprop-resistant chickweed. The majority of farms in the affected area are primarily grassland, with a small area of cereals, so their use of mecoprop has not been intensive. It would appear likely that the chickweed population in this area has been resistant to mecoprop for some time and this was noticed only when cereals were grown and mecoprop was used as the sole broad-leaved weed herbicide. Superficial observations of the plants in the five experiments described indicate that the herbicide resistant plants were as 'fit' (vigorous) as the susceptible ones. This factor would ensure their survival in the fields in years when mecoprop is not used. This absence of a fitness penalty associated with herbicide resistance contrasts with triazine resistance, where resistant plants tend to be less competitive, in the absence of the herbicide, than susceptible ones (Radosevich & Holt 1982).

ACKNOWLEDGEMENTS

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CROSS-RESISTANCE TO PARAQUAT AND ATRAZINE IN CONYZA CANADENSIS

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ABSTRACT

A biotype of Conyza canadensis from a vineyard subjected to repeated paraquat and triazine herbicide treatment, and a wild type from an adjacent ruderal area, were examined for resistance to paraquat and atrazine. A reduction in CO_2 fixation was observed after treatment with paraquat for 1 hour, but thereafter it was strongly stimulated in the resistant biotype while that of the susceptible biotype was inhibited. However CO_2 fixation following glufosinate treatment in paraquat-resistant plants was reduced to a greater extent than in susceptible plants. Glufosinate caused alteration of fluorescence transient in both biotypes. A higher concentration of glufosinate was required to cause inhibition in the susceptible biotype. Cross-resistance to atrazine of the paraquat resistant biotype was demonstrated through slow fluorescence induction measurements.

INTRODUCTION

Paraquat is used as a broad spectrum total-kill herbicide and has been used extensively as a herbicide for foliar treatment in fruit orchards and vineyards. Tolerance to paraquat was first reported by Faulkner (1976) in Lolium perenne. Weed resistance to paraquat has been discovered in Erigeron philadelphicus, in Conyza bonariensis in England, Japan and Egypt (Gressel et al., 1982), in Poa annua in England (Gressel et al., 1982) and in Hordeum glaucum in Australia (Warner & Mackie, 1982). Paraquat had been applied in every case 2 or 3 times annually during the preceding 5 to 11 years. The paraquat-resistant Erigeron spp show cross-resistance to diquat, but are susceptible to glyphosphate, bentazone and MCPA (Watanabe et al., 1982).

Several hypotheses of the paraquat-resistance mechanism have been proposed such as adsorption of the paraquat to lignified areas, lack of penetration due to increased epicuticular wax (Thrower et al., 1965), binding of paraquat to cell walls, restriction of its movements into the chloroplast (Fuerst et al., 1985), alteration of the redox potential of the PS-I, primary electron acceptor and detoxification of the superoxide anion radical by elevated levels of superoxide dismutase, ascorbate peroxidase and glutathione reductase (Youngman & Dodge, 1981; Harvey & Harper, 1982; Shaaltiel & Gressel, 1986).

Unexpectedly, in 1986 we discovered a paraquat-resistant biotype of C. canadensis which was cross-resistant to atrazine. This is an

interesting observation because the action site of atrazine and paraquat are different. Paraquat had also been applied to these vineyards 3 or 4 times annually during the preceding 10 years and atrazine had also been used extensively in this period. The present study involves verification of the reversed resistance to glufosinate in paraquat-resistant C. canadensis and characterisation of the cross-resistance to atrazine.

MATERIALS AND METHODS

Plant material

Resistant plants of C. canadensis were collected from vineyards near Kecskemet (Hungary) where paraquat and atrazine had been applied continuously for 10 years. This population was tested for herbicide-resistance in field experiments. Susceptible plants were collected from adjacent areas where no herbicides had been applied. Plants were collected during the early summer, when 7 - 8 months old and between the rosette and flowering stages. Fully expanded leaves of the same size (6 - 8 cm) were cut from the collected plants and were floated on different concentrations of herbicides.

Field experiments

Small plots (10 m^2) were used in vineyards in a randomised block design with 4 replicates. Applications were made in the summer by knapsack sprayer at a volume 400 l/ha and a pressure of 300 kPa. Paraquat ('Gramoxone', 250 g a.i./l) was used at a range of rates from 4 - 64 l/ha and glufosinate ('Basta', 200 g a.i./l) at 4 l/ha. C. canadensis was treated at the rosette stage. Weed control was assessed visually in comparison with untreated control plots using the EWRC scale, 1 week and 1 month after treatment.

CO_2 fixation

C. canadensis leaves were floated on different concentrations of paraquat and diquat (10^{-4} - 10^{-5} m) at 10 mW illumination intensity. Measurements were made at 1 and 4 hours. The rate of light-induced CO_2 fixation was determined using the method of Lang et al., (1985). Leaves were illuminated with white light of 60 mW/cm^2 for 2 minutes. Fifty discs 5 mm in diameter were cut per treatment, heat denatured and dried by ironing between 2 layers of filter paper at 100 - 150°C, then placed in scintillation vials. The radioactivity of the samples was determined by a liquid scintillation technique. The total or gross photosynthesis was calculated by correction for the rate of dark CO_2 uptake. The samples contained chloroplasts equivalent to 40 µg chlorophyll cm^{-2} in both biotypes.

Fluorescence induction measurements

Excised leaves of both resistant and susceptible biotypes were treated with glufosinate at 10^{-3} to 10^{-5} M concentration for 24 hours. The effect of atrazine and diuron on the PS-II electron transport chain of resistant and susceptible plants was also determined using excised leaves. Fluorescence induction measurements on excised leaves were carried out after a 30-minute dark adaption (Lehoczki et al., 1984). Blue actinic light of 5 mW/cm^2 intensity (generated by a 650 W xenon lamp) was transmitted by a Schott BG 12 filter. Fluorescence emitted at 90°C was detected with a photomultiplier through a red SIF 675 interference filter and recorded with a transient recorder. The dwell time between 1024 samplings was 1 ms and 300 ms in the fast and slow fluorescence induction measurements, respectively. In each experiment, 16 independent curves were recorded and averaged automatically.

RESULTS

Field experiments

The results of the field experiments are shown in Table 1. One week after treatment it was found that paraquat at 4 l/ha and 8 l/ha did not damage the C. canadensis. Paraquat at 16 l/ha and 32 l/ha damaged only the margins of younger leaves. The highest dose (64 l/ha) caused only partial damage. However, glufosinate at 4 l/ha killed the paraquat-resistant C. canadensis in 3 days. One month after treatment the high rates of paraquat were similar to the control plots.

TABLE 1

The effect of post-emergence applications of paraquat and glufosinate on paraquat-resistant C. canadensis in vineyards.

