HGCA

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HERBICIDE RESISTANCE IN BLACK-GRASS (*Alopecurus myosuroides*)

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HERBICIDE RESISTANCE IN BLACK-GRASS
(Alopecurus myosuroides)

by

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CONTENTS

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SUMMARY

1. The combined objectives of the projects were to:
   (a) survey the incidence and study the inheritance of herbicide resistance in black-grass to determine the risk of increase of resistance in the United Kingdom.
   (b) develop the necessary techniques to identify resistance in the laboratory.
   (c) measure under field conditions the efficacy of herbicides identified as being affected by resistance in glasshouse studies.

2. A rating system was devised to define different degrees of resistance, based on the response of plants to chlorotoluron in glasshouse screening tests. Ratings ranged from S, (susceptible, no evidence of resistance) through 1*, (marginal reductions in herbicide activity in pot bioassays) to 5*, where substantial reductions in herbicide activity occurred. A key element in this ranking system was the inclusion of three standard reference populations: Rothamsted (susceptible); Faringdon (partially resistant 2*); Peldon (highly resistant 5*). Only populations classified as 2* or more were deemed to be resistant to chlorotoluron.

3. Resistance to chlorotoluron was detected on 36 new farms (10 in 1988; 14 in 1989; 12 in 1990). These populations came from a wide geographical area. Since 1982, seed samples from a total of 531 fields have been tested for resistance to chlorotoluron. By 1990 resistance had been detected in 67 fields on 46 farms in 19 counties of England.

4. In random surveys an average of 76% of the 267 samples tested for resistance to chlorotoluron between 1988 and 1990 were classified as S, 16% as 1* and 7% as resistant (2* or more). Most of the resistant populations detected were ranked 2*, and therefore exhibited partial resistance to chlorotoluron. More severe resistance, at the 3-5* level was recorded in Buckinghamshire,

Essex, Lincolnshire, Leicestershire, Oxfordshire and Suffolk. The most severe cases of resistance (4*-5*) occurred in the Peldon area of Essex.

5. On 12 farms where resistance to chlorotoluron had been detected, additional samples were collected from other fields. In most cases where resistance was found on one field, it was also present on other fields subjected to similar cultural practices on the same farm.

6. Information on cultural and herbicide history was obtained for 20 fields with resistant populations, representing the period of 6 - 20 years before resistance was detected. All the fields were in a predominantly winter cereal rotation - winter cereals being grown in 89% of the crop years. There was a very low frequency of ploughing - fields were ploughed in only 10% of crop years. Herbicide use was not atypically high - on average 1.7 applications per year of black-grass herbicides.

7. A comparison of the response of plants grown from seeds collected from the same seven fields in 1978 and 1988 provided good evidence for the evolution of resistance during the last 10 years in two fields, and possibly in a third. There was no evidence of development of resistance in the other fields, although the cultural and herbicide histories of all fields was broadly similar. These results demonstrate that current screening tests are detecting resistance which has evolved relatively recently, and are not simply detecting long-standing differences between populations.

8. Comparisons of level of resistance over a period of years showed that resistance to chlorotoluron at the population level developed at a relatively slow rate, despite continued use of herbicides. However, there was no evidence of a decline in resistance level in any field once resistance had been detected. The rate of development of resistance to alternative herbicides may differ to that found with substituted-urea herbicides, such as chlorotoluron.

9. Inheritance studies demonstrated that the gene(s) for chlorotoluron resistance are transmitted via nuclear chromosomes, and not inherited soley through the female line (maternal inheritance) as occurs with triazine resistance. Thus, the resistance trait can be transmitted via pollen, although the importance of this means of spread will depend on the effective dispersal distance of black-grass pollen. The range in response of individual plants to herbicide suggests that the mechanism of inheritance is polygenic, and is therefore controlled by the collective effect of several genes.

10. Chlorotoluron-resistant black-grass showed cross-resistance to other herbicides, such as diclofop-methyl and pendimethalin, which have different modes of action. Patterns of cross-resistance were not consistent between populations, either in terms of the specific herbicides affected or to the degree of resistance. This indicates that more than a single resistance mechanism exists.

11. Experiments conducted in outdoor containers or in the field demonstrated that, while resistance did not cause complete inactivity of herbicides, substantial reductions in activity did occur at normal recommended rates of use of herbicides such as chlorotoluron, isoproturon, diclofop-methyl and fenoxaprop-ethyl. The scale of the reductions in herbicide performance varied substantially between herbicides and populations. Some herbicides, such as fluazifop-P-butyl, quizalofop-ethyl and sethoxydim were effective at normal rates of use. There was a good correlation between the efficacy of chlorotoluron and isoproturon in the field and * ratings obtained from glasshouse screening experiments.

12. Confirmatory testing for resistance demonstrated the difficulty of consistently detecting small differences in response to chlorotoluron in pot experiments at the 1*/2* level. Alternative testing techniques were investigated. Chlorophyll fluorescence looked promising as a technique for detecting resistance to photosynthetic inhibitor herbicides.

INTRODUCTION

Most cases of herbicide resistance have occurred in situations where the same herbicides (or herbicides with the same mode of action) have been used repeatedly, usually associated with intensive agricultural or horticultural systems involving crop monoculture and minimum tillage, in which herbicides have been relied upon to achieve the high level of weed control necessary.

The development of herbicide resistance in weeds is relatively recent, despite the widespread use of selective herbicides for over 40 years. Since the detection of triazine-resistant groundsel (Senecio vulgaris) in the USA in 1968 (Ryan, 1970), there has been a steady increase in the number of resistant species and classes of herbicide to which resistance has evolved. LeBaron (1991, 1992) reported that by 1990, 113 herbicide-resistant weed biotypes had evolved in various locations worldwide. This total includes 58 species (41 dicotyledonous and 17 grass weeds) resistant to triazine herbicides such as atrazine, and 55 species (36 dicotyledonous and 19 grass weeds) resistant to 14 other classes of herbicides. Some species have developed resistance to more than one class of herbicide, so the total number of species recorded with resistance to one or more herbicide was 84 (59 dicotyledonous and 25 grass weeds). Resistant weeds have been recorded in most states of the USA, most provinces in Canada, 18 European and 11 other countries. To date, resistance to the triazine herbicides has been the most widespread type of resistance recorded worldwide.

Triazine herbicides are primarily photosynthetic inhibitors and in the majority of triazine resistant weeds studied, binding of the herbicide to chloroplast membranes is greatly reduced (Fuerst and Norman, 1991). Triazine resistance is usually absolute, and resistant weeds may withstand many times the normally effective herbicide dose. In all weed species so far studied, triazine resistance is inherited via the maternal parent (Souza Machado, 1982) and not via pollen. Therefore all plants derived from seeds shed from resistant plants will also be resistant. Consequently, resistant populations may increase rapidly when selection pressure is favourable. It is relatively easy to identify triazine

resistance because individual plants fall into two distinct categories - resistant or susceptible. At the population level resistance is simply a function of the relative proportions of resistant and susceptible plants.

Black-grass (Alopecurus myosuroides Huds.) is an annual grass weed propagated solely by seeds. These are relatively non-dormant and consequently most germination occurs in the autumn, from September to November. Consequently black-grass is mainly associated with autumn sown crops, especially cereals (Moss, 1980). It is a competitive and fecund weed 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 the past 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. Although 11 different active ingredients are currently available for the control of black-grass in cereals (Ivens, 1991), the most widely used herbicides have been chlorotoluron and isoproturon, which were introduced into the UK in the early 1970's. A survey in 1988 of pesticide usage in England and Wales indicated that chlorotoluron was applied to 10%, isoproturon to 45% and formulated isoproturon mixtures to 17% of the winter cereal area treated with herbicides which control black-grass (Davis, Garthwaite and Thomas, 1990).

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.

In the early 1980's, Niemann and Pestemer (1984) and Moss and Cussans (1985) detected populations of black-grass showing partial resistance to substituted-urea herbicides. The degree of resistance was far less than that found with triazine resistance. Although there was some variation in response between individual plants, the major effect appeared to be an increase in level of resistance of all individuals

within a population, rather than an increase in the proportion of very resistant types. Although the problem appeared to be localised at that time, it was impossible to predict how rapidly resistance would develop elsewhere due to a lack of knowledge of the characteristics of resistant biotypes. Herbicide-resistant black-grass was recognised as a major potential problem and consequently the research studies reported on here were initiated.

THE COMBINED OBJECTIVES OF THE RESEARCH PROJECTS

1. To survey the incidence and study the inheritance of herbicide resistance in black-grass to determine the risk of increase of resistance in the United Kingdom.
2. To develop the necessary techniques to identify resistance in the laboratory.
3. To measure under field conditions the efficacy of herbicides identified as being affected by resistance in glasshouse studies.

SECTION 1.

DISTRIBUTION OF CHLOROTOLURON RESISTANT BLACK-GRASS

INTRODUCTION

Black-grass showing partial resistance to chlorotoluron was first detected in the UK at Faringdon, Oxfordshire, in 1982. More pronounced resistance was found at Peldon, Essex, in 1984 (Moss and Cussans, 1985). By 1987, chlorotoluron-resistant black-grass had been detected on a total of ten farms in three counties of England. Resistance was suspected elsewhere, so additional samples were collected and tested during the course of this project. Some populations were subsequently retested in subsequent years in order to confirm resistance and to determine whether the degree of resistance was increasing. It was recognised that most past sampling had been conducted, not on a random basis, but in response to reports of herbicide failures in the field. It was difficult, therefore, to evaluate how widely resistance occurred. Thus testing of samples collected on a random basis was undertaken in order that the frequency of occurrence of resistance could be estimated.

MATERIALS AND METHODS

Seed samples were collected from cereal fields in July 1988, 1989 and 1990. Some black-grass populations were tested as part of projects funded by MAFF and contract work. However all the populations were included in the same series of screening experiments and so, for completeness, the combined details and results are included below. The sources of the new samples are summarised in Table 1. In addition, IACR tested seed samples from 44 fields between 1988 and 1990 from farms where resistance had previously been identified. These were included to establish whether resistance occurred on other fields on these farms.

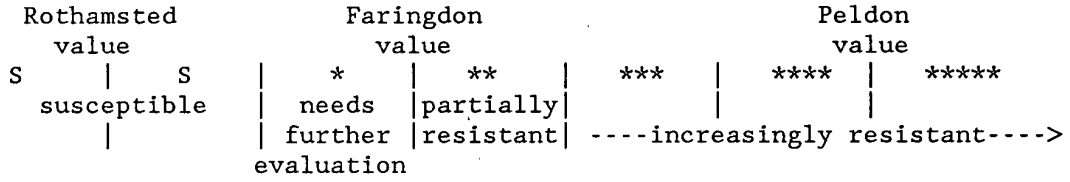
The IACR samples were included in a single screening experiment each year, whereas the large number of ADAS samples necessitated a series of screening experiments to be conducted each year. To enable comparisons to be made between experiments, three standard reference populations were always included: Rothamsted 1987 (susceptible), Faringdon 1987 (partially resistant), Peldon A1 1987 (resistant). The year refers to the year of collection. The Rothamsted population was from the 'no weedkillers' section of the Broadbalk field which has never received any herbicides since its inception in 1843. The Faringdon population was from a cereal field where chlorotoluron and isoproturon have been used regularly since 1974, and where partial resistance was first detected in the UK in 1982. The Peldon A1 population is a cereal field where black-grass highly resistant to chlorotoluron was detected in 1984 (Moss and Cussans, 1985).

Seeds were air dried and cleaned using an air column separator. Seeds were pre-germinated on filter paper in Petri-dishes containing 7 ml of a solution of KNO_3 (2g/l). This was used to increase germination by breaking seed dormancy. Ten pre-germinated seeds were sown in a loam soil (approx 5% organic matter) in each 8.75 cm diameter pot. After emergence, seedlings were thinned to leave six plants/pot. Chlorotoluron ('Dicurane') at a single dose in the range at 2.25 - 3.5 kg a.i./ha was applied at the two-three leaf stage in 200-350 litres water/ha at 210 kPa through either a 'Spraying Systems' 8001 'Tee-jet' (IACR) or a 'Lurmark' 02-F80 (ADAS) nozzle. For each seed stock there was one treated and one untreated pot for each of the five replicates. Pots were placed in a glasshouse and watered from above as necessary. Foliage fresh weight/pot was recorded 3-4 weeks after spraying as a measure of herbicide activity. In some experiments a visual assessment of plant vigour was made at the same time as recording fresh foliage weight. The % reduction in foliage weight was calculated by relating weights in treated and untreated pots for each sample.

