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PROJECT REPORT No. 116

INHERITANCE OF HERBICIDE RESISTANCE IN BLACK-GRASS (*Alopecurus myosuroides*) AND RESPONSES OF THE WEED TO A RANGE OF HERBICIDES

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INHERITANCE OF HERBICIDE RESISTANCE IN BLACK-GRASS *(ALOPECURUS MYOSUROIDES)* AND RESPONSES OF THE WEED TO A RANGE OF HERBICIDES

by

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CONTENTS

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To ensure that the results are set in a wider context, this report includes results of some studies funded by the Ministry of Agriculture, Fisheries and Food and contract work for agrochemical companies.

SUMMARY

1. The combined objectives of the project were:

   a) To study cross-resistance patterns and inheritance of herbicide resistance in black-grass in order to determine the risk of increase of resistance in the United Kingdom.

   b) To determine the response of several populations of resistant black-grass to a range of herbicides applied under simulated field conditions, in order to confirm the relevance of resistance ratings derived from glasshouse tests to field conditions.

2. Resistance to chlorotoluron and fenoxaprop-ethyl was detected on many farms in addition to those previously tested. Chlorotoluron-resistant black-grass was detected on 74 farms between 1982 and 1994 and fenoxaprop-resistant black-grass on 75 farms between 1990 and 1994 in IACR and ADAS tests. Many of these farms contained populations resistant to both herbicides, so the total number of farms identified with black-grass resistant to chlorotoluron and/or fenoxaprop was 117. These farms were located in 23 counties of England. The two counties with the most recorded cases were Essex (23) and Oxfordshire (23).

3. In random surveys conducted between 1988 and 1991, 77% of the 317 samples tested for resistance to chlorotoluron were classified as susceptible, 15% as marginally insensitive (1*) and 7% as resistant (2* or more). Most of the resistant populations detected were ranked 2* and therefore exhibit partial resistance to chlorotoluron.

4. Compared with chlorotoluron, a higher proportion of populations tested for fenoxaprop resistance was classified as resistant, and of these a higher proportion was classified as highly resistant. It should be noted, however, that samples tested for fenoxaprop resistance were not collected on a random basis, but in response to reports of failure of herbicides in the field.

5. A new * rating system for classifying populations for their degree of resistance to both chlorotoluron and fenoxaprop-ethyl was devised. This is based on comparisons of herbicide activity with two standard reference populations, Rothamsted (susceptible) and Peldon (resistant).

6. Studies on cross-resistance involving six populations and 10 herbicides demonstrated that patterns of cross-resistance can differ substantially between populations. All populations tested showed greater resistance to chlorotoluron than isoproturon, demonstrating that the degree of resistance within the same chemical class can vary. There was no consistent relationship between degree of resistance to two of the major herbicides used for black-grass control, isoproturon and fenoxaprop-ethyl. The population showing the greatest resistance to fenoxaprop, Lincs. E1, also showed substantial cross-resistance to diclofop, fluazifop-P-butyl, quizalofop-ethyl and tralkoxydim, but only marginal resistance to chlorotoluron, isoproturon and pendimethalin. At the **population level**, Lincs. E1, as with all other populations tested, showed no resistance to sethoxydim or trifluralin. Subsequent experiments confirmed resistance to sethoxydim in a small proportion of individual plants. The results showed that relating resistance to chemical class is misleading. There is a need to consider herbicides individually, as resistance may occur to some, but not all, herbicides within a class.

7. Experiments in outdoor containers confirmed that resistance can substantially reduce herbicide activity at normal field rates. Fenoxaprop-ethyl at 120 g a.i./ha achieved 98% control of a susceptible standard, but

only 30 - 45% control of five other populations which had also shown resistance in glasshouse tests. One population, Lincs. E1, showed a high level of resistance to fluazifop-P-butyl - only 44% control was achieved by 125 g a.i./ha in contrast to 98% control of the susceptible standard. This was the first proven case of high level resistance to this herbicide in the UK. These container experiments confirmed that none of the six populations studied showed resistance to sethoxydim, 94 - 99% control was achieved by 290 g a.i./ha.

8. A field experiment was conducted to identify the degree to which marginal levels of resistance in black-grass, as identified in glasshouse tests, affect the efficacy of a range of herbicides at different doses and at different weed growth stages. At recommended rates of chlorotoluron there was a poorer level of control on the 1* chlorotoluron resistance rated population (Boxworth) compared to that of a susceptible standard (Rothamsted). There were also differences in response between the two populations with isoproturon and diclofop-methyl, particularly at later timings. These results have important implications as 1* rated populations (marginally insensitive) are not counted as resistant in the current rating system. In random surveys a much higher proportion of samples were rated 1* (15%) than resistant - 2* or more (7%).

9. Evaluation of the progeny from three selection experiments showed that resistance to fenoxaprop can evolve within three generations. The greater the level of selection, the greater the degree of resistance. This supports the view that resistance to fenoxaprop evolves faster when high, rather than low, rates of herbicide are used. The resistance mechanism selected in these experiments was relatively inefficient, but reduced performance at field doses. Resistance developed more rapidly to fenoxaprop than to chlorotoluron.

10. Deselection processes were studied in order to determine how rapidly the level of resistance declines when herbicides are no longer applied. Black-grass plants grown from resistant Peldon seed, and allowed to grow and seed for three generations without herbicide treatment, were less resistant than those plants resulting from continued herbicide selection. However, the shift was modest and the deselected plants still showed significant levels of resistance to both chlorotoluron and diclofop-methyl. It was concluded that any deselection process towards susceptibility is likely to be slow.

11. A small proportion (5%) of plants of a fenoxaprop-resistant population, Oxford S1, survived treatment with sethoxydim and fluazifop-P-butyl. Progeny of these plants showed exceptionally high levels of resistance to sethoxydim, fluazifop and fenoxaprop. However, there was **no** cross-resistance to chlorotoluron or isoproturon. This demonstrated for the first time in black-grass, the existence of a mechanism giving absolute resistance, likely to affect all aryloxyphenoxypropionate ('fop', AOPP) and cyclohexanedione ('dim', CHD) herbicides.

12. Related studies confirmed that this mechanism was target site resistance, caused by an insensitive form of the target enzyme (ACCase) for 'fop' and 'dim' herbicides. This was the first time ACCase target site resistance had been demonstrated in black-grass. A petri-dish assay for detecting target site resistance was developed and appeared to have potential as a diagnostic technique.

13. Target site resistance (ACCase) was detected subsequently on six farms, in Oxfordshire, Lincolnshire and Nottinghamshire. On two fields, Oxford AA1 and Notts A1 this resistance mechanism was present in about 90% of plants, the first time such a high incidence had been found. Glasshouse studies confirmed a high level of resistance to the five 'fop' and 'dim' herbicides tested (fenoxaprop, diclofop, fluazifop, sethoxydim, tralkoxydim) but not to the chemically unrelated substituted urea herbicide, chlorotoluron.

Surprisingly there was cross-resistance to the sulfonyl-urea herbicide chlorsulfuron, for reasons as yet unclear.

14. Inheritance studies were initiated to determine the genetic basis of target site resistance (ACCase). About 50% of F_1 progeny between supposed heterozygous resistant (RS) and susceptible (SS) individuals were resistant, supporting the model of monogenic inheritance. A polycross of resistant F_1 progeny produced F_2 samples which segregated in an approximately 3:1 ratio of resistant:susceptible plants. This also supported the proposed model of monogenic inheritance with the resistant allele dominant. There was no evidence of incomplete dominance.

15. Material characterised at the whole plant level in this project has been used in related biochemical studies of resistance mechanisms. These studies confirmed the existence of at least two mechanisms of resistance in black-grass. One is enhanced metabolism, probably involving P450 enzymes and the second is target site resistance due to an insensitive form of the ACCase enzyme. Enhanced metabolism is a broad-spectrum mechanism affecting the performance of a wide range of chemically unrelated herbicides. This mechanism tends to give partial, rather than absolute, resistance and the degree of resistance varies between populations and different herbicides. Target site resistance (ACCase) is less common at present but appears to severely affect the performance of all 'fop' and 'dim' herbicides in the populations so far detected. It does not affect chemically unrelated herbicides. However, in one population studied in detail (Lincs. E1), these two mechanisms alone cannot explain the degree of resistance to fenoxaprop and fluazifop seen at the whole plant level, and the existence of another mechanism is likely.

INTRODUCTION

Herbicide resistance is the inherited ability of a weed to survive a rate of herbicide which would normally give effective control. This inherited ability contrasts with poor activity resulting from incorrect application or adverse environmental conditions.

There are two ways in which resistance traits may arise within a weed population. A major gene, or major genes, may be present at low frequency, or mutate, so that selection acts to change a population which is initially susceptible. Alternatively, recurrent selection may act on continuous variation and achieve a progressive increase in average resistance from generation to generation, with changes in gene frequency at many loci conferring resistance (Maxwell & Mortimer, 1994).

Herbicide-resistant weeds are an increasing problem. By 1991, herbicide resistance had evolved in 84 weed species in 31 countries worldwide (Moss & Rubin, 1993). Herbicide-resistant biotypes of the following 11 species are present in the UK.

Herbicide	**Latin name**[†]	**Common name**
Triazine resistant:	*Chenopodium album*	Fat hen
	Conyza canadensis	Canadian fleabane
	Epilobium ciliatum	American willowherb
	Matricaria matricarioides	Pineapple weed
	Poa annua	Annual meadow-grass
	Senecio vulgaris	Groundsel
	Solanum nigrum	Black nightshade
Paraquat resistant:	*Poa annua*	Annual meadow-grass
	Epilobium ciliatum	American willowherb
Mecocrop resistant:	*Stellaria media*	Chickweed
Diclofop resistant:	*Lolium multiflorum*	Italian rye-grass
Multiple resistant:	*Alopecurus myosuroides*	Black-grass
	Avena spp.	Wild-oats

[†] based on Clapham, Tutin & Moore (1987).

Herbicide-resistant black-grass is considered to be the most significant resistance problem at present and formed the basis of a previous HGCA-funded research project, (Moss & Clarke, 1992).

Black-grass (*Alopecurus myosuroides* Huds.) is an annual grass weed propagated solely by seeds. These are relatively non-dormant and most seeds germinate in the autumn, from September to November. Consequently black-grass is mainly associated with autumn-sown crops, especially cereals (Moss, 1980). It is competitive and fecund and populations can build up rapidly, especially in reduced cultivation systems. Herbicides have been viewed as the main method of controlling this weed in winter cereals for about 25 years. A high level of control is needed to prevent the weed increasing and consequently many fields have received successive annual applications of herbicides for many years.

Thus, black-grass, as a weed of cereals, has many of the characteristics listed by Harper (1956) which would favour the development of herbicide resistance: high reproductive capacity, absence of a large, dormant seed bank to buffer population changes, association with cereal monoculture and intensive use of a single herbicide type.

The previous HGCA-funded project (Moss & Clarke, 1992) demonstrated that chlorotoluron-resistant black-grass occurred in at least 19 counties of England and was associated with intensive winter cereal growing systems and non-ploughing cultivation techniques. There was no clear association between the occurrence of resistance and intensity of herbicide use. The complexities of cross-resistance were demonstrated very clearly, but patterns of cross-resistance were not consistent between populations, either in terms of the specific herbicides affected or the degree of resistance. The study also demonstrated that resistance did not usually cause complete inactivity of herbicides but could cause substantial reductions in herbicide efficacy at normal recommended rates. Resistance to chlorotoluron developed at a relatively slow rate, despite continued use of herbicides, although there was no evidence of any decline in resistance levels in any field once resistance

had been detected. It was also recognised that the rate of development of resistance to alternative herbicides might differ to that found with the substituted-urea herbicides. Inheritance studies showed that the trait for resistance to chlorotoluron could be transmitted via pollen, and the range in response of individual plants to herbicide suggested that the mechanism of inheritance was polygenic and therefore controlled by the collective effect of several genes.

It was concluded that herbicide resistance in black-grass is a complex problem and poses a serious threat to winter cereal cropping. Consequently, this project was initiated in order to investigate further the complexities of cross-resistance and factors which determine the rate of development of resistance to different herbicide types. It was recognised that more emphasis should be placed on the aryloxyphenoxypropionate herbicides ('fops', AOPP) such as fenoxaprop-ethyl and cyclohexanedione herbicides ('dims', CHD) such as tralkoxydim as these were being used increasingly for grass weed control.

The objectives of the research project

1. To study cross-resistance patterns and inheritance of herbicide resistance in black-grass in order to determine the risk of increase of resistance in the United Kingdom (IACR-Rothamsted).

2. To determine the response of several populations of resistant black-grass to a range of herbicides applied under simulated field conditions in order to confirm the relevance of resistance ratings derived from glasshouse tests to field conditions.

SECTION 1

OCCURRENCE OF HERBICIDE-RESISTANT BLACK-GRASS

INTRODUCTION

Resistance to chlorotoluron was first detected in a UK population of black-grass in 1982. More pronounced resistance was found at Peldon, Essex in 1984 (Moss & Cussans, 1985). By 1990, resistance to chlorotoluron had been found on 46 farms in 19 counties of England (Clarke & Moss, 1991; Moss & Clarke, 1992). Clarke & Moss (1989) proposed a classification system to denote different degrees of resistance to chlorotoluron based on a comparison with the response of three standard reference populations. It was recognised that a system was needed which was appropriate not only for chlorotoluron, but also for other herbicides such as fenoxaprop-ethyl. The experiments reported here present results from further screening tests for resistance, not only to chlorotoluron but also to isoproturon and fenoxaprop-ethyl (+ safener), a herbicide introduced into the UK in spring 1990. For completeness, results from screening tests conducted between 1988 and 1990 are also summarised. These were originally reported in Moss & Clarke (1992) and Clarke & Moss (1989; 1991). A modified resistance classification system is also described which should be appropriate not only for chlorotoluron, but also for fenoxaprop-ethyl.

MATERIALS AND METHODS

Seed samples were collected in July 1991, 1992, 1993 and 1994, mainly from winter cereal fields and tested in glasshouses at ADAS Cambridge, or IACR-Rothamsted. Some black-grass populations were tested as part of projects funded by MAFF or contract work. As all of the populations were included in the same series of experiments, the combined details and results are included below for completeness (Table 1).

TABLE 1 Source of black-grass seed samples used in screening experiments for resistance to chlorotoluron (Farms not previously sampled)

Source		1988	1989	1990	1991	1992	1993	1994
HGCA Survey	(ADAS)	59	49	0	0	0	0	0
Disease Survey	(ADAS)	20	38	101	50	0	0	0
Others	(ADAS)	26	14	7	14	10	0	0
Others	(Roth)	27	11	4	10	12	9	10
Total		132	112	112	74	22	9	10

The 'survey' samples were collected as part of either HGCA-funded random surveys or from fields selected at random from the ADAS Winter Wheat and Winter Barley disease surveys of England and Wales.

The 'other' samples were collected by ADAS or IACR-Rothamsted from fields where poor herbicide performance had been reported or from trial sites.

The test procedure was that described by Moss & Orson (1988) in which seeds were sown in pots of soil and plants sprayed at the 2 - 3 leaf stage with 2.5 -2.75 kg a.i./ha chlorotoluron. Three standard reference populations were used in all tests: Rothamsted 1987 (susceptible); Faringdon 1987 (partially resistant, 2*); Peldon A1 1987 (resistant, 5*). There were five replicates. For the fenoxaprop-ethyl and isoproturon tests a similar methodology was used, but with no, or a different, intermediate population. Plants were sprayed at the 2 - 3 leaf stage with 150 g a.i./ha fenoxaprop-ethyl (+ safener) or 1.25 kg a.i./ha isoproturon. In all tests the % reduction in foliage fresh weight was calculated by relating weights in treated and untreated pots for each sample, 3 - 4 weeks after spraying.

In 1991 a total of 64 populations was collected and subsequently tested by ADAS. Fifty of these samples were collected from fields with black-grass selected at

random, from the ADAS Winter Wheat and Winter Barley disease surveys of England and Wales. Additional samples (14 in 1991, 10 in 1992) were received from fields where clients requested resistance tests.

Forty-one samples were tested by IACR-Rothamsted in 1991, 31 in 1992, 31 in 1993 and 21 in 1994. Most of these samples were from farms where resistance to chlorotoluron had previously been detected and were included to evaluate the response to fenoxaprop-ethyl and isoproturon, and to either ascertain whether resistance occurred on other fields or to determine whether the degree of resistance had changed. Samples from 41 farms never previously found to contain resistant populations were tested (1991 = 10; 1992 = 12; 1993 = 9; 1994 = 10. Nine populations were collected in 1992 from unsprayed areas in fields where herbicides (isoproturon and/or fenoxaprop-ethyl) had given good control of black-grass. These were included to confirm the suitability of the existing susceptible standard population (Rothamsted) for use in screening experiments.

RESULTS AND DISCUSSION

For the chlorotoluron test the classification system described by Clarke & Moss (1989) was used. Samples were classified by a star rating from 1* to 5* or as S (susceptible). Only samples classified as 2* or more were deemed resistant and the higher the star rating the greater the degree of resistance.

Between 1991 and 1994, resistance to chlorotoluron was detected on 28 new farms (Table 2) and was also confirmed on 25 other fields on 15 farms already identified as possessing resistant black-grass. Four samples found to be resistant between 1991 and 1994, Peldon H2, Oxford J1, Oxford G1 and Essex G1, had been collected from fields where black-grass had been rated susceptible when sampled between 1986 and 1988. In addition, the Faringdon 1987 population, rated 2*, showed evidence of further evolution of resistance, as a sample collected in 1992 was rated 3* in comparison. These results demonstrate that resistance to chlorotoluron is continuing to evolve, albeit relatively slowly.

TABLE 2 Classification of level of resistance to chlorotoluron in seeds collected between 1988 and 1994, from farms not previously sampled

Source		Total	S	Number of samples 1*	2*	3*	4*	5*
				<----------Resistant --------->				
1988@	Survey	79	68	7	3	0	1	0
	Others	53	39	8	3	2	1	0
	TOTAL	132	107	15	6	2	2	0
1989#	Survey	87	55	23	8	1	0	0
	Others	25	16	4	5	0	0	0
	TOTAL	112	71	27	13	1	0	0
1990#	Survey	101	81	13	6	0	0	1
	Others	11	2	4	4	0	0	1
	TOTAL	112	83	17	10	0	0	2
1991	Survey	50	42	6	0	2	0	0
	Others	24	13	1	8	1	1	0
	TOTAL	74	55	7	8	3	1	0
1992	Others	22	7	2	7	6	0	0
1993	Others	9	3	5	0	1	0	0
1994	Others	10	7	1	2	0	0	0
	TOTAL	471	333	74	46	13	3	2

@ from Clarke & Moss, 1989
# from Clarke & Moss, 1991

Between 1982 and 1994, seed samples from a total of 660 fields from 606 farms have been tested for resistance to chlorotoluron. Additionally samples have been tested from experimental sites but these are not included in this report. Resistance to chlorotoluron has now been detected in 107 fields on 74 farms. These farms are widely distributed in 22 counties of England: (Bedfordshire (1 farm), Buckinghamshire (5), Cambridgeshire (3), Dorset (1), East Sussex (2), Essex (18), Gloucestershire (1), Hampshire (1), Hertfordshire (2), Kent (1), Leicestershire (1), Lincolnshire (6), Norfolk (2), Northamptonshire (3), Nottinghamshire (1), Oxfordshire (17), Somerset (1), Suffolk (5), Surrey (1), West Sussex (1), Warwickshire (2), Worcestershire (1)). Most of the resistant populations were ranked 2* and therefore exhibit partial resistance to chlorotoluron. However, more severe resistance at the 3 - 5* level, has been recorded in Buckinghamshire, Essex, Lincolnshire, Northamptonshire, Oxfordshire, Suffolk and West Sussex.

In random surveys an average of 77% of the 317 samples tested between 1988 and 1991 were classified as S (susceptible), 15% as 1* and 7% as resistant to chlorotoluron (Table 3). These results have changed little over the years.

TABLE 3 Classification of level of resistance to chlorotoluron for samples from random collections

		% in each category		
Year	Number of samples	S	1*	2* or more resistant
1988#	79	86	9	5
1989#	87	63	26	10
1990#	101	79	13	8
1991	50	84	12	4
Mean (TOTAL 317)		77	15	7

from Clarke & Moss 1991; Moss & Clarke, 1992

In the 1992 Rothamsted tests, better control of 16 chlorotoluron-resistant populations (2* or more) was achieved with isoproturon than chlorotoluron, with one exception. Isoproturon gave levels of control which were 7 - 36% (mean 24%) higher than the corresponding values for chlorotoluron (mean % reduction = 57% for chlorotoluron, 80% for isoproturon). The exception was H/121 from Suffolk which has shown an unusual cross-resistance pattern in previous experiments. The level of control by isoproturon on chlorotoluron-resistant populations was always lower than that of the Rothamsted standard susceptible population (96%). This supports previous results based on tests involving a more limited number of populations, that isoproturon is affected by resistance, but to a lesser extent than chlorotoluron (Moss & Clarke, 1992).

Between 1989 and 1992, ADAS tested 60 samples for resistance to chlorotoluron from non-random sources. These were typically from clients requesting tests from fields where herbicide performance had been inadequate. Of these tests, 59% have been classified as S, 14% as 1* and 27% as 2* or more. This contrasts with the 85 samples tested by ADAS for resistance to fenoxaprop-ethyl. These samples also originated from non-random sources where clients requested tests following poor performance of the herbicide in the field. The results show that of the 85 samples tested, 12% were classified as S, 9% as 1* and 79% as 2* or more (Table 4).

TABLE 4 Classification of level of resistance to fenoxaprop-ethyl from seed collected in 1990, 1991 and 1992 from farms tested by ADAS

Source	Total	S	1*	2*	3*	4*	5*
				<----------Resistant ---------->			
1990	51	3	0	3	6	7	32
1991	25	6	8	3	3	5	0
1992	9	1	0	1	3	4	0
TOTAL	85	10	8	7	12	16	32
% of samples	100	12	9	8	14	19	38

Compared with chlorotoluron, a higher proportion of the fenoxaprop-ethyl tests were classified as resistant and of these a higher proportion were classified as highly resistant. These figures confirm that there are many reasons for poor control of black-grass by chlorotoluron, and other substituted-ureas, other than resistance. However, if control of black-grass by fenoxaprop-ethyl is poor in the field, a higher proportion of these cases can be explained by resistance.