Herbicides	Rate l/ha	Weed control (EWRC scale) 1 week	1 month
Paraquat	4.0	9	9
	8.0	9	9
	16.0	7	9
	32.0	6	9
	64.0	5	8
Glufosinate	4.0	1	1

CO_2 fixation

The data show considerable differences between the paraquat-resistant and susceptible biotypes. CO_2 fixation in the susceptible biotype was inhibited after 1 hour of treatment with either paraquat or diquat (Table 2). In striking contrast, CO_2 uptake in the resistant plants was slightly decreased after 1 hour, but thereafter was stimulated by paraquat. However, treatment with glufosinate at a range of concentrations resulted in a greater reduction in CO_2 fixation in paraquat-resistant plants than in susceptible ones (Table 3). The decrease in CO_2 fixation was considerable after 12 hours treatment. This correlates well with the findings of Kocher (1983) that CO_2 fixation was strongly inhibited in Sorghum halepense plants after 8 hours treatment with glufosinate. Diquat caused partial inhibition after 4 hours treatment.

TABLE 2

Net CO_2 fixation (nmol CO_2 CM^{-2} s^{-1}) of C. canadensis leaves after 1 and 4 hours treatment with paraquat and diquat.

Treatment	Susceptible 1 h	4 h	Resistant 1 h	4 h
Control	1.93	1.89	1.90	1.85
Paraquat (10^{-5} M)	0.60	0.092	1.60	3.45
Diquat (10^{-5} M)	0.54	0.075	1.70	1.55

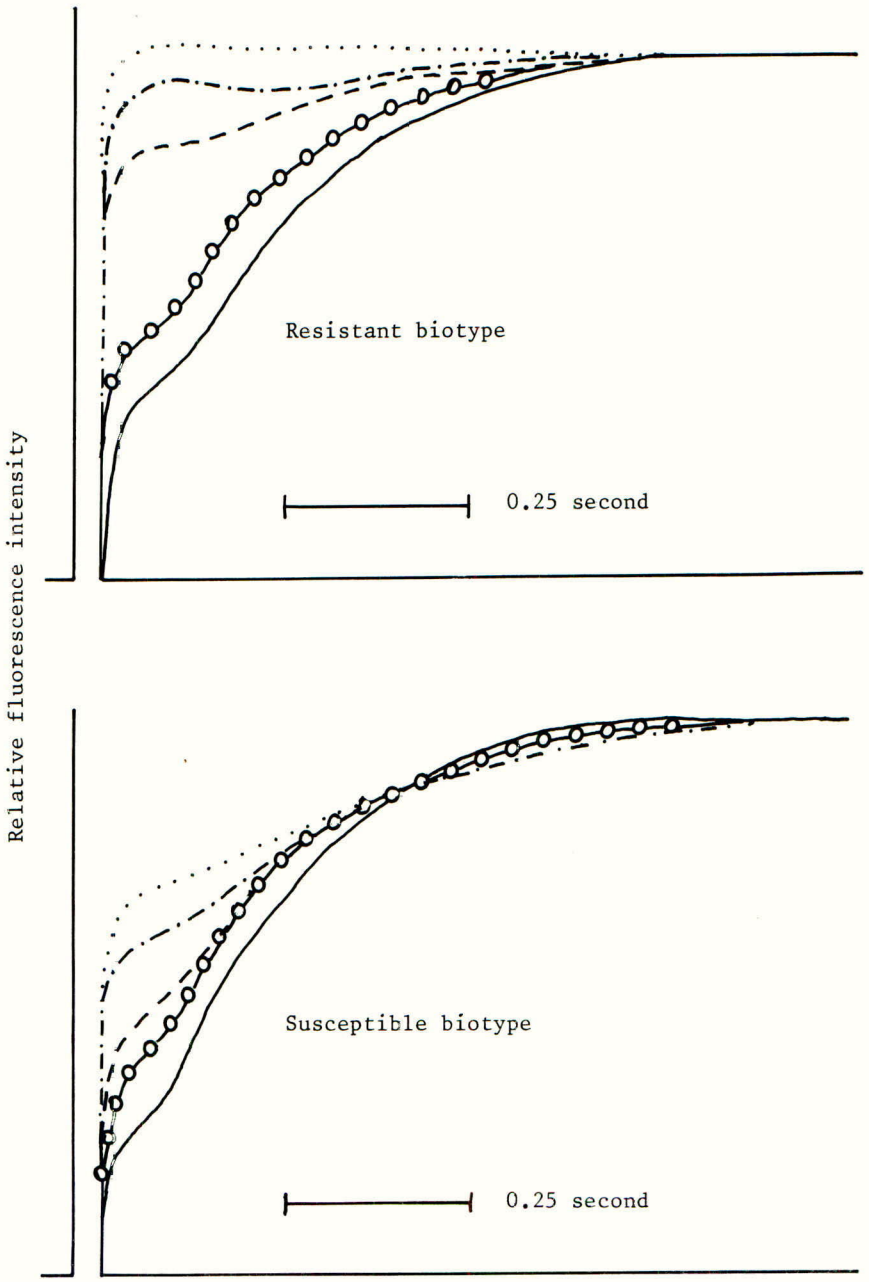

Fig. 1. Fluorescence induction curves of excised leaves of Conyza canadensis treated with glufosinate at 10^{-3}M (·····), 5 x 10^{-4}M (—·—·—), 10^{-4}M (— — —), 10^{-5}M (—o—o—) and untreated (———).