In addition two pots (1990 ADAS) or five pots (1990 IACR) per population were treated at the 2-3 leaf stage with 150 g a.i./ha fenoxaprop-ethyl + safener.

RESULTS AND DISCUSSION

The absolute levels of herbicide activity varied between experiments due to variations in temperature and lighting under greenhouse conditions. Therefore the following arbitrary classification was adopted to identify different degrees of resistance, based on a comparison with the % reduction of the three standard reference populations used in all screening tests. This enabled comparisons to be made between experiments conducted at different times and at different locations.



Typically, the mean % reductions in fresh weight for the three standard populations were: Rothamsted 90%, Faringdon 70%, Peldon 20%. The mid value between Rothamsted and Faringdon was used to separate those populations deemed to be susceptible and those requiring further evaluation to determine whether they exhibit a low level of resistance. Similarly, the populations in the Faringdon to Peldon range were separated into three categories. Populations were only termed 'resistant' (2* or more) if they showed greater resistance than the Faringdon population, which has consistently shown partial resistance to chlorotoluron in previous glasshouse screening tests. Results were analysed statistically but it was not thought appropriate to classify resistance groupings using significance parameters. In a series of notionally identical tests, the standard errors will be influenced by variability, the level of herbicide activity, and the proportion of resistant to susceptible populations included. Therefore the assignation of resistance groupings could change between tests. In addition, because resistance is of a quantitative nature, any dividing line placed across the continuum of varying response must be arbitrary.

Samples classed as 1* showed small reductions in herbicide activity. These marginal levels of resistance might result in reduced field performance, especially under adverse conditions for herbicide

activity. Sites ranked 1* need monitoring to determine whether resistance levels are increasing.

Resistance was detected on 36 new farms (10 in 1988; 14 in 1989; 12 in 1990) (Table 2). These populations came from a wide geographical area (Table 3). Since 1982, seed samples from 531 fields have been tested for resistance to chlorotoluron. By 1990, resistance had been detected in 67 fields on 46 farms. These farms are widely distributed in 19 counties of England: Bedfordshire (1 farm), Buckinghamshire (3), Cambridgeshire (3), Dorset (1), E. Sussex (2), Essex (13), Gloucestershire (1), Hertfordshire (1), Kent (1), Leicestershire (1), Lincolnshire (2), Norfolk (1), Northamptonshire (1), Nottinghamshire (1), Oxfordshire (6), Suffolk (5), Surrey (1), Warwickshire (1), 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, was recorded in Buckinghamshire, Essex, Lincolnshire, Leicestershire, Oxfordshire and Suffolk. The populations showing the greatest resistance to chlorotoluron were from the Peldon area of Essex. We conclude that the incidence of severe resistance is low, but the incidence of more marginal resistance is disturbing in view of the potential for further evolution.

In random surveys an average of 76% of the 267 samples tested between 1988 and 1990 were classified as S, 16% as 1* and 7% as resistant (Table 3). However, between 1988 and 1990, seeds collected from 77 non-random sources, where herbicide performance in the field had been adequate, resulted in 32 % of samples being classified as resistant and 18% as 1*. This shows that poor field performance gives an indication of resistance. However, it is important to note that 50% of samples where a problem in black-grass control was identified were not classified as showing any degree of resistance. This highlights the need to examine all reasons for poor control and not to assume resistance as the only cause.

Resistance was confirmed on 12 farms already identified as possessing resistant black-grass in previous tests (Table 4). In most cases where resistance had been found on one field, it was also present on other fields subjected to similar cultural practices on the same farm (Table 4).

The results for the control of the 1990 samples by fenoxaprop-ethyl were analysed statistically and samples were classified as resistant only if the % reduction in foliage fresh weight was significantly less ($P \leq 0.05$) than the Rothamsted susceptible reference population. Of the 27 populations showing resistance to fenoxaprop-ethyl, 20 showed at least a 2* level of resistance to chlorotoluron. In contrast, 11 of the 137 samples tested were resistant to chlorotoluron but showed no evidence of resistance to fenoxaprop-ethyl. The relative response of the 1990 IACR samples to chlorotoluron and fenoxaprop-ethyl was studied by conducting a regression analysis, based on the data for foliage fresh weight of treated pots. The correlation coefficient ($r=0.47$) was low, but statistically significant at $P \leq 0.05$. There appears to be some relationship between resistance to these two herbicides, which have contrasting modes of action. However, these results support those presented in Sections 5 and 6 which show that the pattern of cross-resistance is not consistent between populations, either in terms of the specific herbicides affected or the degree of resistance (Moss, 1992).

The results presented in this section have been published in two papers by Clarke and Moss (1989, 1991).

TABLE 1. Source of black-grass seed samples used in screening experiments for resistance to chlorotoluron

(fields not previously sampled)

Source	1988	1989	1990
HGCA survey (ADAS)	59	49	101
Disease survey (ADAS)	20	38	0
Others (ADAS)	26	14	7
Others (IACR)	27	11	4
Total	132	112	112

Notes:

1. The samples collected in 1988 (59) and 1989 (49) as part of the HGCA funded random survey were collected within 50 miles of Peldon, Essex, where the most resistant populations occur. The samples collected in 1990 (101) were collected on a random survey basis throughout England.
2. The 'Disease survey' samples collected in 1988 (20) and 1989 (38) were from fields selected at random from the ADAS Winter Wheat and Winter Barley disease survey of England and Wales.
3. The 'other' samples were either collected from trial sites or from fields where poor herbicide performance had been reported.

TABLE 2. Classification of resistance levels for chlorotoluron of 1988, 1989 and 1990 seed collections from farms not previously sampled.

	TOTAL	S	1*	2*	3*	4*	5*
				<-----Resistant---->			
1988	132	107	15	6	2	2	0
1989	112	71	27	13	1	0	0
1990	112	83	17	10	0	0	2
TOTAL	356	261	59	29	3	2	2

TABLE 3. Distribution by county and classification of resistance levels to chlorotoluron for samples collected in 1988, 1989 and 1990 from farms not previously sampled.

County	Number of samples	S	1*	2*	3*	4*	5*
				<-----Resistant---->			
Bedfordshire	16	13	2	1	0	0	0
Berkshire	4	4	4	0	0	0	0
Buckinghamshire	8	5	0	2	0	1	0
Cambridgeshire	55	37	16	2	0	0	0
Dorset	1	0	0	1	0	0	0
Essex	64	51	7	3	2	0	1
Gloucestershire	11	9	1	1	0	0	0
Hampshire	8	6	2	0	0	0	0
Herefordshire	3	1	2	0	0	0	0
Hertfordshire	16	12	3	1	0	0	0
Kent	4	2	1	1	0	0	0
Leicestershire	6	4	1	0	0	0	1
Lincolnshire	20	15	3	2	0	0	0
Norfolk	9	7	1	1	0	0	0
Northamptonshire	6	4	1	1	0	0	0
Nottinghamshire	5	4	0	1	0	0	0
Oxfordshire	21	14	3	3	1	0	0
Suffolk	64	45	14	4	0	1	0
Surrey	4	3	0	1	0	0	0
Sussex	8	6	0	2	0	0	0
Warwicks	3	2	0	1	0	0	0
Wiltshire	6	6	0	0	0	0	0
Worcestershire	12	9	2	1	0	0	0
Yorkshire	2	2	0	0	0	0	0
TOTAL	356	261	59	29	3	2	2

TABLE 4. Resistance rating to chlorotoluron on farms where black-grass samples from several fields were collected.

Population	1988	1989	1990
Bucks C1	4*	3*	4*
Bucks C2	-	4*	3*
Bucks E1	-	2*	-
Bucks E2	-	S	-
Bucks E3	-	S	-
Faringdon standard	2*	-	2*
Faringdon 7 acre	-	-	3*
Lincs. C1	2*	5*	-
Lincs. C2	-	3*	4*
Northants A1	-	-	2*
Northants A2	-	-	2*
Oxford A1	4*	-	3*
Oxford A2	2*	2*	2*
Oxford I1	3*	-	-
Oxford I2	-	3*	-
Oxford I3	-	2*	-
Peldon A1	5*	-	5*
Peldon A2	5*	-	5*
Peldon A3	4*	-	-
Peldon A6	-	-	5*
Peldon B2	5*	-	4*
Peldon B4	4*	-	-
S. Essex A1	3*	-	3*
S. Essex A3	2*	-	3*
S. Essex A4	2*	-	-
S. Essex A5	-	-	2*
Tiptree A1	2*	-	-
Tiptree A2	S	-	-
Worcester A1	-	2*	-
Worcester A2	-	2*	-

Note: Letters refer to a specific farm and numbers refer to individual fields on that farm.

SECTION 2.

CULTURAL AND HERBICIDE HISTORIES OF FIELDS WITH RESISTANT POPULATIONS

INTRODUCTION

Most types of herbicide resistance are associated with the repeated use of the same herbicide, often in situations of crop monoculture. To determine whether there was an association between resistance in black-grass and cultural and herbicide history, information was obtained for fields on 20 farms with resistant populations (Table 5). Information for 264 crop years was obtained, which represented the period of 6 - 20 years before resistance was detected.

RESULTS AND DISCUSSION

All these fields were in a predominantly winter cereal rotation - winter cereals were grown in 236 (89%) of the 264 crop years (range = 64 - 100% for individual fields). Eight fields were in continuous winter cereals. There was a very low frequency of ploughing in only 27 (10%) of the crop years (range 0 - 56%). Non-ploughing tillage systems maximise the proportion of the weed population derived from seeds shed in the previous crop and, therefore, minimise the probability of back-crossing with earlier, unselected generations. Herbicides to control black-grass, mainly chlorotoluron, isoproturon and diclofop-methyl, were used frequently on all fields - on average 1.7 applications per year (range 1.0 - 3.3). However, this is not an atypically high use of herbicides. The main herbicides used were chlorotoluron and isoproturon (56% of applications) and diclofop-methyl (15%). A range of other herbicides were also used for black-grass control, often in sequences or mixtures and sometimes at lower than recommended doses. Every field had a different herbicide history.

The fields on the two farms at Peldon, Essex where resistance is most severe, have received very intensive herbicide treatment. It is difficult to know whether this is a cause or a result of the presence of resistant black-grass. In addition the straw burning/minimum tillage systems used can result in the development of an adsorptive surface layer which can reduce the activity of many soil acting herbicides (Moss, 1985, 1987; Orson and Livingston, 1987). This would encourage the greater use of herbicides even in the absence of resistance.

It is not possible to link directly the occurrence of resistance with intensity of herbicide use. Most of the herbicides used to control black-grass are soil acting and their performance is influenced greatly by climatic and other environmental factors. Thus the selection pressure imposed by these herbicides may be poorly correlated with the amount applied.

Thus resistance to chlorotoluron was mainly associated with continuous, or near continuous, winter cereal cropping, non-inversion tillage systems and regular, but not atypically high use of herbicides to control black-grass.

TABLE 5. Herbicide and cultural histories for 20 fields where resistance to chlorotoluron occurs.