In Rothamsted tests, results for fenoxaprop-ethyl were analysed statistically and samples were classified as resistant only if the % reduction in foliage weight was significantly less ($P \leq 0.05$) than the Rothamsted susceptible reference population. Of the 51 samples collected between 1990 and 1994 from 40 different farms where 'fop' herbicides, (mainly fenoxaprop-ethyl, usually in sequence with isoproturon) had failed to give acceptable control of black-grass, 49 (96%) showed evidence of resistance to fenoxaprop-ethyl in pot tests. Thirty-five of these samples showed greater resistance to fenoxaprop-ethyl than the Peldon standard and 31 of the 51 samples also showed resistance to chlorotoluron. These results show that resistance was a contributory factor to poor field performance, and that resistance to fenoxaprop-ethyl is frequently greater than that found at Peldon, the site with the highest level of resistance to chlorotoluron.

By contrast, the nine populations collected from fields where herbicides had performed well in 1992 were all rated as susceptible to fenoxaprop-ethyl and susceptible or 1* for resistance to chlorotoluron. This demonstrates that the Rothamsted population is not atypically sensitive to herbicides, and is a good representative standard for susceptible populations. While there was some relationship between resistance to chlorotoluron and fenoxaprop-ethyl, the degree of resistance sometimes differed substantially. For example, in Rothamsted tests, the populations showing the greatest resistance to fenoxaprop-ethyl in 1991 and 1992, Lincs. E1 and Notts. A1, were rated respectively as only 2* and 1* for resistance to chlorotoluron.

A compilation of all tests involving fenoxaprop conducted by Rothamsted and ADAS between 1990 and 1994, showed that resistance to fenoxaprop was detected on 103 fields on 75 farms in 16 counties of England. Many, but not all, of these samples also showed resistance to chlorotoluron. Consequently, the distribution of fenoxaprop-resistant black-grass was similar to that for chlorotoluron. The counties and number of farms identified with fenoxaprop-resistant black-grass were: Bedfordshire (1 farm), Buckinghamshire (4), Cambridgeshire (6), Devon (1), Essex (12), Hampshire (3), Hertfordshire (3), Leicestershire (1), Lincolnshire (9), Norfolk (8), Northamptonshire (3), Nottinghamshire (1), Oxfordshire (20), Suffolk (1), W. Sussex (1), Warwickshire (1).

A combined appraisal of all Rothamsted and ADAS tests for resistance between 1982 and 1994 showed that black-grass resistant to chlorotoluron and/or fenoxaprop was detected on farms in 23 counties of England. The distribution of resistance by county is shown in Table 5 and Figure 1.

TABLE 5 Number of farms with black-grass resistant to chlorotoluron and/or fenoxaprop based on Rothamsted and ADAS tests, 1982 - 1994

County	Nos. Farms
Bedfordshire	2
Buckinghamshire	5
Cambridgeshire	9
Devon	1
Dorset	1
Essex	23
Gloucestershire	1
Hampshire	4
Hertfordshire	3
Kent	1
Leicestershire	2
Lincolnshire	12
Norfolk	10
Northamptonshire	4
Nottinghamshire	2
Oxfordshire	23
Somerset	1
Suffolk	6
Surrey	1
East Sussex	2
West Sussex	2
Warwickshire	2
Worcestershire	1
Total	117 farms in 23 counties

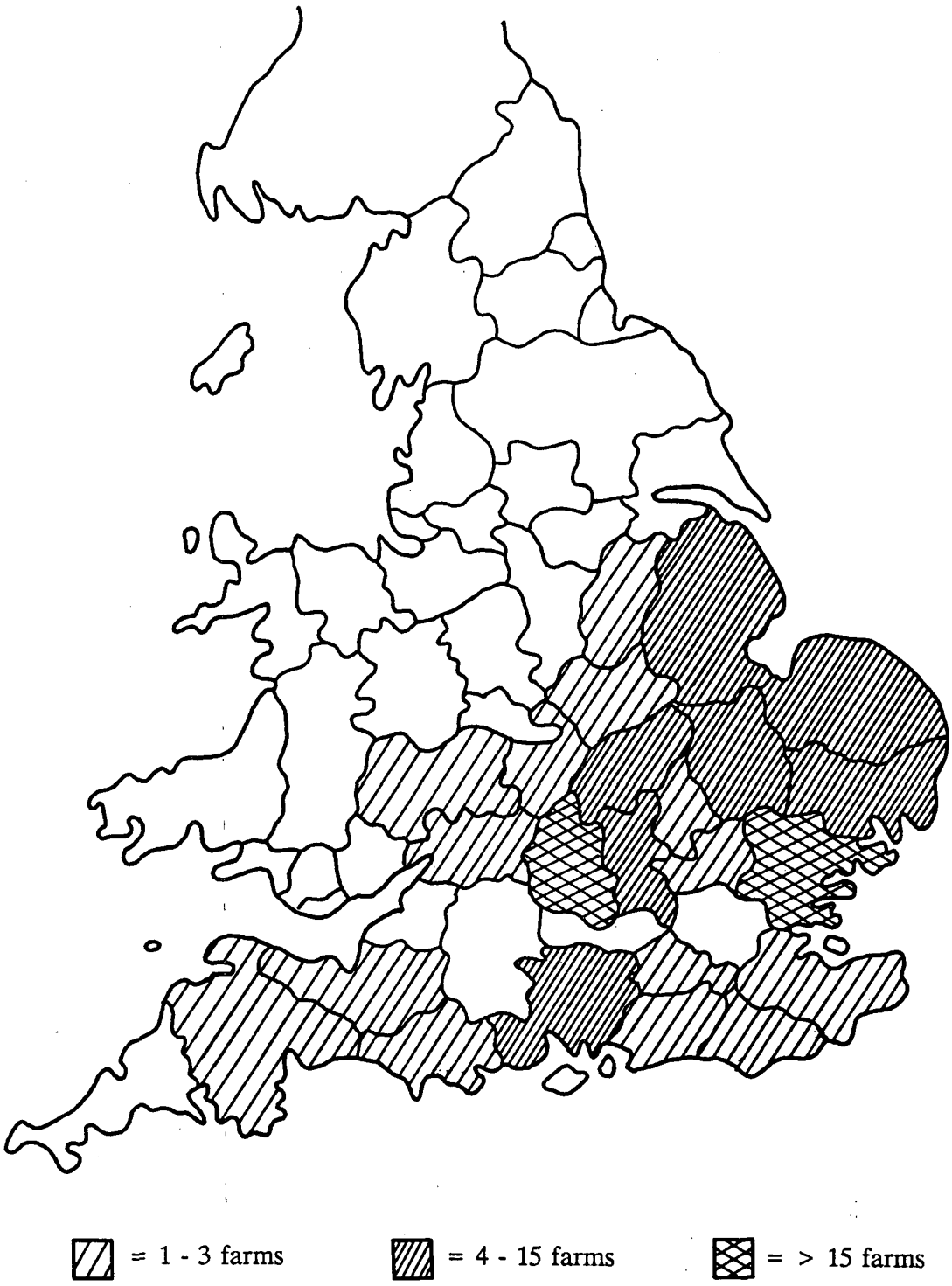


Figure 1. Distribution of farms in England and Wales by county where black-grass resistant to chlorotoluron and/or fenoxaprop had been detected by 1994.

CLASSIFICATION SYSTEM FOR RESISTANCE

Existing system for chlorotoluron

Since 1989, ADAS and Rothamsted have jointly used the classification system developed for the interpretation of results from glasshouse screening tests for chlorotoluron resistance (Clarke & Moss, 1989). This * rating system describes different degrees of resistance to chlorotoluron based on a comparison with the % reduction in foliage weight values of three standard reference populations, Rothamsted 1987 (susceptible), Faringdon 1987 (partially resistant) and Peldon 1987 (resistant). These three reference populations have been included in every screening test. No equivalent system has been described for resistance to fenoxaprop-ethyl, although ADAS has used a similar system but with a different intermediate population and Rothamsted has based resistance ratings on statistical analyses. The Faringdon population was not suitable as an intermediate for fenoxaprop-ethyl tests as it shows relatively more pronounced resistance to this herbicide than to chlorotoluron. In addition, all supplies of Faringdon 1987 seed have been exhausted and more recent collections from the same field show increased levels of resistance to chlorotoluron.

It would be preferable to introduce a classification system appropriate to both chlorotoluron and fenoxaprop-ethyl which did not rely on the selection and maintenance of populations of intermediate resistance. Clarke, Blair & Moss (1994) proposed a modification to the classification system used for chlorotoluron and the introduction of a similar system for fenoxaprop-ethyl.

Proposed resistance classification system for chlorotoluron and fenoxaprop-ethyl

The new rating system involves the use of only two standard reference populations, Rothamsted (susceptible) and Peldon 1993 (resistant) which must be included in every glasshouse screening experiment and is summarised Figure 2.

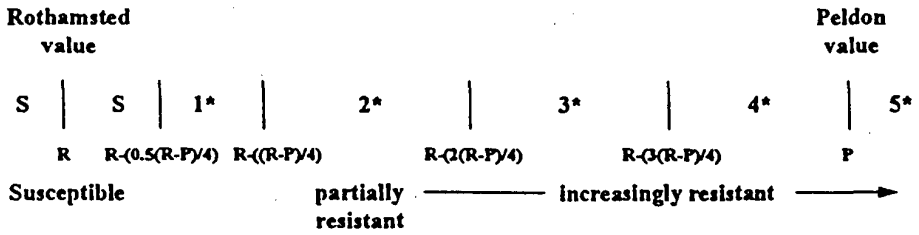


Figure 2. Proposed resistance classification system for both chlorotoluron and fenoxaprop-ethyl (P = % reduction in fresh weight for Peldon population; R = % reduction in fresh weight for Rothamsted population)

The % reduction in foliage fresh weight values between Rothamsted and Peldon are separated into four equal categories. One of these categories, at the Rothamsted end, is subdivided about its mid point into smaller categories, S (susceptible) and 1*. In addition, any populations more sensitive than Rothamsted are also termed susceptible. For example, if the value for Rothamsted is 95% and that for Peldon is 55%, then the susceptible (S) category covers the range from 100% - 90%, 1* = 90 - 85%, 2* = 85 - 75%, 3* = 75 - 65%, 4* = 65 - 55%, 5* = < 55% reduction in foliage weight.

It is important to stress that the determination of the different categories is made using the % reduction in fresh weight values obtained for the two standard reference populations for each individual screening test. The actual values delineating the categories will differ between screening tests. This will occur because the activity of the herbicides is likely to vary between tests due to the influence of environmental factors. For example, chlorotoluron, a photosynthetic inhibitor, tends to be more active in glasshouse tests conducted during the summer months due to the higher light intensities at that time.

The mean and range of the % reduction values for the standard reference populations, which were included in all chlorotoluron screening tests, are presented in Table 6.

TABLE 6 The reduction in foliage weight values for the three standard reference populations of black-grass included in 26 ADAS and Rothamsted screening experiments for chlorotoluron resistance (1987 - 1993)

	Rothamsted	Faringdon	Peldon
Mean	94	84	47
Range	(85 - 99)	(53 - 97)	(7 - 77)
Calculated mean value for 1*/2* boundary	-	81	-
Range	-	(66 - 94)	-

The mean value for the 1*/2* chlorotoluron boundary, calculated as described above using only Rothamsted and Peldon data, was similar to the actual mean Faringdon value. A correlation analysis was conducted to compare the actual % reduction values for Faringdon and the calculated values for the 1*/2* boundaries, using data from all ADAS and Rothamsted tests. The correlation coefficient was 0.54, which was highly significant at $P \leq 0.01$.

The interpretation of the star ratings is the same as in the previous system in that populations are only termed 'resistant' if they receive a rating of 2* or more. An important advantage of the new system is that it can be used for both chlorotoluron and fenoxaprop-ethyl, but every reference to * ratings must specify the herbicide used in the screening evaluation. Comparisons between star ratings for different herbicides must be made with care. The * rating system does, however, encompass the concept of varying degrees of resistance at the population level, and the field performance of chlorotoluron and fenoxaprop-ethyl does relate well with the * ratings based on glasshouse tests (Moss & Clarke, 1992).

SECTION 2

CROSS-RESISTANCE STUDIES

INTRODUCTION

Previous studies demonstrated that chlorotoluron-resistant black-grass shows cross-resistance to a range of other herbicides from different chemical groups. Resistance is not related in any simple way to chemical group or mode of action. The main mechanism of resistance, at least in the case of the substituted-urea herbicides chlorotoluron and isoproturon, is an enhanced ability of resistant plants to metabolise, and hence detoxify, the herbicides (Kemp, Moss & Thomas, 1990; Hall, Moss & Powles, 1995a). This appears to be a broad-spectrum mechanism, at least in the case of Peldon black-grass (the population studied in greatest detail), as a wide range of herbicides is affected. Resistance is dependent on the molecular structure of individual herbicides and hence their vulnerability to the enzyme system involved in this metabolism, probably cytochrome P450 monooxygenases.

Resistance to fenoxaprop-ethyl appears to be due, at least partly, to the presence of other biochemical mechanisms, including target site resistance to ACCase inhibitors, such as aryloxyphenoxypropionate ('fop', AOPP) and cyclohexanedione ('dim', CHD) herbicides (Hall, Moss & Powles, 1993). In target site resistance, an altered form of an essential enzyme involved in lipid biosynthesis is present. This enzyme, acetyl Co-A carboxylase (ACCase), is the target site for all 'fop' and 'dim' herbicides. The altered form of this enzyme still functions, but is insensitive to inhibition by 'fop' and 'dim' herbicides, which are consequently ineffective on resistant plants.

Thus two independent resistance mechanisms have been demonstrated in black-grass - enhanced metabolism and target site. It is probable that other mechanisms exist too.

To achieve a better understanding of cross-resistance patterns, a series of experiments was conducted to investigate cross-resistance to 10 herbicides with contrasting modes of action, in six different black-grass populations. The herbicides used were: the substituted ureas chlorotoluron and isoproturon which are photosynthetic inhibitors; the aryloxyphenoxypropionates ('fops',AOPP) fenoxaprop-ethyl, diclofop-methyl, fluazifop-P-butyl, quizalofop-ethyl and cyclohexanediones ('dims', CHD) sethoxydim and tralkoxydim which are ACCase inhibitors; the dinitroanilines pendimethalin and trifluralin which interfere with cell division.

MATERIALS AND METHODS

The six black-grass populations used were:

(A) **Rothamsted** (susceptible standard). Seed collected in 1990 from winter wheat grown on Section 8 of Broadbalk field, the section which has never received herbicides in its 150 year history. Previous studies have shown that this population is representative of susceptible populations, and is not atypically susceptible to chlorotoluron or fenoxaprop-ethyl.

(B) **Faringdon**. Seed collected in 1990 from a winter wheat field at Faringdon, Oxfordshire. This was the first resistant population found in England and shows partial resistance to chlorotoluron.

(C) **Peldon A1.** Seed collected in 1990 from a winter wheat field at Peldon, Essex. This is the field where black-grass highly resistant to chlorotoluron was first detected in the UK in 1984. This population is the one that has been used for the majority of cross-resistance and biochemical studies in the UK.

(D) **Oxford S1.** Seed collected in 1991 from a winter wheat field in eastern Oxfordshire where two successive applications of fenoxaprop-ethyl had failed to control black-grass. Previous glasshouse studies had confirmed that resistance to fenoxaprop occurred in this population.

(E) **Lincs. E1.** Seed collected in 1991 from a winter wheat field in eastern Lincolnshire where three successive applications of fenoxaprop-ethyl within that crop had failed to control black-grass. The population remaining exceeded 500 heads/m^2. This is the most resistant black-grass population to fenoxaprop-ethyl so far found in glasshouse studies at Rothamsted.

(F) **Oxford I5.** Seed collected in 1991 from a winter wheat field near Abingdon Oxfordshire., where a range of different herbicides had given inadequate control.

These six populations were used in three dose response experiments to determine the degree of resistance to a range of 10 herbicides. The following herbicides were applied:

	Herbicide	**Dose range**
Experiment 1	Chlorotoluron	0.25 - 56.0 kg/ha
	Fenoxaprop-ethyl (+ safener)	15 - 960 g/ha
	Diclofop-methyl	0.29 - 36.48 kg/ha
	Fluazifop-P-butyl (+ "Agral" 0.1%)	7.81 - 500 g/ha
	Sethoxydim + ("Adder" 1%)	9.06 - 580 g/ha
Experiment 2	Isoproturon	0.08 - 10 kg/ha
	Quizalofop-ethyl (+ "Fyzol" 2 l/ha)	4.69 - 300 g/ha
	Tralkoxydim (+ "Adherb" 0.5%)	31.25 - 2000 g/ha

The herbicides were applied in a staggered range of six doses within the ranges given above, depending on the probable level of resistance. In most cases, each dose was twice the preceding dose in the range. Recommended adjuvants were used.

In Experiments 1 and 2 single plants were grown from seed in a Kettering loam soil/grit mixture (5:1) in individual 5 cm square pots in a glasshouse. In both experiments the herbicides were applied at the 2 - 3 leaf stage using a laboratory sprayer delivering approximately 300 l water/ha at 210 kPa through a single 'Lurmark' 01/F110 flat fan nozzle. There were 12 replicate pots per herbicide dose for each population and 40 untreated pots per population. Foliage fresh weight per pot was recorded 21 - 25 days after spraying as a measure of herbicide activity.

In Experiment 3, a petri dish test as described by Moss (1990) was used to assess response to pendimethalin and trifluralin in the six populations. Twenty-five seeds were placed in each 9 cm dish containing three Whatman No. 4 filter papers covered by one glass-fibre paper. There were three replicates of the six concentrations of herbicide (0, 0.4, 1, 3, 10, 50 ppm) and the solutions included 2 g/litres KNO_3 to break seed dormancy and stimulate seedling growth. Seven ml of the appropriate solutions were added to each dish. Dishes were placed in polyethylene bags in an incubator providing a 17°C, 14 h day; 11 °C, 10 h night. After 21 days the length of the primary shoot of each germinated seed was measured.

Foliage fresh weight and shoot length data from the glasshouse (Experiments 1 and 2) and petri dish test (Experiment 3) were analysed using a Maximum Likelihood Programme (Ross, 1987) and $\log_{10}$ ED_{50} values calculated. ED_{50} values were detransformed from the $\log_{10}$ data and represent the herbicide dose required to reduce foliage fresh weight or shoot length by 50%, relative to the no-herbicide controls. Resistance index is a measure of the degree of resistance, and is the ratio of the ED_{50} value for each population to that of the Rothamsted susceptible standard.

RESULTS AND DISCUSSION

The $\log_{10}ED_{50}$ values for statistical comparisons and detransformed values are given in Tables 7 - 9. The resistance indices (the ratio of ED_{50} values relative to the susceptible standard, Rothamsted) are presented in Table 10. Comparisons of the degree of resistance between populations and herbicides can be made most conveniently using these values.

The results demonstrate very clearly that the patterns of cross-resistance can differ substantially between populations. For example, the Peldon population showed the greatest resistance of any population to chlorotoluron, but only modest resistance to fenoxaprop (Table 10). In marked contrast, the Lincs. E1 population, which was collected from a field where three applications of fenoxaprop had failed to give control, was the most resistant population to this herbicide. This population also showed substantial cross-resistance to diclofop, fluazifop, quizalofop and tralkoxydim but only marginal resistance to chlorotoluron, isoproturon and pendimethalin.

The Faringdon, Oxford S1 and Oxford I5 populations generally showed partial resistance to all herbicides, except sethoxydim and trifluralin. All populations showed greater resistance to chlorotoluron than isoproturon, the other substituted urea herbicide tested. This result is consistent with other studies involving a larger number of populations collected in 1992 (Clarke, Blair & Moss, 1994). This demonstrates that the degree of resistance to herbicides within the same chemical group can vary. All populations tested showed varying degrees of resistance to tralkoxydim, but no resistance at the population level to sethoxydim, the other cyclohexanedione herbicide tested. Subsequent studies (see Section 5) showed that a small proportion (5%) of plants of both the Lincs. E1 and Oxford S1 populations is resistant to sethoxydim. Resistance to this herbicide was not demonstrated in this experiment because of the diluting effect of the other 95% of susceptible plants.

All populations showed at least partial resistance to pendimethalin, but not to the other dinitroaniline, trifluralin. This supports other studies which showed that resistance to pendimethalin in the Peldon population is attributable to an oxidative metabolism analogous to that which occurs with chlorotoluron. The lack of cross-resistance to trifluralin appears to be due to differences in its molecular structure (James, Kemp & Moss, 1995). It should be noted, however, that there was some evidence for marginal insensitivity to trifluralin in the Peldon population. This may reflect a gradual loss of sensitivity to this herbicide which could eventually result in resistance. Certainly trifluralin is not immune to resistance, as populations of *Setaria viridis* (Green foxtail), *Eleusine indica* (Goosegrass) and *Amaranthus palmeri* (Palmer amaranth) have evolved resistance to this herbicide in the USA or Canada. At present, no populations of black-grass show unequivocal evidence of resistance to trifluralin in the UK. However, further monitoring is necessary, especially at sites such as Peldon, to determine whether a gradual loss of sensitivity to this herbicide is occurring.

The results show clearly that there is no consistent relationship between the degree of resistance to two of the major herbicides used for black-grass control, isoproturon and fenoxaprop, at least in some populations. The results also show that relating resistance to chemical class is misleading, and there is a need to consider herbicides individually as resistance may occur to some, but not all, of the herbicides within a class.