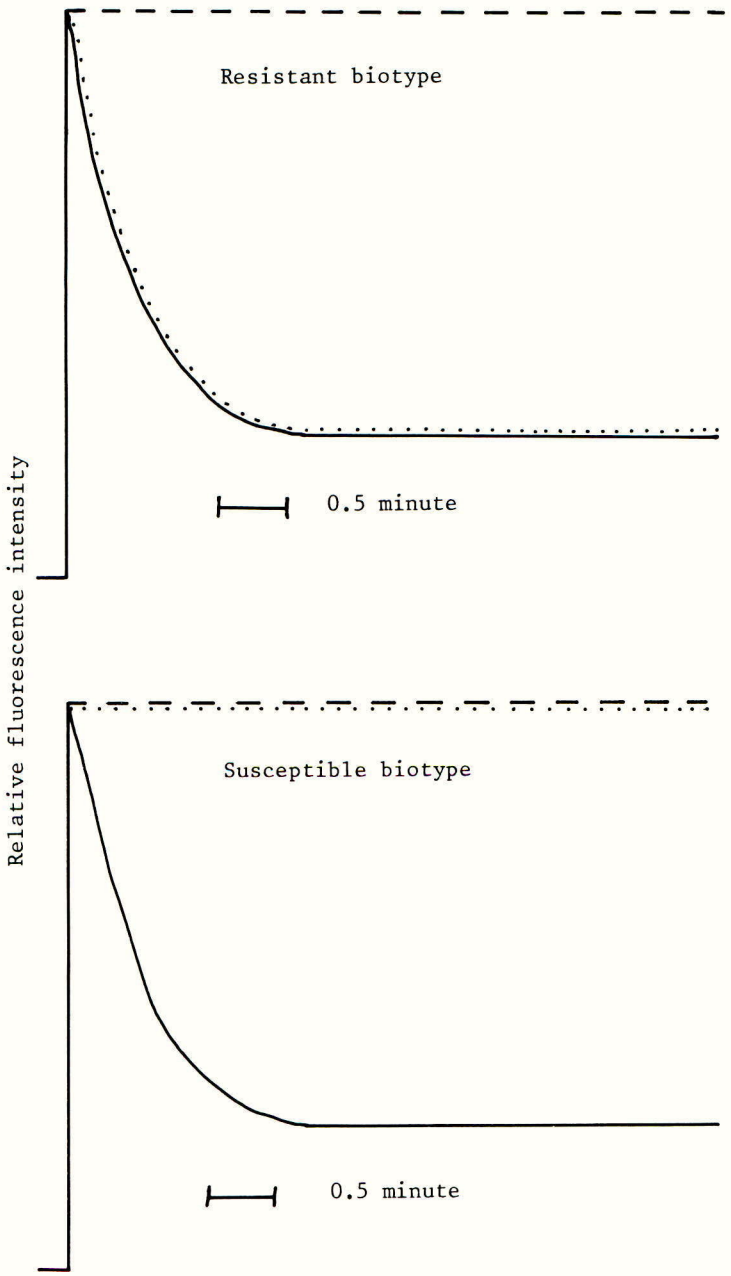

Fig. 2. Slow fluorescence curves for excised leaves of Conyza canadensis treated with 10^{-5}M diuron (– – –), 10^{-4}M atrazine (·····) and untreated (———).

TABLE 3

Net CO_2 fixation (nmol CO_2 cm^{-2} s^{-1}) of C. canadensis leaves after 12 hours treatment with glufosinate.

	Herbicide concentration			
Biotype	Control	10^{-5} M	10^{-4} M	10^{-3} M
Susceptible	2.00±0.10	1.10±0.12	0.35±0.07	0.18±0.08
Resistant	1.90±0.15	0.75±0.10	0.24±0.06	0.095±0.05

Fluorescence induction of excised leaves

The dose-response curves shown in Figure 1 indicate that in vivo, glufosinate directly or indirectly inhibited electron transport. Surprisingly, a higher concentration of glufosinate was required to cause inhibition in the susceptible biotype. We studied electron transport in the presence of atrazine and diuron by measuring the slow fluorescence induction recorded in the time range of 5 minutes (Figure 2). Control and atrazine treated leaves of the resistant biotype exhibited quenching of the fluorescence from the initial P to the terminal T level. After diuron treatment the fluorescence yield was maintained at the P level.

DISCUSSION

The results of CO_2 fixation as a time dependent function of paraquat action in the resistant biotype indicated that paraquat can reach its site of action and then probably stimulate protective processes in the resistant plants. This was also demonstrated by Shaaltiel & Gressel, 1987 and Polos et al., 1987. From the results of the glufosinate experiments, it can be shown that the paraquat-resistant biotype has a reversed resistance to glufosinate.

Glufosinate was more effective as an inhibitor of CO_2 fixation in the resistant biotype than in the susceptible and in field experiments we have found that glufosinate killed paraquat-resistant C. canadensis more rapidly than it killed the sensitive biotype. Glufosinate, a partially systemic contact and non-selective herbicide, seems to be suitable for the replacement of paraquat in post-emergence weed control programmes in vineyards. Glufosinate and its ammonium salts are potent inhibitors of glutamine synthetase (GS), but do not affect glutamate synthetase (GOGAT) or glutamate dehydrogenase, nor do they interfere with enzyme reduction (Wild & Manderscheid, 1983). This results in the rapid accumulation of ammonia in the leaf tissue after herbicide application. It is now generally accepted that higher plants effectively re-assimilate ammonia by the GS/GOGAT system (Wallsgrove et al., 1980; Kocher, 1983):-

$$\text{Glutamate} + \text{ATP} + NH_3 \xrightarrow{(GS)} \text{glutamine} + \text{ADP} + P_i + H_2O$$

$$\text{Glutamine} + \text{L-ketoglutarate} + \text{reduced ferredoxin} \xrightarrow{(GOGAT)} 2\ \text{glutamate} + \text{oxidized ferredoxin}$$

Ammonia metabolism in leaves is directly coupled to photosynthetic electron flow due to the dependence of leaf nitrite reductase and the leaf GS/GOGAT system on reduced ferredoxin (Kocher, 1983).

Our data support the hypothesis that the paraquat resistance mechanism and reversed resistance to glufosinate are connected. It has been concluded that the elevated level of enzymes of the Halliwell-Asada pathway (Shaaltiel & Gressel, 1986) or oversynthesis of substrates is able to reverse the paraquat-induced inhibition in resistant plants (Polos et al 1987). It is probable that as a protective mechanism against paraquat damage, one of the ferredoxin-dependent (decreasing ferredoxin) oversynthesis pathways may be responsible for the decreased capacity for ammonia detoxification. Therefore glufosinate increases ammonia levels more effectively in paraquat resistant plants. This may be indirect evidence of the "oversynthesis" theory for the paraquat resistance mechanism.

From these results it was concluded that C. canadensis displays a cross-resistance to paraquat and atrazine and the data indicate that resistance can be developed to herbicides with different modes of action. Cross-resistance to paraquat and to triazines could be developed independently, or there may be a close connection between them. We are currently investigating these possibilities.

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THE SEED BANK DYNAMICS OF TRIAZINE RESISTANT AND SUSCEPTIBLE BIOTYPES OF *SENECIO VULGARIS* - IMPLICATIONS FOR CONTROL STRATEGIES.

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ABSTRACT

Achene (seed) flux is described in groundsel (*Senecio vulgaris*) populations for triazine-resistant and susceptible biotypes under differing management practices. The respective roles of two achene banks (0 - 2 cm soil depth and > 2cm depth) are examined. It is concluded that achenes of the resistant biotype have greater longevity than the susceptible in the lower bank, whilst in the surface bank biotypes show differential longevity according to management practice.