(Letters identify farms and numbers refer to individual fields)

Site	Harvest years	Years in winter cereals	Number of applications: Chlorotoluron or Isoproturon	Diclofop -methyl	Other black-grass herbicides	Nos. years ploughed
Bucks E1	1976-1988	10/13	10	1	2	1
Bucks C1	1978-1988	11/11	11	0	0	3
Cambridge A1	1971-1988	18/18	13	3	8	0
Faringdon	1972-1983	12/12	10	6	7	0
Lincs. C1	1970-1988	17/19	18	7	10	2
Northants A1	1980-1990	9/11	9	3	8	4
Oxford A1	1975-1988	9/14	14	3	4	2
Oxford I1	1980-1988	9/9	9	3	14	5
Oxford Q1	1985-1990	5/6	10	3	7	0
Oxford R1	1985-1990	6/6	9	3	5	0
Peldon A1	1975-1987	13/13	18	4	6	0
Peldon B1	1975-1987	13/13	22	6	6	0
Peldon C1	1975-1986	12/12	12	0	0	1
Peldon D1	1975-1986	10/12	12	4	4	4
Peldon E1	1973-1987	14/15	11	0	8	0
S.Essex A1	1972-1988	15/17	14	7	7	0
S.Essex B1	1972-1988	14/17	13	6	5	0
Tiptree A1	1974-1988	13/15	12	0	10	0
Warwick C1	1975-1988	12/14	5	4	8	1
Worc. A1	1973-1989	14/17	14	3	8	4

Total crop years = 264
Winter cereals = 236 (89%)

Chlorotoluron/IPU = 246 (56%) applications
Diclofop-methyl = 66 (15%) "
Others* = 127 (29%) "

(* includes terbutryn & metoxuron in early/mid 70's; triallate, chlorsulfuron/metsulfuron in mid 80's; pendimethalin, flamprop-isopropyl; propyzamide & fluazifop-P-butyl in non-cereal crops)

Herbicide applications = 439 ÷ 264 = 1.7 applications per year
Ploughed = 27 (10%)

SECTION 3.

THE RATE OF DEVELOPMENT OF RESISTANCE

INTRODUCTION

Resistance tends to be identified in populations only when it has developed to a level which causes reductions in herbicide performance in the field. Most of the chlorotoluron-resistant populations detected so far show partial resistance, rather than absolute resistance. It is important that the rate of development of resistance from one year to the next is determined, so that the future scale of the problem can be assessed. Such knowledge should also assist in the choice of appropriate control and containment measures.

There is much information available to assist in the prediction of resistance development on a theoretical basis (Gressel and Segel, 1990). However, in practice it is very difficult to predict the rate of development of resistance in individual situations, because of a lack of detailed knowledge of the interactions between the many factors involved.

MATERIALS AND METHODS

Screening for resistance in populations tested in more than one year.

Section 1 gives full details of the methods used in the screening tests for resistance to chlorotoluron. Some populations were tested in more than one year in order to confirm resistance and to determine whether the level of resistance was changing. These samples were collected from the same part of the respective fields, typically from an area of approximately 0.5 ha within each field.

Comparison of seed populations collected in 1978 and 1988

In summer 1988 seeds were collected from 7 fields in central England where seeds had previously been collected in 1978 for a study on the seed production and shedding characteristics of black-grass (Moss, 1983). Fifty plants, derived from seeds from each population, were grown in a nutrient culture system in which the plant roots were immersed in a nutrient solution containing chlorotoluron at 0.25 ppm. An additional 20 plants per population were grown in nutrient solution alone. The system used has been described by Moss and Cussans (1987). Plants were supported by black alkathene beads in individual compartments of polystyrene modular units floating on standard nutrient solution in plastic trays in a controlled environment cabinet (16°C day of 14 h, 75% RH; 10°C night of 10 h, 86% RH). Holes in the base of each compartment allowed roots to develop into the nutrient solution (Hewitt, 1966). Foliage weight was recorded after 24 days to determine whether response to chlorotoluron had changed during the 10 year period.

In addition, a comparison of populations collected in 1985 and 1988 from the same field at Peldon, Essex, where the most severe cases of resistance occur, was made to determine whether resistance levels were changing.

RESULTS AND DISCUSSION

Screening for resistance in populations tested in more than one year.

The resistance rankings for the populations tested in more than one year are summarised in Table 6. The degree of resistance to chlorotoluron, as measured by the star rating, did not change appreciably on any of the fields where seed samples were collected over a period of years. These results show that none of the populations had reverted, and become susceptible. There was no evidence for a rapid increase in the degree of resistance in these fields, despite continued use of herbicides. However, the screening technique used, and the star rating system for resistance, would be unlikely to demonstrate small, subtle, changes in sensitivity between years.

Comparison of seed populations collected in 1978 and 1988

Plants grown from 1988 seed from two populations, Bucks C and Warwick C, were considerably more resistant than those derived from 1978 seeds (Table 7). A third population, Bucks E, showed a marginal change in response to chlorotoluron between 1978 and 1988. However plants grown from the 1978 and 1988 samples of the other four populations were equally sensitive to chlorotoluron, and there was no evidence of any development of resistance in these populations. The sample collected from Peldon in 1988 showed a slightly higher level of resistance to chlorotoluron than the 1985 sample, but the difference was relatively small (Table 7).

The comparison of the 1978 and 1988 populations provides good evidence for the evolution of resistance during the last ten years at two locations, and possibly at a third. This supports results of a monitoring exercise at Faringdon, Oxfordshire, which showed that resistance to chlorotoluron had developed gradually between 1976 and 1985 (Moss and Cussans, 1991). There was no evidence of a change in sensitivity with the other populations, despite continued use of herbicides in the intervening years. The Peldon population showed a small increase in degree of resistance to chlorotoluron between 1985 and 1988. It appears that resistance level is still increasing at Peldon, but at a relatively slow rate despite the continuing use of herbicides.

The herbicide and cultural records available for the fields sampled in 1978 and 1988 showed that the herbicide and cultural histories were broadly similar for all the treated fields (Table 8). All fields were in a predominantly winter cereal rotation and there was a relatively low incidence of ploughing. Herbicide use was relatively modest, averaging 1.1 applications of black-grass herbicides per year. There was no clear relationship between the intensity of herbicide use and the development of resistance. This supports the evidence of the cultural and herbicide histories obtained for a range of fields with resistant black-grass, as detailed in Section 2. Clearly, it is not possible to predict the occurrence of resistance to chlorotoluron simply on the basis of past intensity of herbicide use. It is probable that intensity of herbicide use is only one of the many inter-related factors which are involved in the complex processes involved in the selection for

resistance in the field. Further research is needed to gain a better understanding of these processes in order to improve our predictive ability.

TABLE 6. Resistance rating to chlorotoluron on fields where seed samples were collected in more than one year.

Population	1988	1989	1990
Bucks C1	4*	3*	4*
Bucks C2	-	4*	3*
Faringdon standard	2*	-	2*
Lincs C2	-	3*	4*
Long Ashton stockbed	-	S	S
Oxford A1	4*	-	3*
Oxford A2	2*	2*	2*
Peldon A1	5*	-	5*
Peldon B2	5*	-	4*
Rothamsted standard	S	S	S
South Essex A1	3*	-	3*
South Essex A3	2*	-	2*

Note: Letters refer to a specific farm and numbers refer to individual fields on that same farm.

See Section 1 for details of method for determining resistance ratings.

TABLE 7. Response to chlorotoluron of plants grown from seeds collected from seven winter cereal fields in 1978 and 1988.
(sites listed in order of insensitivity to chlorotoluron based on 1988 samples)

Year seed collected	% reduction in foliage weight (0.25 ppm chlorotoluron) 1978	1988
Bucks C	96	31
Warwick C	97	78
Bucks E	96	87
Oxford J	95	94
Bucks D	95	94
Oxford L	94	96
Stockbed[1]	97	96
	% reduction in foliage fresh weight (0.75 ppm chlorotoluron)	
	1985	1988
Peldon	67	43

[1] = a seed production plot, established from seed collected from a field in 1975. No herbicide applied 1975-1988.

TABLE 8. Herbicide and cultural histories for 7 fields where seeds were collected in 1978 and 1988 for determination of change in response to chlorotoluron.
(sites listed in order of insensitivity to chlorotoluron based on 1988 samples, as in previous table)

Site	Harvest years	Years in winter cereals	Mean number of herbicide applications	Nos. years ploughed
Bucks C	1982-1988	7/7	1.0	2 out of 7
Warwick C	1978-1988	9/11	1.4	1 out of 11
Bucks E	1978-1988	9/11	1.1	1 out of 11
Oxford L	1978-1988	9/11	0.8	no information
Oxford J	1985-1988	4/4	1.3	no information
Bucks D	1978-1988	10/11	1.0	3 out of 11
Stockbed[1]	1978-1988	No crop	0.0	Never

[1] = a seed production plot, established from seed collected from a field in 1975. No herbicide applied 1975-1988.

SECTION 4.

THE INHERITANCE OF RESISTANCE

INTRODUCTION

The genetic basis of resistance is likely to be one of the key factors determining the rate of development and spread of resistance. In triazine resistance, resistance is due to a mutation altering the triazine binding site in the thylakoid membrane in the chloroplast (Fuerst and Norman, 1991). This, the most common type of triazine resistance, is not inherited by nuclear inheritance, but by maternal inheritance of cytoplasmic DNA (Souza Machado, 1982). Consequently triazine resistance is not transmitted via pollen, but all progeny of a resistant plant are highly resistant. Therefore, some initial breeding studies on herbicide resistant black-grass were undertaken in order to gain an insight into the genetic basis of resistance and the likely influence of this on the spread and future development of resistance.

MATERIALS AND METHODS

Breeding experiments

During summer 1987, plants of chlorotoluron-resistant (Peldon) and susceptible (Rothamsted) populations of black-grass were grown in separate pots in three groups - Rothamsted (susceptible) alone, Peldon (resistant) alone, and an equal mixture of Rothamsted and Peldon plants. There were a total of 60 plants in each group. Each group was isolated to prevent cross-pollination between groups, but cross pollination between plants within each group was encouraged by gently tapping heads at regular intervals to release pollen. After flowering, seeds were collected by gently tapping the heads over a container to encourage the seeds to shed. In the mixed group, seeds were collected separately from Peldon and Rothamsted mother plants. One hundred and fifty plants

derived from each of these four collections of seeds were grown in nutrient culture (using the method described in Section 3). The progeny of Rothamsted plants, either grown in isolation or mixture were exposed to 0.1 ppm chlorotoluron, whereas progeny of all Peldon plants were exposed to 0.5 ppm chlorotoluron. In addition 24 plants from each collection were grown in nutrient culture alone. Foliage fresh weight was recorded 24 days after the introduction of herbicide. In the results section and tables, the progeny of Rothamsted and Peldon plants grown in isolation are termed 'Rothamsted' or 'Peldon', whereas the progeny from the Rothamsted or Peldon plants grown in mixture are termed 'Rothamsted (+Peldon)' or 'Peldon (+Rothamsted)' respectively.

RESULTS

The mean and range of foliage fresh weights for each set are given in Table 9. The mean plant weights for the progeny of Rothamsted and Peldon plants grown in mixture were respectively higher and lower than the corresponding values for the progeny of plants grown in isolation. The values for untreated plants were similar for all four sets. A chi-squared test was performed on the results. This test provides for a simple comparison of the distribution of the foliage fresh weight values of two different samples by dividing each distribution into two, but not necessarily equal, parts. The segregation value used was 1200 mg/plant. The results are summarised below. The numbers refer to the number of plants (out of a total of 150 treated plants per set) which had a foliage fresh weight above or below 1200 mg per plant.

Source population	Rothamsted	Rothamsted (in mixture with Peldon)	Peldon	Peldon (in mixture with Rothamsted)
Foliage fresh weight > 1200 mg/plant	1	40	96	48
Foliage fresh weight < 1200 mg/plant	149	110	54	102

χ Roth v Roth(+P) = 42.97 Significant at $P \leq 0.001$
χ Peld v Peld(+R) = 30.77 Significant at $P \leq 0.001$

The results show that there was a significant shift towards resistance to chlorotoluron in the progeny of the Rothamsted plants grown in mixture with Peldon plants. There was almost a two-fold difference in mean foliage weight (Table 9) and about 25% of the progeny collected from the susceptible plants showed higher levels of resistance than the progeny of susceptibles only (Figure 1). In contrast, there was a marked shift towards susceptibility in the progeny of the Peldon plants grown in mixture with Rothamsted plants.

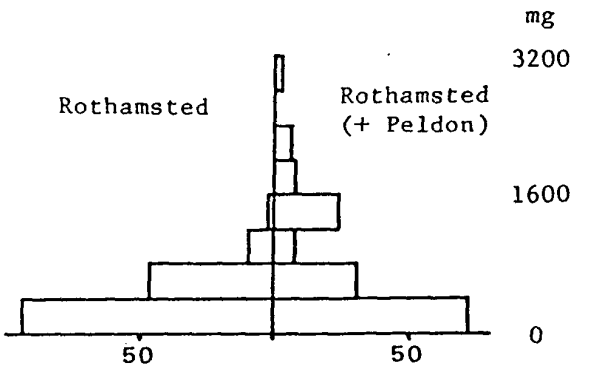
The results show that the gene(s) for chlorotoluron resistance is/are nuclearly inherited, and not maternally inherited as in triazine resistance. One consequence of this is that the resistance trait can be transmitted via pollen, although the importance of this means of spread will depend on the effective dispersal distance of black-grass pollen. The range in response of individual plants to the herbicide suggests that the mechanism of inheritance is polygenic. However, it is possible that a single gene is involved if the phenotypic expression of resistance is modified by other factors.