Using mixtures, sequences or rotations of different types of herbicides is often proposed as a means of delaying the evolution of resistance. This is good advice in many situations, but may be ineffective where broad-spectrum resistance mechanisms exist, as selection for the same detoxification process may occur despite using a wide range of herbicide types. However, until we have a more detailed understanding of the frequency of occurrence of different resistance mechanisms, using mixtures or rotations of herbicides from different herbicide groups is advisable (Moss & Clarke, 1994).

TABLE 7. Experiment 1 - Effects of five herbicides on six black-grass populations

	Log_{10} ED_{50} Values				
	Chlorot.	**Fenox.**	**Diclofop**	**Fluaz.**	**Sethox.**
Rothamsted	- 0.403	1.575	- 0.216	1.141	1.083
Faringdon	0.103	1.880	0.587	1.591	0.884
Peldon	1.043	2.166	0.785	1.900	1.272
Oxford S1	0.112	2.264	0.895	1.843	1.229
Lincs E1	0.015	3.009	0.913	1.983	1.368
Oxford I5	0.128	2.029	0.788	1.755	1.077
S.E. ±	0.055	0.110	0.093	0.086	0.138
L.S.D. ($P \leq 0.05$)	0.159	0.322	0.273	0.252	0.403

	ED_{50} values* (detransformed)				
	Chlorot.	**Fenox.**	**Diclofop**	**Fluaz.**	**Sethox.**
Rothamsted	0.4	37.6	0.6	13.9	12.1
Faringdon	1.3	75.8	3.9	39.0	7.7
Peldon	11.0	146.7	6.1	79.5	18.7
Oxford S1	1.3	183.5	7.9	69.7	16.9
Lincs E1	1.0	1022.0	8.2	96.2	23.3
Oxford I5	1.3	106.9	6.1	56.9	11.9

* = kg a.i./ha for chlorotoluron and diclofop-methyl.
g a.i./ha for fenoxaprop-ethyl, fluazifop-P-butyl and sethoxydim.

ED_{50} values are the herbicide doses required to reduce foliage fresh weight by 50%, relative to the no herbicide controls for the same population

TABLE 8. Experiment 2 - Effects of three herbicides on six black-grass populations

	Log_{10} ED_{50} values		
	Isoproturon	**Quizalofop**	**Tralkoxydim**
Rothamsted	- 0.784	1.089	0.931
Faringdon	- 0.544	1.410	1.856
Peldon	- 0.056	1.825	2.033
Oxford S1	- 0.481	1.638	2.164
Lincs E1	- 0.692	2.006	2.129
Oxford I5	- 0.345	1.666	1.543
S.E. ±	0.051	0.110	0.148
LSD ($P \leq 0.05$)	0.150	0.321	0.433

	ED_{50} values * (detransformed)		
	Isoproturon	**Quizalofop**	**Tralkoxydim**
Rothamsted	0.16	12.3	8.5
Faringdon	0.29	25.7	71.8
Peldon	0.88	66.9	107.8
Oxford S1	0.33	43.4	145.8
Lincs E1	0.20	101.5	134.5
Oxford I5	0.45	46.4	34.9

* = kg a.i./ha for isoproturon; g a.i./ha for quizalofop and tralkoxydim

TABLE 9. Experiment 3 - Effects of two herbicides on six black-grass populations

	Log_{10} ED_{50} values	
	Pendimethalin	**Trifluralin**
Rothamsted	- 0.236	0.665
Faringdon	0.219	0.581
Peldon	0.900	0.929
Oxford S1	0.271	0.710
Lincs E1	0.164	0.566
Oxford I5	0.142	0.652
S.E. ±	0.073	0.049
LSD ($P \leq 0.05$)	0.218	0.145

	ED_{50} values* (detransformed)	
	Pendimethalin	**Trifluralin**
Rothamsted	0.6	4.6
Faringdon	1.7	3.8
Peldon	7.9	8.5
Oxford S1	1.9	5.1
Lincs E1	1.5	3.7
Oxford I5	1.4	4.5

* = ppm

TABLE 10. Resistance Indices
(ratio of ED_{50} values relative to Rothamsted susceptible standard)

Experiment 1	Chlorot.	Fenox.	Diclofop	Fluaz.	Sethox.
Rothamsted	1.0	1.0	1.0	1.0	1.0
Faringdon	3.2	2.0	6.3	2.8	0.6
Peldon	27.6	3.9	10.0	5.7	1.5
Oxford S1	3.2	4.9	12.9	5.0	1.4
Lincs E1	2.6	27.2	13.4	6.9	1.9
Oxford I5	3.4	2.8	10.1	4.1	1.0

Experiment 2	Isoproturon	Quizalofop	Tralkoxydim
Rothamsted	1.0	1.0	1.0
Faringdon	1.8	2.1	8.4
Peldon	5.5	5.5	12.6
Oxford S1	2.1	3.5	17.1
Lincs E1	1.3	8.3	15.8
Oxford I5	2.8	3.8	4.1

Experiment 3	Pendimethalin	Trifluralin
Rothamsted	1.0	1.0
Faringdon	2.9	0.8
Peldon	13.7	1.8
Oxford S1	3.2	1.1
Lincs E1	2.5	0.8
Oxford I5	2.4	1.0

SECTION 3

INFLUENCE OF RESISTANCE ON HERBICIDE EFFICACY IN FIELD CONDITIONS

INTRODUCTION

Resistance is only one of many factors which influence herbicide activity in the field. Consequently, in field experiments it is difficult to determine to what extent poor herbicide performance is due to resistance compared to other unrelated factors such as soil or climatic conditions. This is a particular problem if resistance is partial, rather than absolute.

It is important that the effects of resistance are measured at doses recommended for application in the field, so that the likely impact of resistance can be assessed. This can best be achieved by comparing the performance of herbicides on different populations grown in a standard soil at one site. In this way soil and climatic variables are eliminated.

Two experiments were conducted. The first involved the same six populations as used in the glasshouse cross-resistance experiments (Section 2). The objective was to compare performance in glasshouse and outdoor conditions. The objective of the second experiment was to identify the way in which marginal levels of resistance, as identified in glasshouse tests, affect the efficacy of a range of herbicides at different doses and at different growth stages of black-grass in the field.

MATERIALS AND METHODS

Container Experiment

This technique involves growing plants of different populations in a standard soil in outdoor containers in order to simulate field conditions. Seeds (300 per container) of the six populations used in the glasshouse cross-resistance experiments (see Section 2) were incorporated into the top 5 cm of a silty loan soil/grit mix (about 4% organic matter) in separate plastic containers (27 x 18 x 10 cm) on 7 October 1991 and immediately sunk into an outdoor sand plunge bed at Rothamsted. The experiment comprised a randomised block design with four replicates. There were two untreated containers per replicate for each population.

The herbicide treatments were the field recommended rates of: chlorotoluron 3.5 kg a.i./ha; fenoxaprop-ethyl (+ safener), 120 g a.i./ha; fluazifop-P-butyl, 125 g a.i./ha (+ 'Agral', a non-ionic wetter 0.1%); sethoxydim, 290 g a.i./ha (+ 'Adder', a mineral adjuvant oil at 1% of total spray volume). The herbicides were applied post-emergence on 23 December 1991 when the black-grass plants were at the 3 - 3½ leaf stage and the first tiller just emerging. Trays were removed from the sandbed temporarily and herbicides applied using a single nozzle laboratory sprayer delivering 315 l water/ha at 210 kPa through a single 'Lurmark' 01-F110 flat fan nozzle.

Herbicide activity was assessed by determining numbers of surviving plants and foliage fresh weight per container on 3 - 5 March 1992, when untreated black-grass was at the 4 tiller stage.

ADAS Boxworth Field Experiment

Two populations were used. The Rothamsted population was a standard susceptible population and the Boxworth population had a resistance rating of 1* for chlorotoluron, which would be classified as being only marginally resistant (Clarke & Moss, 1989). Seeds were sown in three rows of two metre length (10 cm apart and 30 cm between different populations) in a chalky boulder clay soil on 16

October 1992. There were four replicates of each treatment. Four herbicides (chlorotoluron, isoproturon, diclofop-methyl and fenoxaprop-ethyl (+ safener)) each at three doses (recommended, double recommended, half recommended), were sprayed on 4 February 1993 at the three-leaf stage, or on 11 March 1993 at the three-tiller stage, using an Oxford Precision Sprayer delivering 225 l water/ha through 02-F80 nozzles at 200 kPa. Black-grass control was assessed by counting the number of plants on 4 February 1993 (before treatment) and 15 April 1993 (at least one month after treatment) and expressed as the % reduction of the number in that plot before treatment.

RESULTS AND DISCUSSION

Container Experiment

The mean foliage weight in untreated containers was: Rothamsted - 31.1 g; Faringdon - 57.0 g; Peldon - 42.8 g; Oxford S1 - 42.5 g; Lincs. E1 - 46.9 g; Oxford I5 - 36.1 g. To avoid the effects of herbicide activity being confounded with differences in growth between populations, data were converted to % reductions in foliage weight relative to untreated for the same population (Table 11).

The Rothamsted population (susceptible standard) was controlled very effectively by all four herbicides - over 97% reduction in foliage fresh weight (Table 11). This demonstrates that the environmental conditions and method of application were conducive to high levels of herbicide activity. Chlorotoluron gave reduced levels of control for all other populations. The poorest control occurred with the Peldon population. Fenoxaprop-ethyl gave poor control of all populations in comparison with Rothamsted. However, the level of control was of a similar order for all these non-Rothamsted populations (29.7 - 45.4%). In contrast with the chlorotoluron results, Peldon did not show greater resistance to fenoxaprop compared to the other populations. This demonstrates that resistance to chlorotoluron and fenoxaprop is not always directly correlated. Fluazifop gave good control of four populations (> 94%) including Peldon. However, with these four populations, fewer Rothamsted plants survived treatment than the other populations (0 v 4 - 7 plants

per container) although most (71 - 89%) of the survivors did show severe herbicidal symptoms (Table 12). Fluazifop gave significantly poorer control of the Oxford S1 and especially the Lincs. E1 populations, compared with Rothamsted. In addition, fewer of the surviving plants (Oxford S1 - 32%; Lincs. E1 - 16%) showed severe herbicidal symptoms. This was the first time that a high degree of resistance to fluazifop had been demonstrated in the UK.

Sethoxydim gave good control (> 93%) of all populations. However while there were no surviving, healthy plants with four of populations, all the Oxford S1, and most (76%) of the Lincs. E1 survivors were healthy and showed few herbicidal symptoms. Plants of Oxford S1 were grown on for seed production in order to determine the degree of resistance of the progeny of the survivors. Results of these evaluations are given in Section 5. The subsequent studies showed, for the first time, that the black-grass plants surviving sethoxydim had a different resistance mechanism, namely target site resistance (insensitive ACCase). This contrasts with the enhanced metabolism detected in the Peldon population.

The results of the container experiment confirm that the level of resistance to chlorotoluron and fenoxaprop is sufficient to substantially reduce herbicide activity in field conditions. The findings also indicate that the poor control achieved by application of fenoxaprop in cereal fields in 1990 and 1991 at the five non-Rothamsted sites was due, at least partly, to resistance.

The results of the container experiment also broadly agree with those from the glasshouse dose response experiments (Section 2), and the * ratings for resistance based on previous glasshouse screening tests (Clarke, Blair & Moss, 1994). Thus with chlorotoluron, Peldon (5*) was clearly the most resistant population, Rothamsted was susceptible (S) with the other four populations intermediate (2 - 3*). With fenoxaprop the correlations between glasshouse and outdoor performance were good, but not quite as clear cut as for chlorotoluron. The most resistant population in the glasshouse dose response experiment, Lincs. E1 (5 *), was also the least well controlled outdoors, Rothamsted was fully susceptible and

the other populations intermediate (3 - 5 *). The Lincs. E1 population also showed the greatest resistance to fluazifop in the dose response experiment and this was confirmed in the container experiment. Some populations (e.g. Peldon, Oxford I5) showed evidence of resistance to fluazifop in the glasshouse experiment, but not in the containers. This was probably a reflection of the good activity of the single dose used outdoors, as other studies have demonstrated small reductions in fluazifop performance on the Peldon population at reduced doses (Moss & Clarke, 1992). This demonstrates that differences detected in glasshouse experiments may not always be evident in the field when conditions are favourable for herbicide activity. It should be noted however that a small number of plants did survive fluazifop treatment with all populations except Rothamsted. The container results confirmed that, **at the population level**, there was no evidence of resistance to sethoxydim. However, subsequent studies (Section 5) show that the resistance status of individual plants needs to be considered, as other mechanisms of resistance can be present in a small proportion of plants.

ADAS Boxworth Field Experiment

The Boxworth population of black-grass had a resistance rating of 1* to chlorotoluron, as detected in glasshouse tests, indicating that it is likely to show only a marginal level of resistance. It has been previously reported (Clarke & Moss, 1991) that there can be a reduction in the efficacy of chlorotoluron at the recommended rate (3.5 kg a.i./ha), when the 1* population is compared to a susceptible standard population (Rothamsted). The results of this experiment confirm this finding (Table 13).

There were statistically significant differences between the means of all treatments for each population, indicating that the marginal level of resistance within the Boxworth population did have an effect on herbicide efficacy. There were, however, no significant differences when considering the treatment x population x growth stage interaction.

However, within each treatment there were noticeable differences, particularly at the later timing. These showed that all rates of isoproturon and diclofop-methyl, double rate chlorotoluron, and half and full rates of fenoxaprop-ethyl, were all more effective on the Rothamsted population, than on the 1* population. At the earlier timing, all rates of isoproturon and fenoxaprop-ethyl, full and double rates of chlorotoluron, and half rates of diclofop-methyl were all more effective on the Rothamsted population.

The results associated with isoproturon and diclofop-methyl at the later timing, appear to be contrary to the findings of Clarke & Moss (1991), who found very little difference in control between the two different populations. However, this may be due to the conditions affecting herbicide activity at the time of treatment. The results also show that, when averaged over all treatments, there was a much better level of control in the susceptible population, which may be associated with resistance in the Boxworth population. This is an important finding, as recent random surveys have found that 15% of populations were classified as 1* (Clarke, Blair & Moss, 1994; see Section 1). The results also show the importance of early control, as the plants treated later showed a much better level of survival. All reduced rates of herbicide gave much poorer control than did full and double doses.

The field experiment showed that populations of black-grass which show only marginal levels of resistance to chlorotoluron in glasshouse tests, can cause a reduction in its efficacy in the field. It also showed that there is potentially a reduction in efficacy of isoproturon, diclofop-methyl and, to a lesser extent, fenoxaprop-ethyl in populations showing marginal levels of resistance to chlorotoluron, and that reduced rates of herbicide resulted in poorer control.

CONCLUSIONS

These results demonstrate that, in most cases, glasshouse-based assessments of resistance do relate quite well to herbicide performance in the field. However, the results of glasshouse tests may, in some cases, overstate the likely impact of resistance. Conversely, small differences detected in the glasshouse should not be dismissed, as such marginal resistance may cause significant reductions in herbicide performance in the field, especially in sub-optimal conditions. Clearly, glasshouse evaluations for resistance have a useful role to play in investigations of cross-resistance, but care is needed in interpreting the results in terms of the likely effects on herbicide performance in the field.

TABLE 11. The effect of four herbicides on six black-grass populations grown in outdoor containers

	% Reduction in foliage fresh weight			
	Chlorotoluron	Fenoxaprop-ethyl	Fluazifop-P-butyl	Sethoxydim
	3.5 kg/ha	120 g/ha	125 g/ha	290 g/ha
Rothamsted	99.9	98.1	97.7	99.0
Faringdon	67.0	45.0	94.5	97.6
Peldon	8.2	39.1	94.3	98.1
Oxford S1	73.8	41.0	84.5	93.1
Lincs. E1	79.7	29.7	44.4	94.3
Oxford I5	81.0	45.4	95.3	98.2

S. E. ± 4.59
LSD ($P \leq 0.05$) 13.0

TABLE 12. The effect of four herbicides on six black-grass populations grown in outdoor containers

	Nos. of surviving plants per container				
	Nil	Chlorotoluron	Fenoxaprop-ethyl	Fluazifop-P-butyl	Sethoxydim
		3.5 kg/ha	120 g/ha	125 g/ha	290 g/ha
Rothamsted	52	0	2	0	1
Faringdon	72	48	54	4	0
Peldon	70	75	53	7	0
Oxford S1	63	43	53	6	4
Lincs. E1	77	43	62	58	3
Oxford I5	57	30	42	4	0

TABLE 13. ADAS Boxworth field experiment: Mean percentage reduction of black-grass plants/m²

Herbicide	Rate of a.i. kg/ha	Sprayed at 3 leaf		Sprayed at 3 tiller	
		Rothamsted (S)	Boxworth (1*)	Rothamsted (S)	Boxworth (1*)
chlorotoluron	1.75	7.2	10.2	2.5	3.2
chlorotoluron	3.5@	77.8	57.8	38.2	37.8
chlorotoluron	7.0	83.3	79.1	81.3	53.8
isoproturon	1.25	61.6	33.9	27.4	0.0
isoproturon	2.5@	77.7	65.9	63.1	41.3
isoproturon	5.0	96.5	90.3	99.4	81.5
diclofop-methyl	0.567	92.4	78.0	23.0	0.0
diclofop-methyl	1.134@	95.5	98.4	73.0	39.5
diclofop-methyl	2.268	100.0	100.0	93.7	74.1
fenoxaprop-ethyl	0.075	99.1	86.1	35.2	31.3
fenoxaprop-ethyl	0.15@	99.1	92.8	96.5	88.5
fenoxaprop-ethyl	0.30	99.0	92.8	94.4	95.4
			S.E.D. ± 12.26	(72 d.f.)	
Mean		82.5	74.2	60.5	44.7
			S.E.D. ± 3.54	(72 d.f.)	

@ = Recommended rate
* = Rating for resistance

SECTION 4

SELECTION AND DESELECTION STUDIES

INTRODUCTION

The development of herbicide resistance is an evolutionary process. The **rate** at which resistance evolves at the population level is important. More time and greater opportunities for adopting strategies to minimize its impact are likely to be available in situations where rate of selection is slow. Conversely, where resistance builds up rapidly, a major resistance problem may develop so quickly that absolute failure of herbicides may occur within a few years. There is much theoretical information on the factors influencing selection for resistance, but much less practical knowledge. Thus, there is still considerable debate about whether high or low doses favour resistance.

Previous studies indicated that rate of development of resistance to substituted-urea herbicides, such as chlorotoluron and isoproturon, appeared to be relatively slow (Moss & Clarke, 1992). However, there were suspicions that resistance to aryloxyphenoxypropionate herbicides ('fops',AOPP) such as fenoxaprop-ethyl, could develop more rapidly. Consequently, a series of three linked experiments was conducted to investigate the rate of development of resistance to contrasting herbicides, chlorotoluron or isoproturon (substituted ureas), and fenoxaprop-ethyl (a 'fop'). One of these experiments also incorporated different levels of herbicide efficacy in order to study how herbicide dose may influence the rate of selection for resistance. In addition an experiment was conducted to investigate rate of deselection in the absence of herbicides.

MATERIAL AND METHODS

Selection Experiments

Three experiments were conducted to study changes in response to chlorotoluron or isoproturon and fenoxaprop-ethyl over three generations. The experiments were conducted in the glasshouse, except for the first generation of Experiment 3, which was on part of the black-grass stockbed at Long Ashton Research Station.

The populations and degree of selection imposed are detailed below.

Experiment	Population	Herbicides		Degree of selection[a] Year 1	2	3
Experiment 1	Rothamsted	chlorotoluron		2.4%	2.5%	3.3%
		fenoxaprop		2.5%	2.5%	3.3%
Experiment 2	Rothamsted	chlorotoluron	(H)	2.2%	1.9%	3.3%
			(L)	14.9%	14.4%	15.1%
		fenoxaprop	(H)	2.2%	0.6%	2.9%
			(L)	15.1%	16.8%	14.7%
Experiment 3	Long Ashton	isoprot./chlorot.[b]		0.52%[c]	2.5%	3.2%
		fenoxaprop		0.52%[c]	2.5%	3.3%

[a] The proportion of treated plants which were grown on to produce seed
[b] year 1 = isoproturon; year 2 and 3 = chlorotoluron
[c] field selection
H = high selection
L = low selection

The Rothamsted population comprised seeds collected in 1987 from the 'no weedkillers' section of Broadbalk field which has never received herbicides. The Long Ashton population comprised the 1990 stockbed which was originally sown with seeds collected from the stockbed at the Weed Research Organisation. That stockbed was established with seeds collected in 1975 from the field at Faringdon where resistance was subsequently detected in 1982. The original 1975 population was susceptible to chlorotoluron. The Long Ashton stockbed population had been

grown in the absence of exposure to herbicides since 1975. Consequently both populations had been subjected to no, or minimal herbicide selection prior to these experiments.

The initial population size in year 1 of Experiments 1 and 2 averaged 492 plants for each herbicide/selection level (range = 456 - 576). In years 2 and 3, selection was based on a similar number of plants, averaging 472 plants (range = 225 - 585). In Experiment 3, the initial population size on the outdoor plots was approximately 6858 plants for each herbicide, and in years 2 and 3 (glasshouse) averaged 402 plants (range 363-480).

Pre-germinated black-grass seeds were sown in individual 5 cm pots containing a Kettering loam/grit 5:1 mix. Plants were examined and only healthy plants of similar growth stage were retained for spraying.

Herbicides were applied at the 2-3 leaf stage using a laboratory sprayer delivering 200 -300 l water/ha at 210 kPa through a single flat fan nozzle. The dose rates used were in the ranges: chlorotoluron 1.0 - 1.5 kg a.i./ha; fenoxaprop-ethyl 70 - 125 g a.i./ha. In a few cases, repeat applications were made when the initial level of activity was inadequate. The doses were chosen to give partial, rather than complete, kill so that the least affected plants could be selected visually. This procedure was performed 3 - 4 weeks after spraying, and the selected plants transplanted into larger pots. Sets of pots for each herbicide and selection level were isolated in compact groups when black-grass heads started to emerge, to encourage cross pollination within, but not between, sets. Mature seeds were collected and the cycles of selection repeated for a total of three generations.