INTRODUCTION

Groundsel (*Senecio vulgaris*) is a monocarpic composite which has an ephemeral or annual life cycle and functions as an opportunistic species (Harper 1981). Population renewal is achieved by seed germination from a soil reservoir, the production of achenes by surviving adult plants and their return dispersal to the soil. The population dynamics of triazine-susceptible and resistant biotypes of *S. vulgaris* was examined by Putwain et al. (1982) and Mortimer (1983). Study of the behaviour of seedling and adult plant populations demonstrated the influence of alternative weed management practices on the phenology and life history of plants Opportunistic plant species plants have short life expectancy. The long term persistence and abundance of the species in the adult phase is determined by the size of the seed bank. This paper reports an experimental investigation into the flux of triazine-susceptible and resistant achenes of *S. vulgaris* under different management practices.There were three experimental approaches.

1. The incorporation of achenes and their zonation in soil;
2. The survivorship of achenes within the soil profile;
3. The loss of achenes from the soil through seedling establishment.

MATERIALS AND METHODS

Study site

Experiments were conducted in 1984 and 1985 in blackcurrant plantations at the University of Liverpool Botanic Gardens at Ness, Wirral. Three weed management programmes had been applied over the previous seven years. These comprised ; a) spring application of simazine at a rate of of 2.5 kg a.i./ ha. ; b) spring simazine (as in a)) together with the use of paraquat in summer as a directed spray ; and c) a soil rotovation in spring which disturbed the soil profile to a depth of 15 cm, but did not invert it, and destroyed existing vegetation.

The incorporation of achenes and their zonation in soil.

The immediate fate of dispersed achenes

Achenes of *S. vulgaris* were sown at a rate of 6.10^4 m^{-2} (viability 98%) into spring simazine and rotovation treated areas in summer 1985. This was the same period when achenes were being naturally dispersed from plants in the locality. The fates and depth distribution of achenes were then monitored during the subsequent 38 days. On each occasion that soil cores were taken counts were made of emerging seedlings. Soil cores were sectioned depthwise into 1 cm slices, and spread thinly on sterile peat. Soil was then incubated in a heated glasshouse for 15 weeks during which emerging seedlings were counted, removed and the soil stirred on a weekly basis.

The distribution of achenes overwintering within the soil profile and the influence of soil disturbance on the soil distribution of achenes

In spring 1985, soil cores were taken from all three treatment areas where achene dissemination of both bioytpes had occurred during the previous spring and summer 1984. The distribution of achenes was estimated from counts of seedlings from cores as described above. The effects of soil disturbance on the soil profile distribution of achenes were determined as follows. Soil cores were taken from plots sown with *S. vulgaris* at a rate of 6.10^4 m^{-2} before and immediately after rotovation. Achene distribution was again estimated from emerging seedlings in core samples.

The survivorship of achenes within the soil profile.

Samples of 50 achenes of both biotypes were buried in nylon mesh packets (200 µm aperture) at depths of 1 and 7 cm in 1984 in each of the three management regimes : a) spring simazine treatment (2.5 kg a.i./ ha); b) soil rotovation to a depth of 15 cm; and c) a control in which neither herbicide application nor soil disturbance occured and a dense vegetation cover persisted. Packets were retrieved periodically over an 18 month period and their contents examined. The number of seedlings (dead and alive) were recorded and the remaining achenes were placed on moistened germination paper in petri-dishes and incubated at 20°C under a 12h photoperiod. Germination was recorded weekly, and achenes which germinated under these conditions were classified as being in enforced dormancy (Harper,1957). The viability of achenes which did not germinate after 28 days was tested with 1% 'tetrazolium chloride solution (Moore,1962). Achenes displaying stained embryonic tissue were classified as viable and to have been in a state of induced or innate dormancy. During rotovation treatment, packets which were to remain buried longer were temporally removed, stored and then replaced in darkness using a light-proof box to prevent exposure of seeds to direct light.

The loss of achenes from the soil through seedling establishment.

The emergence of seedlings of both biotypes was monitored in permanent plots located within the three management regimes, over twelve months from March 1984. Emerging seedlings were tagged with fine, coloured wire and their fates followed.

RESULTS AND DISCUSSION

The incorporation of achenes and their zonation in soil.

The immediate fate of dispersed achenes

Achene dispersion down the soil profile occurred immediately on sowing although the majority of achenes remained at or near the surface 1 cm of the profile in both simazine treated and rotovated plots. During the subsequent 5 weeks immediately after dissemination little movement of achenes occurred in simazine treated plots but after 38 days in rotovation treated areas, 24 % were at depths greater than 1 cm.

The fates of sown achenes over the immediate period after dissemination are illustrated in Fig 1. Soil samples removed on the same day as sowing (day 0) in both treatments resulted in a recovery of approximately 50% of achenes sown. This fraction however increased on subsequent sampling dates in simazine treated areas to fall after 40 days. In rotovated areas (Fig 1 b) the majority of achenes were found 5 days after sowing but then declined to 30 % after 25 days. Since 98% of sown achenes were viable, these results suggest that seed dormancy was not fully broken in the sample period. Breaking of innate or induced seed dormancy may have also occurred. The decline in the recovered fraction in rotovated plots may be either due to invertebrate predation and achene decay or to the induction of dormancy in achenes in the field, that was not broken during the period of assessment. Seedling emergence was observed in simazine treated plots by day 5 and resulted in a cumulative total of 43 % of achenes sown within 5 weeks. Little seedling emergence was noted in rotovated areas. Hilton (1983) has suggested that the germination requirements of *S. vulgaris* are not tightly defined and may change with time. In contrast , Popay and Roberts (1970) concluded that a light stimulus was usually required for germination. Watson (1987) found that seedling emergence in the field from depths greater than 2 cm was rare.

The distribution of achenes within the soil profile and the influence of soil disturbance

The distribution of *S. vulgaris* achenes in the profile under all three management regimes seven months after original dissemination was not significantly different from the depth distribution observed immediately post disperal. At least 90% of achenes were present in the top 2 cm of the soil in all three treatments. Soil rotovation evenly distributed achenes over the 0 - 2 cm layer which were initially in the top 1 cm of the profile. A small fraction was also distributed deeper within the profile to a depth of 5 cm. (Fig. 2) The differences in the proportion of seeds in the 0 - 2 cm and 2 - 5 cm layers before and after rotovation was statistically significant ($P \leq 0.001$ and $P \leq 0.01$ respectively).

The survivorship of achenes within the soil profile.

The decline in numbers of viable achenes buried at both 1 and 7cm was found to be exponential when graphically examined as arithmetic plots. Therefore survivorship was measured as half lives (Table 1), calculated by linear regression of logarithmically transformed numbers of viable achenes against time.