TABLE 9. Response of different populations and crosses between populations to chlorotoluron.
(values are fresh weight of foliage in mg/plant)

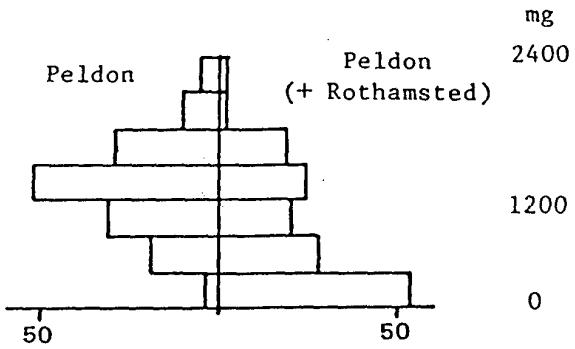
Population		Rothamsted	Rothamsted (+Peldon)
No herbicide	mean	1549	1634
	range	715 - 2359	806 - 2704
0.1 ppm	mean	387	698
	range	18 - 1222	23 - 2960

Population		Peldon	Peldon (+Rothamsted)
No herbicide	mean	1679	1648
	range	592 - 2953	876 - 2616
0.5 ppm	mean	1370	815
	range	153 - 2929	12 - 3326

FIGURE 1. Response to chlorotoluron of uncontrolled crosses within and between a susceptible (Rothamsted) and a resistant (Peldon) population.



Numbers of plants in each weight category
(0.1 ppm chlorotoluron)



Numbers of plants in each weight category
(0.5 ppm chlorotoluron)

SECTION 5.

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 and with contrasting modes of action (Moss, 1992). The population studied in greatest detail, Peldon, shows varying degrees of resistance to 23 herbicides. Resistance is not related in any simple way to chemical grouping or mode of action. Resistance to chlorotoluron and isoproturon in the Peldon population appears to be due mainly to enhanced herbicide metabolism (Kemp, Moss and Thomas, 1990). Cross-resistance to other herbicides may be the result of detoxification via similar oxidative processes. There is evidence that cross-resistance patterns are not consistent between populations (Moss, 1990). An experiment was conducted to investigate the degree of cross-resistance to three herbicides with contrasting modes of action, in a range of black-grass populations. The herbicides used were: chlorotoluron, a phenyl-urea herbicide which inhibits photosynthesis; diclofop-methyl, an aryloxyphenoxypropionate herbicide which inhibits Acetyl Co-A carboxylase, an enzyme involved in fatty acid biosynthesis; pendimethalin, a dinitroaniline herbicide which interferes with cell division.

MATERIALS AND METHODS

Five black-grass populations were assessed for resistance to chlorotoluron and diclofop-methyl in a glasshouse pot experiment. The same populations were also tested for resistance to pendimethalin in a Petri-dish test.

Single plants were grown from seeds of the five populations in a Kettering loam soil/grit mixture (5:1) in individual 5 cm square pots in a glasshouse. Chlorotoluron (dose range = 0.38 - 28.00 kg a.i./ha) and diclofop-methyl (0.30 - 38.40 kg a.i./ha) were applied, each at eight doses, at the 3 leaf stage using a laboratory sprayer delivering 278 l water/ha at 210 kPa through a single 'Spraying Systems' 8001 'Teejet' nozzle. There were 12 replicate pots per herbicide dose for each population. In addition, there were 25 untreated pots per population. Foliage fresh weight per pot was recorded 24 days after spraying.

A Petri-dish test as described by Moss (1990) was used to assess resistance to pendimethalin in the same five 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 seven concentrations of herbicide (0, 0.15, 0.4, 1, 3, 10, 50 ppm) and the solutions included 2 g/litre KNO_3 to break seed dormancy and stimulate seedling growth. Each dish was wetted with 7 ml of the appropriate solution. Dishes were placed in polyethylene bags in a controlled environment cabinet (18°C 14 h day, 12°C 10 h night). The lengths of primary shoots were recorded for each germinated seed after 3 weeks.

Foliage fresh weight and shoot length data from the glasshouse and Petri-dish experiments was analysed using a Maximum Likelihood Programme (Ross, 1987) and $\log_{10}ED_{50}$ values calculated. ED_{50} values were detransformed from the log data and represent the herbicide dose required to reduce foliage fresh weight or shoot length by 50%, relative to untreated.

RESULTS AND DISCUSSION

The ED_{50} values and the ratio to the Rothamsted reference susceptible population are given in Table 10. The Peldon population showed the greatest resistance to all the herbicides tested. The Bucks C population also showed clear evidence of resistance to all herbicides but at a lower level than the Peldon population. In marked contrast, H/121 from Suffolk

showed a high degree of resistance to chlorotoluron but no clear resistaance to diclofop-methyl or pendimethalin. The Faringdon population showed partial resistance to chlorotoluron, but a much higher level of resistance to diclofop-methyl.

These results demonstrate the complexities in cross-resistance patterns which exist between different populations. These differences do not appear to be related to previous history of herbicide use. Most of the detailed studies which have been conducted on mechanisms of resistance have involved the Peldon population. These studies have demonstrated that enhanced herbicide metabolism is the main mechanism involved. However, the detection of resistance patterns which contrast with that of the Peldon population, suggests that other resistance mechanisms are present in other populations.

It is sometimes suggested that farmers should alternate herbicides as a means of avoiding the development of resistant weed populations. However, if cross-resistance to herbicides with different mechanisms occurs, then selection for the same detoxification process may still occur despite using a range of herbicide types. The presence of different resistance mechanisms in different populations is a further complication. It is important that further work is conducted in order to elucidate the range of cross-resistance patterns which exist in different black-grass populations. This information is an essential pre-requisite for the formulation of widely applicable control strategies.

TABLE 10. Effects of three herbicides on five black-grass populations

	$Log_{10}ED_{50}$ values		
	Chlorotoluron	Diclofop-methyl	Pendimethalin
Peldon 1987	0.884	0.715	0.812
Bucks C1	0.374	0.350	0.177
H/121	0.538	-0.109	-0.166
Faringdon 1987	0.117	0.529	0.026
Rothamsted 1987	-0.497	-0.196	-0.264
S.E. ±	0.084	0.123	0.078
L.S.D.(P≤0.05)	0.238	0.347	0.220

	ED_{50} values* (detransformed)		
	Chlorotoluron	Diclofop-methyl	Pendimethalin
Peldon 1987	7.65	5.18	6.49
Bucks C1	2.36	2.24	1.50
H/121	3.45	0.78	0.68
Faringdon 1987	1.31	3.38	1.06
Rothamsted 1987	0.32	0.64	0.54

* = ED_{50} values - kg a.i./ha for chlorotoluron and diclofop-methyl and ppm for pendimethalin. All detransformed from log_{10} values.

	Ratio of ED_{50} values relative to Rothamsted		
	Chlorotoluron	Diclofop-methyl	Pendimethalin
Peldon 1987	24.0	8.1	11.9
Bucks C1	7.4	3.5	2.8
H/121	10.8	1.2	1.3
Faringdon 1987	4.1	5.3	2.0
Rothamsted 1987	1.0	1.0	1.0

SECTION 6.

EFFICACY OF HERBICIDES IN FIELDS AT PELDON, ESSEX

INTRODUCTION

Resistant black-grass populations were first detected as a result of reports of inadequate control by herbicides in the field. The most resistant populations found so far occur in fields on farms at Peldon, Essex. Consequently, four field trials were conducted in order to evaluate the efficacy of a range of herbicides and sequences on fields known to contain chlorotoluron-resistant black-grass.

MATERIALS AND METHODS

Two individual herbicide evaluation trials were conducted to evaluate, in the field, single applications of herbicides which showed promising results in previous glasshouse experiments. Some of these herbicides were not selective in wheat. The individual herbicide evaluation trials conducted in 1987/88 and 1988/89 were located in fields on two different farms at Peldon, Essex. The 1987/88 trial was conducted in the absence of any crop.

The herbicides which appeared most effective in the individual herbicide evaluation trial were included in two herbicide sequences trials, with one pre-emergence followed by two post-emergence treatments. The two sequences trials were conducted in 1987/88 and 1988/99 on the same two farms at Peldon as the single herbicide evaluation trials, but on different fields.

Plot size for all trials was 6 x 3 m and there were three replicates in a randomised block design. Treatments were applied using a modified

Van de Weij pressurised knapsack sprayer delivering 200 l water/ha using 02-F80 nozzles at 200 kPa. The full list of treatments and dates of application are given in the results tables. Plant and head densities of black-grass were assessed using ten randomly placed 0.1 m^2 quadrats per plot.

RESULTS AND DISCUSSION

INDIVIDUAL HERBICIDE EVALUATION TRIALS

The maximum control achieved in the herbicide evaluation trials (Table 11) was 75%. Trifluralin, ethofumesate, propyzamide, fluazifop-P-butyl, carbetamide and tri-allate gave the best levels of control. For the 1987/8 results, when there was no crop and for products which are non-selective in cereals (such as carbetamide, fluazifop-P-butyl, ethofumesate and propyzamide) level of control was hindered in these trials by lack of crop competition. For these treatments especially, % reduction of plant numbers is more likely to reflect their capabilities.

The generally poor control achieved by herbicides may have been due to several factors, apart from resistance. The absence of a crop in the 1987/88 trial because of the very wet autumn would have allowed for a more complete recovery of plants, and particularly flowering heads. The wet weather conditions may have also caused poor control by reducing herbicide efficacy. The adsorptive capacity of the soil, measured as Kd values, was sufficiently high, despite ploughing, to affect herbicide performance (Cussans *et al.* 1982). Fixed quadrat counts indicated that poor herbicide performance was not due to delayed emergence of weeds.

The field results do, however, tend to confirm the results obtained in previous glasshouse experiments, except for the relatively better performance of chlorsulfuron/metsulfuron and the relatively poorer performance of fluazifop-P-butyl. The relatively good performance of chlorusulfuron has been noted previously (Orson and Livingston, 1987).

HERBICIDE SEQUENCES TRIALS

In the herbicide sequences trials higher levels of control were achieved in 1988/9 than the previous year (Table 12). However despite the equivalent of using four chemical treatments the highest level of control was only 86% (pre-emergence tri-allate followed by early post-emergence) isoproturon/trifluralin followed by isoproturon). Both years trials demonstrate the benefit of a pre-emergence treatment. The 1987/8 trial demonstrated a benefit from applying tralkoxydim rather than isoproturon as the late post-emergence spray, but this was not repeated in 1988/9.

The very wet autumn in 1987, and the relatively high adsorption levels (Kd) on both fields may have contributed to the inadequate levels of control achieved. In addition, weed densities were so high that a relatively good control of plants could have been masked by the ability of survivors to tiller and produce many more heads per plant than on untreated plots, where intra-specific competition would have been intense. It was not possible to assess plant numbers in the sequences trials because of they very wet ground conditions and the very high weed densities present.

CONCLUSIONS

These field trials confirm the difficulty in achieving satisfactory control of black-grass in fields at Peldon. In any one season, as the number or frequency of active ingredients applied increased, the level of control improved slightly. However, even sequences of four herbicides, costing at least £85/ha, were insufficient to achieve acceptable levels of control. It would appear that reasonable levels of control can be achieved with some herbicides which are not selective in cereals, but this would mean a change of cropping. The most sensible strategy to control black-grass at Peldon, and other farms with similar problems, is likely to be a combination of ploughing and the inclusion in the arable rotation of crops on which fluazifop-P-butyl, propyzamide, ethofumesate or carbetamide can be used. Delayed drilling and growing

spring or other crops which discourage black-grass would also be beneficial.

Finally, it is important to emphasise that other factors, apart from resistance, probably contributed to poor herbicide activity at Peldon. It is very difficult to critically assess the relative contribution of these various factors in field experiments. Consequently, additional studies in more controlled conditions were undertaken, as described in the next section.