For the first generation of Experiment 3, two plots (each 68 m^2) of the Long Ashton stockbed were treated with either isoproturon (2.5 kg a.i./ha) or fenoxaprop-ethyl (150 g a.i./ha) on 30 March 1990 using a hand held plot sprayer. The number of black-grass plants present before herbicide treatment was assessed by counting plants in 20 x 0.1 m^2 quadrats per plot. The 18 most healthy survivors

on each plot were dug up on 8 May 1990 and transferred to pots of soil. Each set was isolated in a glasshouse and grown on until seeds could be collected. The second and third cycles of selection in Experiment 3 were conducted in the glasshouse in the same manner as Experiments 1 and 2.

Evaluation of progeny of selected plants

Separate evaluations were conducted for each selection experiment, but a similar procedure was adopted. Pre-germinated seeds from the source populations, Rothamsted 1987 or Long Ashton stockbed 1990 (untreated area), and from the third generation of selected plants were sown in individual 5 cm square pots containing a Kettering loam/grit mixture (5:1) and slow release fertilizer.

Each evaluation comprised a completely randomised design with 24 (Experiment 3) or 30 (Experiments 1 and 2) replicate pots (1 plant/pot) at each of six (Experiment 2) or eight (Experiments 1 and 3) doses per herbicide treatment. In addition, there were 40 untreated pots per population. The dose ranges used were 0.0547 - 14.0 kg a.i./ha chlorotoluron and 15 - 3840 g a.i./ha fenoxaprop-ethyl. The herbicides were applied in a staggered range of doses within the ranges given above. Plants were sprayed at the 2 - 3 leaf stage using a laboratory sprayer fitted with a single Lurmark 01-F110 flat fan nozzle delivering 216 - 249 l/ha (depending on experiment) at 210 kPa.

Fresh weight of foliage per pot was recorded three weeks after spraying. Dose response data were analysed in the same manner as for the cross-resistance experiments (see Section 2).

Deselection Experiment

A black-grass population from Peldon, Essex is known to be highly resistant to chlorotoluron and diclofop-methyl (Moss & Clarke, 1992). An experiment was conducted in which the Peldon population was grown on for three generations in the absence of herbicides. The first generation of seeds was collected from untreated plots of a field experiment in 1987. These seeds were sown in pots in a

glasshouse, and 50 plants established. These were grown on in isolation and seeds collected. These in turn were sown and 70 plants established and allowed to produce seed. This sample represented seeds produced after three generations of deselection in the absence of herbicides.

A dose response experiment was conducted in which the response of four populations to chlorotoluron and diclofop-methyl was compared. The populations were: Rothamsted (susceptible standard), Peldon 1986 field collection, Peldon 1992 field collection, Peldon deselected sample as described above. Plants were established in individual 5 cm square pots. Plants were sprayed at the three-leaf stage with chlorotoluron (dose range 0.109 - 56.0 kg a.i./ha) or diclofop-methyl (0.142 - 72.6 kg a.i./ha). A staggered range of seven doses was used within the ranges detailed above. There were 20 replicate pots per herbicide dose and 40 untreated pots per population. Herbicides were applied using a laboratory sprayer as described in Section 2. Application volume = 200 l water/ha. Foliage fresh weight was recorded three weeks after spraying and dose response data analysed as described in Section 2.

RESULTS AND DISCUSSION

Selection Experiments

In all three experiments the progeny of plants selected using fenoxaprop-ethyl for three generations showed a significant loss of sensitivity to this herbicide (Tables 14 - 16, Figures 3 - 8). The ED_{50} values (the dose required to reduce foliage weight by 50% relative to untreated) were respectively 2x, 3.5x (higher level selection) and 3.8x that of the susceptible parent populations. This level of resistance, although quantitative rather than absolute, was sufficient to cause poor levels of control (75%, 10% and 25%, for the three experiments) at the field recommended rate of 120 g a.i./ha, compared with 81 - 91% control of the parent populations.

Even at twice the field recommended rate, reduced activity of fenoxaprop on selected material was detectable, with % reductions of 89%, 60% and 51%

respectively compared with 90 - 96% control of the susceptible parent populations.

The progeny of plants from the higher level of selection were more resistant than those from the lower level of selection in Experiment 2. Thus ED_{50} values were respectively 3.3x and 2.0x those of the susceptible parent population for the higher and lower levels of selection to fenoxaprop in Experiment 2. The fenoxaprop selected populations showed no, or only marginal, resistance to chlorotoluron, with ED_{50} ratios 0.9 - 1.3x those of the susceptible parent.

In contrast to selection by fenoxaprop, the progeny of plants selected with chlorotoluron for three generations showed no clear evidence of increasing resistance to either chlorotoluron or fenoxaprop. Compared to the susceptible parent population, ED_{50} ratios ranged from 0.9 - 1.6 and in most instances were not statistically significant.

The changes in sensitivity detected in these experiments were relatively modest, but sufficient to reduce activity at field rates. It is probable that the resistance mechanism operating is enhanced metabolism. There was no evidence for the presence of even a few highly resistant plants. The size of the initial population was small, at about 400 - 500 plants. Consequently it was unlikely that rare, highly resistant, individuals would be present, especially as the populations received no or minimal previous selection. Consequently, selection probably operated on processes present in most, if not all, plants. All black-grass plants have the ability to metabolize herbicides such as chlorotoluron and fenoxaprop to a limited degree, but in susceptible plants this ability is insufficient to prevent the plants being killed. If, as seems probable, herbicide metabolism is polygenic and dependent on the additive effect of many genes each having a small effect, then recurrent selection may have acted on such a continuous variation and achieved a progressive increase in average resistance from generation to generation. The enzyme system most likely to be involved is cytochrome P450. Many cytochrome P450 enzymes exist in plants, but few have been isolated. At present, we do not know whether a single, or many different enzymes are involved in enhanced herbicide metabolism.

In these selection experiments, response to chlorotoluron was not correlated with resistance to fenoxaprop. Thus, the population selected with fenoxaprop in Experiment 3 showed the greatest insensitivity to fenoxaprop of any of those evaluated, but was as sensitive to chlorotoluron as the parent population. This may imply that different P450 enzymes are involved in resistance to chlorotoluron and fenoxaprop, and that resistance to these two herbicides can operate independently. However, other mechanisms of resistance to fenoxaprop are known to exist, so this aspect can only be answered by more detailed biochemical studies.

The results support the view that resistance to 'fops' such as fenoxaprop can develop more rapidly than resistance to substituted-urea herbicides such as chlorotoluron. At a similar level of imposed selection the loss of sensitivity to fenoxaprop was significantly greater than with chlorotoluron. There was also evidence that the higher the level of selection, the greater the loss of sensitivity. The practical implication of this is that **high** doses are more likely to favour resistance than **low** doses, at least with fenoxaprop.

Deselection Experiment

The results are given in Table 17 and Figures 9 and 10. The population deselected for three generations was less resistant to both herbicides than the parent Peldon 1986 field population. This indicates that an appreciable amount of deselection did occur. However, even with three generations of deselection, this population showed clear evidence of resistance to both herbicides relative to the Rothamsted susceptible standard. The Peldon 1992 field population showed a similar level of chlorotoluron resistance and a slightly increased level of diclofop resistance compared to the 1986 population. This indicates that resistance to both herbicides in the field has either not increased (chlorotoluron) or has increased slowly (diclofop) during the six year period 1986 - 1992. The results also show that

resistance has not declined in the field, almost certainly because of the continued annual use of herbicides.

CONCLUSIONS

The results from the three linked selection experiments were very consistent. In each experiment it was demonstrated that resistance to fenoxaprop can evolve within three generations. Although resistance was quantitative, the performance of the field recommended rate was substantially reduced. The higher the level of selection, the higher the level of resistance. This supports the view that resistance to fenoxaprop is more likely to develop where high, rather than reduced, rates of herbicides are used. The failure to select for the same degree of resistance to chlorotoluron supports other experimental and field observations, that resistance to fenoxaprop increases at a faster rate. The precise reasons for this remain unclear, but may be linked to differences in the rate of metabolism between herbicides.

The results for the deselection experiment indicate that, in the absence of herbicide application, some reversion towards susceptibility may occur. However, in practice this will be difficult to achieve and resumption of herbicide treatment is likely to re-impose selection for more resistant individuals such that the benefits of deselection are short lived.

The main practical implications of this work are: (a) repeated application of 'fop' herbicides poses a risk of rapid evolution of resistance. (b) reduced doses of 'fop' herbicides reduce, rather than increase the rate of development of resistance. (c) the absence of spraying will result in some reversion towards susceptibility, but this is unlikely to be a rapid process.

TABLE 14. Experiment 1 Response of black-grass after three generations of selection

	Log_{10} ED_{50} values		ED_{50} values (ratio to Rothamsted in brackets)	
	Chlorotoluron	**Fenoxaprop**	**Chlorotoluron**	**Fenoxaprop**
Rothamsted	- 0.462	1.579	0.346 (1.0)	37.9 (1.0)
Chlorotoluron survivors	- 0.269	1.690	0.538 (1.6)	49.0 (1.3)
Fenoxaprop survivors	- 0.396	1.878	0.402 (1.2)	75.5 (2.0)
S. E. ±	0.036	0.065	kg/ha	g/ha
LSD ($P \leq 0.05$)	0.106	0.192		

TABLE 15. Experiment 2 - Response of black-grass after three generations of selection at two levels (low and high[a])

	Log_{10} ED_{50} values		ED_{50} values (Ratio to Rothamsted in brackets)	
	Chlorotoluron	**Fenoxaprop**	**Chlorotoluron**	**Fenoxaprop**
Rothamsted	- 0.460	1.764	0.347 (1.0)	58.0 (1.0)
Chlorotoluron low selection	- 0.335	1.970	0.462 (1.3)	93.3 (1.6)
Chlorotoluron high selection	- 0.274	1.911	0.532 (1.5)	81.4 (1.4)
Fenoxaprop low selection	- 0.347	2.116	0.450 (1.3)	130.7 (2.3)
Fenoxaprop high selection	- 0.389	2.308	0.408 (1.2)	203.0 (3.5)
S. E. ±	0.075	0.074	kg/ha	g/ha
LSD ($P \leq 0.05$)	0.222	0.219		

[a] low = 14.4 - 16.8% and high = 0.6 - 3.3% of plants were grown on to produce seed (see Materials and Methods).

TABLE 16 Experiment 3 - Response of black-grass after three generations of selection

	Log_{10} ED_{50} values		ED_{50} values (ratio to LARS in brackets)	
	Chlorotoluron	**Fenoxaprop**	**Chlorotoluron**	**Fenoxaprop**
LARS	- 0.714	1.680	0.193 (1.0)	47.8 (1.0)
Chlorotoluron survivors	- 0.734	1.861	0.184 (1.0)	72.7 (1.5)
Fenoxaprop survivors	- 0.708	2.255	0.196 (1.0)	179.8 (3.8)
S. E. ±	0.055	0.060	kg/ha	g/ha
LSD ($P \leq 0.05$)	0.155	0.168		

Fig. 3 Expt 1: Response to chlorotoluron

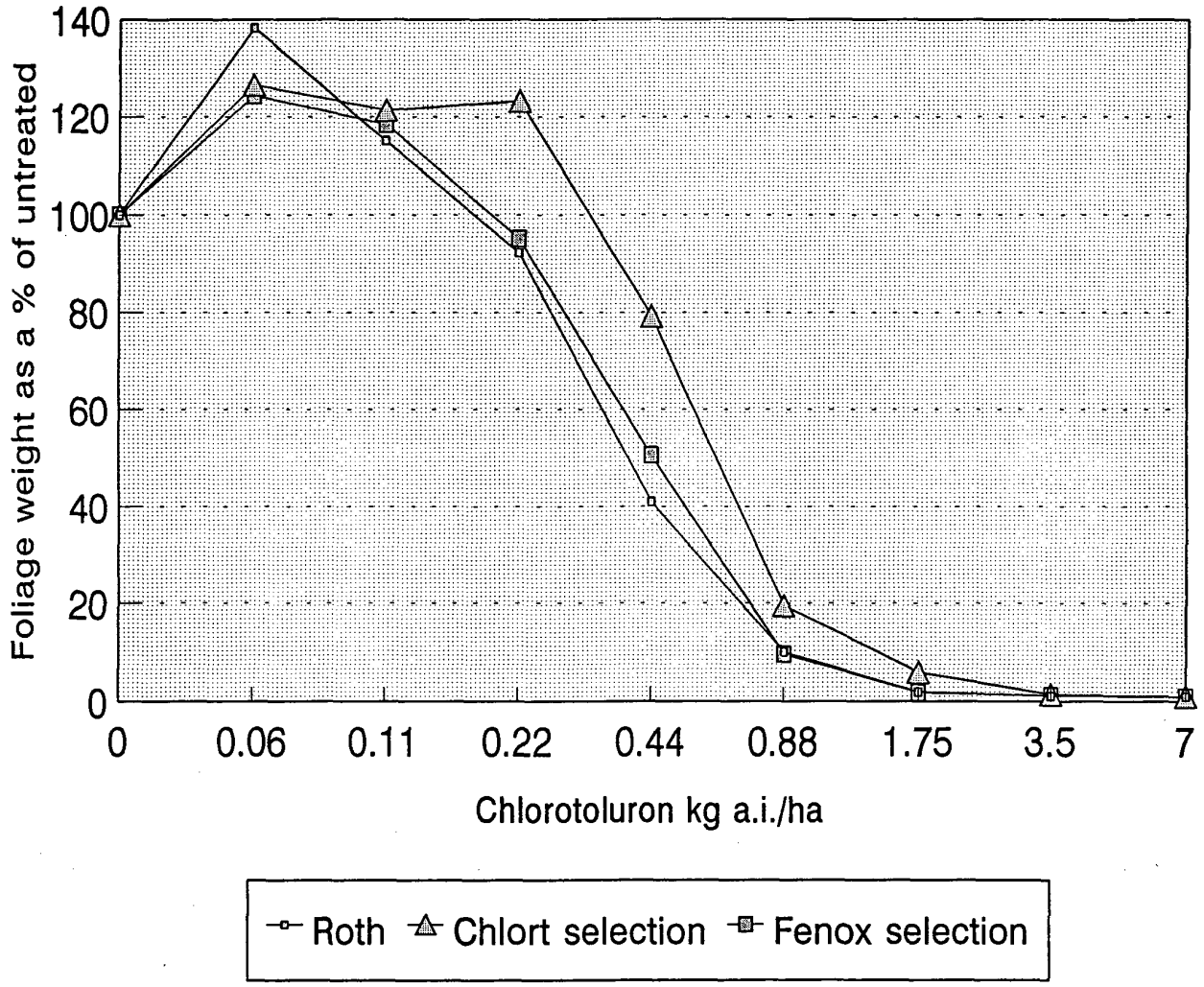


Fig. 4 Expt 1: Response to fenoxaprop

Fig. 5 Expt 2: Response to chlorotoluron

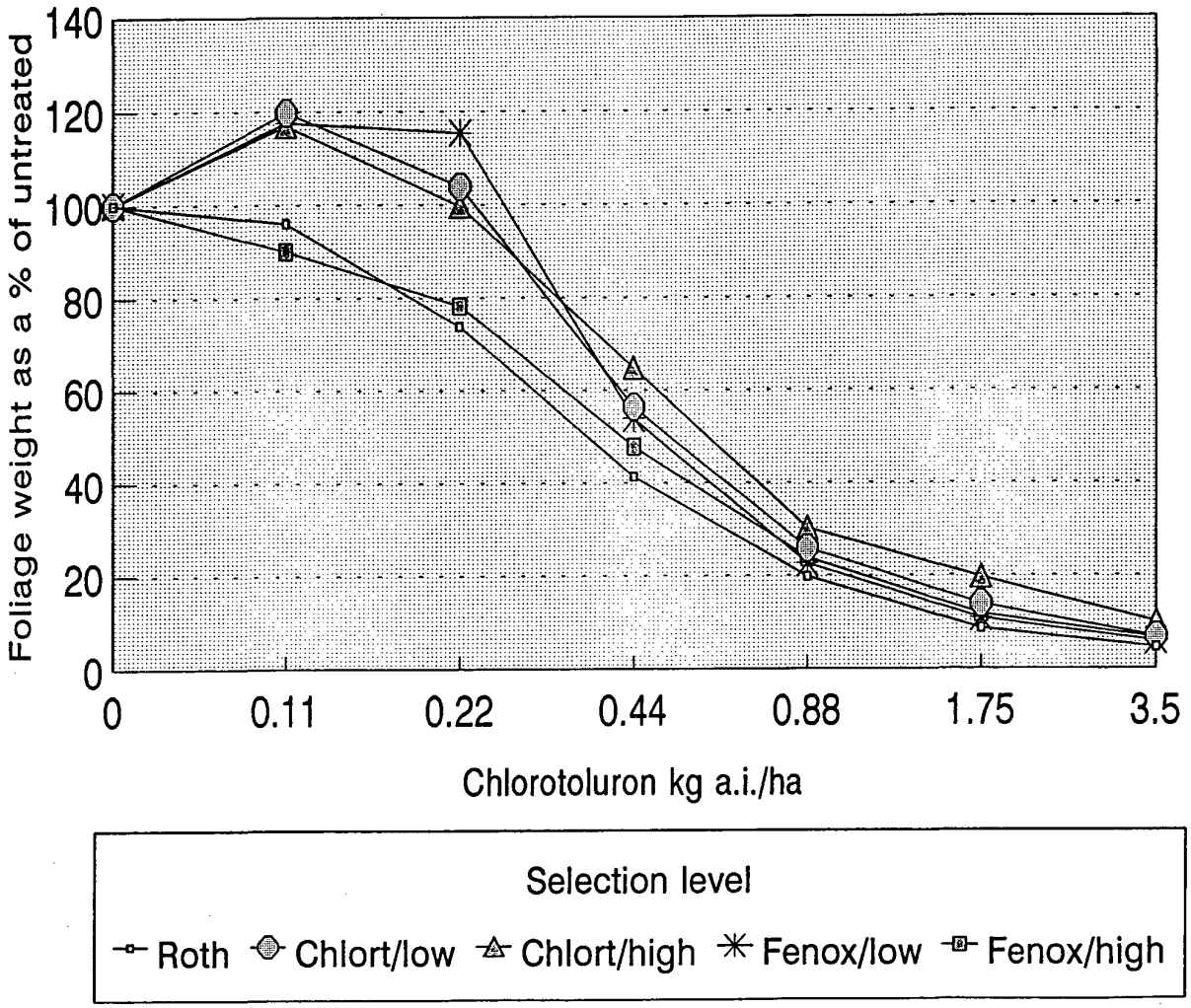


Fig. 6 Expt 2: Response to fenoxaprop

Fig. 7 Expt 3: Response to chlorotoluron

Fig. 8 Expt 3: Response to fenoxaprop

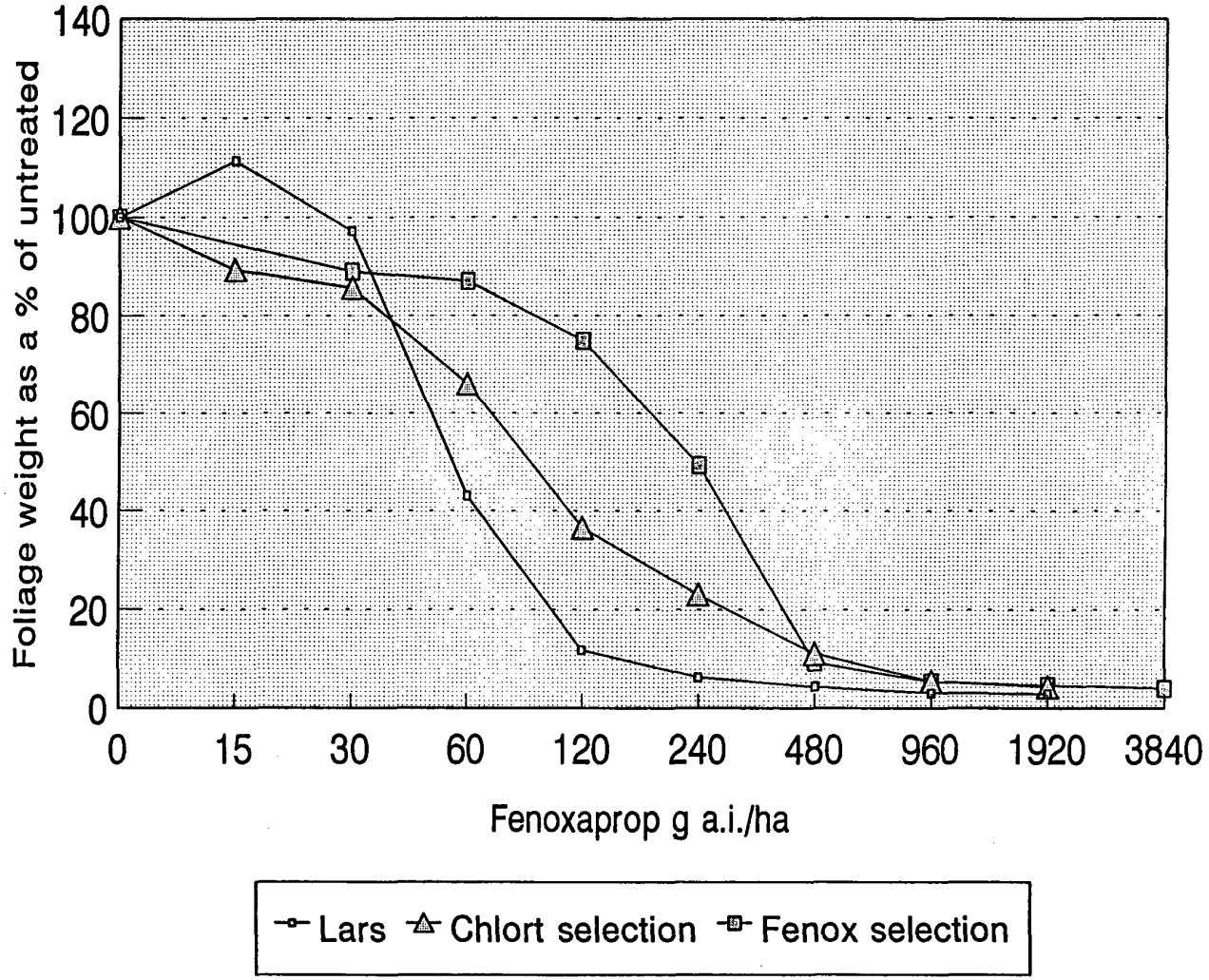


TABLE 17 Deselection experiment

	Log_{10} ED_{50} values	
	Chlorotoluron	Diclofop-methyl
Rothamsted 1992	- 0.904	- 0.620
Peldon 1986 field	0.240	0.266
Peldon 1992 field	0.239	0.506
Peldon deselected	- 0.293	- 0.054
S.E. ±	0.086	0.161
L.S.D. ($P \leq 0.05$)	0.241	0.451