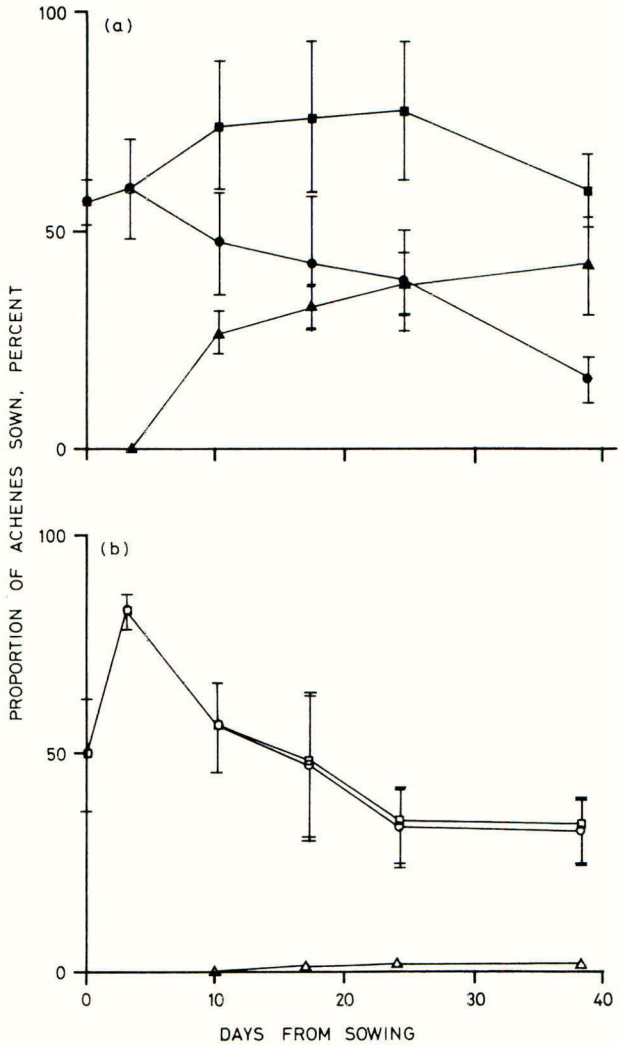

Fig. 1 The fates of achenes of *Senecio vulgaris* during a 38 day period immediately after sowing into (a) simazine treated areas, and b) rotovated areas. Squares - the proportion of achenes accounted for; circles - the proportion of sown achenes remaining in the achene bank; triangles - the cumulative proportion of achenes emerging. Confidence estimates are 2 s.e.'s.

TABLE 1

The survivorship of buried achene populations of *Senecio vulgaris* in a blackcurrant plantation under differing management regimes (see text for details). Data are mean rates of decline expressed as a half life in days for simazine resistant and susceptible biotypes and in parentheses are the % loss per annum.

Management regime	Depth of achene burial (cm)			
	1		7	
	Resistant	Susceptible	Resistant	Susceptible
Soil rotovation	329 (53.7)	454 (42.7)	884 (24.9)	696 (30.5)
Spring simazine	245 (64.4)	273 (60.4)	986 (22.6)	527 (38.2)
Control	501 (39.6)	433 (44.2)	1633 (14.4)	1189 (19.2)

With burial at 7 cm depth, there was a significantly ($P \leq 0.01$) lowered death risk to achenes. Fewer resistant biotype achenes died in all three treatments,at this depth, than those of the susceptible biotype ($P \leq 0.05$). There was a significantly ($P \leq 0.05$) higher mortality of the resistant biotype when achene burial occurred at 1cm in rotovated plots. In the undisturbed control plots at both depths, the death risk was least and at 1cm was similar for both biotypes. Rotovation disturbance probably encouraged germination but since the emergence of seedlings of the resistant biotype was less successful from depths of 1.0 cm and 2.0 cm, this might explain the higher mortality of the resistant achenes under rotovation.

In situ death together with germination were found to be approximately equal as causes of loss of viable achenes. Of achenes buried and later retrieved, only 0.01% were classified as being in a state of innate or induced dormancy.

Seedling establishment

Germination and emergence of seedlings represent a loss of achenes from the soil.Two distinct flushes of seedling emergence were observed, a 'spring' flush with peak emergence in April and May and an 'autumn' flush with peak emergence in August and September as described previously (Putwain et al.,1982). The two biotypes did not not differ in the timing of seedling emergence but significantly more seedlings ($P \leq 0.01$) of the resistant biotype emerged during the spring flush in comparison to the susceptible in both of the simazine treated areas. There was a significant ($P \leq 0.01$) interaction between bioype and management regime. Emergence of the susceptible biotype was similar in the three management regimes whilst significantly less resistant biotypes emerged in the rotovated treatment . Susceptible plants produced on average 59.5 achenes in rotovated plots and failed to set seed under simazine. The resistant biotype was substantially more fecund in

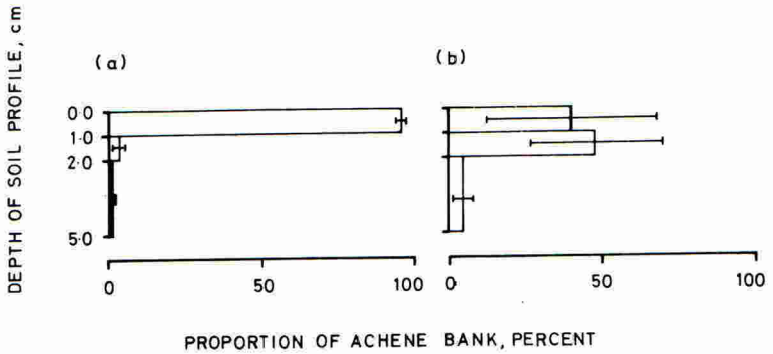

Fig. 2. The effect of rotovation on the depth distribution of achenes of *Senecio vulgaris* a) before and b) after rotovation. Confidence limits are 2 s.e.'s.

simazine treated plots than in rotovated ones, producing on average 39.1 achenes per plant in simazine treated plots compared with 5.7 in the rotovation plots. The probability of a seedling surviving to flower is given in Table 2. As expected the probability of survival of the susceptible biotype in simazine treated plots was very low (0.03). Survivorship of the susceptible biotype in the rotovation plots was considerably greater (0.3) than survival of the resistant biotype (0.12).