TABLE 11. Results of individual herbicide evaluation trials

		1987/88 Church Field Brickhouse Farm, Peldon		1988/89 Lower 16 Acre Peldon Hall	
Cultivation method		Ploughed		Minimum cultivated	
Kd (chlorotoluron)		5.3		5.3	
Herbicide (rate a.i./ha)		% Reduction black-grass Plants	Heads	% Reduction black-grass Plants	Heads
Pre-emergence		20/10/87		30/9/88	
trifluralin	(1.1 kg)	48	62	53	33
chlorsulfuron + metsulfuron-methyl	(15g) (5g)	35	55	-	-
tri-allate	(2.25 kg) (c)	42	39	68	33
pendimethalin	(2.0 kg)	50	38	29	50
Early post-em. (GS 12/13)		14/12/87		24/10/88	
isoproturon	(2.5 kg)	26	38	34	31
chlorotoluron	(3.5 kg)	5	27	31	43
diclofop-methyl	(1.14 kg)	19	35	25	29
tralkoxydim	(200 g) (a)	1	31	32	38
SMY 1500	(1.6 kg) (b)	34	45	33	35
isoproturon + trifluralin	(1.95 kg) (1.3 kg)	-	-	31	29
carbetamide	(2.1 kg)	57	62	51	30
fluazifop-P-butyl	(187 g) (a)	75	48	57	31
ethofumesate	(2.0 kg)	70	62	55	22
propyzamide	(700 kg)	69	53	65	24
barban	(312 g)	46	57	-	-
Late post-em. (GS 25-29)		18/4/88			
fluazifop-P-butyl	(187 g) (a)	34	53	-	-
tralkoxydim	(350 g) (a)	7	23	-	-
S.E.D. +/-		19	18	13	13
(Untreated population/m^2)		(20.3)	(362)	(958)	(1247)

(a) tank-mixed with 0.1% non-ionic wetter ('Agral')
(b) 4-amino-6-tert-butyl-3-ethylio-1,2,4-triazin-5(4H)-one in formulation UK 220.
(c) granular formulation

TABLE 12. Results of herbicide sequences trials

1987/8 Twitch Field, Peldon Hall
(minimum cultivated Kd (chlorotoluron) 8.7)

Early post-em. 9/3/88 (GS 11/13)	Late post-em 18/4/88 (GS 23/25)	Pre-em. applied 10/11/87 Nil	chlorsulf./m-methyl
		% black-grass control (heads)	
IPU	IPU	21	25
IPU	IPU/tralkoxydim	19	12
IPU	tralkoxydim	35	45
IPU/tralkoxydim	IPU	0	18
IPU/tralkoxydim	IPU/tralkoxydim	0	26
IPU/tralkoxydim	tralkoxydim	20	39
tralkoxydim	IPU	0	24
tralkoxydim	IPU/tralkoxydim	20	19
tralkoxydim	tralkoxydim	21	31

S.E.D. +/- 12.5
(Untreated population 1773 heads/m^2)

1988/9 Melondowns Field, Brickhouse Farm, Peldon
(minimum cultivated Kd (chlorotoluron) 11.4)

Early post-em 2/11/88 (GS 11/13)	Late post-em. 24/1/89 (GS 22)	Pre-em. applied 14/10/88 Nil	trifluralin	tri-allate
		% black-grass control (heads)		
IPU	IPU	56	60	79
IPU	tralkoxydim	51	69	69
IPU/trifluralin	IPU	55	77	86
IPU/trifluralin	tralkoxydim	73	72	81
IPU/tralkoxydim	IPU	64	78	74
IPU/tralkoxydim	tralkoxydim	47	78	77

S.E.D. +/- 13.4
(Untreated population 2206 heads/m^2)

Abbreviations and rates of active ingredient/ha

IPU = isoproturon (2.5 kg except 1989 when 2.1 kg late post-em); tralkoxydidm (200 g); chlorsulf./m-methyl = chlorsulfuron (15 g)/metsulfuron-methyl (5 g), trifluralin (1.1 kg); tri-allate granules (2.25 kg); Nil = no pre-em. herbicide applied.

SECTION 7.

EFFECT OF RESISTANCE ON HERBICIDE ACTIVITY IN SIMULATED FIELD CONDITIONS

INTRODUCTION

Experiments investigating the response of black-grass populations to herbicides under glasshouse conditions show clearly that resistance is not absolute, and that herbicides do have some effect on plant growth. 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. No herbicide treatments or sequences gave consistently good control of black-grass in field experiments conducted at Peldon, Essex, where the most resistant populations to chlorotoluron occur (Section 6; Clarke and Moss, 1989; Orson and Livingston, 1987). While such field experiments are useful, they have the limitation that it is difficult to determine how much poor herbicide performance is due to resistance and how much to other unrelated factors (Moss, 1987). This is a particular problem with the soil acting herbicides used for black-grass control, as the performance of these is greatly influenced by soil and climatic conditions (Orson, 1991).

An alternative method of assessing comparative performance of herbicides is to grow plants of different populations in a standard soil at one site. Thus soil and climatic variables are eliminated.

MATERIALS AND METHODS

Container Experiments 1 and 2

This technique involves growing plants of different populations in a standard soil in outdoor containers in order to simulate field conditions. Seeds (300/container) of the different populations were

incorporated into the top 5 cm of a silty loam soil (3 - 5 % o.m.) in separate plastic containers (27 x 18 x 10 cm) on 5 October 1987 (Expt. 1) or 4 October 1990 (Expt. 2) and placed outdoors. Both experiments comprised randomised block designs with five (Expt. 1) or four replicates (Expt. 2). There were three untreated containers per replicate for each population. In Experiment 1, pre-emergence herbicides were applied on 6 October 1987 and post-emergence herbicides on 4 January 1988 when black-grass plants had 3 leaves. In Experiment 2, seven herbicides, each at three doses were applied on 19 November 1990 at the 3½ leaf stage. See Tables 13 and 14 for details of herbicides and doses. All herbicides were applied using a laboratory sprayer delivering 250 l water/ha at 210 kPa through as single 'Spraying Systems' 8001 Teejet nozzle.

Herbicide activity was recorded by assessing foliage fresh weight per container on 13 March 1988 (Expt. 1) and 11 April 1991 (Expt. 2).

Boxworth field experiment

Seeds of 5 populations of black-grass with a range of ratings of resistance to chlorotoluron (Table 15) were sown in 4m long paired rows (10 cm apart, 30 cm between populations) in a chalky boulder clay at ADAS Boxworth, Cambridgeshire on 19 October 1989. There were 4 replicates. Three herbicides, each at 4 doses, were sprayed on 16 February at the 2 tiller stage using a modified Van de Weij pressurised knapsack sprayer in 200 l water/ha using 02-F80 nozzles at 200 kPa. Black-grass control was assessed by counting the number of plants one month after treatment, and expressed as the % reduction of the number in that plot before treatment.

RESULTS AND DISCUSSION

Container Experiment 1.

The Rothamsted population (susceptible standard) was well controlled by many herbicides, especially those applied post-emergence (Table 13). All herbicides gave some control of the Peldon populations but significantly ($P \leq 0.05$) poorer control, compared with Rothamsted, was achieved with barban, chlorotoluron, cyanazine, isoproturon, metazachlor,

metribuzin, simazine, tralkoxydim and SMY 1500. In contrast, carbetamide, ethofumesate, fluazifop-P-butyl, propyzamide, quizalofop-ethyl, sethoxydim and trifluralin gave similar levels of control of both populations.

With the exception of trifluralin, all the herbicides that can be used in cereals showed reduced activity on the Peldon population. In contrast, several of the herbicides that can only be used in non-cereal crops, e.g. ethofumesate, fluazifop-P-butyl, propyzamide, quizalofop-ethyl, and sethoxydim gave a good control. Thus black-grass at Peldon should be easier to control by herbicides in non-cereal than in cereals crops.

A limitation of experiments involving single dose rates is that excellent levels of control achieved by some herbicides can mask small differences in sensitivity. This has been confirmed in subsequent studies with fluazifop-P-butyl (see results for Container Experiment 2), which have demonstrated that this herbicide is marginally less effective on the Peldon population. It is important to emphasise that the results obtained with a single resistant population may not be generally applicable, as other populations may have different cross-resistance patterns.

Container Experiment 2

The Rothamsted susceptible standard population was controlled very effectively by the recommended rate of all herbicides (Table 14). With the exception of chlorotoluron and tralkoxydim, the lower rates also gave over 90% control. In contrast all rates of chlorotoluron, isoproturon, fenoxaprop-ethyl and tralkoxydim gave substantially poorer levels of control of the Peldon population. This population was well controlled by (>95%) by the recommended rates of fluazifop-P-butyl, quizalofop-ethyl and sethoxydim. The lower rate of quizalofop-ethyl and sethoxydim also gave a high level of control but the lower rate of fluazifop-P-butyl was significantly poorer. This supports previous studies which indicated that there is partial resistance to fluazifop-P-butyl in the Peldon population (Moss, 1987). There was no evidence of differential efficacy

between the Rothamsted and Peldon populations with sethoxydim. Control of the Bucks population by the three herbicides tested was also poorer than Rothamsted, but similar to Peldon.

Boxworth Field Experiment

The results demonstrated that resistance can cause substantial reductions in herbicide performance, even at higher than recommended dose rates (Table 15). There was a good correlation between the efficacy of chlorotoluron in the field and the * ratings obtained from the glasshouse screening experiments. At equivalent doses, isoproturon gave better control than chlorotoluron and again there was a good correlation with the * ratings. There was a poorer correlation between field performance of diclofop-methyl and * rating for chlorotoluron.

Control of the Boxworth population (1*) was poorer than Rothamsted (susceptible) when treated at the label recommended rate of chlorotoluron (3.5 kg a.i./ha). This result is of concern since 16% of the populations of black-grass were ranked 1* in the random surveys detailed in Section 1. The results for H/121 confirm that it shows a high level of resistance to chlorotoluron and isoproturon, but no resistance to diclofop-methyl. The apparently higher level of control of H/121 by diclofop-methyl than the susceptible standard (Rothamsted) warrants further study to establish whether negative cross-resistance exists. Other glasshouse studies have confirmed the contrasting pattern of cross-resistance, relative to the Peldon population, but have showed no evidence of negative cross-resistance (see Section 5).

CONCLUSIONS

The results of all experiments conducted in simulated field conditions, in which populations with differing resistance ratings to chlorotoluron were included, are summarised in Table 16. This includes results of other experiments additional to those described above. The * ratings for chlorotoluron resistance, obtained from pot screening experiments as described in Section 1, were well correlated with the

activity of this herbicide in the simulated field conditions. Reductions in the activity of isoproturon, diclofop-methyl and fenoxaprop-ethyl were also clearly demonstrated. However, the relationship between * rating for chlorotoluron and the performance of the other herbicides was poorer, for reasons that are only partially understood.

The H/121 population from Suffolk had shown a high degree of resistance to chlorotoluron, but no resistance to diclofop-methyl in the glasshouse experiments described in Section 5. In contrast, the Norfolk A population was shown to be susceptible to chlorotoluron in two previous screening tests (Clarke and Moss, 1989; Moss and Orson, 1988), but had shown a low level of resistance to diclofop-methyl. In a dose response experiment conducted in the glasshouse, the diclofop-methyl ED_{50} value for the Norfolk A population was 3.8 fold higher than that of the Rothamsted standard (1.18 v 0.31 kg a.i./ha). Thus, these contrasting patterns of cross-resistance were confirmed in simulated field conditions.

The results of these experiments show that, while resistance does not cause complete inactivity of herbicides, substantial reductions in activity can occur. However, the scale of the reductions in herbicide performance varies substantially between herbicides and populations for reasons that are, at present, only partially understood.

TABLE 13. The effect of herbicides on two black-grass populations grown in outdoor containers in 1987/1988 (Expt. 1).