	ED_{50} values (kg a.i./ha)	
	Chlorotoluron	Diclofop-methyl
Rothamsted 1992	0.12	0.24
Peldon 1986 field	1.74	1.85
Peldon 1992 field	1.73	3.21
Peldon deselected	0.51	0.88

	Resistance Index (ratio of ED_{50} values to Rothamsted)	
	Chlorotoluron	Diclofop-methyl
Rothamsted 1992	1.0	1.0
Peldon 1986 field	14.5	7.7
Peldon 1992 field	14.4	13.4
Peldon deselected	4.3	3.7

Fig. 9 Deselection : response to chlorotoluron

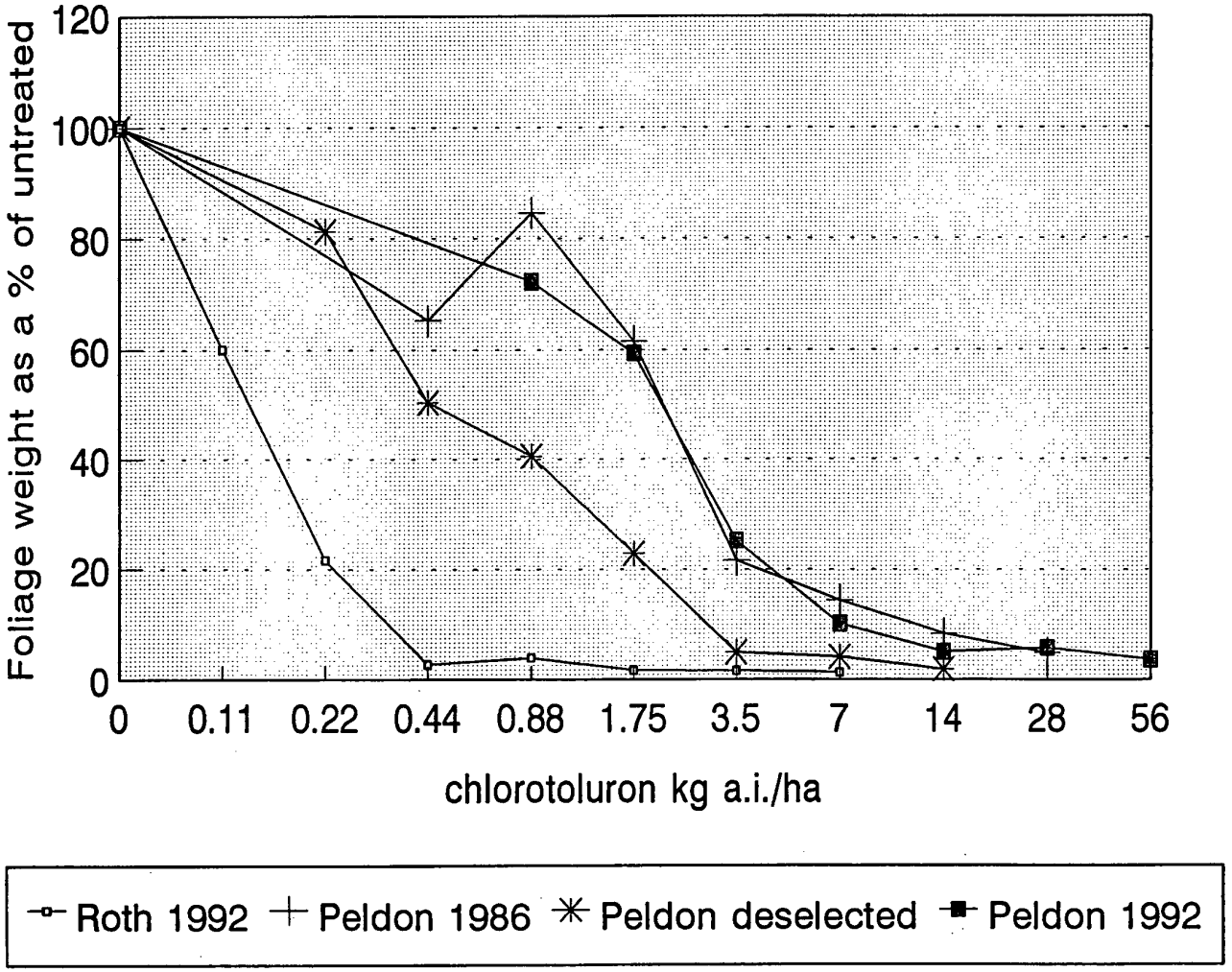
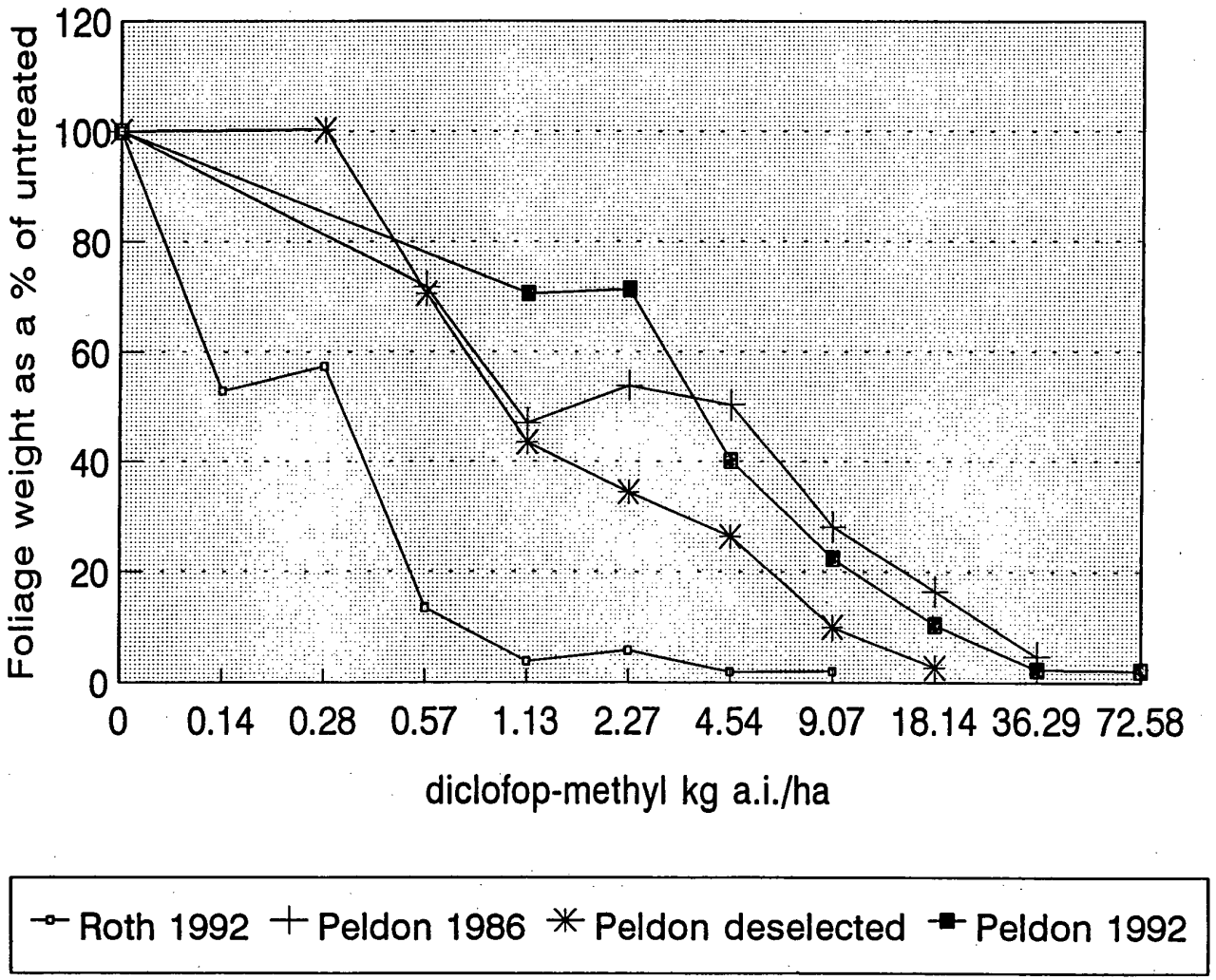


Fig. 10 Deselection : response to diclofop-methyl



SECTION 5

TARGET SITE RESISTANCE (ACCase)

INTRODUCTION

The main mechanism of resistance in black-grass, at least in the case of the substituted-urea herbicides chlorotoluron and isoproturon, is an enhanced ability of resistant plants to metabolise and detoxify the herbicides (Kemp, Moss & Thomas, 1990; Kemp & Caseley, 1987; Hall, Moss & Powles, 1995a; Menendez *et al.*, 1994). These studies, involving UK and Spanish populations, have found no evidence for differential uptake or translocation between resistant and susceptible populations. More recent studies indicate that enhanced metabolism can also explain cross-resistance to the dinitroaniline pendimethalin (James, Kemp & Moss, 1995) and to the aryloxyphenoxypropionate ('fop') herbicides diclofop-methyl and fenoxaprop-ethyl (Menendez *et al.*, 1993); Hall, Moss & Powles, 1995b). However, other mechanisms of resistance may occur, as happens in the multiple-herbicide resistant annual rye-grass *(Lolium rigidum)* in Australia (Powles & Matthews, 1992).

One of the other mechanisms of resistance in annual rye-grass is target site resistance to aryloxyphenoxypropionate ('fop') and cyclohexanedione ('dim') herbicides. These herbicides, such as fenoxaprop-ethyl and tralkoxydim, inhibit an essential enzyme, acetyl-coenzyme A carboxylase (ACCase) which is involved in fatty acid biosynthesis in plants. Thus, this enzyme is the target site of action for 'fop' and 'dim' herbicides. Target site resistance occurs when a modified form of this enzyme is present which, while still fully functional in the plant, is not inhibited by 'fop' or 'dim' herbicides. Consequently, activity of the modified enzyme is not impaired even in the presence of the herbicides, and the plants are resistant.

It is clear that some herbicides, such as fenoxaprop-ethyl are vulnerable to enhanced metabolism and target site resistance. Consequently, in the field it is impossible to determine which mechanisms are present if a herbicide such as fenoxaprop fails to give good control.

The suspicion that target site resistance could be present in some black-grass populations was first considered when a few plants of two populations, Lincs. E1 and Oxford S1, survived treatment with sethoxydim in a container experiment (see Section 3). Sethoxydim killed all plants of the Peldon population in the same experiment. The Peldon population is known to be resistant to many herbicides due to enhanced metabolism, so it appears that sethoxydim is not vulnerable to this mechanism. Consequently, an alternative reason for the plants surviving sethoxydim treatment was sought.

MATERIALS AND METHODS

Seed collection from Oxford S1 plants surviving sethoxydim and fluazifop

The container experiment conducted in 1991/92, (described in detail in Section 3) included two populations, Oxford S1 and Lincs. E1, where two - three applications of fenoxaprop-ethyl had failed to control black-grass in the field. Containers were sown with seeds collected from those fields in 1991. The treatment with sethoxydim at the field rate of 290 g a.i./ha killed virtually all plants of the other populations, but a small number of Oxford S1 and Lincs. E1 plants survived.

With Oxford S1, 14 out of 253 (5.5%) and with Lincs. E1 13 out of 307 (4.2%) sethoxydim treated plants survived, and most showed no herbicidal symptoms. With fluazifop-P-butyl treatment, 22 out of 253 (8.7%) Oxford S1 and 233 out of 307 (76%) Lincs. E1 plants survived. This high survival rate for Lincs. E1 treated with fluazifop is probably due to the presence of a third, as yet uncharacterised, mechanism.

Eight Oxford S1 plants which had survived sethoxydim and 10 Oxford S1 plants which had survived fluazifop treatment were transferred from containers to pots containing compost. These were grown on and the two sets of pots isolated in separate glasshouses. Plants were allowed to cross-pollinate within, but not between, sets and seeds were collected during June 1992.

Evaluation of progeny of plants surviving sethoxydim and fluazifop

The response of four seed populations to five herbicides was evaluated in order to determine the degree of resistance and cross-resistance. The four populations were: Rothamsted (susceptible standard); Oxford S1 bulk seed sample collected from the field in 1991; progeny of the eight Oxford S1 plants surviving sethoxydim in the 1991/92 container experiment (= sethoxydim survivors); progeny of the ten Oxford S1 plants surviving fluazifop (= fluazifop survivors).

Pre-germinated seeds were sown in individual 5 cm square pots containing a Kettering loam/grit (5:1) mix. A single plant was established in each pot. Sethoxydim (+ "Adder" oil) (dose range = 4.53 - 1160 g a.i./ha), fluazifop-P-butyl (+ "Agral" wetter) (3.91 - 2000 g a.i./ha), fenoxaprop-ethyl (+ safener) (15 - 3840 g a.i./ha), isoproturon (0.039 - 10 kg a.i./ha) and chlorotoluron (0.055 - 14 kg a.i./ha) were applied as a staggered range of eight doses within the ranges given above. Recommended adjuvants were used as detailed in Section 2. Plants were sprayed at the 2½ - 3 leaf stage using a laboratory sprayer delivering 207 l water/ha at 210 kPa through a single Lurmark 01/F110 flat fan nozzle. There were 14 replicate pots per herbicide dose and 40 untreated pots for each population. Three weeks after spraying, the fresh weight of foliage per pot and vigour of each plant was recorded. Data were analysed as described in Section 2 and ED_{50} values calculated.

Detection of target site (insensitive ACCase) resistance in a range of populations

Other studies indicated that treatment with sethoxydim can be used as a means of detecting plants with target site resistance to ACCase inhibitors. A series of three experiments was conducted to evaluate this concept in 31 different populations. Some were collected in more than one year. Apart from the susceptible standards (Rothamsted and Long Ashton stockbed = LARS), all populations were from fields where resistance to fenoxaprop-ethyl had been identified in screening tests. The samples were mainly collected in 1993 or 1994. Samples collected in 1991 from the Oxford S1 and Lincs E1 fields were included in order to determine whether any subsequent change in frequency of target site resistance had occurred.

The technique used involved sowing approximately 200 pre-imbibed seeds in each germination tray containing a Kettering loam:grit (5:1) mix. Seeds were spread out over the soil surface and then covered with the same soil mix to about 1 cm depth. There were four or five replicate trays per population. When emerging plants were at the 2 - 3 leaf stage, numbers per tray were counted and then all trays sprayed with sethoxydim at 145 g a.i./ha + a mineral oil adjuvant ("Adder" at 1% spray volume). This dose is half the field recommended rate. Applications were made using a laboratory sprayer as described above. To ensure good coverage, two successive applications were made, each at half the target dose. The numbers of surviving plants were determined by visual assessment, 20 - 26 days after treatment. Sets of six surviving plants of the Notts. A1 and Oxford AA1 1993 populations were transferred to pots and treated with the field recommended rates of 10 different herbicides.

Cross resistance in populations with target site resistance (ACCase)

The response of three populations, Rothamsted susceptible standard, Notts. A1 1993 and Oxford AA1 1993, to seven herbicides was determined in a dose response experiment. The latter two populations comprised a high proportion of individuals with apparent target site resistance, based on previous studies. The same technique

as described in the 'Evaluation of progeny' section above was used. Sethoxydim (+ "Adder") (dose range = 4.53 - 4640 g a.i./ha), tralkoxydim (+ "Adherb" + "Agral") (10.94 - 5600 g a.i./ha), fluazifop-P-butyl (+ "Agral")(3.91 - 2000 g a.i./ha), fenoxaprop-ethyl (+ S) (7.5 - 3840 g a.i./ha), diclofop-methyl (0.142 - 36.29 kg a.i./ha), chlorotoluron (0.109 - 3.5 kg a.i./ha), chlorsulfuron (2.5 - 160 g a.i./ha) were applied as a staggered range of six doses within the ranges given above. Plants were sprayed at the two leaf stage using the laboratory sprayer delivering 223 l water/ha. There were 20 replicate pots per herbicide dose and 40 untreated pots per population. Foliage fresh weight and plant vigour were assessed four weeks after spraying.

Petri dish test for target site resistance

The effects of sethoxydim and fenoxaprop-ethyl on seed germination and early seedling growth were investigated. Two black-grass populations were used, Rothamsted (susceptible) and Oxford AA1 (about 90% of plants had shown evidence of target site resistance in previous studies). Twenty-five seeds were placed in each 9 cm petri dish containing three Whatman No. 1 filter papers covered with a single glass fibre filter paper. The following concentrations of herbicide were used: 0, 0.1, 1, 10, 100, 1000, 10,000 ppm sethoxydim and fenoxaprop-ethyl. There were two replicates. Seven mls of solutions were pipetted into each petri dish. The dishes were placed in an incubator set at a 17°C 14 h day: 11°C 10 h night. Two weeks later, the shoot length of each germinated seed was measured.

A dose response analysis was conducted as previously described (Section 2).

RESULTS

Evaluation of progeny of plants surviving sethoxydim and fluazifop

The ED_{50} values and ratios to the Rothamsted susceptible standard (Resistance Index) are given in Table 18. The full dose responses for sethoxydim and

isoproturon are presented in Figures 11 and 12. The response of the Oxford S1 population to the different herbicides was similar to that found in the cross-resistance studies (see Section 2). Thus the resistance indices were: sethoxydim (1.2 this test v 1.4 cross-resistance test); fluazifop (4.8 v 5.0); fenoxaprop (3.6 v 4.9); isoproturon (1.4 v 2.1); chlorotoluron (4.6 v 3.2). The excellent agreement between the two experiments demonstrates the consistency of the technique used. The level of resistance to 'fop' and 'dim' herbicides in the progeny of Oxford S1 plants surviving sethoxydim or fluazifop was much greater. The ED_{50} levels were so high in some instances, that they could not be estimated precisely, and many plants survived at the highest doses used which were 4 - 32 x the field recommended rates. These plants represent the most resistant black-grass plants so far detected and are also the first plants found which show resistance to the cyclohexanedione ('dim') herbicide sethoxydim. These results demonstrate very convincingly that the progeny of the few plants surviving sethoxydim or fluazifop were very highly resistant to both these herbicides, and fenoxaprop. In marked contrast, the levels of resistance to isoproturon and chlorotoluron were relatively low and very similar to those in the Oxford S1 field population. These results suggest that at least two independent mechanisms of resistance are present in Oxford S1. One is enhanced metabolism, giving a low level of resistance to chlorotoluron, isoproturon, fenoxaprop and fluazifop, but not sethoxydim, at the population level. In addition, a small proportion (5%) of plants have a second mechanism of target site resistance (insensitive ACCase) which gives a high level of insensitivity to 'fops' and 'dims', but not to herbicides with other modes of action. It is probable that these two resistance mechanisms are also present in the Lincs. E1 population.

The ED_{50} values were based on population responses. The response of individual plants provides additional information relating to the inheritance of resistance. Of the 84 plants of Oxford S1 sprayed with the six highest rates of sethoxydim (rates which kill all susceptibles), two plants (2.4%) were clearly unaffected (Fig. 13). These were evidently those types which survived in the 1991/92 container experiment (Section 3). Of the 84 plants which were the progeny of sethoxydim survivors and were treated with the top six rates of sethoxydim in the dose response

experiment, 63 were virtually unaffected (Fig. 14), while the rest were killed or severely affected. This segregation seemed very clear cut, so the fact that 75% survived appears important. This suggested that this resistance mechanism is based on a single dominant gene, and that the original survivors were heterozygous RS. Thus, when crossed amongst themselves, they produced 25% RR, 50% RS and 25% SS. Thus, 75% of the progeny were either RR or RS, all resistant phenotypes if the resistance allele R is dominant. The genetics of target site resistance was studied in greater detail to determine whether this theory was correct (see Section 6).

Detection of target site resistance (insensitive ACCase) in a range of populations

The results for all experiments are presented in Table 19. Most populations showed no evidence of target site resistance, including Peldon and Bucks C1 which are highly resistant to chlorotoluron. Many of the populations had shown a high level of resistance to fenoxaprop in screening trials, but clearly this was not correlated with presence of target site resistance.

However, six populations showed some evidence of target site resistance. The populations with single plants surviving have not been included in this total. The Notts. A1 and Oxford AA1 showed very high levels - over 80% were unaffected by sethoxydim regardless of year of collection. Over 50% of the Oxford X1 population collected in 1994 survived, and this proportion appeared to have increased since 1992. Three other populations showed evidence of the presence of target site resistance at a low level - Lincs. E1, Oxford S1 and Lincs. I1. The samples collected in 1993 from Lincs E1 and Oxford S1 confirmed the results obtained with the 1991 samples, which were used in the initial cross-resistance studies (Section 2), container experiment (Section 3) and in the target site resistance evaluations detailed previously in this section. However, there was no evidence that the level of target site resistance had changed appreciably between 1991 and 1993/94. This was almost certainly due to reduced use of 'fops' and 'dims' during this period. Only one such application (alloxydim on Lincs. E1 and cycloxydim on Oxford S1) was applied to each field between 1991 and 1994. The high survival levels of Notts.

A1 and Oxford AA1 probably reflect intensive use of 'fops' and 'dims'. On Notts. A1 there were eight applications of 'fops' (fenoxaprop or fluazifop) in the five years prior to 1993. This intensity of treatment is very likely to result in a rapid increase in the level of target site resistance, analogous to that demonstrated with the Oxford S1 population described in a previous part of this section.