CONCLUSIONS

The retention of a bank of propagules within the soil is a requisite for the persistence of *S.vulgaris* in perturbed habitats such as orchards and tree nurseries. In such habitats where resistance to triazine herbicides has evolved, a knowledge of the persistence and flux of resistant achenes is crucial in determining management strategies. Achenes suffered different fates according to depth of burial. The results suggest that achenes in soil effectively comprise two banks with different effects on species maintenance. The probability of seedling emergence in the field from > 2 cm depth is very low (Watson,1987) and depletion of the achene bank below this depth is continual (14 -38 % per annum, Table 1), it is the flux of achenes in the surface seed bank (0 - 2 cm) that is of prime importance to the annual renewal of adult plant populations. Components of this flux during a calendar year are given in Table 2. The analysis suggests that the differences between the biotypes in this bank that are of overiding importance are a) the probability of seedlings surviving to produce achenes and b) the fecundity of those plants. There were substantial net gains to the achene bank by the resistant biotype in simazine treated plots and by the susceptible in rotovated ones. Conversely susceptible biotypes under simazine treatment would eventually become extinct as would the resistant biotype under rotovation but at a slower rate.

Achene burial lower in the profile (7 cm) gave rise to a reservoir from which seedling recruitment was rare but in which the rate of loss of achenes was lowered. Furthermore burial at a depth of 7 cm resulted in a reduced rate of achene loss of the resistant biotype (Table 1).

TABLE 2

Flux of achenes in the surface seed bank of *S. vulgaris* under two management regimes .

Biotype	Proportion of achenes remaining dormant.	Probability of seedling surviving to flower.	Achenes produced per adult plant.	Achene return per achenes lost.	Proportion of achenes lost.
Simazine treated plots					
Resistant	0.36	0.24	39.1	9.4	0.64
Susceptible	0.40	0.03	0.0	0.0	0.60
Rotovated plots					
Resistant	0.46	0.12	5.7	0.68	0.54
Susceptible	0.57	0.30	59.5	17.85	0.43

The overiding implications of these findings are that weed management practices that place achenes at depth will result in a depletion of the susceptible biotype at a faster rate than the resistant.

This will only be of practical significance if inversion or mixing of a soil profile by ploughing or deep rotovation places resistant achenes at or close to the soil surface. Use of a triazine herbicide would then lead to a rapid increase in the population of resistant biotypes. Otherwise a superior strategy would be to leave soils undisturbed and if necessary use residual herbicides unrelated to the triazines. In the surface seed bank however, it is the fate of adult plants that determine the relative success of the two biotypes. The rate of movement of achenes between the two zones and particularly the upward return to the surface bank was not covered in detail in this work. However the minor alteration in depth distribution between the zones as shown earlier suggests that upward achene movement under shallow rotovation was slight. Complete profile inversion (as with ploughing) would be required for recruitment of seedlings from achenes residing at depth with the consequent potential for reactivating a resistant population.

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THE RESPONSE OF SIMAZINE-RESISTANT AND SUSCEPTIBLE BIOTYPES OF CHAMOMILLA SUAVEOLENS, EPILOBIUM CILIATUM AND SENECIO VULGARIS TO OTHER HERBICIDES

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ABSTRACT

A simazine-resistant biotype of Chamomilla suaveolens discovered at Luddington, Warwickshire was shown in pot experiments to tolerate a pre-emergence dose of simazine 1000 times that lethal to a susceptible biotype. Resistant and susceptible biotypes were found in close proximity at this site, the resistant types occurring only on land with a history of regular simazine treatment. Compared with the susceptible biotype the simazine resistant type was also somewhat more resistant to metribuzin and lenacil but not to diuron, diphenamid, napropamide and pendimethalin. The same pattern and extent of resistance to simazine, metribuzin and lenacil was shown by biotypes of Senecio vulgaris. The simazine-resistant biotype of Epilobium ciliatum was appreciably more susceptible to post-emergence applications of oxyfluorfen, paraquat and pyridate compared with the simazine-susceptible biotype.

INTRODUCTION

Simazine-resistant biotypes of weed species previously controlled by simazine have become a serious problem in fruit and ornamental crops and on non-cropped areas in England. Resistant S.vulgaris, (groundsel), Poa annua (annual meadowgrass) and E.ciliatum (American willowherb) were reported in 1982 (Putwain 1982, Bailey et al. 1982) and Erigeron canadensis (Canadian fleabane) by Clay (1987). Other species are reported to have resistant biotypes but no experimental evidence is available.

On experimental plots at Luddington Experimental Horticulture Station, Warwickshire, C.suaveolens (pineapple weed) was found to tolerate pre-emergence applications of simazine at 4 kg/ha in spring 1983 (Clay, unpublished data). The response of this biotype in pot experiments to higher doses of simazine and to other pre-emergence herbicides is described in this paper along with the simazine susceptibility of biotypes from adjacent areas. The response of simazine -resistant and susceptible biotypes of E.ciliatum and S.vulgaris to a range of herbicides was also tested in pot experiments. A biotype of E.ciliatum on experimental plots at East Malling Research Station, Kent was found in 1983 to be tolerant to repeated paraquat applications at recommended doses but was not present on plots treated with low doses of simazine (Clay, unpublished data). The effect of paraquat and other post-emergence herbicides was tested on this and a simazine-resistant biotype in pot experiments.

MATERIALS AND METHODS

The experiments were carried out in glasshouses at the Weed Research Organization, Begbroke Hill, Oxford (WRO) in 1984/5 and at Long Ashton Research Station (LARS) in 1985/6. Simazine-resistant (R) and susceptible (S) biotypes were obtained from the following locations:-

C.suaveolens, R : Luddington EHS, Warwicks, (fruit plantation)
S : Germoe, Cornwall (waste ground), WRO, Oxford (arable field)
E.ciliatum R : WRO Oxford, (ex fruit farm, Wokingham, Berks).
S : East Malling R.S., Kent (fruit plantation)
S.vulgaris R : WRO, Oxford (fruit plantation). S:Headington, Oxford (garden)

Generally seed was taken from plants growing at the original location, sown and grown on in pots in the glasshouse and the seed collected from these plants used for subsequent experiments. Where a series of experiments was done on one species, seed stock was replenished from further glasshouse-grown plants. For Experiment 3, single plants were obtained from sites at Luddington EHS which had been treated with simazine for >10 years or had no previous treatment. These plants were grown-on in the glasshouse and seed harvested and used for the experiment. For pre-emergence experiments, 7.5 cm diam. pots, were filled with sandy loam (WRO) or sandy clay loam soil (LARS) and sown, covered with a 2 mm layer of coarse sand and watered overhead to uniformly moisten the soil. For post-emergence herbicide experiments, seed was sown in a peat/sand compost in seed trays and single plants transferred to 9 cm diam. pots of soil at the seedling stage. Supplementary lighting was given from October to March.