Herbicide	Rate of application (kg a.i./ha)	% reduction in foliage fresh weight	
		Rothamsted	Peldon
Pre-emergence			
Cyanazine	2.25	47	31
Ethofumesate	1.40	99	99
Simazine	1.15	84	73
Metazachlor	1.25	99	82
Metribuzin	1.05	42	31
Trifluralin	1.20	43	47
Post-emergence			
Barban	0.31	81	43
Carbetamide	2.10	94	90
Chlorotoluron	3.50	100	20
Fluazifop-P-butyl[1]	0.094	97	93
"	0.188	98	95
Isoproturon	2.50	100	63
Propyzamide	0.35	96	92
"	0.70	90	91
Quizalofop-ethyl[2]	0.125	98	97
Sethoxydim[3]	0.34	98	97
Tralkoxydim[1]	0.20	95	58
SMY 1500	1.75	100	63
		S.E. ± 3.9	

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

2 & 3 = adjuvant oil (2 ='Fyzol', 3 ='Adder') was used with these treatments.

TABLE 14. Effect of seven herbicides on different black-grass populations grown in outdoor containers in 1990/91 (Expt. 2).

Herbicide	Dose kg a.i./ha		% reduction in foliage fresh weight: Rothamsted (S)	Peldon (5*)	Bucks Cl (4*)
chlorotoluron	1.17		53.0	-9.6	-12.6
chlorotoluron	3.50	@	99.0	-3.6	4.0
chlorotoluron	10.50		99.9	11.9	12.7
isoproturon	0.83		92.3	-3.9	5.6
isoproturon	2.50	@	98.9	14.7	22.8
isoproturon	7.50		99.3	73.3	61.7
fenoxaprop-ethyl (+ Safener)	0.04		98.3	47.2	45.8
fenoxaprop-ethyl (+ Safener)	0.12	@	97.7	61.0	45.8
fenoxaprop-ethyl (+ Safener)	0.36		97.9	66.8	66.8
fluazifop-P-butyl + Agral 0.1%	0.042		99.6	88.2	-
fluazifop-P-butyl + Agral 0.1%	0.125	@	98.8	99.2	-
fluazifop-P-butyl + Agral 0.1%	0.375		99.0	99.6	-
quizalofop-ethyl + Fyzol 11E 21/ha	0.025		97.9	94.5	-
quizalofop-ethyl + Fyzol 11E 21/ha	0.075	@	99.5	97.3	-
quizalofop-ethyl + Fyzol 11E 21/ha	0.225		99.9	99.9	-
sethoxydim + Adder 1%	0.97		99.4	99.4	-
sethoxydim + Adder 1%	0.290	@	99.4	99.7	-
sethoxydim + Adder 1%	0.869		99.7	99.9	-
tralkoxydim + Adherb 0.5%	0.083		75.4	23.4	-
tralkoxydim + Adherb 0.5%	0.250		93.6	47.1	-
tralkoxydim + Adherb 0.5%	0.750		98.9	69.0	-
				LSD (P≤0.05) =	9.60

@ = label recommended rate
* = ratings for resistance to chlorotoluron based on glasshouse screening tests (see Section 1)

TABLE 16. The efficacy of four herbicides applied at recommended field rates to a range of black-grass populations grown in outdoor containers or in field plots at one site, 1985-1991.
(from Moss, 1992)

	% reduction in foliage fresh weight							
Population[1] Resistance rating[2]	Roth. susc.	Norf. susc.	Box. 1*	Far. 2*	Tip. 3*	H/121 4*	Bucks 4*	Peld. 5*
Chlorotoluron 3.5 kg a.i./ha	82-100 (5)	-	61 (1)	42-50 (2)	55 (1)	38 (1)	4 (1)	0-30 (5)
Isoproturon 2.5 kg a.i./ha	66-100 (5)	-	95 (1)	84 (1)	-	45 (1)	23 (1)	15-63 (5)
Diclofop-methyl 1.14 kg a.i./ha	53-100 (4)	83 (1)	46 (1)	30-51 (2)	-	85 (1)	-	8-55 (4)
Fenoxaprop-ethyl 0.12 kg a.i./ha	98 (1)	-	-	-	-	-	46 (1)	61 (1)
Trifluralin 1.2 kg a.i./ha	43-87 (3)	-	-	-	-	-	-	47-86 (3)

1 = Roth. = Rothamsted; Norf. = Norfolk A; Box. = Boxworth; Far. = Faringdon; Tip. = Tiptree A; H/121 = Suffolk; Bucks = Bucks C; Peld. = Peldon

2 = Rating for resistance to chlorotoluron based on glasshouse tests as described in Section 1.

( ) = Number of experiments

TABLE 15. Effect of three herbicides on five black-grass populations sown in a field experiment at Boxworth, Cambridge

Herbicide	Rate of a.i. kg/ha	% reduction in numbers of black-grass plants/m^2 Rothamsted (S)	Boxworth (1*)	Faringdon (2*)	H/121 (4*)	Peldon (5*)
chlortoluron	1.75	43.8	18.6	5.3	34.7	12.2
chlortoluron	3.5 @	86.9	60.5	42.0	38.2	28.1
chlortoluron	7.0	95.5	82.7	80.8	41.2	12.4
chlortoluron	14.0	100.0	95.5	88.1	74.9	29.4
isoproturon	1.25	60.0	42.7	38.0	38.5	16.6
isoproturon	2.5 @	95.5	94.9	84.0	44.5	53.7
isoproturon	5.0	100.0	95.6	98.2	70.9	94.1
isoproturon	10.0	100.0	100.0	100.0	84.7	100.0
diclofop	0.567	22.6	7.8	0.0	27.9	6.9
diclofop	1.134 @	53.4	46.1	29.8	84.5	9.7
diclofop	2.268	64.3	58.9	46.6	88.4	56.1
diclofop	4.536	93.7	91.5	71.7	92.7	41.1
Mean (LSD (P≤0.05) = 16.81)		78.0	68.5	59.6	58.2	42.2

@ = maximum label recommended rate

* = ratings for resistance to chlorotoluron based on glasshouse screening tests (see Section 1)

diclofop = diclofop-methyl

SECTION 8.

CONFIRMATORY TESTS FOR RESISTANCE

INTRODUCTION

The screening tests for resistance conducted by ADAS and IACR consist of spraying a single discriminating dose of herbicide onto plants grown in pots of soil in the glasshouse. However, small differences in methodology may influence the degree of activity of herbicides. For example factors such as the soil type used, the light intensity, temperature regime, method of watering, growth stage at spraying and amount of time before assessing may all affect herbicide activity both between sites and between successive experiments at the same site. This is one of the main reasons why standard reference populations were included in all tests conducted by ADAS and IACR. In order to gain more information on the consistency of the pot screening test, samples tested by ADAS, and found to be resistant to chlorotoluron were retested by IACR.

MATERIALS AND METHODS

1987, 1988 and 1989 samples

Twenty-one seed populations collected in 1987, 1988 and 1989 were included in a pot dose response experiment, in which six doses of chlorotoluron (range 0.3125 - 10.0 kg a.i./ha) were applied post-emergence to black-grass plants grown in pots in a glasshouse. There were four replicates and other experimental details were the same as those described in Section 1. Foliage fresh weight was recorded three weeks after spraying.

The following reference populations were used: Rothamsted 1987, Faringdon 1987, Peldon 1987. In addition a population collected from the same field as the Faringdon 1987 population was included (Faringdon

1990). For details of the reference populations and the * rating system refer to Section 1 of the report. All the 17 ADAS samples were ranked as 2* for resistance to chlorotoluron in previous tests conducted at Cambridge, except for B/4 (Boxworth) = 1*, and H/17 = 3*. The reference populations were rated S, 2* and 5* respectively.

A dose response analysis was carried out on the fresh weight data using a maximum likelihood program (MLP) and ED_{50} values calculated. The ED_{50} values given in the table are the doses of chlorotoluron needed to reduce fresh weight by 50%, relative to mean weight of all untreated pots.

1990 samples

Fifteen seed populations were included in a pot experiment in which a single dose of chlorotoluron (2.5 kg a.i./ha) was applied post-emergence to plants growing in pots in a glasshouse using the system described in Section 1. There were four replicates and foliage fresh weight and plant vigour were assessed three weeks after spraying. Plant vigour was assessed by giving each plant a score from 1 - 4. These four categories were:

1. Plant unaffected by herbicide.
2. Visibly reduced growth or herbicidal effects but plants growing well.
3. Severe reductions in growth or obvious symptoms.
4. Plant dead with no, or very little, visible green leaf.

The same reference populations as described above, and Faringdon 1990, were included. Eleven ADAS populations were tested - seven had been ranked 2*, two ranked 5* (C/112 and D/74), one ranked 1* (Boxworth) and one ranked as susceptible to chlorotoluron (D/105). The D/105 population had showed the greatest resistance to fenoxaprop-ethyl of any of the populations included in ADAS tests.

Mean foliage fresh weight of untreated pots ranged from 6.080 - 8.649 g/pot. The lowest weights were of C/112 and D/51, - the poorest plants at spraying. Foliage fresh weight data were therefore converted to % reduction relative to the mean untreated weights for the same

population, prior to analysis.

RESULTS AND DISCUSSION

1987, 1988 and 1989 samples (Table 17)

Even the highest dose did not reduce foliage fresh weight of the Peldon standard by 50%, so a precise ED_{50} could not be calculated. H/17 was the most resistant population after Peldon, with an ED_{50} value 6.3 x that of Rothamsted. A further 12 of the ADAS populations were less sensitive to chlorotoluron on an ED_{50} basis than Faringdon 1987, and so could be termed 'resistant'. These included B/4 (Boxworth), which was ranked 1* in the previous ADAS test. Four ADAS samples (D/41, D/53, D/56 and D/48) were more susceptible than Faringdon 1987 on a comparison of ED_{50} values. D/41 was only just below Faringdon 1987, and would thus warrant a 1* rating.

Comparisons of populations based on their response at the 2.5 kg a.i./ha dose (as used in screening tests) are shown also in Table 17 in terms of % reduction in fresh weight relative to untreated, and appropriate * rating. On this basis, eight of the ADAS populations gained a 2* or 3* rating, four populations were 1*, and five populations were susceptible.

Eight ADAS populations were resistant (2* or more) on both an ED_{50} basis and on a comparison at 2.5 kg a.i./ha (H/17, H/10, C/61, H/111, C/108, H/79, H/96, Cambridge A1). One ADAS population (D/41) warranted a 1* rating on both comparisons. Three populations (D/53, D/56, D/48) were susceptible, regardless of method of comparison. Five ADAS populations gave less consistent results. B/4 (Boxworth), D/59, H/89, T/116 and D/42 were less sensitive than Faringdon 1987 on an ED_{50} basis (i.e. were 'resistant'), but rated only 1* (B/4, D/59, H/89) or were susceptible (T/116, D/42) on a comparison at 2.5 kg a.i./ha.

Most of the nine ADAS populations which were susceptible, 1* or gave inconsistent results in the Rothamsted test were close to the 1*/2* boundary in the ADAS tests (mean difference on the Faringdon standard =

2%). In contrast, most of the seven ADAS populations which were consistently resistant in the Rothamsted test, were less close to the 1*/2* boundary in the ADAS tests (mean difference on Faringdon = 7%).

It is perhaps significant that in the Rothamsted test, several populations were resistant (above Faringdon 1987) on an ED_{50} basis, but not resistant (susceptible or 1*) on the single dose comparison. This is not unexpected, as a dose response comparison should be more discriminating, and be capable of detecting small differences between populations which are not detectable at a single, fairly high, dose.

Faringdon 1990 was clearly more resistant than Faringdon 1987. This is of concern as it indicates that the level of resistance has increased in this field, and also that Faringdon 1990 is not a suitable substitute for Faringdon 1987 as a 'marker' for resistance.

1990 samples (Table 18)

The % reduction values ranged from 9 - 95%. This indicates that the single doses achieved a good discrimination in response between populations. The response to chlorotoluron of the 1987 standard reference populations was similar to previous tests - Rothamsted - 93% ; Faringdon - 86% ; Peldon - 11%. Of the nine populations resistant (2* or more) to chlorotoluron in ADAS tests, four were confirmed as resistant in this test (D/74, D/79, C/123, C/112). Two of the other five were 1* (D/75 ; D/91) and three were susceptible (D/51 ; D/84 ; D/92). The two populations which gave 5* resistance results in ADAS tests were rated 2* (D/74) and 4* (C/112) in this experiment. D/105 was resistant to chlorotoluron (2*) in this experiment, although it was susceptible in the ADAS test. Boxworth 1991 was rated 2* in this experiment. Faringdon 1990 was more resistant to chlorotoluron than Faringdon 1987, as found in the previous test above.