Most of the Notts. A1 and Oxford S1 survivors which were subsequently transferred to pots, survived treatment with fenoxaprop-ethyl, fluazifop-P-butyl, quizalofop-ethyl, clodinafop-propargyl, cycloxydim and tralkoxydim. This confirms that target site resistance is likely to affect all 'fop' and 'dim' herbicides. In contrast, all plants were killed by isoproturon, glyphosate and paraquat, and severely affected by imazamethabenz. Although only six plants were sprayed with each herbicide (3 Notts. A1 + 3 Oxford AA1) the results indicate that target site resistance affects **all** 'fops' and 'dims', but not herbicides with other modes of action.

Cross resistance in populations with target site resistance (ACCase)

The ED_{50} values are presented in Table 20 and full dose responses for sethoxydim, fenoxaprop, chlorotoluron and chlorsulfuron in Figures 15 - 18. The Notts A1 and Oxford AA1 populations had shown a high level of resistance to sethoxydim in the previous evaluation test, and this was confirmed. Indeed over 80% of plants of both populations survived applications of 4640 g a.i./ha, 16 x the recommended field dose. Exceptionally high levels of resistance to all the other 'fops' and 'dims' tested were also confirmed. However, resistance to the sulfonyl-urea herbicide chlorsulfuron was evident in the Notts. A1 and especially the Oxford AA1 population. This was unexpected, as chlorsulfuron has a different site of action to the ACCase inhibitors. It is probable that a different mechanism is responsible, possibly enhanced metabolism, but this needs confirmation. Both Notts. A1 and Oxford AA1, showed very modest levels of insensitivity to the substituted urea chlorotoluron, confirming results from screening experiments which rated both as non-resistant (Oxford AA1 - 1*; Notts. A1 - S).

Petri dish test for target site resistance

There was little effect on germination except at very high doses, but effects on shoot length were much greater. There was a large difference in the response of the two populations, especially with sethoxydim (Table 21, Fig. 19 and 20).

With Rothamsted, all seedlings were extremely stunted at 1 ppm sethoxydim whereas this dose had no effect on Oxford AA1. It required over 1000 x that dose to achieve complete suppression in growth of Oxford AA1. The difference with fenoxaprop-ethyl was less, but still highly significant. Thus, the resistance index for effects on shoots was over 1000 for sethoxydim and 15 for fenoxaprop-ethyl.

These results indicate that such a petri dish assay may provide a relatively rapid diagnostic test for target site resistance. It will not be suitable for freshly collected seeds which are likely to be dormant. However, as innate dormancy in black-grass usually lasts only 2 -3 months, it should be feasible to obtain a result within 3 - 4 months of collection. Thus, with seeds collected in mid July, test results should be available by October/November. It might be possible to achieve a faster result if seed dormancy breaking treatments were used, but further studies will be needed to ensure that such chemical or physical treatments do not interact with the resistance test.

CONCLUSIONS

These studies detected suspected target site resistance in black-grass. This was the first time such a resistance mechanism had been found in this weed. Subsequent studies at the biochemical level confirmed that the mechanism in the Lincs. E1, Oxford S1, Notts. A1 and Oxford AA1 populations was due to insensitive ACCase (Hall, Moss & Powles, unpublished). Consequently, two distinct mechanisms of resistance have now been confirmed in black-grass - enhanced metabolism and target site resistance (insensitive ACCase). The presence of target site resistance gives a very high degree of resistance to all 'fops' and 'dims' in all the populations so far studied. Its presence in a high proportion of plants means that the use of any 'fop' or 'dim' is likely to be ineffective. The 'fops' and 'dims' are being used increasingly for grass-weed control in a variety of crops. It is important that the presence of this mechanism is detected when only a small proportion ($< 5\%$) of plants is affected. It may then be possible to apply the **occasional** 'fop' or 'dim' herbicide without increasing the proportion of plants with target site resistance. This has been achieved at the Oxford S1 and Lincs. E1 sites, at least for two to three years. However, if 'fops' and 'dims' are used repeatedly, then a small proportion may soon become a high proportion of the population, and at this stage applications of any 'fop' or 'dim' will be ineffective. This would very seriously limit the herbicide options available, especially if restrictions are placed on some alternative herbicides (e.g. isoproturon) due to concerns over leaching to groundwater. Target site resistance has only been found on six fields in England so far, and appears to be much less common than other resistance mechanisms at present. However, it is almost certain to become a more widespread problem, especially if 'fop' and 'dim' herbicides are used more intensively.

TABLE 18. Evaluation of progeny of plants surviving sethoxydim and fluazifop

	Log_{10} ED_{50} values				
	Sethox.	Fluaz.	Fenox.	Isoprot.	Chlorot.
Rothamsted	0.952	0.810	1.469	- 0.976	- 0.827
Oxford S1	1.019	1.493	2.021	- 0.822	- 0.162
Sethoxydim survivors	> 3.065	> 3.301	3.602	- 0.831	- 0.429
Fluazifop survivors	> 3.065	2.814	2.959	- 0.882	- 0.445
S.E. ±	0.094	0.099	0.186	0.065	0.088
L.S.D. ($P \leq 0.05$)	0.324	0.280	0.525	0.183	0.249

	ED_{50} values*				
	Sethox.	Fluaz.	Fenox.	Isoprot.	Chlorot.
Rothamsted	9.0	6.5	29.4	0.11	0.15
Oxford S1	10.5	31.1	104.9	0.15	0.69
Sethoxydim survivors	> 1160	> 2000	4000	0.15	0.37
Fluazifop survivors	> 1160	652	910	0.13	0.36

* g a.i./ha for sethoxydim, fluazifop and fenoxaprop-ethyl
kg a.i./ha for isoproturon and chlorotoluron

	Resistance Indices (ratio of ED_{50} values relative to the Rothamsted susceptible standard)				
	Sethox.	Fluaz.	Fenox.	Isoprot.	Chlorot.
Rothamsted	1.0	1.0	1.0	1.0	1.0
Oxford S1	1.2	4.8	3.6	1.4	4.6
Sethoxydim survivors	> 130	> 310	136	1.4	2.5
Fluazifop survivors	> 130	101	31	1.2	2.4

Fig. 11 Response of four black-grass populations to sethoxydim

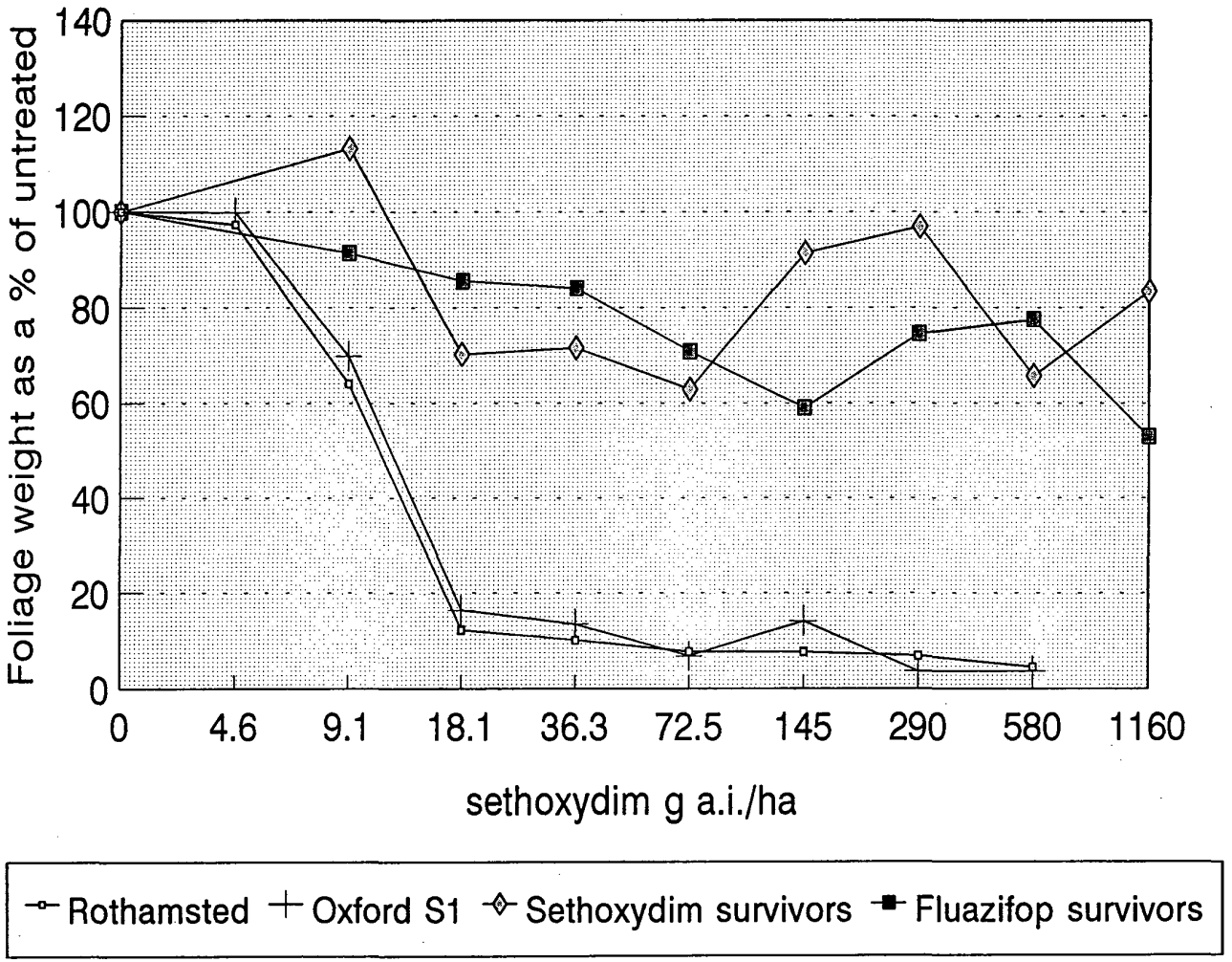
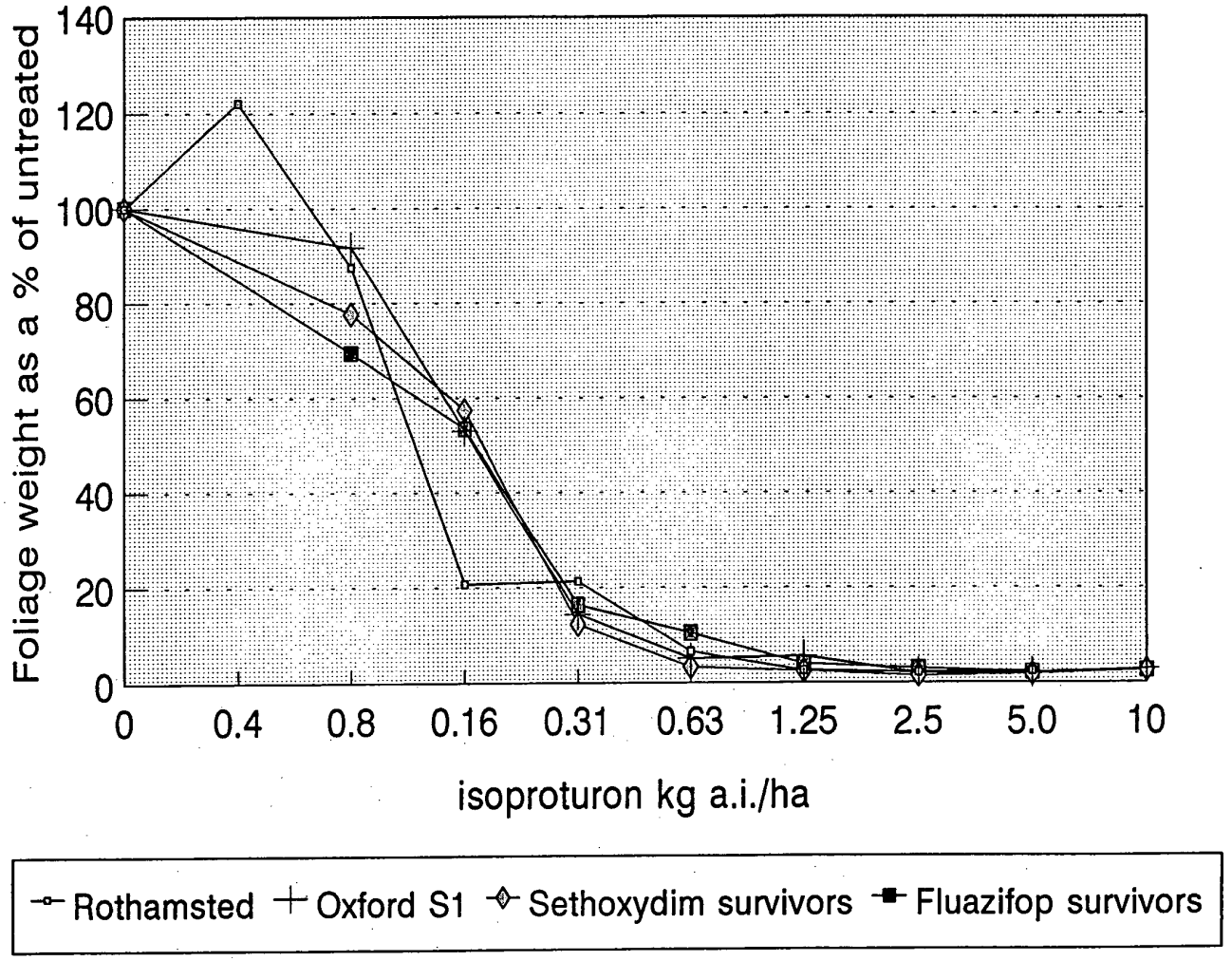


Fig. 12 Response of four black-grass populations to isoproturon



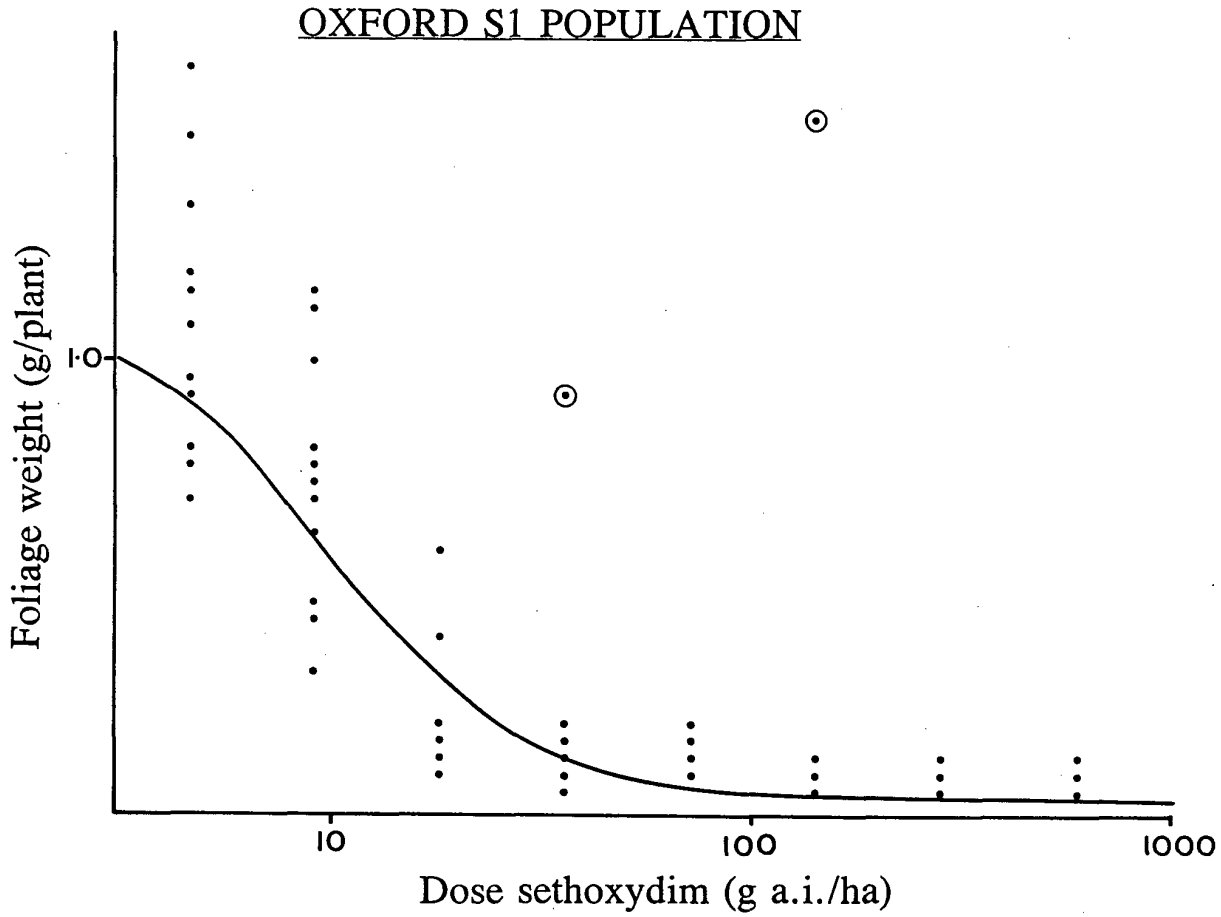


Fig. 13 Response of Oxford S1 population to sethoxydim.

(each point represents the foliage weight for an individual pot but not all points are shown as some are superimposed)

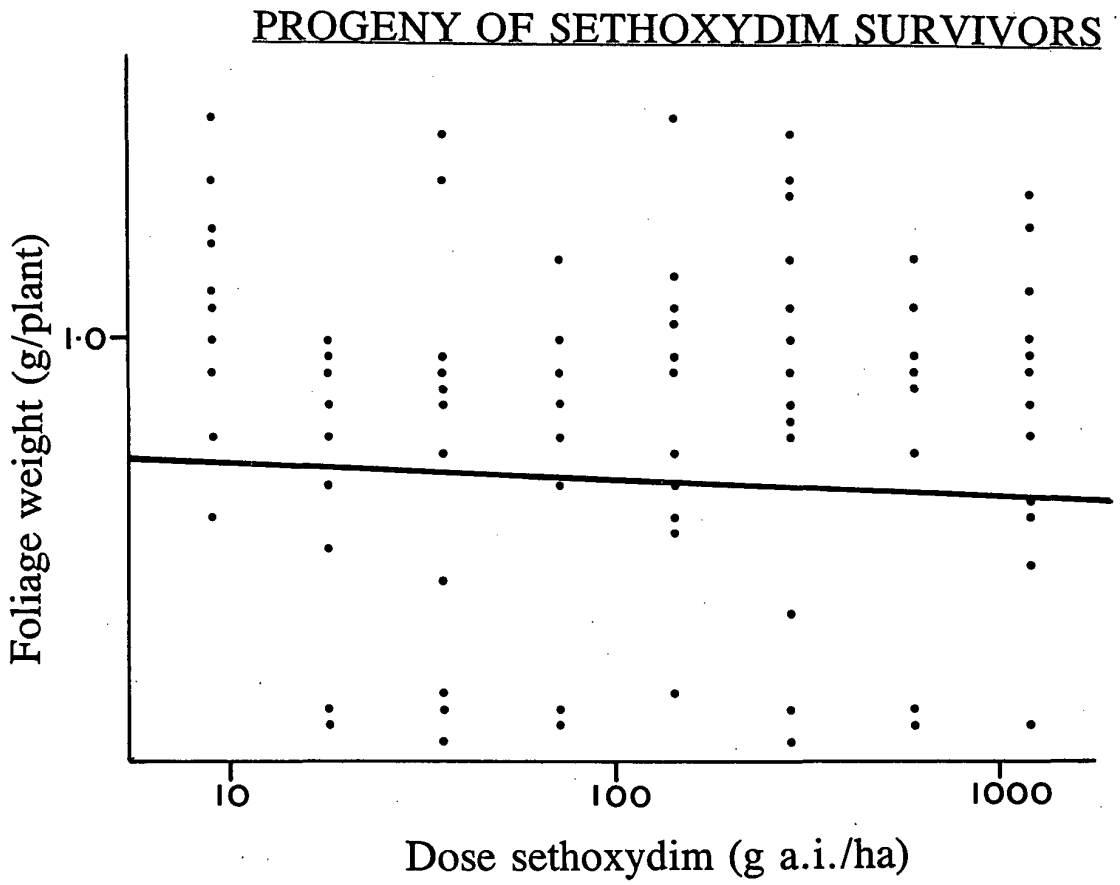


Fig. 14 Response of progeny of Oxford S1 plants surviving sethoxydim.