Herbicides were applied with a laboratory track sprayer at 210kPa pressure, pre-emergence treatments 1 day after sowing and post-emergence to established plants. For Experiment 7, a pressurized knapsack sprayer was used. Herbicide doses and formulations are shown in Tables 1-7, all doses referring to kg a.i./ha. Experimental details were:-

	Location	Spray date	Replication	Seeds/pot	Spray vol.rate(l/ha)
Expt 1	WRO	22 May 84	6	40	390
" 2	LAR	26 June 86	6	100	490
" 3	WRO	24 Oct. 85	4	40	390
" 4	LARS	8 Aug. 86	4	20	390
" 5	WRO	18 Feb. 85	6	40	390
" 6	WRO	19 Mar. 85	4	1 plant	240
" 7	LARS	15 Nov. 85	4	1 plant	300

There were two or three untreated control treatments in each experiment. After spraying pre-emergence experiments, the pots were set out in foil dishes in randomised blocks, watered initially with a fine rose overhead and subsequently from below. Post-emergence experiment pots were watered on the soil only or placed on capillary matting.

Plant response was assessed by measurements of shoot fresh weight and plant numbers/pot. For E.ciliatum post-emergence experiments maximum shoot height was recorded and plant vigour on a 0-9 scale where 0=plant dead, 5=50% growth inhibition, 9=plant healthy.

RESULTS

Experiment 1. The growth of the simazine-resistant biotype (R) of C.suaveolens from Luddington was unaffected by doses of simazine up to 24 kg/ha (Table 1). The susceptible biotype (S) was killed by a dose of 0.07 kg/ha and the 0.01 kg/ha dose reduced shoot fresh weight by 30%. Experiment 2. Numbers surviving and shoot fresh weight of R biotypes of C.suaveolens and S.vulgaris were not reduced by a dose of 100 kg/ha simazine (Table 2) whereas doses of 0.1 kg/ha or more killed the S biotypes and 0.01 kg/ha reduced shoot fresh weight and numbers surviving. Simazine doses of 0.1 kg/ha or more killed S E.ciliatum and 0.01 kg/ha reduced shoot growth. With the R biotype doses of 10 kg/ha or more reduced shoot weight but plants appeared healthy. With all species there was no difference between the biotypes in the number of untreated plants surviving or shoot weight/plant except that plant number was 60% less with the S C.suaveolens (Germoe).

TABLE 1

The effect of simazine* applied pre-emergence to a resistant (R) and susceptible (S) biotype of C.suaveolens (Experiment 1)

Shoot Fresh wt (% untreated) 5 weeks after treatment								
Dose (kg/ha)	0.01	0.07	0.49	3.45	12.0	24.0	0	Actual value**
Biotype R	80	102	103	106	91	84	100	3.82
S	69	0	0	0	0	0	100	4.29

* 50% a.i., s.c. in all experiments **g/pot

TABLE 2

The effect of simazine applied pre-emergence on resistant (R) and susceptible (S) biotypes of C.suaveolens (Cs), E.ciliatum (Ec) and S.vulgaris (Sv)(Experiment 2)

		% untreated (3 weeks after treatment)						
Dose (kg/ha)		0.01	0.10	1.0	10.0	100	0	Actual value
Spp.	Biotype							(untreated)
				Seedling Number				Number/pot
Cs	R	101	95	94	85	102	100	82
	S	80	0	0	0	0	100	35
Ec	R	119	78	78	107	105	100	58
	S	48	0	0	0	0	100	54
Sv	R	99	99	100	102	101	100	102
	S	58	1	0	0	0	100	96
				Shoot fresh wt				wt/plant
Cs	R	108	83	95	59	85	100	156
	S	53	0	0	0	0	100	150
Ec	R	118	73	70	36	27	100	44
	S	15	0	0	0	0	100	46
Sv	R	113	79	92	88	99	100	119
	S	47	0	0	0	0	100	111

TABLE 3

The effect of simazine at different doses on C.suaveolens from different sites at Luddington E.H.S, (L), Begbroke (B) and Germoe(G) assessed 5 weeks after treatment (Experiment 3)

Dose (kg/ha)	Site	L1*	L2*	L3	L4	L5	L6*	L7*	L8*	L9*	L10	L11*	B	G
		Seedling Number (% untreated)												
0.02		169	100	148	116	93	130	152	103	113	97	150	101	130
0.14		174	110	1	7	0	110	102	106	98	0	136	0	0
0.98		333	148	0	1	0	99	119	104	163	0	94	0	0
6.86		272	118	0	0	0	76	146	83	138	0	119	0	0
48.0		139	103	0	0	0	89	104	99	98	0	84	0	0
Untreated		100	100	100	100	100	100	100	100	100	100	100	100	100
Actual no./pot		6.6	22.1	27.0	28.5	36.9	15.1	15.9	39.0	17.8	36.7	10.1	32.1	6.9
		Shoot fresh wt (% untreated)												
0.02		93	110	87	98	77	92	85	101	92	80	134	74	28
0.14		155	97	1	1	0	95	115	113	117	0	91	0	0
0.98		109	74	0	2	0	69	102	88	79	0	56	0	0
6.86		88	81	0	0	0	95	88	117	70	0	43	0	0
48.0		69	67	0	0	0	90	77	88	78	0	59	0	0
Untreated		100	100	100	100	100	100	100	100	100	100	100	100	100
Actual wt/plant(mg)		59	441	176	217	328	516	453	318	417	231	273	340	636

* Sites receiving annual application of simazine for >10 years

Experiment 3. Growth of C.suaveolens plants from seven out of 11 locations at Luddington was not inhibited by simazine doses up to 48 kg/ha (Table 3). Plants from the remaining four sites were killed or severely inhibited by doses of 0.14 kg/ha or more but largely unaffected by 0.02 kg/ha. Numbers of plants emerging were variable between replicates and particularly between locations; mean shoot weight/plant in untreated pots also varied considerably but this was not related to the response of that biotype to simazine.

Experiment 4. The simazine resistant biotypes of C.suaveolens and S.vulgaris were also more resistant to metribuzin and lenacil although killed or severely inhibited at the higher doses used (Table 4). The S biotype tended to be slightly more susceptible to diuron but there was no difference in the response of the two biotypes to diphenamid, napropamide and pendimethalin.

Experiment 5. Vigour of the R biotype of E.ciliatum was little affected by simazine at up to 64 kg/ha (Table 5), although final shoot weight was reduced. A dose of 0.06 kg/ha killed the S biotype. Diuron was somewhat less toxic to the R biotype but there was little difference in biotype response to diphenamid.

Experiment 6. Oxyfluorfen, paraquat and pyridate caused leaf necrosis and growth reduction to both biotypes of E.ciliatum (Table 6) but effects were more severe on the simazine resistant biotype. Pyridate at 6 kg/ha killed the R biotype.

Experiment 7. Atrazine at 1 and 4 kg/ha severely damaged the S but not the R biotype of E.ciliatum (Table 7). Oxyfluorfen caused more damage to the R than the S biotype. Paraquat at 1 kg/ha caused only transient damage to the S biotype but killed the R biotype. At 4 kg/ha it resulted in a 40% growth reduction of the S biotype.