Although plants of C/112 and D/51 were poorer than the other populations, this did not prevent resistance to both herbicides being detected in C/112. This demonstrates that small differences in plant size or vigour at spraying, are unlikely to seriously affect the

response to these herbicides. The number of plants in the categories 1 & 2 for vigour correlated well with the fresh weight data, but tended to increase the differences in response between populations compared with the fresh weight data.

The results confirm resistance to chlorotoluron in four of the nine populations which were rated as resistant in the ADAS tests. Two others were 1* in this test, confirming at least a marginal level of insensitivity. However, the other three populations appeared to be as susceptible as the Rothamsted standard. While a high degree of resistance to chlorotoluron in C/112 was confirmed (5* - ADAS; 4* this test), resistance level in D/74 differed between tests (5* ADAS; 2* this test). In addition, D/105 was resistant in this test, but was as susceptible as the Rothamsted standard in the ADAS test (95.1% v 95.3%). While differences of a single * should be expected between tests, some of these differences are greater than this.

CONCLUSIONS

The populations which were ranked 3* or more in the ADAS tests (H/17 1989; D/74 1990; C/112 1990) showed up clearly as resistant in these confirmatory tests, albeit not always at the same * rating. However, the results emphasise the difficulty of consistently detecting small differences in response to chlorotoluron in pot tests at the 1*/2* level. The partial insensitivity of the Faringdon population has been consistently detected in pot screening experiments, which demonstrates the level of discrimination possible.

It is clear that marginal differences between populations are unlikely to be detected consistently on a single dose comparison, and may also be hard to detect on a dose response basis. At present, there is no evidence that alternative tests, such as chlorophyll fluorescence or petri-dish tests, will be better at discriminating between populations. In addition, the same alternative tests are unlikely to be appropriate for resistance to contrasting types of herbicides. The pot test is likely to remain the most appropriate method for screening for

resistance. A major advantage is that it is suitable for determining the activity of a wide range of herbicides, unlike many more specialized tests. The major disadvantages are the time taken to obtain results, and the inconsistencies in results at the 1*/2* level. Hopefully these can be resolved, by refining the protocol.

RECOMMENDATIONS

The following three factors need particular consideration in relation to screening tests for resistance.

(a). Choice of herbicide dose.

In IACR screening tests, conducted between 1985 - 1991, 2.5 - 4.0 kg chlorotoluron/ha (mostly 2.75 kg/ha) has given the following levels of control of the reference populations: 87 - 99% (mean = 93%) control of Rothamsted; 70 - 96% (mean = 83%) control of Faringdon and 9 - 51% (mean = 26%) control of Peldon. The % difference between Rothamsted and Faringdon has ranged from 3 - 20% (mean = 10%). These tests have been conducted in autumn each year. The appropriate dose to use at other times of year, and in other soils, may differ.

It is important that the dose of herbicide used is sufficiently discriminating, so that relatively small differences between populations can be detected. The use of more than a single dose is desirable, but increases the size of any screening test considerably. Small differences in sensitivity between populations can be important as studies in the field have shown reductions in herbicide performance in populations showing only marginal levels of resistance in pot screening tests (see Section 6).

The critical factors determining the level of control in pots are dose, soil type - especially organic matter level, light intensity, temperature, growth stage at spraying and watering method. The most appropriate dose to use can only be chosen in the light of experience. The possibility of overspraying if too low a dose has been used initially can be considered.

(b). Choice of standard reference population

This is of particular importance in the interpretation of different screening experiments and in assessing the results in terms of likely affect on field performance of herbicides. The three chlorotoluron standards Rothamsted, Faringdon and Peldon have been very useful in this respect but may not be appropriate for use in the evaluation of resistance to other herbicides.

(c). Assessments

Measurement of fresh weight, 3 - 4 weeks after spraying gives a good assessment of herbicide activity. Additional information can be obtained by assessing individual plant vigour. The four categories used in this experiment seem adequate. Generally, categories 1 & 2 include plants which would survive, and recover from herbicide applications. This information can be a useful addition to the fresh weight per pot data, and can highlight small differences between populations which tend to be masked by taking combined weights of 6 plants per pot. (Assessing individual plant weight is not appropriate due to time involved and effects of intra-specific competition.)

TABLE 17. Response of 21 black-grass populations to chlorotoluron, 1987, 1988 and 1989 samples.
(Listed in order of resistance based on ED_{50} comparisons)
Reference populations underlined)

Population	* Rating in ADAS tests	ED_{50}[1] kg ai/ha	Ratio ED_{50} to Rothamsted	% reduction[2] at 2.5 kg ai/ha	
1. Peldon 1987	5*	>10.0	>26	33	5*
2. H/17 1989	3*	2.410	6.3	59	3*
3. H/10 1989	2*	2.263	5.9	59	3*
4. C/61 1989	2*	1.750	4.6	68	3*
5. H/111 1988	2*	1.245	3.2	73	2*
6. C/108 1989	2*	1.228	3.2	66	3*
7. Faringdon 1990	-	1.199	3.1	68	3*
8. H/79 1989	2*	1.015	2.6	81	2*
9. H/96 1988	2*	0.906	2.4	87	2*
10. B/4 1988	1*	0.760	2.0	90	1*
11. D/59 1989	2*	0.689	1.8	90	1*
12. H/89 1988	2*	0.683	1.8	88	1*
13. Camb. A1 1987	2*	0.672	1.8	87	2*
14. T/116 1989	2*	0.628	1.6	91	S
15. D/42 1989	2*	0.526	1.4	93	S
16 Faringdon 1987	2*	0.511	1.3	87	2*
17. D/41 1989	2*	0.505	1.3	89	1*
18. D/53 1989	2*	0.401	1.0	92	S
19. D/56 1989	2*	0.387	1.0	94	S
20. Rothamsted 1987	S	0.383	1.0	93	S
21. D/48 1989	2*	0.375	1.0	96	S

[1] = ED_{50} value (kg ai/ha) detransformed from $\log_{10}$ values.

[2] = % reduction in fresh weight, and * ratings, at the 2.5 kg ai/ha. This is equivalent to the standard resistance test as described in Section 1.

TABLE 18. Response of 15 black-grass populations to chlorotoluron, 1990 samples unless other wise indicated.
(Listed in order of resistance based on % reduction in foliage weight, reference populations underlined)

Population	* rating in ADAS tests	% reduction in foliage weight and * rating[1]		Nos. Plants alive (total in vigour categories 1 & 2, maximum = 24)
1. Peldon 1987	5*	9	5*	24
2. C/112	5*	10	4*	24
3. Faringdon	-	67	2*	12
4. D/105	S	72	2*	17
5. D/79	2*	83	2*	0
6. C/123	2*	83	2*	6
7. D/74	5*	85	2*	0
8. Boxworth 1991	1*	85	2*	1
9. Faringdon 1987	2*	86	2*	2
10. D/75	2*	89	1*	0
11. D/91	2*	89	1*	0
12. D/51	2*	93	S	0
13. Rothamsted 1987	S	93	S	0
14. D/92	2*	95	S	0
15. D/84	2*	95	S	0

Note: [1] * rating as described in Section 1.

SECTION 9.

NEW TECHNIQUES FOR DETECTION OF RESISTANCE

The current method used for the detection of resistance to chlorotoluron consists of collecting a seed sample in July from the field in question, sowing the seeds in pots of soil in the glasshouse, applying herbicide post-emergence at the 2-3 leaf stage and assessing the effects about 3 weeks after spraying. This is the technique described in greater detail in Section 1. The main limitations of this technique are the necessity of obtaining a seed sample and the length of time needed to complete a test. The initial dormancy of seeds prevents tests being conducted immediately after seed collection, so in practice tests cannot be started until 2-3 months later. A test takes about 7 - 8 weeks to complete so results are not normally available to assist in choice of herbicide or other control measures in the autumn following collection. However, the technique is relatively simple to conduct, requires no complex equipment, is applicable to a wide range of different herbicides, and mimics to some degree at least the conditions which exist in the field.

A more rapid test for resistance would be useful. This might either be conducted on seed samples or on plants collected directly from the field following a failure of a herbicide to control black-grass. Two alternative techniques were investigated:

1. chlorophyll fluorescence using detached leaves.
2. a Petri-dish test in which the early development of seedlings was assessed following seed germination in the presence of pendimethalin, a dinitroaniline herbicide.

CHLOROPHYLL FLUORESCENCE

INTRODUCTION

Chlorophyll fluorescene is a well established technique for studying photosynthetic activity and has been used for the identification of triazine resistant weeds (Rubin, 1992; van Oorschot, 1991). In collaboration with a visiting scientist (Baruch Rubin, Professor of Weed Science, Hebrew University of Jerusalem, Israel) this technique was investigated as a means of detecting differences in photosynthetic activity in detached black-grass leaves exposed to chlorotoluron, a herbicide which inhibits photosynthesis.

When leaves are illuminated, fluorescence rises rapidly from a low level $F(_0)$ via an intermediate level (I) to a peak level (M). Values for M and I were recorded using the Hansatech equipment and the ratio (M - I)/M calculated. The less photosynthesis that occurs, the closer the I value is to M, and consequently the smaller the calculated ratio.

MATERIALS AND METHODS

Plants were dug up in March 1990 from five fields containing black-grass populations which had been tested in previous screening experiments for resistance to chlorotoluron. The plants were grown on in pots in a glasshouse for three weeks and then newly emerged leaves from 30 plants per population were removed. A Hansatech recorder was used to measure fluorescence induction curves of the detached leaves after standing in chlorotoluron solution (10^4 molar) for two hours. The leaves were then placed in distilled water in the dark, and further readings were taken 24 and 48 hours later. Assessments were also made on untreated leaves.

RESULTS AND DISCUSSION

The fluorescence measurements measured immediately after leaves had been treated showed that photosynthesis was suppressed in all populations

(Table 19). Differences between populations emerged after a 24 hour recovery period. The Peldon sample showed complete recovery while photosynthesis in the Rothamsted leaves remained suppressed. The other populations showed intermediate responses. The values for untreated leaves remained at a high level for 24 hours, but showed a slight decline with some populations after 48 hours.

The differences in the recovery rates correlated quite well with the resistance * rating of the populations in the glasshouse tests. The small differences in the ranking order may be due to differences in the samples used. In the fluorescence test plants dug up for the field were assessed whereas in the screening experiment seed samples were used.

The fluorescence technique show considerable promise as a research tool, but much more developmental work will be needed if it is to be used as a screening technique. The uniformity of the leaves and the number required per sample make the technique quite labour intensive at present. The technique is unlikely to be appropriate for detection of resistance to herbicides which are not photosynthetic inhibitors e.g. fenoxaprop-ethyl, although this needs to be evaluated. Thus chlorophyll fluorescence is unlikely to replace the standard pot evaluation technique, but may be useful for the relatively rapid diagnosis of resistance in advance of a definitive pot test.

EVALUATION OF A PROCEDURE FOR RESISTANCE TESTING IN PETRI-DISHES

INTRODUCTION

Previous studies demonstrated that measuring the length of shoots of seeds germinating in the presence of the dinitroaniline herbicide pendimethalin, had potential as a method for testing populations for resistance (Moss, 1990). Seeds of susceptible and resistant populations germinated normally, but shoot development was inhibited to a much greater degree in susceptible populations. Dinitroaniline herbicides interfere with cell division, and therefore have a different mode of action to substituted urea herbicides such as chlorotoluron, which

inhibit photosynthesis. However, cross-resistance between herbicides with contrasting modes of action has been demonstrated in some populations of black-grass (Moss, 1990), and consequently an assessment of resistance to pendimethalin might be useful as a measure of more general resistance. Tests in Petri-dishes can be conducted more rapidly than a glasshouse pot tests and can be conducted in incubators which provide more standard conditions than those occurring in the glasshouse. Consequently it was decided to evaluate a range of populations, mainly collected in 1988, which had previously been included in a glasshouse screening test for resistance to chlorotoluron, as described in section 1.