(each point represents the foliage weight for an individual pot but not all points are shown as some are superimposed)

TABLE 19. Detection of target site resistance (insensitive ACCase)

Site	Year of collection	Total plants before spraying	Total plants surviving	% Surviving
Notts A1	1994	470	447	95.1
Notts A1	1993	581	534	91.9
Notts A1	1992	489	413	84.5
Oxford AA1	1994	535	445	83.2
Oxford AA1	1993	564	501	88.8
Oxford X1	1994	474	243	51.3
Oxford X1	1992	533	154	28.8
Oxford S1	1993	634	13	2.1
Oxford S1	1991	768	39	5.1
Lincs E1	1994	631	12	1.9
Lincs E1	1993	699	50	7.2
Lincs E1	1991	832	32	3.8
Lincs I1	1994	521	4	0.8
Essex F1	1994	563	1	0.2
Lincs H1	1993	848	2	0.2
Oxford A2	1994	595	1	0.2
Oxford W1	1994	606	1	0.2
Bucks C1	1994	532	0	0
Bucks E5	1994	567	0	0
Bucks G1	1994	579	0	0
Essex G1	1994	557	0	0
S Essex A3	1994	593	0	0
Faringdon	1994	514	0	0
Lincs C2	1994	562	0	0
Lincs D1	1990	492	0	0
Lincs F1	1991	530	0	0
Lincs J1	1994	547	0	0
Northants B1	1994	513	0	0
Northants C1	1994	528	0	0
Oxon 15	1994	497	0	0
Oxon DD1	199?	539	0	0
Peldon A1	1993	462	0	0
Peldon A5	1994	571	0	0
Peldon H2	1994	473	0	0
Sussex A1	1994	364	0	0
LARS S/B	1993	747	0	0
LARS S/B	1994	556	0	0
Roth. Clay.	1994	527	0	0
Rothamsted	1993	528	0	0

TABLE 20. Cross-resistance evaluation in populations with target site resistance (ACCase)

	Log_{10} ED_{50}						
	Sethox.	Tralk.	Fluaz.	Fenox.	Diclo.	Chlorot.	Chlors.
Rothamsted	1.10	< 1.04	1.06	1.50	- 0.31	- 0.61	- 0.21
Oxford AA1	3.76	2.63	2.82	3.56	1.01	- 0.42	1.36
Notts. A1	4.20	2.17	2.64	3.50	0.79	- 0.47	0.82
S.E. ±	0.37	0.12	0.08	0.04	0.06	0.06	0.24
L.S.D. ($P \leq 0.05$)	1.05	0.34	0.21	0.11	0.17	0.16	0.68

	ED_{50} values*						
	Sethox.	Tralk.	Fluaz.	Fenox.	Diclo.	Chlorot.	Chlors.
Rothamsted	12.5	< 10.9	11.5	31.7	0.49	0.25	0.62
Oxford AA1	> 5000	422	654	3630	10.3	0.38	22.7
Notts. A1	> 5000	149	432	3189	6.1	0.34	6.7

* g a.i./ha for sethoxydim, tralkoxydim, fluazifop, fenoxaprop and chlorsulfuron
kg a.i./ha for diclofop and chlorotoluron

	Resistance Index (ratio of ED_{50} values to Rothamsted)						
	Sethox.	Tralk.	Fluaz.	Fenox.	Diclo.	Chlorot.	Chlors.
Rothamsted	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Oxford AA1	> 400	> 39	57	115	21	1.6	37
Notts. A1	>400	> 14	37	101	13	1.4	11

Fig. 15 Response of three black-grass populations to sethoxydim

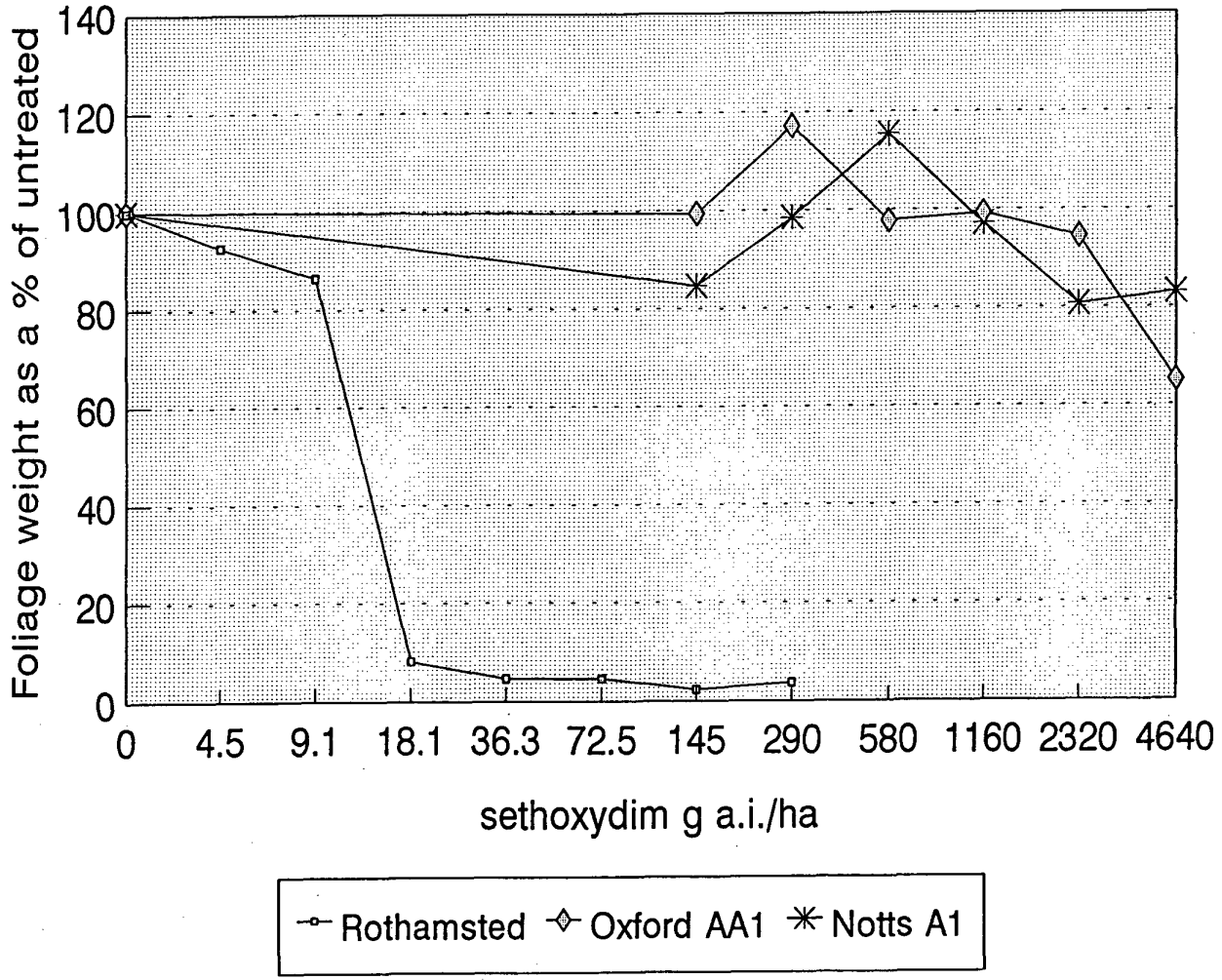


Fig. 16 Response of three black-grass populations to fenoxaprop-ethyl

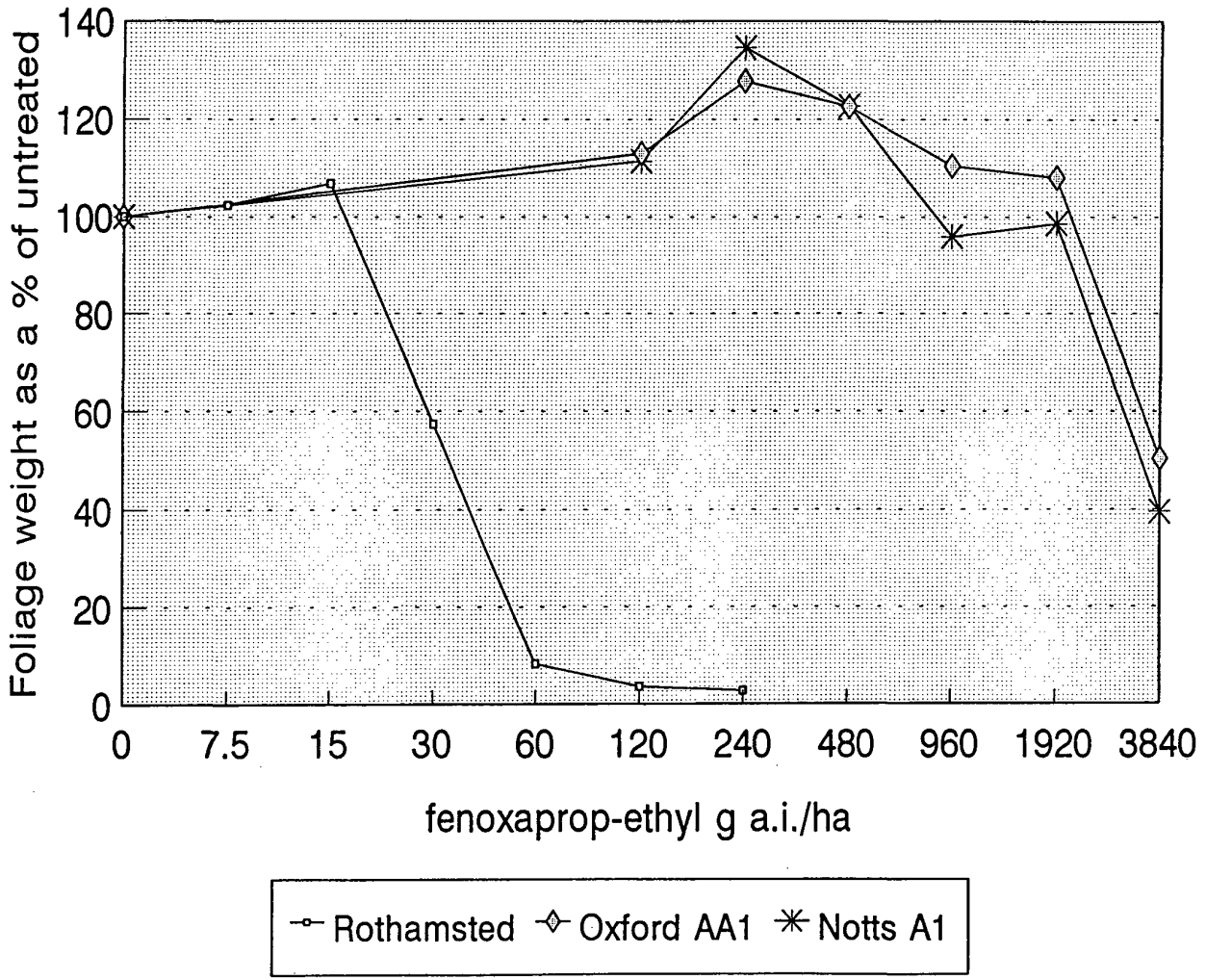


Fig. 17 Response of three black-grass populations to chlorotoluron

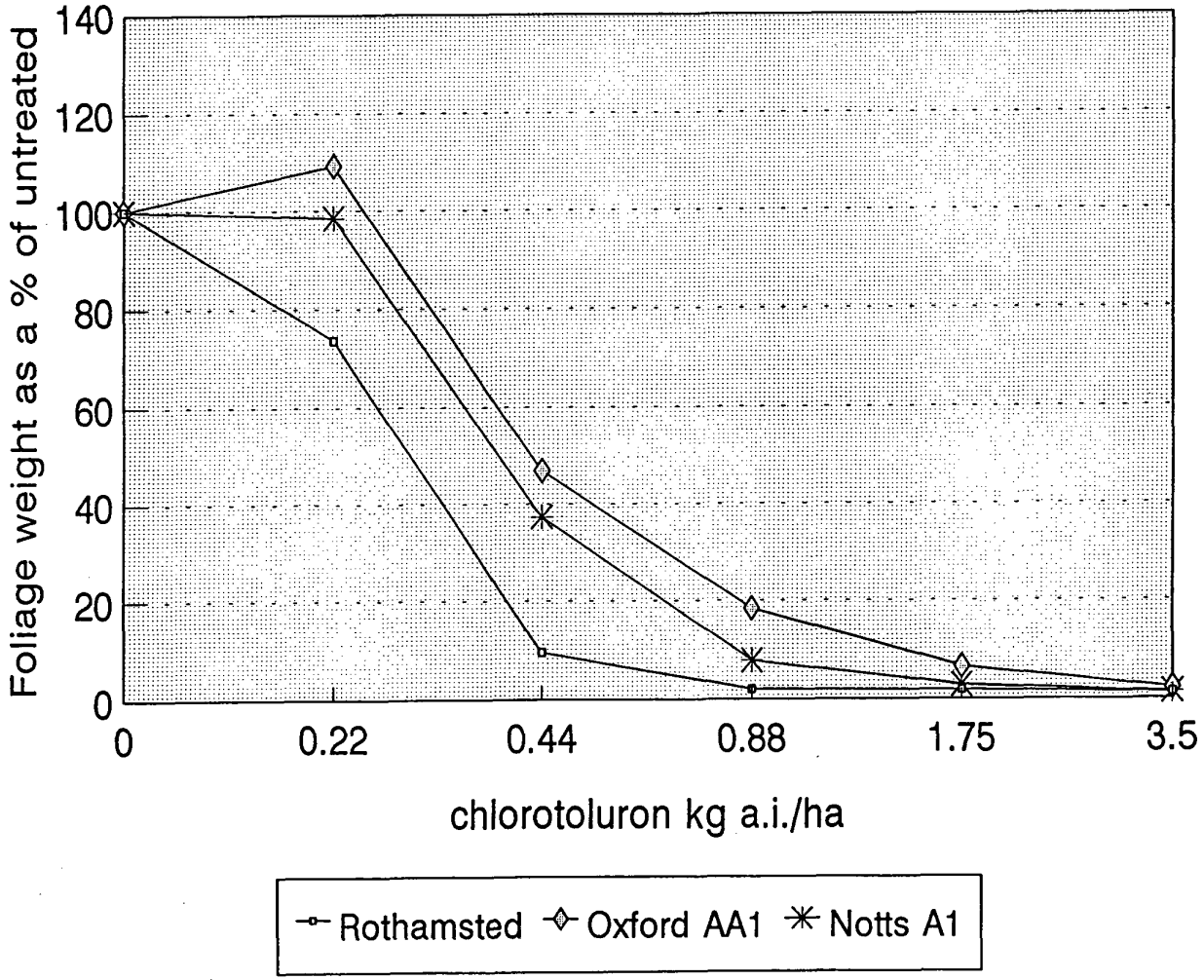


Fig. 18 Response of three black-grass populations to chlorsulfuron

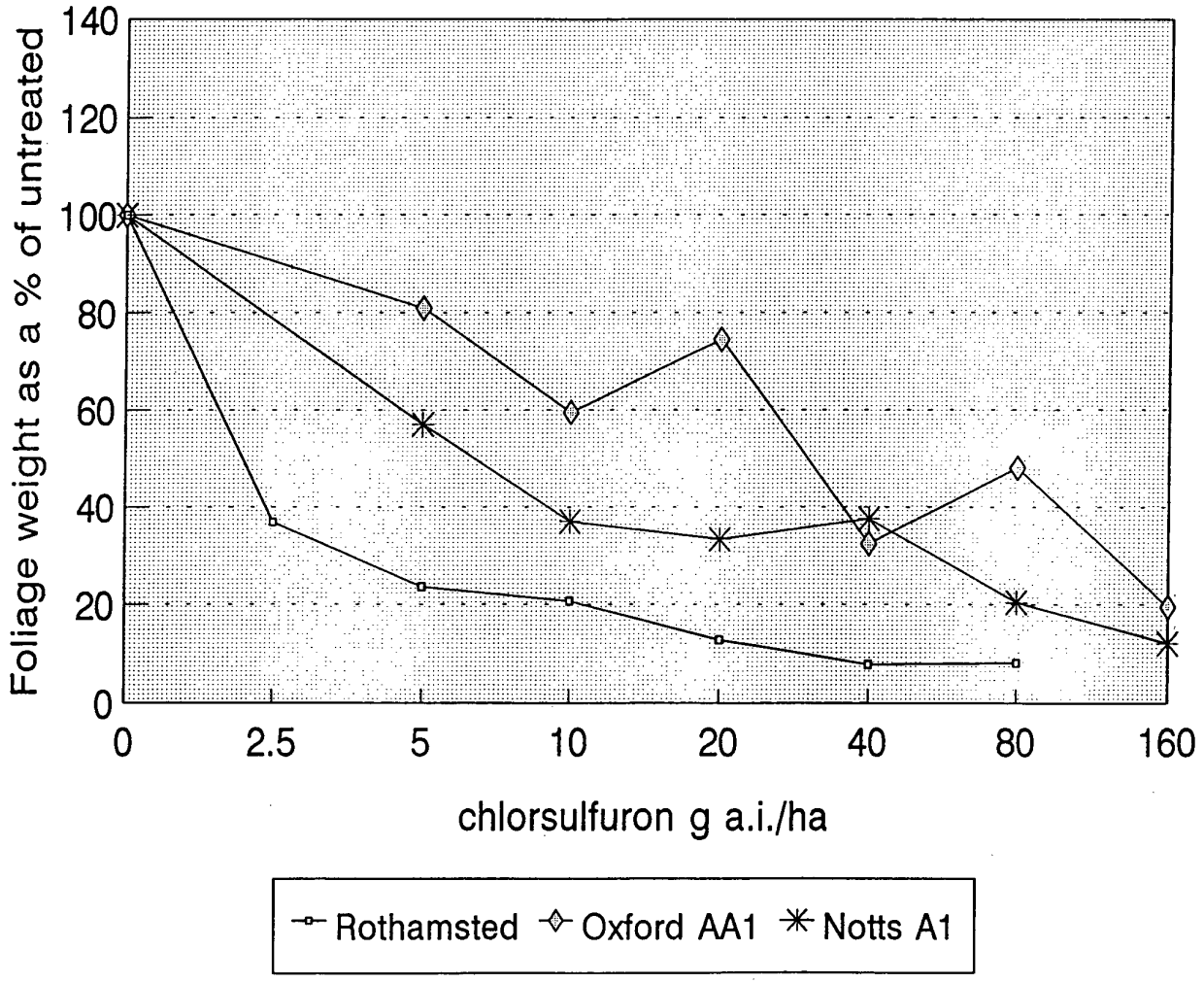


TABLE 21. Effect of sethoxydim and fenoxaprop-ethyl on the shoot growth of two black-grass populations

	Rothamsted	Oxford AA1	S.E.D.	L.S.D ($P \leq 0.05$)
Sethoxydim				
Log_{10} ED_{50}	-1.066	1.971	0.250	0.530
ED_{50} (g/ha)	0.086	93.4	-	-
Resistance Index	1.0	1086	-	-
Fenoxaprop				
Log_{10} ED_{50}	0.026	1.190	0.318	0.686
ED_{50} (g/ha)	1.06	15.47	-	-
Resistance Index	1.0	15	-	-

Fig. 19 The effect of sethoxydim on the shoot length of two black-grass populations

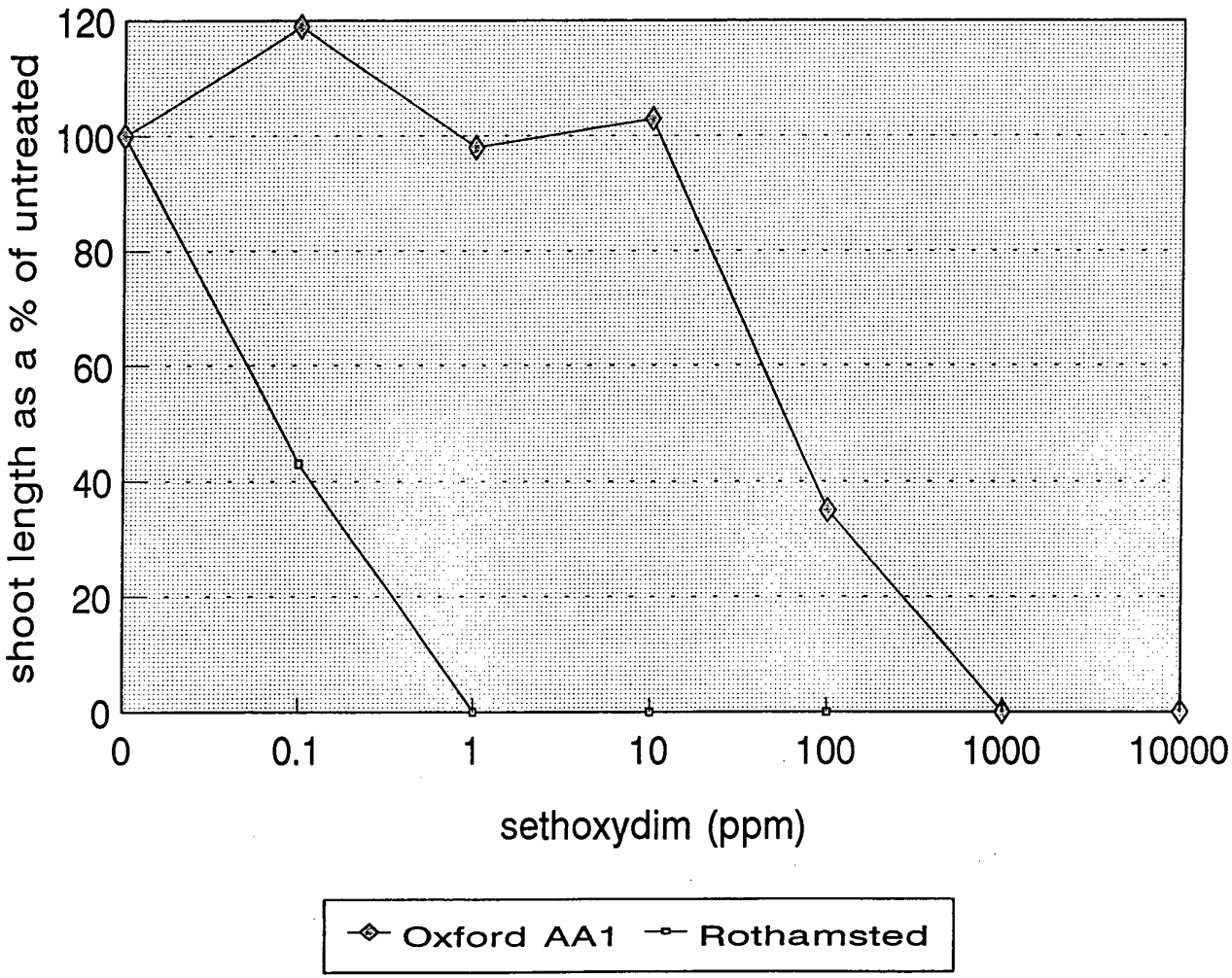
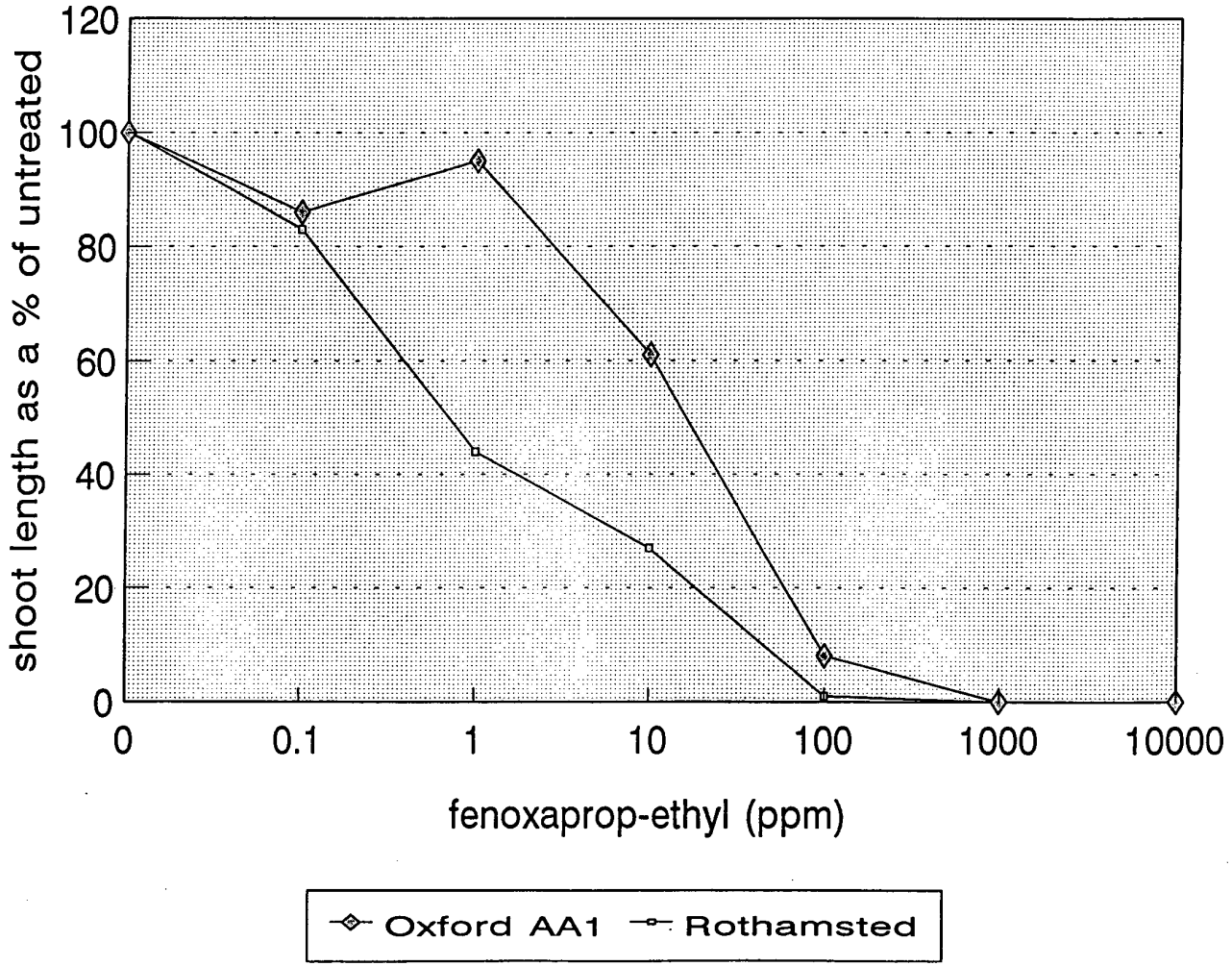