TABLE 4

The effect of herbicides applied pre-emergence to simazine resistant (R) and susceptible (S) biotypes of C.suaveolens and S.vulgaris (Experiment 4)

		Fresh wt shoots (% untreated) 3 weeks after treatment					
		C.suaveolens			S.vulgaris		
		Dose (kg/ha)					
Herbicide formulation	Biotype	0.25	1.0	4.0	0.25	1.0	4.0
Simazine	R	94	67	74	80	63	62
(50% SC)	S	0	0	0	0	0	0
Metribuzin	R	62	1	0	50	11	0
(70% WP)	S	0	0	0	0	0	0
Lenacil	R	10	15	8	39	28	12
(80% WP)	S	0	0	0	0	0	0
Diuron	R	12	0	0	78	24	0
(50% WP)	S	1	0	0	42	47	0
Diphenamid	R	20	3	6	11	5	6
(70% WP)	S	16	3	4	7	2	2
Napropamide	R	12	8	2	15	7	6
(45% SC)	S	15	2	1	26	5	3
Pendimethalin	R	12	2	1	97	60	17
(33% EC)	S	18	0	1	64	59	15

TABLE 5

The effect of herbicides applied pre-emergence to simazine-resistant (R) and susceptible (S) biotypes of E.ciliatum (Experiment 5)

Herbicide	Dose (kg/ha)	Results as % untreated Vigour score (6 weeks)** R	S	Fresh weight (8 weeks)** R	S
Simazine	0.06	108	0	114	0
"	0.25	94	0	78	0
"	1.0	100	0	94	0
"	4.0	96	0	74	0
"	16.0	94	0	77	0
"	64.0	84	–	53	–
Diuron	0.016	104	97	112	88
"	0.012	46	0	36	0
"	0.25	0	0	0	0
Diphenamid	0.04	98	101	103	106
"	0.20	22	19	24	20
"	1.00	12	11	7	4
Untreated		100	100	100	100
Actual value		8.33*	8.75*	9.6	15.3 g/pot
SE±		4.3	2.5	7.0	6.0

* Vigour score, 0–9 scale ** Weeks after treatment

TABLE 6

The effect of oxyfluorfen, pyridate and paraquat on a simazine-resistant (R) and simazine-susceptible (S) biotype of E.ciliatum* (Experiment 6)

Herbicide	Dose (kg/ha)	Results as % untreated 4 weeks after treatment Vigour score R	S	Max. plant height R	S	Shoot fresh wt R	S
Oxyfluorfen (24% EC)	0.25	42	54	40	58	33	52
	1.0	35	41	40	54	34	47
	4.0	26	51	28	60	18	41
Paraquat (20% a.c.)	0.25	55	89	68	102	70	107
	1.0	45	83	52	95	51	83
	4.0	26	54	16	55	8	40
Pyridate (50% WP)	0.375	68	73	80	78	68	54
	1.50	26	73	24	80	21	65
	6.0	0	57	0	62	0	37
Untreated		100	100	100	100	100	100
Actual value		7.7**	7.8**	222	309mm	6.8	5.0g/pot
S.E.±		6.8		10.0		10.4	

* Plant height 80–120 mm at spraying ** Vigour score, 0–9 scale

TABLE 7

The effect of atrazine, oxyfluorfen and paraquat on a simazine-resistant (R) and a susceptible (S) biotype of E.ciliatum* (Experiment 7)

Herbicide	Dose (kg/ha)	Results as % untreated (4 weeks after treatment) Max.plant height R	Max.plant height S	Shoot fresh wt R	Shoot fresh wt S
Atrazine**	1	96	49	100	25
"	4	89	5	89	1
Oxyfluorfen	1	31	44	14	43
"	4	28	44	12	43
Paraquat	1	0	93	0	83
"	4	0	59	0	65
Untreated		100	100	100	100
Actual value		735	819 mm	28.4	27.7g/pot
SE±		15.3	14.2	17.0	15.3

* plant height 160 to 250 mm at spraying ** 50% S.C.

DISCUSSION

Triazine-resistant weeds have become widespread in perennial crops in England since the first confirmation of resistance in S.vulgaris and P.annua (Putwain 1982) and E.ciliatum (Bailey et al. 1982). Triazine-resistance in C.suaveolens has not previously been described in the UK or elsewhere; a few populations resistant to bromacil and simazine are also reported from E. Anglia (A.J.Greenfield, personal communication). The pattern of resistance appears similar with all these species. Very high doses of simazine were tolerated in these experiments compared with other tests (Putwain 1982; Bulcke et al. 1987); this may have resulted from the smaller amounts of simazine available to the plants from applying the herbicide as a spray after sowing compared with pre-planting incorporation. It does however demonstrate that very high doses of simazine will not overcome the resistance. The partial resistance of the R biotypes to metribuzin and lenacil and their susceptibility to herbicides of other chemical groups agrees with earlier work on these and other weed species (Parochetti et al 1982; Clay & Bailey 1985; Clay 1987) and indicates that a number of different herbicides are available to control the weeds albeit at greater cost than simazine.

The correlation between previous simazine use and resistance seen in the plants from different sites at Luddington confirm earlier suggestions that resistant biotypes are unlikely to spread into 'wild' populations possibly because they are less fit (Radosevich & Holt 1982). Not being wind-dispersed C.suaveolens is less likely to be transferred far on seeding but could be spread by machinery etc. The locations sampled at Luddington were all within an area of 0.5 km square. There was no evidence in these experiments of poorer growth of simazine-resistant biotypes of C.suaveolens or S.vulgaris but growth of E.ciliatum was significantly reduced at higher simazine doses (Tables 2,5). Bulcke et al. (1987) found considerable variation in leaf form and vigour of E.ciliatum biotypes but this was not correlated with their triazine resistance.

The occurrence of paraquat resistance in the simazine-susceptible biotype of E.ciliatum from East Malling, Kent agreed with the observations at the site. Paraquat resistance has been reported in other species notably P.annua and Conyza (Erigeron) species (LeBaron & Gressel 1982). The apparent greater tolerance to oxyfluorfen of the paraquat resistant biotype may be explained by its increased capacity to detoxify active oxygen species (Shaaltiel & Gressel 1986). The result with pyridate is unexpected since it is active in photosynthesis on photosystem 11 and therefore could be expected to be less toxic to the simazine-resistant biotype. Pyridate has given unexpected results in other work in that mixtures with simazine have given better post-emergence control of simazine-resistant E.ciliatum (Bailey & Clay 1985) and E.canadensis (Clay 1987) than pyridate alone.

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