MATERIALS AND METHODS

Seed samples of 14 populations, mostly collected in 1988, were cleaned on an air column separator to improve the proportion of viable seeds. The three reference populations, Rothamsted 1987, Faringdon 1987 and Peldon 1987, which were used in the pot screening tests (see Section 1 for details) were included. Twenty-five seeds were placed in each 9 cm diameter Petri-dish containing three Whatman No. 4 filter papers covered by one glass-fibre paper. Seven ml of a solution containing either 0, 0.4, 1, 3, 10 or 50 ppm pendimethalin were added to each dish. All concentrations contained KNO_3 (2 g/litre in order to break dormancy and so increase % seed germination. There were three replicates. Dishes were placed in polyethylene bags in a controlled environment cabinet (18°C 14 h day, 12°C 10 h night). After 3 weeks the numbers of germinated seeds with primary shoots more than 25 mm long were assessed. Dose response data were analyzed using a Maximum Likelihood Programme and $\log_{10}ED_{50}$ values determined (Ross, 1987). These values represent the concentration of herbicide required to reduce mean shoot length by 50% relative to untreated.

RESULTS AND DISCUSSION

Germination in untreated dishes was generally good - over 75% for all populations except Worcester A (51%), and Cambridge A1 (60%). There was no evidence that the herbicide had any effect on germination, even at the highest concentration. To compensate for differences in germination %, the number of seeds with shoots of over 25 mm was expressed as a % of the number of germinated seeds in that same dish. In dishes containing no herbicide, over 90% of germinated seeds had shoots over 25 mm long.

The Peldon population was clearly the most resistant population tested (Table 20). If Faringdon 1987 is used as a reference population for resistance, then most of the populations identified as being resistant to chlorotoluron in a previous pot screening test, (2* or more) also showed cross-resistance to pendimethalin. The Bucks C population was the most resistant population, apart from Peldon, in both the Petri-dish test and in the pot screening experiment. The Bedford A population gave conflicting results in the Petri-dish and pot screening test, but the differences were comparatively small. The dose responses of the Warwick C and Lincs. C populations, which were both rated 2* resistance to chlorotoluron in the pot screening test, were very similar to that of the Faringdon reference population.

A comparison of resistance to chlorotoluron and pendimethalin was made by calculating a correlation coefficient for the response of the 13 populations which had been included in a previous pot screening experiment for resistance to chlorotoluron (see Section 1 for details of methods), in which a single discriminating dose of herbicide was applied post-emergence and fresh weight of foliage was recorded three weeks after spraying. Comparisons were made using mean foliage weight per pot, as a measure of response to chlorotoluron, and total numbers of germinated seeds with shoots over 25 mm long in Petri-dishes at the 3 ppm concentration (total of three reps), as a measure of response to pendimethalin (Figure 2). The correlation coefficients was 0.846, which was statistically significant at $P \leq 0.05$.

These results support those obtained from the pot screening tests in which chlorotoluron was used. Peldon was clearly the most resistant population to both pendimethalin and chlorotoluron. The results provide evidence that populations resistant to chlorotoluron are also likely to show cross-resistance to pendimethalin, despite the very different modes of action of these two herbicides. However, some populations have been identified which show more specific types of resistance. For example the population H/121 from Suffolk, included in the cross-resistance experiments described in Section 5, showed resistance to chlorotoluron but no resistance to pendimethalin or diclofop-methyl.

The Petri-dish test has potential as a fairly rapid test for resistance to dinitroaniline herbicides. However, it is unlikely to be suitable for use immediately after collecting seeds in July, as black-grass seeds have some degree of innate dormancy. Also, it would be unwise to assume that resistance ratings obtained from Petri-dish experiments can be applied directly to other herbicides.

TABLE 19. Evaluation of chlorophyll fluorescence for the detection of resistance to chlorotoluron in black-grass plants collected from five fields.

Fluorescence measurements recorded on detached leaves immediately after 2 h treatment with chlorotoluron (0 time) and after a subsequent 24 and 48 hours recovery period in water.

	(M-I)/M x 100 (see text)						
	Untreated			Treated			
	0	24	48	0	24	48	hours
Rothamsted (S)	298	269	188	31	42	49	
Oxford A1 (2*)	312	303	268	37	90	110	
Faringdon (2*)	325	251	249	48	100	109	
S.Essex A1 (2*)	306	328	262	67	184	180	
Peldon (5*)	308	299	254	92	291	249	

Note: * ratings for resistance to chlorotoluron based on pot screening test. See Section 1 for full details.

TABLE 20. Response of 14 black-grass populations to pendimethalin in a Petri-dish test.

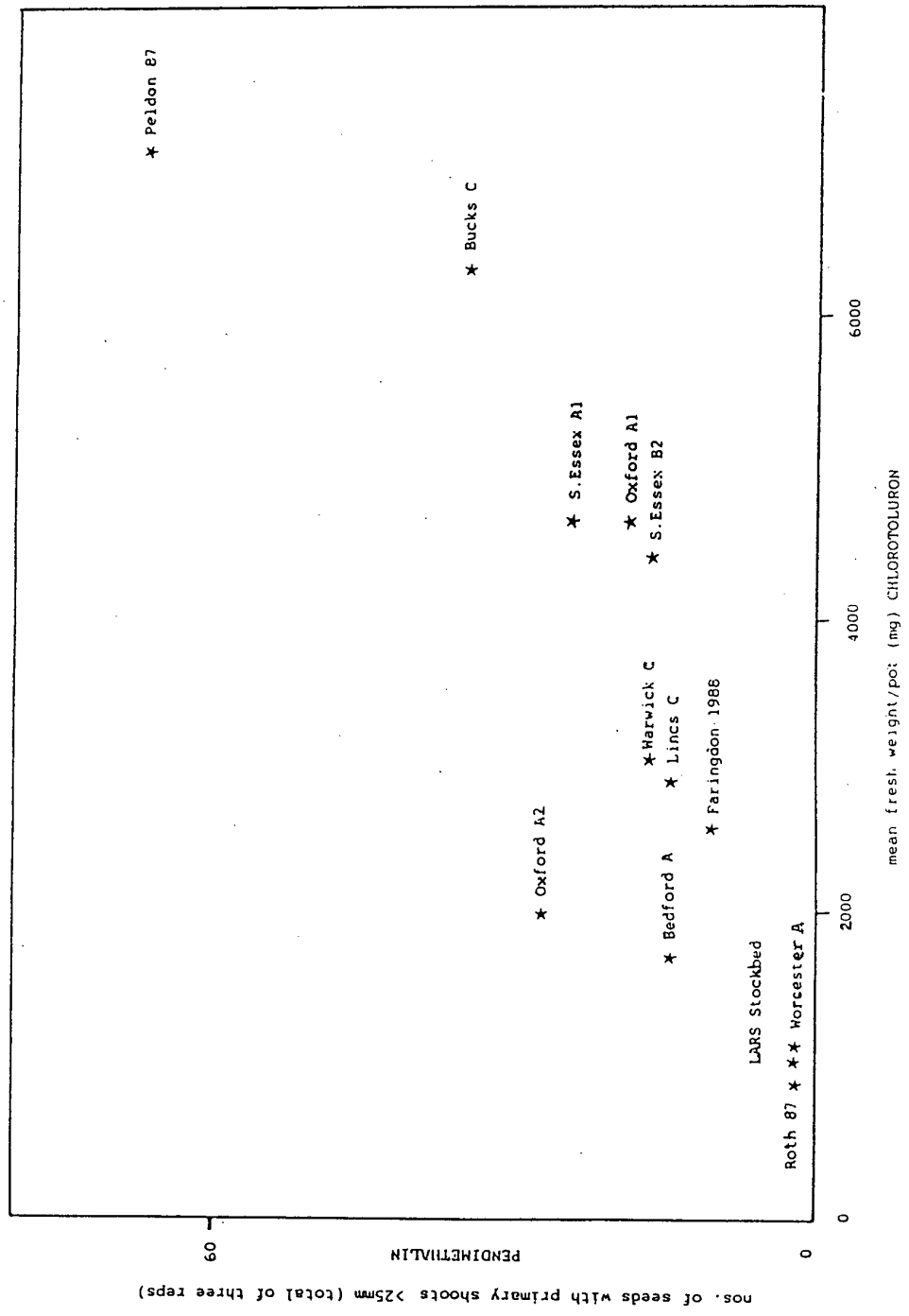
(Populations listed in descending order of resistance to pendimethalin, based on ED_{50} values.)

Population and * rating for chlorotoluron[1]		Pendimethalin $log_{10}ED_{50}$ ppm	ED_{50}[2] ppm	Ratio to Rothamsted
Peldon 1987	5*	1.602	39.99	54.0
Bucks C1	4*	0.620	4.16	5.6
Oxford A2	2*	0.488	3.07	4.1
S. Essex A1	3*	0.329	2.14	2.9
Bedford A1	1*	0.308	2.03	2.7
Oxford A1	4*	0.259	1.82	2.5
S. Essex B1	3*	0.238	1.73	2.3
Lincs. C1	2*	0.188	1.54	2.1
Faringdon 1987	2*	0.152	1.42	1.9
Warwick C1	2*	0.125	1.34	1.8
Faringdon 1988	2*	0.104	1.27	1.7
Worcester A1	S	0.023	1.06	1.4
LARS stockbed	S	-0.047	0.90	1.2
Rothamsted 1987	S	-0.130	0.74	1.0
		S.E. ± 0.076		
		L.S.D. 0.215		
		($P \leq 0.05$)		

[1] = * rating based on pot screening test for resistance to chlorotoluron. See Section 1 for full details.

[2] = detransformed from the $log_{10}ED_{50}$ data.

Figure 2. Comparison of the response of 13 black-grass populations to chlorotoluron in a pot experiment and to pendimethalin in Petri-dishes.



CONCLUSIONS

The project showed that chlorotoluron-resistant black-grass occurred in at least 19 counties of England. Resistance was associated with intensive winter cereal growing systems and non-ploughing cultivation techniques. Herbicides were used regularly on all fields with resistant black-grass, but the use of herbicides was not atypically intensive. There was no clear relationship between the occurrence of resistance and intensity of herbicide use.

Although the occurrence of populations showing a high degree of resistance was relatively low, the more widespread incidence of marginal resistance is disturbing in view of the potential for further evolution. The rate of development of resistance to substituted urea herbicides, such as chlorotoluron and isoproturon, appeared to be relatively slow, although there was no evidence of a rapid decline in resistance levels in any field once resistance had been detected. Therefore, it appears that it will be difficult to eliminate resistant populations once they have developed. It is important to recognise that the rate of development of resistance to alternative herbicides may differ to that found with the substituted-urea herbicides, currently the most widely used herbicides for black-grass control in England. Indeed preliminary studies indicate that resistance to aryloxyphenoxypropionate herbicides such as fenoxaprop-ethyl, which are being used increasingly for grass weed control in a variety of crops, can develop more rapidly.

The complexities of cross-resistance were demonstrated very clearly. Patterns of cross-resistance were not consistent between populations, either in terms of the specific herbicides affected or to the degree of resistance. The use of a wide variety of different herbicide types makes it difficult to assess the potential risk of resistance development and likely cross-resistance patterns in any individual field. Resistance did not cause complete inactivity of herbicides, but substantial reductions in activity of a wide range of herbicides were demonstrated at normal, and higher than recommended, rates of use in the field.

Information from this project has been used in the publication of provisional 'Guidelines for the Prevention and Control of Herbicide-resistant Black-grass' which were produced by the Weed Resistance Action Group (WRAG) in 1991. It is clear that a broader based approach to black-grass control is needed, in which herbicide use is integrated with non-chemical methods of weed control.

New techniques for the identification of resistant populations were investigated. Chlorophyll fluorescence showed promise as a rapid test for resistance detection for photosynthetic inhibitors. However, further refinement will be needed before it can be used for routine screening purposes and other techniques will be needed to determine resistance to other types of herbicides.

Herbicide resistance in black-grass is a complex problem but poses a serious potential threat to winter cereal cropping. Clearly a better understanding of the complex interactions between the herbicide mode of action, weed ecology and the genetics and mechanisms of resistance is needed. Further studies are in progress which are supported by the HGCA research levy (Project 0047/91), which aim to investigate further the complexities of cross-resistance and the factors which determine the rate of development of resistance to different herbicide types. Greater emphasis is being placed on the aryloxyphenoxpropionate herbicides, such as fenoxaprop-ethyl. This research should improve our ability to devise effective measures, both for the prevention of resistance, and the control of existing resistant populations.

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