Fig. 20 The effect of fenoxaprop-ethyl on the shoot length of two black-grass populations



SECTION 6

GENETICS OF TARGET SITE RESISTANCE

INTRODUCTION

The studies relating to target site resistance in Section 5 provided some evidence that inheritance of this trait might be monogenic involving a single gene. This contrasts with the enhanced metabolism mechanism which is probably polygenic, involving at least two genes (Chauvel, 1991). The heritability of resistance is an important element in determining the rate of selection. Thus, detailed experiments were conducted to determine the genetics of target site resistance (ACCase).

MATERIALS AND METHODS

Seeds from Oxford S1 1991 (resistant) and Rothamsted 1990 (susceptible) were used as source material. Seeds of Oxford S1 were sown in germination trays and plants sprayed at the 2½ leaf stage with 290 g sethoxydim/ha. As expected, few plants (4%) survived confirming results found before (Section 5).

Eight surviving plants were removed and each plant split to provide five separate tillers (clones) which were each planted in pots of compost. To confirm the resistance status, one tiller of each plant was treated with sethoxydim at 580 g a.i./ha. All survived this treatment confirming that they were highly resistant. Rothamsted 1990 seeds were sown in individual pots and when plants were well tillered, split to provide separate tillers (clones) which were planted in pots of compost.

F_1 crosses

Four pots of individual Oxford S1 clones were placed together with four pots of individual Rothamsted clones in pollen-proof chambers when heads started to emerge. Black-grass is mainly an obligate cross-pollinator, so it was assumed that any viable

seeds produced would be the result of crosses between the Oxford S1 and Rothamsted parents. Seeds were collected when mature and kept separate for each parental clone. To confirm the self-incompatibility of each clone, the remaining plants were isolated in separate pollen-proof bags. Seeds were collected when mature and were dissected to determine the proportion containing caryopses.

Seeds from the 16 samples from the crosses, eight Oxford S1 and eight Rothamsted parent clones, were pre-germinated and sown in individual 5 cm pots containing a Kettering loam/grit (5:1) mix. Plants were sprayed at the three-leaf stage with 145 g sethoxydim/ha. The number of surviving plants were determined four weeks after spraying.

F_2 crosses

Surviving plants were grown on and transplanted into larger pots. Each of the 16 sets, (average 40 plants), was isolated in a pollen-proof chamber and plants were allowed to polycross within, but not between sets. Mature seed was collected as a bulk sample from each set. Seeds were sown in individual 5 cm pots and plants sprayed at the four leaf stage with 145 g sethoxydim/ha. Numbers of survivors were determined 14 days after spraying and these were divided into two equal sets. One set was sprayed with 1158 g and the other set with 9264 g sethoxydim/ha. These are very high doses, representing 4 x and 32 x the recommended field dose. The aim was to determine whether there was any evidence for incomplete dominance.

RESULTS AND DISCUSSION

F_1 analysis

The technique required that the black-grass clones used were self-incompatible. Most of the 'selfed' plants produced seeds (spikelets) which contain no, or very few, caryopses. In most cases the proportion of seeds with caryopses was $< 1\%$, confirming almost total self-incompatibility. However, two Oxford S1 clones and one Rothamsted clone showed evidence of self-fertility and produced appreciable numbers of viable

seeds. These clones were excluded from further analysis. One set of crosses, Rothamsted 3 x Oxford 5 produced plants which all survived sethoxydim (Rothamsted 3 = 24 alive, 0 dead; Oxford 5 = 52 alive, 0 dead). These too were excluded from the analysis. It is probable that the Oxford 5 clone was homozygous resistant, RR, so when crossed with a homozygous susceptible clone SS (Rothamsted), produced heterozygous progeny RS, all of which were resistant. This contrasts with the results for all the other clones as detailed below.

The results for the remaining clones are presented in Table 22 and Figure 21. The overall ratio of resistant:susceptible (R:S) individuals was close to a 1:1 for both Rothamsted (1.01) and Oxford S1 (1.18) parent plants. This ratio would be that predicted if the original Oxford S1 clones were heterozygous RS and Rothamsted homozygous for susceptibility, SS. Thus, crosses would be expected to segregate into RS, RS, SS, SS, giving a 1:1 ratio if the resistance allele was dominant. The similarity in the ratios of Oxford S1 and Rothamsted parents shows that resistance is nuclearly inherited in a Mendelian manner, with no evidence for maternal inheritance. Hence, the resistance genes can be transmitted via pollen, which is not the case with some other forms of resistance. The tests for goodness of fit demonstrated that the observed ratios did not differ significantly ($P \leq 0.05$) from a 1:1 ratio.

F_2 analysis

The results for the evaluation of the F_2 progeny resulting from the polycross of the F_1 plants surviving sethoxydim treatment, are given in Table 23 and Figure 22. The data for Rothamsted 3 and Oxford 5 have been included, as the genotype of the F_1 progeny (RS) should be the same as that of the **survivors** of the F_1 progeny of the other clones following treatment with sethoxydim.

The overall ratios of resistant:susceptible (R:S) individuals was very close to a 3:1 ratio. This segregation ratio would be expected if the individuals in the polycross were all heterozygous RS. Hence random crossing would produce RR, RS, RS, SS, giving a 3:1 ratio if the resistance allele was dominant. If incomplete dominance occurred, then the RR individuals would be expected to be more resistant than RS

heterozygotes, and so at high herbicide doses a segregation of 1:2:1 would be expected. The plants surviving the initial herbicide treatment were subjected to much higher doses to see if any differential response occurred. Very few (0.7%) plants were killed even by the exceptionally high dose equivalent to 32 x field rate. At this dose most plants showed some leaf scorch, but there were no obvious differential effects. At the lower dose, there was no evidence of leaf scorch and over 92% plants were indistinguishable to untreated controls. Hence, there was no evidence of incomplete dominance, so heterozygous (RS) plants appear as resistant as homozygous (RR) individuals.

CONCLUSIONS

These results provide good evidence that target site resistance to ACCase inhibitors is monogenic, in contrast to the probable polygenic enhanced metabolism mechanism. Consequently, target site resistance involves a single gene. The resistant allele (R) is completely dominant to the susceptible (S). The implications of this are that heterozygous (RS) plants will be as completely resistant to field applications of 'fop' and 'dim' herbicides as homozygous (RR) plants. The practical consequence of these results is that target site resistance could build up very rapidly if 'fop' or 'dim' herbicides are used repeatedly. Although this type of resistance has been found on a limited number of fields, it almost certainly occurs elsewhere. Selection pressure can be reduced by minimising the use of 'fops' and 'dims', as this form of resistance appears to be specific to these herbicide types. Further studies are needed to determine the extent of target site resistance and to develop improved methods for its detection.

TABLE 22 Analysis of F_1 progeny

Clone	Nos. plants Alive	Dead	Clone	Nos. plants Alive	Dead
Roth 1	53	42	Oxford 1	40	42
Roth 2	54	47	Oxford 2	59	36
Roth 3	40	40	Oxford 3	58	46
Roth 4	27	39	Oxford 4	4	4
Roth 5	18	24	Oxford 5	52	52
Roth 6	30	27			
Total	222	219		213	180
Ratio	1.01			1.18	

X^2 test for goodness of fit with Yates' correction

Rothamsted

$$X^2 = \frac{[(222-220.5)-\frac{1}{2}]^2}{220.5} + \frac{[(220.5-219)-\frac{1}{2}]^2}{220.5}$$

X^2 = 0.010 n.s. Tabulated P 0.05 = 3.84 at 1 d.f.

Oxford

$$X^2 = \frac{[(213-196.5)-\frac{1}{2}]^2}{196.5} + \frac{[(196.5-180)-\frac{1}{2}]^2}{196.5}$$

X^2 = 2.606 n.s. Tabulated P 0.05 = 3.84 at d.f.

Conclusion

Ratio of alive:dead plants for both maternal populations do not differ significantly from 1.0.

TABLE 23 Analysis of F_2 Progeny

Clone	Nos. plants Alive	Dead	Clone	Nos. plants Alive	Dead
Roth 1	69	20	Oxford 1	82	31
Roth 2	56	21	Oxford 2	56	21
Roth 3	77	24	Oxford 3	41	24
Roth 4	82	29	Oxford 4	75	30
Roth 5	68	18	Oxford 5	90	35
Roth 6	69	18	Oxford 6	108	37
Roth 7	83	28			
Total	504	158		452	178
Ratio	3.19			2.54	

X^2 test for goodness of fit with Yates' correction

Rothamsted

$$X^2 = \frac{[(504-496.5)-\frac{1}{2}]^2}{496.5} + \frac{[(165.5-158)-\frac{1}{2}]^2}{165.5}$$

X^2 = 0.39 n.s. Tabulated P 0.05 = 3.84 at 1 d.f.

Oxford

$$X^2 = \frac{[(472.5-452)-\frac{1}{2}]^2}{472.5} + \frac{[(178-157.5)-\frac{1}{2}]^2}{165.5}$$

X^2 = 3.39 n.s. Tabulated P 0.05 = 3.84 at 1 d.f.

Conclusion

Ratio of alive:dead plants for both maternal populations do not differ significantly from 3.0

Fig. 21 Number of alive and dead plants in analysis of F1 progeny

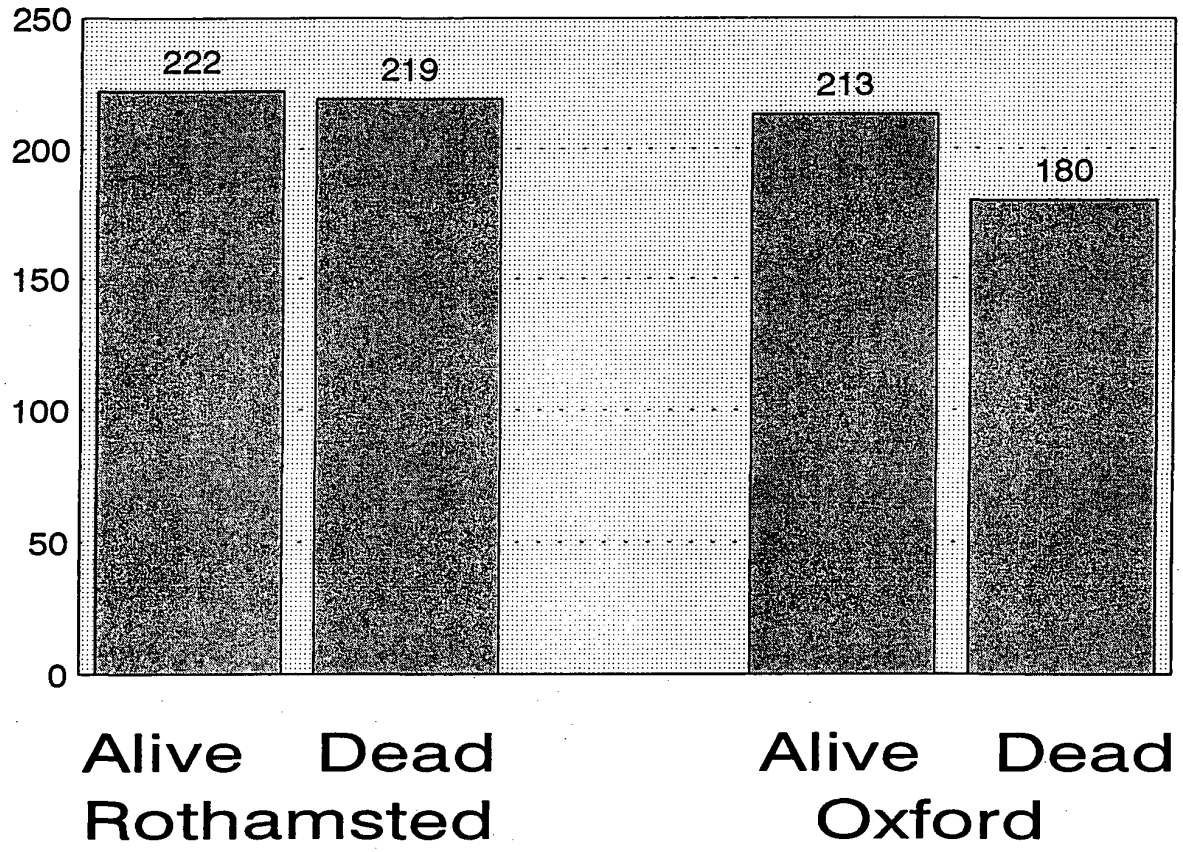
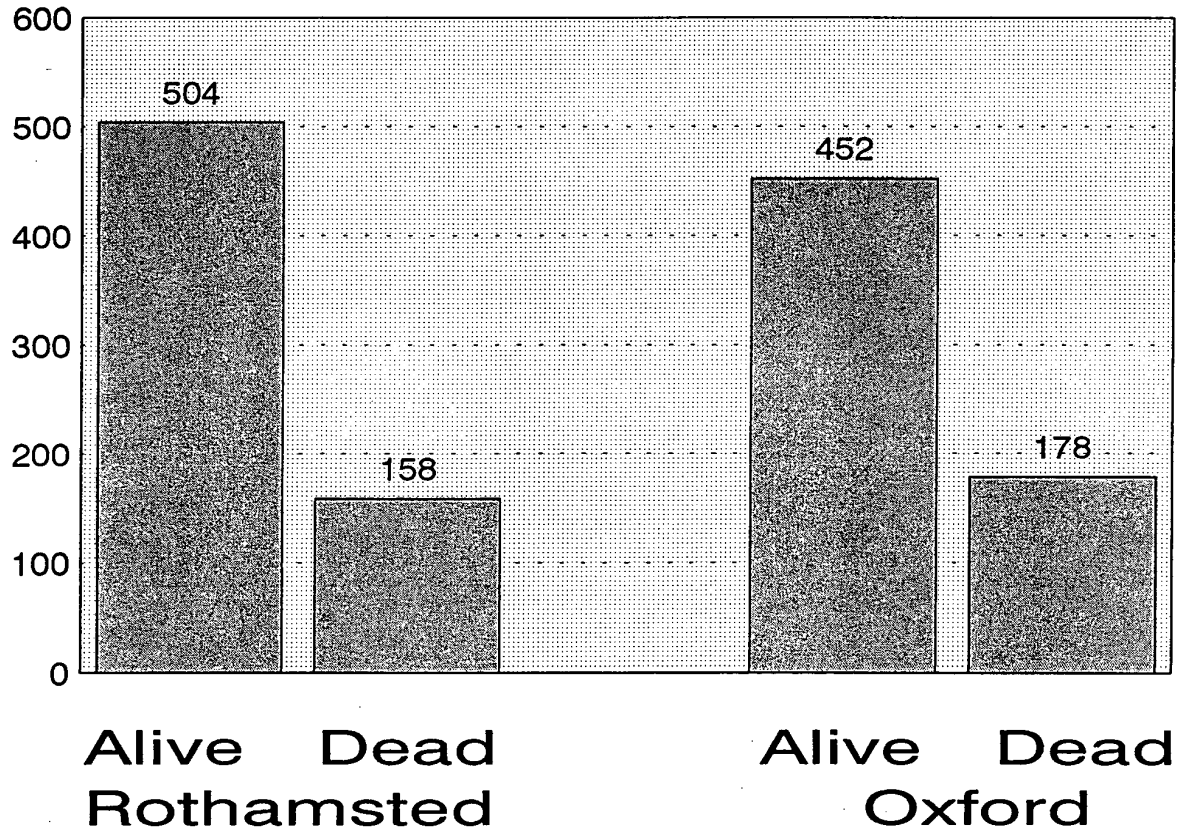


Fig. 22 Number of alive and dead plants in analysis of F2 progeny



CONCLUSIONS

The project demonstrated that herbicide resistant black-grass is widely distributed in England and is present in at least 23 counties. Resistance to chlorotoluron tended to be partial rather than absolute, but more pronounced resistance to fenoxaprop was detected more frequently. No random surveys on the incidence of fenoxaprop resistance were conducted but resistance to this herbicide was confirmed in 16 counties. There was a good correlation between herbicide performance in the glasshouse and in outdoor field conditions. This demonstrates the value of glasshouse studies in the initial appraisal of the impact of resistance.

The experiments demonstrated that even resistance ratings of 1* can be associated with reduced herbicide activity in the field. This finding has important implications as 1* rated populations (marginally insensitive) are not classed as resistant in the current rating system. In random surveys a much higher proportion of samples were rated 1* (15%) than resistant - 2* or more (7%).

The complex nature of cross-resistance was demonstrated very clearly. This is due, at least partly, to the presence of multiple mechanisms of resistance. It is unlikely that the extent of cross-resistance can be characterised routinely on individual fields. Even if feasible, this may change within a relatively short period. Consequently, anti-resistance strategies must be robust, and be appropriate for a wide range of situations. Knowledge gained from testing of samples from individual fields can be used to 'fine tune' more general advice.

Results from selection studies confirmed that resistance to fenoxaprop can evolve more rapidly than resistance to chlorotoluron. The results also indicated that high, rather than low, doses favour the development of resistance. Deselection may occur if herbicide use ceases, or if herbicides are used which impose no selection pressure. However, the evidence from these studies was that this would be a relatively gradual process.

A major finding was the demonstration of target site resistance to ACCase inhibiting herbicides such as 'fops' and 'dims'. Although only detected on six fields so far, this type of resistance has the potential to be a major problem as it can seriously affect performance of all 'fops' and 'dims'. When target site resistance affects a high proportion of plants in a population, it is probable that 'fop' or 'dim' herbicides cannot be used effectively for the foreseeable future. The loss of such herbicides would have serious implications for grass weed control. It is vital that target site resistance is detected early, while only a small proportion of plants is affected. If not, then levels can increase rapidly given further selection with 'fop' or 'dim' herbicides. The simple monogenic nature of target site resistance helps explain why this type of resistance can build up rapidly, in contrast with the more gradual development of polygenically-based enhanced metabolism.

Herbicide resistance in black-grass is a complex problem and continues to pose a serious threat to winter cereal cropping. Resistance to the 'fop' and 'dim' herbicides poses a particular threat because resistance can develop rapidly and cause substantial reductions in efficacy of some herbicides. The future threat posed by target site resistance must also be recognised. Any restrictions on the use of isoproturon due to concerns about leaching to groundwater, will almost inevitably result in increased use of 'fop' or 'dim' herbicides. While these may have a better environmental profile, their use is likely to exacerbate resistance problems.

Information from this project has been used in the formulation of anti-resistance strategies, as detailed in the *Guidelines for the Prevention and Control of Herbicide Resistant Black-grass* produced by the Weed Resistance Action Group with HGCA funding (Moss & Clarke, 1993; 1994). These emphasise the need to integrate herbicide use with non-chemical methods of weed control. The key to combatting the resistance problem is to adopt resistance prevention strategies at an early stage and to reduce reliance on herbicides (Moss, 1995).

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